Human Cancer Biology

Clinical Cancer Research

Molecular Diagnosis of Activating EGFR Mutations in Non–Small Cell Lung Cancer Using Mutation-Specific Antibodies for Immunohistochemical Analysis

Akihiko Kawahara[1], Chizuko Yamamoto[4], Kazutaka Nakashima[1], Koichi Azuma[2], Satoshi Hattori[3], Masaki Kashihara[4], Hisamichi Aizawa[2], Yuji Basaki[4], Michihiko Kuwano[5], Masayoshi Kage[1], Tetsuya Mitsudomi[6], and Mayumi Ono[4]

Abstract

Purpose: Therapeutic responses of non–small cell lung carcinoma (NSCLC) to epidermal growth factor receptor (EGFR)–targeted drugs, such as gefitinib and erlotinib, are closely associated with activating EGFR mutations. The most common mutations are delE746-A750 in exon 19 and L858R in exon 21, accounting for ~90% of all EGFR mutations. Recently, EGFR mutation-specific antibodies were developed and did well in immunohistochemical analysis, giving a sensitivity of ~90%. We have investigated whether this method detects activating EGFR mutations with sensitivity comparable with direct DNA sequencing, which is used to detect these mutations in NSCLC.

Experimental Design: We used antibodies specific for the E746-A750 deletion mutation in exon 19 and the L858R point mutation in exon 21 in Western blot analysis and immunohistochemistry to determine the presence of these mutations in NSCLC cell lines. We also examined these EGFR mutations in NSCLC tumor samples from 60 patients by immunohistochemically and direct DNA sequencing.

Results: We were able to identify EGFR mutations in NSCLC tumor samples immunohistochemically with a sensitivity of 79% using the anti–delE746-A750 antibody and 83% using the anti-L858R antibody. Additional DNA sequencing markedly improved the sensitivity obtained by immunohistochemistry.

Conclusions: This simple and rapid assay for detecting EGFR mutations, even in the small bronchial biopsies obtained in stage IV NSCLC patients, will be useful for diagnosing responsiveness to EGFR-targeted drugs in patients with NSCLC. Combining this with DNA sequencing is recommended for the development of improved personalized EGFR-targeted therapeutics. *Clin Cancer Res; 16(12); 3163–70. ©2010 AACR.*

Lung cancer is the most common cause of death from cancer worldwide. Non–small cell lung carcinoma (NSCLC) is the major type of lung cancer and is classified into three histologic types: adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (1, 2). Since the introduction of the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib and its approval for clinical use in the treatment of advanced NSCLC (3), a critical question has been how to optimize its therapeutic efficacy in NSCLC patients. Subsequent studies have shown a significant association between the presence of EGFR-activating mutations in lung tumors and their sensitivity to gefitinib and another EGFR tyrosine kinase inhibitor, erlotinib. Most of these mutations occur in exons 18 to 21 in the tyrosine kinase domain, the most common being deletions in exon 19, such as delE746-A750, and the L858R point mutation in exon 21 (4–6). These mutations are found more frequently in female patients, individuals who have never smoked, and patients of East Asian ethnicity (7–11).

Of the various molecular mechanisms that bring about EGFR activation and that affect responses to gefitinib, erlotinib, and other EGFR-targeted drugs (12), activating EGFR mutations, especially delE746-A750 and the L858R point mutation, are closely associated, with favorable clinical outcomes in ~80% of patients with NSCLC, especially in patients from East Asia (13, 14). The delE746-A750 mutation in exon 19 and the L858R mutation in exon 21 are the most common mutations found in NSCLC, accounting for ~90% of all EGFR mutations. The presence

Authors' Affiliations: [1]Department of Diagnostic Pathology, Kurume University Hospital; [2]Division of Respirology, Neurology, and Rheumatology, Department of Internal Medicine, Kurume University School of Medicine; [3]Biostatistics Center, Kurume University, Kurume, Japan; [4]Department of Pharmaceutical Oncology; [5]Laboratory of Molecular Cancer Biology, Department of Pharmaceutics, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan; and [6]Department of Thoracic Surgery, Aichi Cancer Center Research Institute, Nagoya, Japan

Note: A. Kawahara, C. Yamamoto, and K. Nakashima contributed equally to this work.

Corresponding Author: Mayumi Ono, Department of Pharmaceutical Oncology, Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka 812-8582, Japan. Phone: 81-92-642-6296; Fax: 81-92-642-6296; E-mail: mono@phar.kyushu-u.ac.jp.

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Translational Relevance

Activating mutations in the kinase domain of the epidermal growth factor receptor (*EGFR*) gene are critical for determining the therapeutic efficacy of EGFR-targeted drugs for patients with non–small cell lung carcinoma. DNA sequencing of the EGFR tyrosine kinase domain has been used to determine treatment strategies for these patients. Recently, mutation-specific anti-EGFR antibodies recognizing delE746-A750 in exon 19 and L858R in exon 21 have been developed and used immunohistochemically to identify EGFR mutations in cancer cells. The identification of EGFR mutations immunohistochemically and in Western blots is further investigated in this article. Our results suggest that a simple immunohistochemical diagnosis using these antibodies can provide important quantitative and tissue-specific expression data to complement DNA sequence results. We show that the sensitivities of the immunohistochemical and DNA tests are comparable and that the two methods show good correlation in determining the EGFR mutations present in non–small cell lung carcinoma in a Japanese population.

of these activating EGFR mutations is often determined by direct PCR-based sequencing of seven exons of the EGFR tyrosine kinase domain, exons 18 to 24. Yu and colleagues (15) have developed specific antibodies recognizing the delE746-A750 and L858R mutations, which can be used to identify the EGFR status of tumor samples and provide a simple immunohistochemical method for diagnosing EGFR mutations in human tissue. In this study, we have further investigated the use of these mutation-specific antibodies in immunohistochemistry and their application to the diagnostic screening of lung cancer patients and their responsiveness to EGFR-targeted drugs.

Materials and Methods

Cell lines and tissue culture

PC9 and QG56 cells were kindly provided by Dr. Yukito Ichinose (Kyushu Cancer Center, Fukuoka, Japan) and 11-18 cells were kindly provided by Dr. Kazuhiko Nakagawa (Kinki University, Osaka, Japan). LK2 cells were purchased from the Japanese Collection of Research Bioresources, and H1975 and HeLa cells were purchased from the American Type Culture Collection. PC9, QG56, LK2, H1975, and 11-18 cells were cultured in RPMI supplemented with 10% fetal bovine serum. HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum as described previously (16). The cells were maintained under standard cell culture conditions at 37°C in a humid environment in 5% CO_2.

Western blot analysis

Cells were rinsed with ice-cold PBS and lysed in 50 mmol/L HEPES, 150 mmol/L NaCl, 1% Triton X-100, and 10% glycerol containing 5 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL aprotinin, 10 μg/mL leupeptin, and 1 mmol/L sodium orthovanadate (Triton X-100 buffer). Cell lysates were subjected to SDS-PAGE and transferred to Immobilon membranes (Millipore Corp.). After transfer, the membrane was incubated with blocking solution, probed with primary antibodies, and then washed. The primary antibodies were mutation-specific anti-EGFR antibodies recognizing the wild-type (WT) EGFR (D38B1; 16), the delE746-A750 mutation in exon 19 (6B6), and the L858R mutation (43B2) in exon 21 (15), all kindly provided by Cell Signaling Technology. The protein content was visualized using horseradish peroxidase–conjugated secondary antibodies, followed by enhanced chemiluminescence (Amersham).

Tumor samples

We retrospectively examined 45 primary NSCLC adenocarcinomas showing moderate to strong expression of total EGFR that had been completely removed surgically from patients at the Department of Surgery, Kurume University Hospital, between 1995 and 2005 (Kurume tumor samples). We also examined 15 primary NSCLC tumors surgically removed from patients at the Aichi Cancer Center (Nagoya tumor samples).

DNA extraction and direct DNA sequencing

Exon 19 (delE746-A750) and exon 21 (L858R) mutations in the *EGFR* gene were identified by direct DNA sequencing. In brief, genomic DNA was purified from paraffin-embedded tissues using a QIAamp DNA Micro kit (QIAGEN). The exon sequences of the EGFR kinase domain were amplified by nested PCR using specific primers, and exons 19 and 21 were done.

Immunohistochemistry for activating EGFR mutations in cultured lung cancer cells

Cells cultured on slides were rinsed with ice-cold PBS and fixed with 4% paraformaldehyde in PBS for 30 minutes. After fixation, the slides were washed briefly in water and boiled in a microwave for 30 minutes in 1 mmol/L EDTA (pH 9.0) target retrieval solution (DakoCytomation) to recover antigens. Intrinsic peroxidase activity was blocked by treatment with peroxidase-blocking reagent (DakoCytomation) for 5 minutes. After washing in TBS (DakoCytomation) for 5 minutes, primary antibodies, as used for Western blotting, were diluted 1:100 and applied to the cells. The slides were incubated at room temperature for 30 minutes, washed in TBS for 5 minutes, and incubated with labeled polymer–horseradish peroxidase secondary antibody (ChemMate ENVISION Kit, DakoCytomation) for 30 minutes at room temperature. After washing in TBS for 10 minutes, the slides were visualized using 3,3′-diaminobenzidine.

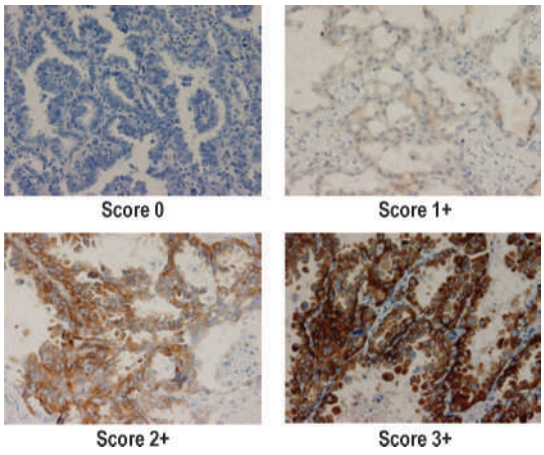

Fig. 1. Immunohistochemical staining and scores for NSCLC adenocarcinoma tumor samples labeled with anti–delE746-A750 EGFR antibody. Staining intensity was scored as 0, 1+, 2+, and 3+ (Materials and Methods).

Immunohistochemistry for activating EGFR mutations in clinical samples from NSCLC patients

Paraffin-embedded tissue samples of human lung cancer tissues were used to cut 4-μm sections, which were mounted on coated glass slides and incubated with the same mutation-specific anti-EGFR antibodies used for Western blotting 4°C overnight. We used DAKO autostainer (DakoCytomation). In evaluating the expression of EGFR mutations as biomarkers, we assumed that the staining intensity of the cancer cell membranes or cytoplasm with the mutation-specific antibodies represented the level of EGFR expression in the cancer specimens. The intensity of staining was scored using the following scale: no staining, 0; weak staining, 1+; moderate staining, 2+; and strong staining, 3+ in >10% of cancer cells (Fig. 1). We classified scores of 0 and +1 as negative and scores of 2+ and 3+ as positive.

Results

Immunocytochemical analysis of activating EGFR mutations in human lung cancer cell line

We first determined whether the mutation-specific antibodies can specifically recognize EGFR mutations in Western blots (Fig. 2) using five human lung cancer cell lines (QG56, LK2, PC9, 11-18, and H1975) and HeLa cells, a cervical cancer cell line. DNA sequence analysis showed that PC9 carried the delE746-A750 in exon 19 of the EGFR, and 11-18 and H1975 carried the L858R mutation in exon 21, whereas the QG56, LK2, and HeLa cell lines carried no EGFR mutations in either of these two exons. In Western blots, five of these cell lines showed comparable levels of EGFR expression when labeled with the control anti-EGFR antibody, but LK2 showed a lower level of expression, consistent with a previous study (16). The deletion-specific antibody recognized the mutant EGFR with a E746-A750 deletion in PC9 cells, whereas the antibody specific for the L858R mutation recognized EGFR in the 11-18 and H1975 cell lines (Fig. 2A). However, the deletion-specific antibody did not recognize EGFR in 11-18 and H1975 cells carrying the L858R mutation, and the antibody specific for the L858R mutation did not recognize EGFR in PC9 cells with the E746-A750 deletion,

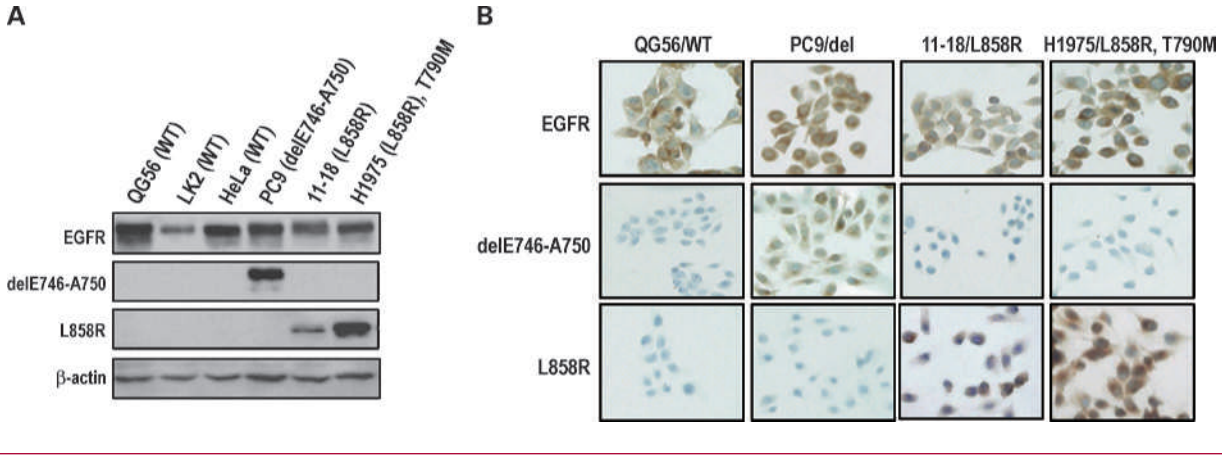

Fig. 2. Identification of the EGFR mutations delE746-A750 and L858R in NSCLC lines. The cervical cancer cell line HeLa was used as a control. A, Western blots showing the expression of EGFR, the delE746-A750 mutation, and L858R mutation in five NSCLC lines (QG56, LK2, PC9, 11-18, and H1975) under normal cell culture conditions. EGFR expression was identified using a control anti-WT EGFR antibody. Anti–delE746-A750 antibody labeled only PC9 carrying an exon 19 deletion. Anti-L858R antibody labeled 11-18 and H1975 cells carrying point mutations in exon 21. Expression of EGFR protein was determined in immunoblots using 100 μg protein of each cell lysate per lane. The loading control used was β-actin. B, immunohistochemical analysis of four NSCLC lines (QG56, PC9, 11-18, and H1975). Anti-WT EGFR antibody stained all cell lines, anti–delE746-A750 antibody stained only PC9 cells, and anti-L858R antibody stained 11-18 and H1975 cells.

Table 1. Immunohistochemistry and DNA sequence analysis of 60 NSCLC tumor samples

Patient no.	DNA sequencing of EGFR mutations	Immunohistochemistry		
		delEGFR	L858R	WT EGFR
1	E746-A750	2+	0	3+
2	E746-A750	3+	0	3+
3	E746-A750	3+	0	3+
4	E746-A750	1+	0	2+
5	E746-T751>A*	3+	1+	3+
6	E746-A750	2+	0	3+
7	E746-A750	2+	0	3+
8	E746-A750	2+	0	2+
9	E746-A750	2+	0	3+
10	E746-A750	1+	0	3+
11	E746-A750	1+	0	3+
12	E746-A750	3+	0	3+
13	S752-I759*	1+	2+	3+
14	L747-T751>P*	1+	1+	3+
15	L858R	0	3+	3+
16	L858R	1+	3+	3+
17	L858R	0	2+	3+
18	L858R	0	3+	3+
19	L858R	0	2+	3+
20	L858R	0	3+	3+
21	L858R	0	1+	2+
22	L858R	0	3+	3+
23	L858R	0	3+	3+
24	L858R	1+	3+	3+
25	L858R	1+	1+	3+
26	L858R	0	3+	3+
27	L858R	0	1+	3+
28	L858R	0	3+	3+
29	L858R	0	2+	3+
30	L858R	0	2+	2+
31	L858R	0	1+	3+
32	L858R	0	2+	3+
33	L858R	0	3+	3+
34	No mutation	0	0	3+
35	No mutation	0	0	3+
36	No mutation	0	0	3+
37	No mutation	0	0	3+
38	No mutation	0	0	3+
39	No mutation	0	0	3+
40	No mutation	0	0	3+
41	No mutation	0	0	3+
42	No mutation	0	0	3+
43	No mutation	0	0	3+
44	No mutation	0	0	3+
45	No mutation	0	0	3+
46	T751-I759>NKA*	2+	0	3+
47	L747-P753>S*	1+	0	2+
48	L747-T751>Q*	0	0	3+
49	L747-A750>P*	1+	0	3+
50	E746-A750	3+	0	3+

(*Continued on the following page*)

Table 1. Immunohistochemistry and DNA sequence analysis of 60 NSCLC tumor samples (Cont'd)

Patient no.	DNA sequencing of EGFR mutations	Immunohistochemistry		
		delEGFR	L858R	WT EGFR
51	E746-A750	3+	0	3+
52	E746-A750	3+	0	2+
53	L858R	0	2+	3+
54	L858R	0	3+	2+
55	L858R	0	3+	3+
56	L858R	0	3+	3+
57	No mutation	0	0	2+
58	No mutation	0	0	3+
59	No mutation	0	1+	3+
60	No mutation	0	0	2+

*Rare exon 19 deletion mutations.

confirming that these two antibodies were specific for the two mutations and would function in Western blots.

We next asked whether these mutation-specific antibodies were able to recognize mutant EGFRs in cultured lung cancer cells in immunohistochemical tests (Fig. 2B). Apparent expression of EGFR was seen in all four lung cancer cell lines, QG56, PC9, 11-18, and H1975, when labeled with the control anti-WT EGFR antibody. The deletion-specific antibody labeled only PC9 cells carrying the delE746-A750 EGFR mutation, whereas the antibody specific for the EGFR point mutation labeled only 11-18 and H1975 cells carrying the L858R mutation. Therefore, Western blotting and immunohistochemical analysis consistently showed that each mutation-specific antibody was able to identify the appropriate EGFR-activating mutation present in lung cancer cell lines.

Immunohistochemical analysis of activating EGFR mutations in NSCLC patients

We investigated EGFR mutation status in NSCLC adenocarcinomas showing moderate to strong total EGFR expression from 45 patients who were treated at the Kurume University Hospital by direct DNA sequence analysis (Table 1, patients 1-45). Deletion mutations in exon 19, including delE746-A750, delE746-T751>A, delS752-I759, and delL747-T751>P, were present in 14 patients, the L858R point mutation in exon 21 was present in 19 patients, and WT EGFR was present in 12 patients (Table 1). We then labeled paraffin-embedded samples of the NSCLC adenocarcinomas immunohistochemically for the EGFR mutations. Figure 3A shows representative images of two examples in each case of cancers carrying the delE746-A750 mutation, the L858R mutation, and WT EGFR. The two cases carrying the delE746-A750 mutation were stained strongly by the anti–delE746-A750 and anti-WT EGFR antibodies but not with the anti-L858R antibody; those carrying the L858R mutation were stained strongly by the anti-L858R and anti-WT EGFR antibodies but not with the anti–delE746-A750 antibody; those carrying only WT EGFR were only stained by the control anti-EGFR antibody (Fig. 3A).

Among the 45 cases of primary NSCLC, 12 samples had been shown to carry delE746-A750 deletion mutation, including a delE746-T751>A mutation (patient 5), and 75% (9 of 12) of these were stained by the deletion-specific antibody with a score of 2+ or 3+. Of the three samples that were identified by DNA sequencing as carrying rare exon 19 deletion mutations (patients 5, 13, and 14), the tumor sample carrying a delS752-I759 mutation (patient 13) and a delL747-T751>P mutation (patient 14) was not positively stained by the delE746-A750–specific antibody (Table 1).

All of the tumor samples from the 19 patients shown to carry the exon 21 L858R point mutation by direct DNA sequencing analysis (patients 15-33) were also stained by the anti-L858R antibody. Fifteen of 19 cases were positively stained with a score of 2+ or 3+ (Table 1). Twelve patients (patients 34-45), whose tumors carried WT EGFR according to DNA sequencing, were not stained by either of the mutation-specific antibodies. As shown in Fig. 3B, in one tumor (patient 23), the cancer cells and bronchial epithelial cells in the sample were strongly stained by the control EGFR antibody, but only the cancer cells were strongly stained by the anti-L858R antibody.

We further investigated whether these two mutation-specific antibodies can be useful for diagnosing rare exon 19 deletion mutations in 15 NSCLC patients who had been treated at the Aichi Cancer Research Hospital (patients 46-60). Paraffin-embedded tissue samples, which included four rare exon 19 deletions (patients 46-49), three delE746-A750 mutations (patients 50-52), four L858R mutations (patients 53-56), and four WT EGFR (patients 57-60), were examined immunohistochemically (Table 1). Of rare exon 19 deletion mutation, L747-P753>S (patient 47), L747-T751>Q (patient 48), and L747-A750>P (patient 49) were negatively stained by the anti–delE746-A750 antibody, and only T751-I759>NKA

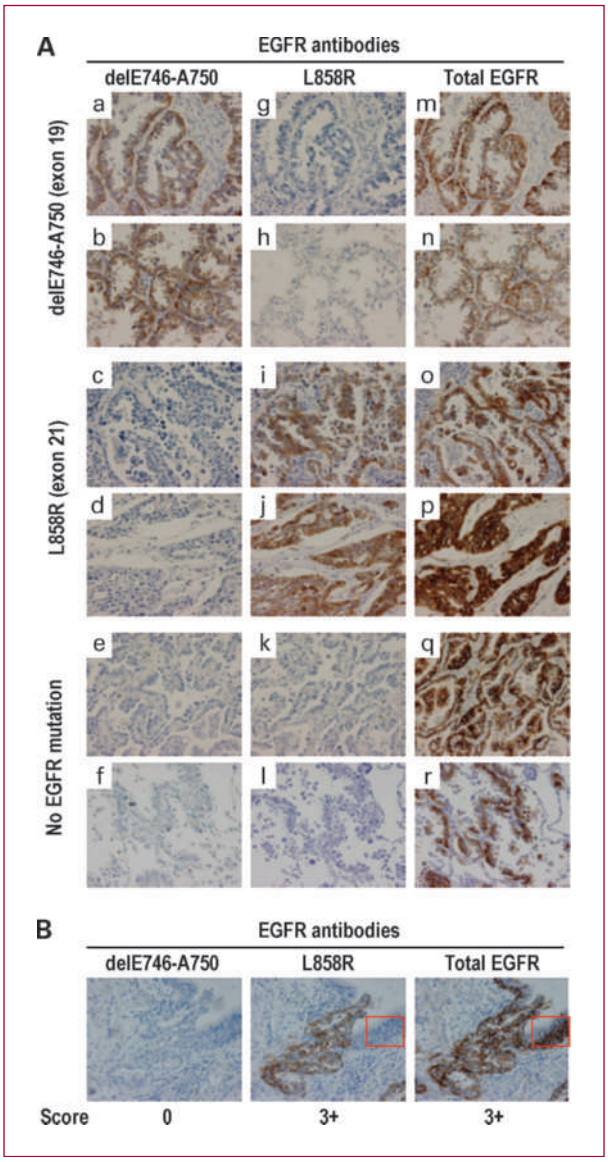

Fig. 3. A, immunohistochemical analysis of human NSCLC tumor samples. Control EGFR antibody stained all six tumor samples shown, the EGFR deletion-specific antibody stained cancer cells only in the two samples with delE746-A750 mutations (a and b), and the L858R-specific antibody stained only the cancer cells in the two samples with L858R mutations (i and j). B, differential diagnosis by immunohistochemical analysis of WT and mutant EGFRs in a NSCLC patient. In one tumor sample (Table 1, patient 23), labeling for total EGFR showed strong EGFR expression in both bronchial epithelial cells (red) and cancer cells. However, labeling with the anti-L858R antibody only stained the cancer cells and did not stain the bronchus.

(patient 46) was moderately stained (+2). None of these four samples carrying deletions were stained by the anti-L858R antibody, but tumor samples carrying L858R mutations (patients 53-56) by DNA sequencing were positively stained by the anti-L858R antibody.

The diagnostic data in Table 1 for EGFR mutations identified by immunohistochemistry are summarized in Table 2. We observed a high correlation between the results from DNA sequencing and immunohistochemistry. When staining +2 and +3 were determined as positive, EGFR mutation-specific antibodies detected delE746-A750 mutations in 79% (11 of 14) of cases identified by DNA sequencing, including patients from Kurume University Hospital and Aichi Cancer Research Hospital, and detected L858R mutations in 83% (19 of 23) of cases, indicating that this type of immunohistochemical analysis would be capable of diagnosing activating EGFR mutations. Thus far, rare exon 19 deletion mutations were examined using the anti–delE746-A750 antibody (Table 2); shorter (patients 14, 48, and 49) or longer (patients 13 and 47) deletion mutations than 15 bp were not positively stained. One (T751-I759>NKA, patient 46) harboring six–amino acid deletion, which was moderately (2+) stained and 1 (E746-T751>A, patient 5) was positively stained. Furthermore, of the samples without these EGFR mutations, immunohistochemistry with the two specific antibodies identified 100% (16 of 16) as negative for the deletion and point mutations in EGFR.

We next investigated whether these two mutation-specific antibodies can be useful in the small bronchial biopsies from stage IV NSCLC patients. Each stage IV patient harboring delE746-A750 or L858R showed strongly positive staining with the anti–delE746-A750 and anti-L858R, respectively (Fig. 4). In contrast, a stage IV patient without EGFR mutations showed strongly positive staining with anti-WT EGFR antibodies but not with both the anti–delE746-A750 and anti-L858R antibody.

Discussion

The ability to selectively administer EGFR-targeted drugs, such as gefitinib and erlotinib, to NSCLC patients carrying activating EGFR mutations is essential to the establishment of personalized anticancer therapy. A recent study has shown favorable clinical outcomes for patients with NSCLC adenocarcinoma carrying EGFR mutations after administering gefitinib compared with cisplatin-paclitaxel (17). The diagnosis of the activating EGFR mutations that are closely associated with the therapeutic efficacy of EGFR-targeted drugs is clearly essential to this strategy. The development of rapid and precise diagnostic techniques for activating EGFR mutations is particularly important for personalizing therapeutics in East Asian patients because these activating EGFR mutations (delE746-A750 and L858R) are significantly more frequent in this ethnic group. These EGFR mutations have been observed in 27.0% NSCLC patients in Japan, 36.8% in China, 19.2% in Korea, and 38.6% in Taiwan (8, 18–20).

The identification of EGFR mutations using mutation-specific antibodies would be a very useful diagnostic method for use in conjunction with DNA sequencing. Yu et al. (15) have generated antibodies specific for delE746-A750 and L858R mutations in EGFR and reported that the sensitivity of immunohistochemical assays using these antibodies was 92% in tests on 340 paraffin-embedded NSCLC tumor samples compared with a sensitivity of

Table 2. Summary of immunohistochemistry and DNA sequence analysis of NSCLC tumor samples

(A) Exon 19 deletions

Immunohistochemistry	DNA sequencing								
	delE746-A750	delE746-T751>A	delS752-I759	delL747-T751>P	delT751-I759>NKA	delL747-P753>S	delL747-T751>Q	delL747-A750>P	WT*
delE746-A750 (+)	11	1	0	0	1	0	0	0	0
delE746-A750 (–)	3	0	1	1	0	1	1	1	16

(B) L858R

Immunohistochemistry	DNA sequencing	
	L858R	WT*
L858R (+)	19	0
L858R (–)	4	16

*The same tumor samples were tested immuhistochemically in (A) and (B).

99% for DNA sequencing. This suggested that this simple immunohistochemical approach can be useful for establishing a rapid, sensitive, and cost-effective method to identify NSCLC patients responsive to EGFR-targeted therapeutics (15). In one tumor sample (patient 23) from the series used in this study, tumor cells, but not normal bronchial epithelial cells, were specifically immunostained with anti-L858R antibody (Fig. 3B), indicating that differential diagnosis between the WT and mutant EGFR in a single pathologic section was possible using this immunohistochemical approach. This result also suggested that a somatic EGFR mutation was present in cancer cells but not in normal cells.

In this study we have confirmed the usefulness of EGFR mutation-specific antibodies for the identification of activating EGFR mutations. The sensitivity of the delE746-A750– and L858R-specific antibodies was found to be 79% to 83% when all samples from Kurume University Hospital and Aichi Cancer Research Hospital were scored.

Several other rare deletion mutations are also known to occur close to the E746-A750 deletion in exon 19 (21). In this study, to further investigate the presence of other EGFR mutations in detail, we also carried out direct DNA sequencing of exons 19 and 20 and confirmed that several deletion mutations occurred close to delE746-A750. We identified rare deletions in exon 19 of the *EGFR*

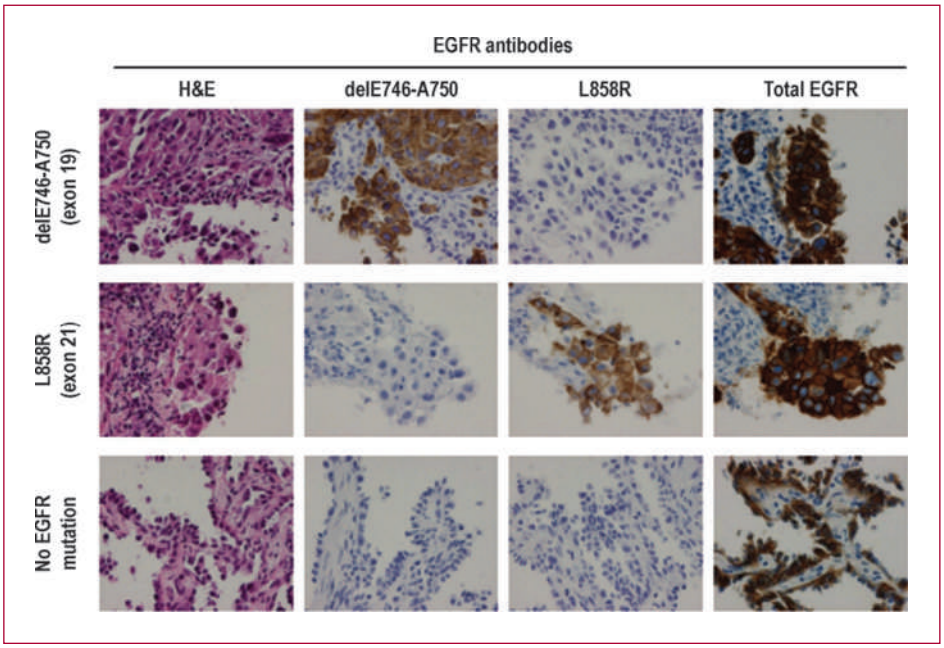

Fig. 4. Immunohistochemical analysis of bronchial biopsy samples of stage IV NSCLC patients. A sample with delE746-A750 mutation was stained with anti delE746-A750–specific antibody, and a sample with L858R mutation was stained with L858R-specific antibody. These samples were stained with WT antibody. No sample without EGFR mutations were stained with these two mutation-specific antibodies. Samples were stained with H&E.

gene in seven tumor samples: one 8–amino acid deletion (S752-I759, patient 13), two 6–amino acid deletion (T751-I759.NKA, patient 46; L747-P753>S, patient 47), one 5–amino acid deletion (E746-T751>A, patient 5), two 4–amino acid deletion (L747-T751>P, patient 14; L747-T751>Q, patient 48), and one 3–amino acid deletion (L747-A750>P, patient 49). Of these seven rare exon 19 deletion mutations, five samples (patients 13, 14, and 47-49) were not positively stained, one sample (patient 46) was moderately stained, and one sample (patient 5) that harbor 5–amino acid deletion with T751A was positively stained by the anti–delE746-A750 antibody (Tables 1 and 2), suggesting that the del E746-A750 antibody may not be useful for identification of these rare exon 19 deletion mutations. Yu et al. (15) also reported rare deletion mutations in exon 19, one of which (E746-T751) was stained by the anti–delE746-A750 antibody, whereas the other (L747-A750) was not. Further refinement of these mutation-specific antibodies will be required to encompass these rare exon 19 deletion mutations and to improve the sensitivity of molecular diagnosis using immunohistochemistry.

Our immunohistochemical data described here for EGFR mutation-specific antibodies suggest that this approach will be very useful for identifying the EGFR mutations that are known to be closely associated with the therapeutic efficacy of EGFR-targeted drugs. One particular merit of immunohistochemical diagnosis is that it provides a measure of the expression levels of mutant EGFRs in the cancer cells in tumors from individual patients. Combining DNA sequencing and immunohistochemistry will be very useful for further refining personalized diagnoses for patients with NSCLC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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