ORIGINAL ARTICLE

Increased risk of recurrence in resected *EGFR*-positive pN0M0 invasive lung adenocarcinoma

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Abstract

Background: This study was conducted to evaluate the prognostic and recurrent impact of *EGFR* mutation status in resected pN0M0 lung adenocarcinoma with consideration of the histological subtype.

Methods: Following retrospective analysis of whole 474 consecutive pathological N0M0 lung adenocarcinoma patients, the prognostic significance of *EGFR* mutation status was evaluated in limited 394 subjects. Overall survival and recurrence-free interval (RFI) were estimated using the Kaplan–Meier method and compared using a log-rank test. Univariate and multivariate analyses were performed using Cox proportional hazard models.

Results: The five-year RFI was 85.7% and 93.3% for *EGFR* positive ($n = 176$) and negative ($n = 218$) cases, respectively (hazard ratio [HR] 1.992, 95% confidence interval [CI] 1.005–3.982; $P = 0.048$). Following the exclusion of specific subtypes free from recurrence or *EGFR* mutation (adenocarcinoma in situ, minimally invasive adenocarcinoma, and invasive mucinous adenocarcinoma), the five-year RFI was obviously poorer in *EGFR* positive compared to negative cases (80.7% and 92.1%, respectively; HR 2.163, 95% CI 1.055–4.341; $P = 0.035$). Multivariate analysis excluding the specific subtypes confirmed that male sex, age, current or Ex-smoking status, pleural invasion, and *EGFR*-positive status were independently associated with shorter RFI. No significant differences in five-year overall survival were found between the *EGFR* mutation positive and negative groups (88.7% and 93.7%, respectively; HR 1.630, 95% CI 0.787–3.432; $P = 0.2$).

Conclusion: *EGFR* mutations are associated with recurrence in pN0M0 lung adenocarcinoma. *EGFR* mutation status and histological subtype should be considered when evaluating the risk of recurrence in resected lung adenocarcinoma patients.

Introduction

EGFR mutations represent one of the most frequent genetic aberrations in lung adenocarcinoma. The frequency in Asia is reported at 40–60%.[1,2] Somatic mutations in *EGFR* are more likely to occur in Asian, female, and adenocarcinoma patients.[1,3] A number of studies have estimated the prognostic impact of *EGFR* mutation in resected non-small cell lung cancer (NSCLC) patients; however, most of them included several types of histology and advanced stage. The prognostic significance of *EGFR*

mutations as oncogenic driver mutations in resected pN0M0 lung adenocarcinoma is yet to be determined. Herein, we evaluated the oncological consequences of *EGFR* mutations in pN0M0 lung adenocarcinoma. In addition to overall survival (OS), the recurrence-free interval (RFI) was utilized to estimate the oncological impact of *EGFR* mutation on recurrence. By using the RFI, death unrelated to lung cancer and the therapeutic effect of *EGFR*-tyrosine kinase inhibitors (TKIs) were excluded.

Methods

Study design

We performed a retrospective cohort study of consecutive patients with pN0M0 lung adenocarcinoma at a single institution. Patient medical records were reviewed, and clinicopathological data including *EGFR* mutation status were obtained. The frequency of *EGFR* mutations was assessed in relation to different clinicopathological characteristics. The impact of *EGFR* mutation status on OS and RFI was evaluated in resected pN0M0 lung adenocarcinomas in all cases, as well as in a limited subset. In analysis of the limited subset, adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and invasive mucinous adenocarcinoma (IMA) were excluded as there is no risk of recurrence in AIS/MIA and *EGFR* mutation does not occur in IMA.

The effects of *EGFR* mutation status on OS and RFI were initially estimated in all 394 tumors. Subsequently, OS and RFI were calculated for specific histological subtypes by considering the risk of recurrence and positive *EGFR* mutation status. After excluding AIS, MIA, and IMA, the analyzed cases included those ≤ 5 cm in tumor diameter and classified as pathological stage IA1–IIA, according to the International Association for the Study of Lung Cancer (IASLC) tumor node metastasis (TNM) classification system (8th edition).[4] All included cases underwent complete resection. Pathological diagnoses were conducted by two pathologists according to IASLC/American Thoracic Society (ATS)/European Respiratory Society (ERS) classification.[5] If multiple tumors were resected in a single patient, they were identified as intrapulmonary metastases or independent tumors according to the latest IASLC proposals.[6,7] In cases where tumors were diagnosed independently of one another, each was considered a primary tumor. Observations of non-relapsed tumors were censored at the time of recurrence in patients with multiple primary tumors.

The Institutional Review Board of Hiroshima University (Hiroshima, Japan) approved the retrospective design of this study for utilizing resected specimens and collecting and analyzing patient data (E-12). All study participants provided informed written consent. The research was conducted in accordance with the 1964 Declaration of Helsinki and its later amendments.

Patients

The data of 474 consecutive lung adenocarcinoma patients who underwent surgical resection for pN0M0 disease at the Hiroshima University Hospital (Hiroshima, Japan) between January 2007 and December 2013 were retrospectively analyzed. The records of patients who underwent surgical resection with therapeutic intent were reviewed, and only completely resected cases were enrolled in the study. In cases treated with lobectomy (*n* = 232) or segmentectomy (*n* = 94), the lack of lymph node metastasis was confirmed by systematic or lobe-specific lymphadenectomy. In cases where lymph node metastasis was not suspected by preoperative computed tomography (CT), positron-emission tomography (PET)-CT, and curative resection by wedge resection was deemed achievable intraoperatively, lymph node resection was omitted. Regardless of whether evaluation was performed by an external examination body or the institutional laboratory, *EGFR* mutation status was determined using a peptide nucleic acid (PNA)-locked nucleic acid (LNA) PCR clamp-based detection test as previously described.[8] *EGFR* mutation status was usually established during the clinical course by an external examination body (LSI Medience Corp., Tokyo, Japan) with specimens obtained from bronchoscopic biopsy, frozen tissue, or formalin-fixed paraffin-embedded (FFPE) tissue. If *EGFR* mutation was not evaluated during the clinical course, DNA was extracted from frozen tissue sections using a QIAamp DNA Micro Kit or from FFPE tissue sections using a QIAamp DNA FFPE Tissue Kit (Qiagen GmbH, Hilden, Germany) in the institutional laboratory. The PNA-LNA PCR clamp-based detection test was conducted using a 7900HT Fast Real-Time PCR System (Thermo Fisher Scientific Inc., Waltham, MA, USA). Primers and probes were supplied by Integrated DNA Technologies Inc. (Coralville, IA, USA). Details of the PCR conditions and oligonucleotide sequences of each primer and probe are provided in Supplementary Table S1.

Statistical analyses

Overall survival and RFI were used to evaluate the oncological significance of *EGFR* mutation status. OS was defined as the interval between the date of surgery and the date of death from any cause. RFI was defined as the interval between the date of surgery and the date of radiologically detected recurrence. Patients who died from causes other than lung cancer were censored from RFI analysis. OS and RFI were estimated using the Kaplan–Meier method, and differences between survival curves were analyzed using the log-rank test. The significance of frequencies was evaluated by chi-square test. Patient age and pathological tumor size were compared as continuous variables using Mann–Whitney *U* tests. Univariate analyses were built with *EGFR* mutation status and clinicopathological factors regarding prognosis, and multivariate analysis was conducted using a Cox proportional hazards model with a backward stepwise procedure. *P* values calculated in two-tailed tests were employed and < 0.05 was considered

Table 1 Clinicopathological characteristics of the enrolled lung adenocarcinoma cases (*n* = 394)

Clinicopathological characteristic	Cases (*n* = 394)
Age, years	
Median	67
Interquartile range	14.0
Sex, N (%)	
M	195 (49.5)
F	199 (50.5)
Smoking status, N (%)	
Current or Ex-smoker	182 (46.2)
Never-smoker	212 (53.8)
Surgical procedure, N (%)	
Lobectomy	232 (58.9)
Segmentectomy	94 (23.9)
Wedge resection	68 (17.3)
Pathological tumor size, N (%)	
≤ 1.0 cm	77 (19.5)
> 1.0 and ≤ 2.0 cm	163 (41.4)
> 2.0 and ≤ 3.0 cm	97 (24.6)
> 3.0 cm	57 (14.5)
Predominant subtype, N (%)	
AIS	77 (19.5)
MIA	27 (6.9)
Lepidic	67 (17.0)
Papillary	177 (44.9)
Acinar	17 (4.3)
Solid	14 (3.6)
Micropapillary	4 (1.0)
IMA	11 (2.8)
EGFR mutation status, N (%)	
Positive	176 (44.7)
Negative	218 (55.3)
EGFR mutant variants, N (%)	176 (100)
Ex18 MUT	11 (6.3)
Ex19 DEL	68 (38.6)
Ex21 MUT	95 (54.0)
L858R	92 (52.3)
L861Q	3 (1.7)
Double mutation	2 (1.1)
Ex18 MUT and L858R	1 (0.6)
Ex19 DEL and L858R	1 (0.6)
Pleural invasion, N (%)	
Y	53 (13.5)
N	341 (86.5)
Lymphatic invasion, N (%)	
Y	35 (8.9)
N	359 (91.1)
Vascular invasion, N (%)	
Y	50 (12.7)
N	344 (87.3)
Recurrence, N (%)	
Y	33 (8.4)
N	361 (91.6)

AIS, adenocarcinoma in situ; DEL, deletion; Ex, exon; F, female; IMA, invasive mucinous adenocarcinoma; L, leucine; M, male; MIA, minimally invasive adenocarcinoma; MUT, mutation; N, no; Q, glutamine; R, arginine; Y, yes.

significant. Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA) and StatMate V (ATMS Co., Ltd., Tokyo, Japan).

Results

The CONSORT diagram of the study is shown in Figure 1. Of the 474 lung adenocarcinoma cases reviewed, 80 were excluded according to the following criteria: intrapulmonary metastases (*n* = 18); parietal pleural invasion (*n* = 14); specimen unavailability (*n* = 14); post neoadjuvant chemotherapy/radiotherapy (*n* = 13); loss during postoperative follow-up (*n* = 5); accompanied by another histological component (*n* = 5); incomplete resection (*n* = 3); proven to be recurrence (*n* = 2); simultaneously administered therapies for carcinoma of another organ (*n* = 2); potential to be a non-primary lung adenocarcinoma (*n* = 2);
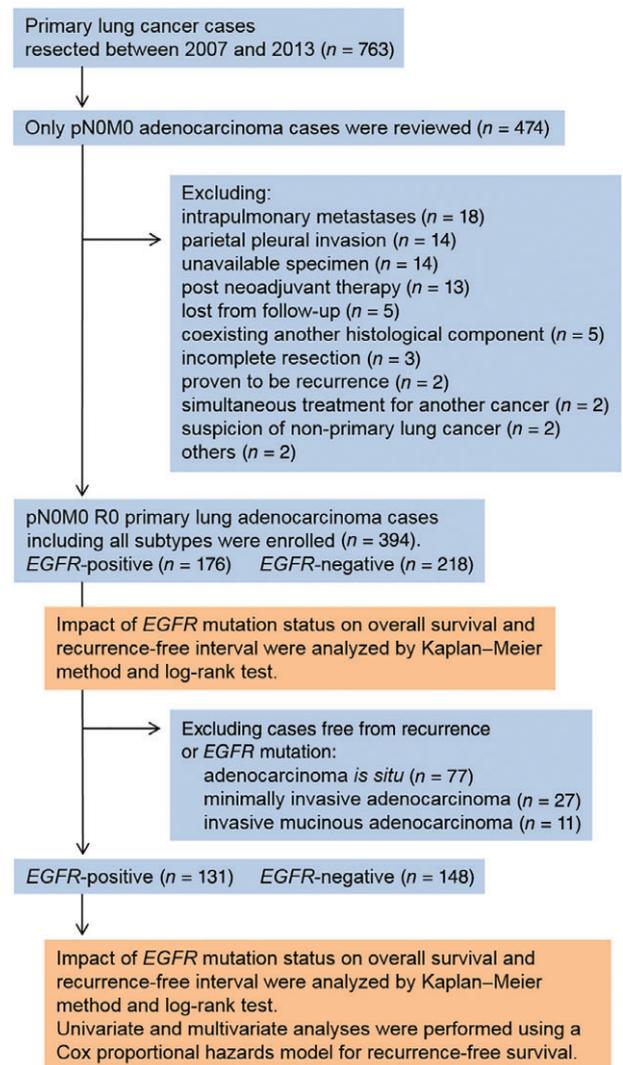

Figure 1 Consort diagram of the study.

recurrence of uncertain origin because of repeated surgery for several types of cancer (*n* = 1); and lung pleomorphic carcinoma occurring within a short interval after lung adenocarcinoma resection (*n* = 1).

Twenty-six patients underwent resection for multiple lung adenocarcinomas (55 tumors in total) that were diagnosed as independent primary adenocarcinomas. Among patients with multiple lung adenocarcinomas, three tumors recurred, including one at the surgical margin and two intrapulmonary recurrences. In the latter two cases, the resected primary tumors were invasive adenocarcinoma and AIS or MIA. Therefore, we determined that invasive carcinoma was responsible for the recurrence. In the whole cohort, 33 patients recurred: local (lung or intrapulmonary lymph node) in 15, regional (mediastinal or intrapleural region) in 12, and distant (extra-thoracic lymph node or another organ) in 6 patients.

The prognostic impact of *EGFR* mutation status was evaluated in 394 tumors from 365 patients. The median follow-up was 50.4 months (interquartile range [IQR] 28.1 months). A summary of the clinicopathological characteristics of the 394 cases enrolled in this study is provided in Table 1. *EGFR* mutation status was positive in 176 cases (44.7%). The frequency of *EGFR* mutation positivity was significantly higher in female patients; nonsmokers; patients with larger tumors (median 2.0 cm [IQR 1.2 cm] vs. 1.6 cm [IQR 1.3 cm] for *EGFR* positive and negative cases, respectively); papillary predominant subtypes; and cases accompanied by a lepidic or micropapillary component. Conversely, *EGFR* mutation positive status was observed significantly less frequently in the acinar and solid predominant histological subtypes. None of the IMA cases harbored an *EGFR* mutation (Table 2). The frequencies of *EGFR* mutations in different histological subtypes are summarized in Figure 2. Among the *EGFR* mutation positive cases, the proportions with an exon 19 deletion and L858R mutation were 39.2% (*n* = 69) and 53.4% (*n* = 94), respectively (Table 1).

The overall five-year OS was 88.7% and 93.7% for the *EGFR* positive and negative cases, respectively (hazard ratio [HR] 1.630, 95% confidence interval [CI] 0.787–3.432; *P* = 0.2). The overall five-year RFI was 85.7% and 93.3% for the *EGFR* positive and negative cases, respectively (HR 1.992, 95% CI 1.005–3.982; *P* = 0.048).

After excluding AIS, MIA, and IMA cases, the five-year OS was 86.3% and 94.1% for *EGFR* positive and negative cases, respectively (HR 1.503, 95% CI 0.685–3.312; *P* = 0.3). The five-year RFI was 80.7% and 92.1% for *EGFR* positive and negative cases, respectively (HR 2.163, 95% CI 1.055–4.341; *P* = 0.035) (Fig 3).

Univariate analysis after excluding AIS, MIA, and IMA cases identified age, pathological tumor size (cm), a highly malignant subtype (micropapillary/solid predominant

adenocarcinoma), pleural/lymphatic/vascular invasion, and positive *EGFR* mutation status as significantly associated with shorter RFI. Multivariate analysis confirmed that male sex (HR 0.273, 95% CI 0.087–0.860; *P* = 0.027), age (HR 1.078, 95% CI 1.032–1.125; *P* = 0.001), current or Ex-Smoking status (HR 3.056, 95% CI 1.087–8.590; *P* = 0.034), pleural invasion (HR 5.454, 95% CI 2.250–13.222; *P* < 0.001), and positive *EGFR* mutation status (HR 2.607, 95% CI 1.042–6.523; *P* = 0.041) were independently associated with poor RFI (Table 3).

Discussion

EGFR mutations represent one of the major somatic mutations in lung adenocarcinoma and are a therapeutic target in advanced lung adenocarcinoma. Several randomized phase III trials have demonstrated their clinical advantage over cytotoxic chemotherapy agents.[9,10] However, the oncological significance of *EGFR* mutation status in stage I lung adenocarcinoma is controversial.[11] We retrospectively reviewed completely resected lung adenocarcinoma cases and evaluated the prognostic significance of *EGFR* mutation status with respect to specific histological features.

Although previous studies have shown that *EGFR* mutations are of significant prognostic value in lung adenocarcinoma, these studies included various histologic subtypes or pStage.[12–18] Systematic meta-analyses have shown that *EGFR* mutation status is not a prognostic factor in patients with surgically resected NSCLC.[11] Kobayashi *et al.* demonstrated that positive *EGFR* mutation status correlates with growth in early-stage lung adenocarcinoma cases with a ground-glass component of ≥ 50%.[19] This report supports the unfavorable effects of *EGFR* mutation status in early-stage lung adenocarcinoma. However, studies limited to stage I cases indicated better prognostic tendencies in patients with *EGFR* mutations[20–22] which contrasts with our results. The discrepancy can be explained as follows. The frequency of *EGFR* mutations is higher in adenocarcinoma with a concomitant lepidic component (formerly known as a bronchioloalveolar carcinoma component).[23–25] Previous studies did not consider histological subtypes or negative prognostic factors, such as pleural, lymphatic, and vascular invasion in their analyses.[12,20–22,26,27] AIS and MIA are constantly accompanied by the lepidic component and their distributions is higher in stage I. Thus, *EGFR* mutation in stage I adenocarcinoma is likely to be AIS or MIA. On the contrary, *EGFR* wild-type tumors are likely to be invasive adenocarcinoma cases without a lepidic component, and therefore, more often accompanied by pleural and/or lymphovascular invasion. Without considering histological subtypes, comparing *EGFR* mutation positive to wild-type cases is akin to comparing noninvasive or preinvasive cases to invasive tumors. In a cohort with extremely

Table 2 Clinicopathological characteristics of the enrolled lung adenocarcinoma cases (*n* = 394) according to *EGFR* mutation status

Clinicopathological characteristic	Cases (*n* = 394)	*EGFR* mutation status		*P*
		Positive (*n* = 176)	Negative (*n* = 218)	
Age, years				
Median	67	67	66	
Interquartile range	14.0	13.0	13.25	0.5
Sex, N (%)				
M	195	75 (42.6)	120 (55.0)	
F	199	101 (57.4)	98 (45.0)	0.01*
Smoking status, N (%)				
Current or Ex-smoker	182	68 (38.6)	114 (52.3)	
Never-smoker	212	108 (61.4)	104 (47.7)	0.007*
Surgical procedure, N (%)				
Lobectomy	232	110 (62.5)	122 (56.0)	0.2
Segmentectomy	94	42 (23.9)	52 (23.9)	1.0
Wedge resection	68	24 (13.6)	44 (20.2)	0.09
Pathological tumor size, cm				
Median	1.85	2.0	1.6	
Interquartile range	1.4	1.2	1.3	0.03*
Pathological tumor size, N (%)				
≤ 1.0 cm	77	27 (15.3)	50 (22.9)	0.06
> 1.0 and ≤ 2.0 cm	159	68 (38.6)	91 (41.7)	0.5
> 2.0 and ≤ 3.0 cm	98	50 (27.9)	48 (22.0)	0.1
> 3.0 cm	60	31 (17.6)	29 (13.3)	0.2
Predominant subtype, N (%)				
AIS	77	33 (18.8)	44 (20.2)	0.7
MIA	27	12 (6.8)	15 (6.9)	1.0
Lepidic	67	30 (17.0)	37 (17.0)	1.0
Papillary	177	94 (53.4)	83 (38.1)	0.002*
Acinar	17	3 (1.7)	14 (6.4)	0.04*
Solid	14	2 (1.1)	12 (5.5)	0.04*
Micropapillary	4	2 (1.1)	2 (0.9)	0.8
IMA	11	0 (0.0)	11 (5.0)	0.007*
Lepidic component, N (%)				
Y	309	149 (84.7)	160 (73.4)	
N	85	27 (15.3)	58 (26.6)	0.007*
Micropapillary component, N (%)				
Y	85	49 (27.8)	36 (16.5)	
N	309	127 (72.2)	182 (83.5)	0.007*
Solid component, N (%)				
Y	37	12 (6.8)	25 (11.5)	
N	357	164 (93.2)	193 (88.5)	0.1
Mucinous component, N (%)				
Y	51	10 (5.7)	41 (18.8)	
N	343	166 (94.3)	177 (81.2)	< 0.001*
Pleural invasion, N (%)				
Y	53	19 (10.8)	34 (15.6)	
N	341	157 (89.2)	184 (84.4)	0.2
Lymphatic invasion, N (%)				
Y	35	14 (8.0)	21 (9.6)	
N	359	162 (92.0)	197 (90.4)	0.6
Vascular invasion, N (%)				
Y	50	23 (13.1)	27 (12.4)	
N	344	153 (86.9)	191 (87.6)	0.8
Recurrence, N (%)				
Y	33	19 (10.8)	14 (6.4)	
N	361	157 (89.2)	204 (93.6)	0.12

*P < 0.05. AIS, adenocarcinoma in situ; F, female; IMA, invasive mucinous adenocarcinoma; M, male; MIA, minimally invasive adenocarcinoma; N, no; Y, yes
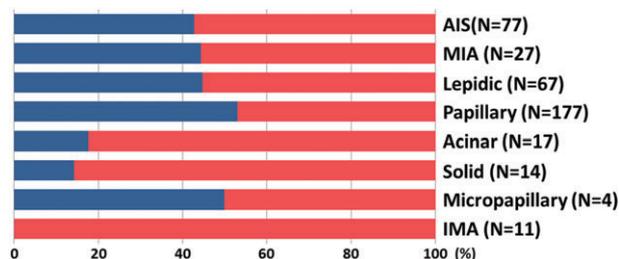
Figure 2 Frequency of *EGFR* mutation status according to predominant histological subtype. *EGFR* positive cases are represented by the blue bar, and *EGFR* negative by the red bar. (■) positive, and (■) negative.

high five-year OS of 98% and a low rate of *EGFR* mutations (20.2%),[20] most cases harboring *EGFR* mutations might be AIS and MIA. AIS and MIA were first described in the IASLC/ATS/ERS classification[5] and are free from recurrence after complete resection.[28,29] They must be considered separate from invasive adenocarcinoma as so in the newly revised TNM staging: Tis (AIS) and T1a (mi) (MIA).[4] When evaluating recurrence risk in pN0M0 cases, it is important to exclude cases that are curable by complete resection. IMA should likewise be excluded from such analyses because this subtype is a variant of invasive adenocarcinoma[5] that is moderately to highly

Figure 3 Kaplan–Meier curves for overall survival (OS) and recurrence-free interval (RFI) according to *EGFR* mutation status. Confidence limits are shown as colored shaded areas. (**a**) OS and (**b**) RFI curves of all histological subtypes (*n* = 394). (**c**) OS and (**d**) RFI curves of specific subtypes, excluding adenocarcinoma in situ, invasive mucinous adenocarcinoma, and minimally invasive adenocarcinoma (*n* = 279). (**a**) (——) Negative 93.7%, (——) Positive 88.7%, p = 0.2, (**b**) (——) Negative 93.3%, (——) Positive 85.7% p = 0.048, (**c**) (——) Negative 94.1%, (——) Positive 86.3% p = 0.3, (**d**) (——) Negative 92.1%, and (——) Positive 80.7% p = 0.035.
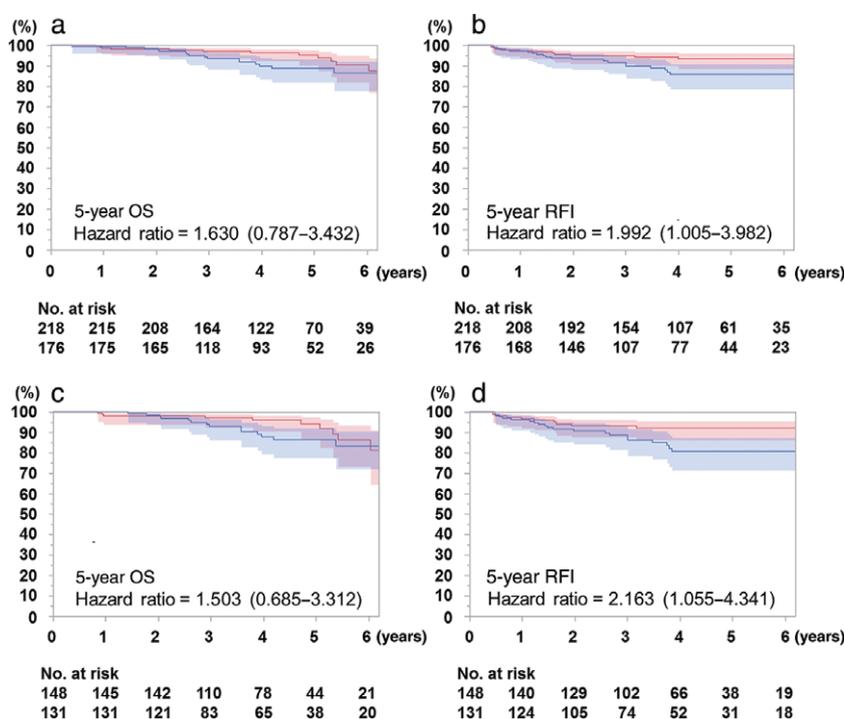

Table 3 Univariate and multivariate analyses of recurrence-free interval in pN0M0 lung adenocarcinoma cases, excluding AIS, MIA, and IMA (*n* = 279)

Prognostic factor	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Male sex	0.616 (0.302–1.257)	0.183	0.273 (0.087–0.860)	0.027*
Age	1.063 (1.020–1.108)	0.004*	1.078 (1.032–1.125)	0.001*
Current or ex-smoking history	1.025 (0.507–2.074)	0.945	3.056 (1.087–8.590)	0.034*
Pathological tumor size	2.082 (1.437–3.015)	< 0.001*	1.513 (0.966–2.370)	0.071
High malignancy subtype	3.405 (1.305–8.882)	0.012*	0.984 (0.301–3.220)	0.979
Sublobar resection	0.633 (0.273–1.470)	0.287	1.071 (0.391–2.930)	0.894
Pleural invasion	7.599 (3.716–15.539)	< 0.001*	5.454 (2.250–13.222)	< 0.001*
Lymphatic invasion	3.471 (1.580–7.624)	0.002*	0.745 (0.279–1.985)	0.556
Vascular invasion	5.342 (2.573–11.093)	< 0.001*	2.412 (0.991–5.874)	0.052
Positive *EGFR* mutation status	2.165 (1.037–4.519)	0.040*	2.607 (1.042–6.523)	0.041*

*P < 0.05. AIS, adenocarcinoma in situ; CI, confidence interval; HR, hazard ratio; IMA, invasive mucinous adenocarcinoma; MIA, minimally invasive adenocarcinoma.

malignant[30,31] but does not harbor *EGFR* mutations.[30,32,33] Cases with relatively high malignancy that do not harbor *EGFR* mutations are not suitable for inclusion in analyses of the prognostic impact of *EGFR* mutation status. In fact, the proportion of *EGFR* mutation-positive cases among AIS and MIA were high, while the recurrence rate of IMA was not particularly low (*n* = 2, 18.2%) in this study. Thus, the prognostic significance of *EGFR* mutations is underestimated unless AIS, MIA, and IMA are excluded. The more IMA cases included, the poorer the OS and RFI of the *EGFR* mutation-negative cohort.

Apart from the histological distinctions, another reason for the discrepancy in results between previous studies and our study is the different methodology employed to estimate the prognostic impact. We employed OS and RFI as prognostic parameters. In the latter, non-cancer-specific deaths were censored. RFI censoring of deaths from causes other than lung cancer is preferable to directly estimate oncological impact. OS usually includes death from a non-cancer-specific event. A previous study included six patient deaths as prognostic events for OS[15] however, five of them were *EGFR* wild-type carriers who died without experiencing recurrence. Additionally, OS is generally improved in *EGFR* mutation-positive cases after receiving EGFR-TKIs.[13,34] Previous studies concluded that *EGFR* mutation is a favorable prognostic indicator and suggest that OS is prolonged by EGFR-TKIs.[14–16,22] In summary, the distribution of *EGFR* mutation and potential curability varies considerably among sub-histologies. The therapeutic effect of TKIs must be excluded to precisely estimate the prognostic impact of *EGFR* mutation. Thus, we report contradictory results on the prognostic impact of *EGFR* mutation compared to those in published literature.

In addition to distinguishing histological subtypes and utilizing cancer-specific parameters, ethnic characteristics may also account for our results. The frequency of *EGFR* mutations is reported to be higher among Asian populations; some studies have reported incidence as high as 40–60%.[1,2] Conversely, the frequency of *EGFR* mutations in non-Asian populations is reported to be lower, at 10–20%.[35,36] Among the latter populations, the significance of *EGFR* mutation status may be weaker, and another somatic mutation or a combination of several genetic aberrations may be important to estimate prognosis. Thus, ethnicities should also be taken into account in such analyses.

This study has some limitations, including its retrospective, single-institutional design and limited sample size. The inclusion of cases with multiple tumors or those treated by wedge resection might be also a weakness of this study. Although second primary tumors and metastases in cases with multiple tumors were diagnosed according to the IASLC proposals, the absolute methodology with solid evidence to distinguish second primary from metastasis

has not yet been established. In all wedge resection cases, although the potential of metastatic lymph nodes was excluded intraoperatively as well as preoperative CT/PET-CT, pathological confirmation by systematic or lobe-specific lymphadenectomy is preferred. Large-scale prospective studies examining several genetic variations at multiple institutions are therefore warranted. As a clinical background, patients undergoing lobectomy or segmentectomy who have undergone adequate lymph node dissection are preferable.

In conclusion, *EGFR* mutations are associated with recurrence in pN0M0 lung adenocarcinoma, particularly in types other than AIS, MIA, and IMA. The ratio of *EGFR* mutation and the risk of recurrence vary among histological subtypes. *EGFR* mutation status should be considered together with histological subtype when estimating the risk of recurrence in resected pN0M0 lung adenocarcinoma patients.

Acknowledgments

Disclosure

No authors report any conflict of interest.

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Supporting Information

Additional Supporting Informationmay be found in the online version of this article at the publisher's website:

Table S1. PCR conditions and oligonucleotide sequences for peptide nucleic acid (PNA)-locked nucleic acid (LNA) PCR clamp method.