Human Pathology (2008) 39, 316–323

ELSEVIER

Human PATHOLOGY

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Original contribution

Phosphorylation status of epidermal growth factor receptor is closely associated with responsiveness to gefitinib in pulmonary adenocarcinoma

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Received 1 August 2007; revised 26 October 2007; accepted 26 October 2007

Keywords:
Epidermal growth factor receptor;
Gefitinib;
Immunohistochemistry;
Phosphorylation;
Predictive marker

Summary Twenty-one cases of primary lung carcinoma were analyzed for correlations between the presence of somatic mutations of the *epidermal growth factor receptor* (*EGFR*) gene and the phosphorylation status of EGFR, which was analyzed by immunohistochemistry with antibodies recognizing the phosphorylated form of EGFR. Somatic mutations were detected in 11 (52.4%) of the 21 cases. Immunohistochemistry with an antibody recognizing EGFR phosphorylated at tyrosine (pEGFR-tyr) 992 and an antibody recognizing EGFR phosphorylated at tyrosine 1173 (pEGFR-tyr1173) revealed that 12 (57.1%) and 21 (100%) of the 21 cases were positive, respectively. Interestingly, the mutation status of the *EGFR* gene was strongly correlated with immunoreactivity for pEGFR-tyr992 ($P = .0019$). pEGFR-tyr992 immunoreactivity was significantly correlated with clinical responsiveness to gefitinib ($P = .0011$). These findings suggest that immunohistochemical evaluation with anti–pEGFR-tyr992 antibody is useful for prediction of responsiveness to gefitinib.

1. Introduction

Lung cancer is the leading cause of cancer-related death, accounting for 1.18 million deaths annually worldwide [1]. The overall 5-year survival rate after diagnosis of lung cancer is only 15% [2]. This may be largely due to the practical difficulties in diagnosing lung cancer at an early stage. In addition, even at an early stage, some tumors will already have acquired potential for invasion or systemic metastasis. Therefore, advances in multidisciplinary therapy, especially chemotherapy, are essential in order to improve the survival of patients with lung cancer.

Gefitinib is an orally active molecular-targeted drug that inhibits epidermal growth factor receptor (EGFR)–dependent growth of cancer cells in patients with non–small cell

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0046-8177/$ – see front matter © 2008 Published by Elsevier Inc.
doi:10.1016/j.humpath.2007.10.027

lung carcinoma (NSCLC). It is known to be a competitive inhibitor of the adenosine triphosphate–binding cleft within the tyrosine kinase domain of EGFR [3]. Although gefitinib was initially considered to be more effective and less toxic than conventional cytotoxic agents, recent phase III clinical trials [4-6] have failed to demonstrate its effectiveness in patients with NSCLC overall. Furthermore, as severe adverse events of gefitinib such as interstitial lung disease have been reported [7], its administration has become restricted. However, some patients who have certain personal or histologic characteristics, such as being Asian, female, a nonsmoker, and diagnosed as having adenocarcinoma, are reportedly responsive to gefitinib [8-11]. Furthermore, recent reports have stressed that patients with somatic mutations in the *EGFR* gene have exhibited good responsiveness to gefitinib [12-18]. Mitsudomi et al [15] showed that gefitinib was effective in 83% of patients with *EGFR* mutations, whereas it was effective in only 10% of patients without *EGFR* mutations ($P < .0001$). Similarly, Taron et al [18] showed that gefitinib was effective in 94.1% of patients with *EGFR* mutations, compared with only 12.6% of patients without *EGFR* mutations ($P < .0001$). These findings strongly suggest that it is possible to select patients who would be responsive to gefitinib according to whether they have *EGFR* gene mutation.

In this study, we analyzed 20 cases of primary pulmonary adenocarcinoma and 1 case of adenosquamous carcinoma for the phosphorylation status of EGFR protein and the mutation status of the *EGFR* gene. Immunohistochemistry with antibodies specifically recognizing the phosphorylated form of EGFR revealed that positive immunoreactivity for EGFR phosphorylated at tyrosine (pEGFR-tyr) 992 was significantly correlated with the presence of mutation of the *EGFR* gene, suggesting that immunohistochemistry with the anti–pEGFR-tyr992 antibody may be a convenient tool for predicting the presence of EGFR gene mutation.

2. Materials and methods

2.1. Patients and tissues

Tissue samples were obtained from 21 Japanese patients who underwent surgical resection for primary lung carcinoma at Oita University Hospital or Oita Prefectual Hospital. The specimens were routinely fixed in 10% formalin and embedded in paraffin. Serial sections cut out from paraffin-embedded blocks were used for routine histopathology, mutation analysis of the *EGFR* gene, and immunohistochemistry.

Clinical and pathologic data for the patients are shown in Table 1. The median age of the 21 patients was 71 years, ranging from 45 to 78 years, and the male-female ratio was 11/10. Nine patients were current or former smokers. Pathologically, 8, 2, 10, and 1 of the 21 cases were categorized into stage I, II, III, and IV, respectively. Of the total 21 cases, 20 were histopathologically diagnosed as adenocarcinoma, and the remaining 1 case was diagnosed as

Table 1 Patient characteristics of clinical and histologic analysis

No.	Age/ sex	Smoking	Stage	Histology (histologic subtype)	PS	Recurrent site		Response to gefitinib
						Lung	Others	
1	75/F	−	pT2N0M0, IB	AdSq (solid > acinar)	2	+	+	PR
2	76/F	−	pT1N0M0, IA	Ad (papillary > acinar)	1	+	−	PR
3	63/F	−	pT2N1M0, IIB	Ad (papillary > acinar > BAC)	1	−	+	PR
4	71/F	−	pT1N2M0, IIIA	Ad (papillary > acinar)	0	+	+	PR
5	64/M	−	pT4N2M0, IIIB	Ad (papillary > acinar)	0	+	−	PR
6	77/F	−	pT2N0M0, IB	Ad (papillary > BAC > acinar)	0	+	−	PR
7	72/F	−	pT1N0M0, IA	Ad (papillary > acinar > BAC)	0	+	−	PR
8	64/M	−	pT2N0M0, IB	Ad (papillary > acinar)	0	+	+	PR
9	59/M	−	pT4N2M0, IIIB	Ad (papillary > BAC > acinar)	2	+	+	PR
10	76/M	+	pT2N0M0, IB	Ad (BAC > acinar)	1	+	+	SD
11	45/F	+	TxN3M0, IIIB	Ad (papillary > solid)	1	−	+	SD
12	75/F	−	pT3N2M0, IIIA	Ad (papillary > acinar > BAC)	2	+	−	SD
13	47/M	+	pT1N2M0, IIIA	Ad (acinar > papillary)	1	−	+	SD
14	77/M	+	pT1N0M0, IA	Ad (papillary)	2	−	+	SD
15	49/F	−	pT4N2M0, IIIB	Ad (papillary)	0	+	+	SD
16	77/M	+	pT1N0M0, IA	Ad (papillary)	3	−	+	PD
17	71/M	+	pT3N0M0, IIB	Ad (papillary > acinar)	2	+	−	PD
18	51/M	+	pT4N2M0, IIIA	Ad (solid > acinar)	1	+	+	PD
19	76/M	+	pT4N2M0, IIIB	Ad (solid > acinar)	2	−	+	PD
20	78/M	+	pT2N2M1, VI	Ad (papillary > acinar)	1	+	−	PD
21	51/F	−	pT1N2M0, IIIA	Ad (papillary > acinar)	1	+	+	PD

Abbreviations: F, female; M, male; AdSq, adenosquamous carcinoma; Ad, adenocarcinoma; BAC, bronchioloalveolar carcinoma; PS, performance status.