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Positive *EGFR* mutation status is a risk of recurrence in pN0–1 lung adenocarcinoma when combined with pathological stage and histological subtype: A retrospective multi-center analysis

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ABSTRACT

Objectives: Recurrence risk of resected lung adenocarcinoma is represented by pathological stage (pStage), histological subtype, and potentially by *EGFR* mutation. However, the relationship among these factors and their combined impact on prognosis are unclear.

Materials and Methods: Using a multicenter database, we retrospectively investigated the prognostic impact of *EGFR* mutation status in relation to pStage and histological subtype in resected pN0–1M0 lung adenocarcinoma.

Results: Among 1155 pN0–1M0 adenocarcinoma cases, pStage 0 and IA1–IB were confirmed predominantly in *EGFR*-positive cases. AIS, MIA, and lepidic predominant adenocarcinoma were also more frequently found in *EGFR*-positive cases and showed no/little recurrence regardless of *EGFR* mutation status. The 5-year recurrence-free survival (RFS) of papillary, acinar, solid, and micropapillary predominant adenocarcinoma was stratified by pStage (IA1–IB, IIA–IIIA) or histological malignant subtype (intermediate or high malignant subtype), and more finely subdivided by *EGFR* mutation status. Positive *EGFR* mutation cases showed worse RFS in both classifications. Low malignant subtype and pStage IA1–IB intermediate malignant subtype showed low frequency of recurrence. Whereas, in pStage IA1–IB high malignant subtype and pStage IIA–IIIA cases, *EGFR*-positive cases showed poorer 5-year RFS than *EGFR*-negative (49.6% and 75.6%, respectively, hazard ratio [HR] = 1.84, 95% CI = 1.38–7.42, $p < 0.01$) and multivariate analysis indicated positive *EGFR* mutation status was significantly related to poorer PRF (HR = 2.005, 95% CI = 1.029–3.906, $p = 0.041$).

Conclusion: EGFR mutation harbored primarily in early-stage or low-malignant histological subtypes with no/little recurrence. In pN0–1M0 adenocarcinoma with higher risk of recurrence, positive *EGFR* mutation cases showed worse RFS. *EGFR* mutation status enables better stratification of recurrence risk when considering pStage and histological malignant subtype.

1. Introduction

Lung adenocarcinoma without metastasis to the mediastinal lymph node or distant site is generally an indication for surgical resection. Recurrence can occur even after complete resection, and pathological stage (pStage) and/or histological subtype classification are utilized to predict the risk of recurrence. Epidermal growth factor receptor (*EGFR)* mutations are one of the most common oncogenic driver mutations of lung adenocarcinoma [1,2] especially in Asia [3,4], and their prognostic implication in lung adenocarcinoma remains controversial. We previously suggested that the risk of recurrence is high with positive *EGFR* mutation status in resected pN0M0 adenocarcinoma of non-variant histological subtypes with recurrence risk. [5] Currently, the impact of the relationship among pStage, histological subtype and *EGFR* mutation on recurrence risk in resected cases remains unclear. Herein, we used a multicenter database to retrospectively investigate the

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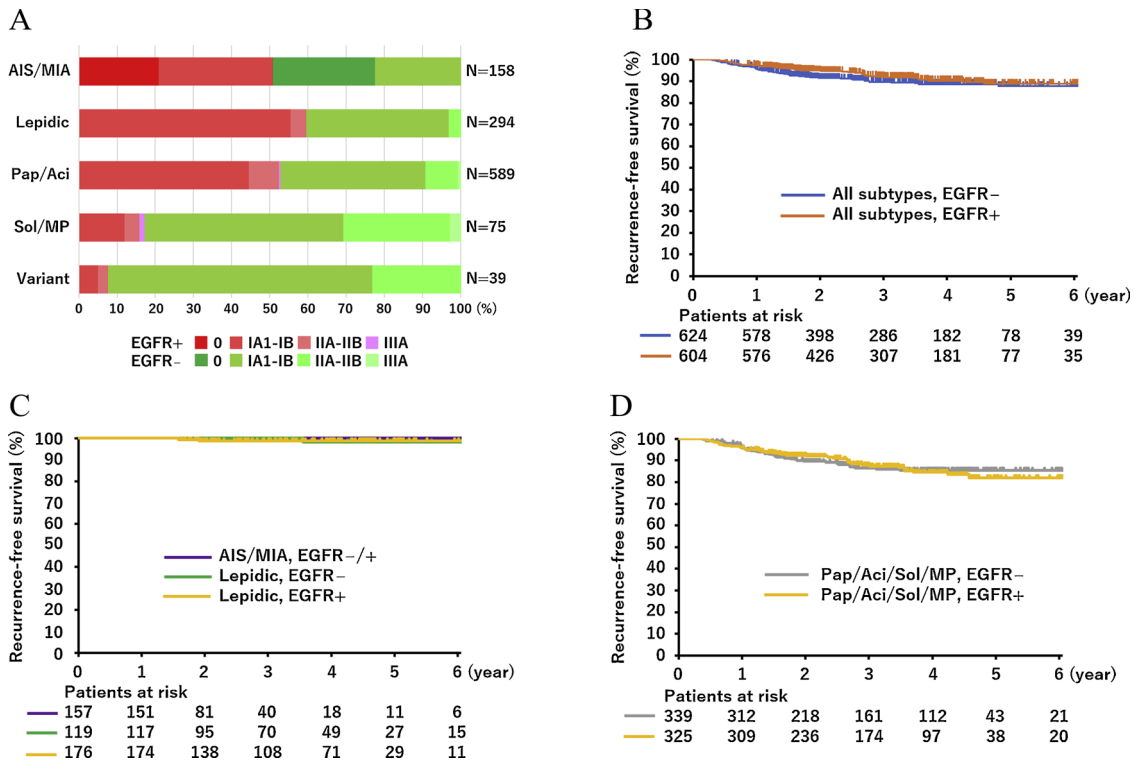

Fig. 1. Distribution of *EGFR* mutation status according to pathological stage and histological subtype. RFS curves by histological subtypes based on *EGFR* mutation status. (A) Distribution of *EGFR* mutation status according to histological subtypes and pathological Stage. (B) RFS curves according to *EGFR* mutation status in 1228 pN0–1M0 adenocarcinoma cases.(C) RFS curves of AIS/MIA or lepidic predominant subtypes according to *EGFR* mutation status. (D) RFS curves of ≤ 5 cm cases according to *EGFR* mutation status after excluding AIS/MIA/lepidic predominant/variant subtypes.
Abbreviations: Aci, acinar predominant adenocarcinoma; AIS, adenocarcinoma *in situ*; MIA, minimally invasive adenocarcinoma; MP, micropapillary predominant adenocarcinoma; Pap, papillary predominant adenocarcinoma; Sol, solid predominant adenocarcinoma.

recurrence risk of pN0–1M0 non-variant invasive lung adenocarcinoma based on pStage, histological subtype, and *EGFR* mutation status.

2. Materials and methods

2.1. Study design

This was a retrospective 3-center database analysis including 2836 cases resected between January 2010 and December 2016 at Kanagawa Cancer Center, Tokyo Medical University Hospital, and Hiroshima University Hospital. The exclusion criteria included: non-adenocarcinoma, incomplete resection or palliative surgery, metastasis in contralateral lung or other organ, mediastinal or extrathoracic lymph node metastasis, omission of intraoperative lymph node dissection, lack of or inappropriate status in clinicopathological data [i.e., follow-up, standard uptake value (SUV) in positron emission tomography-computed tomography (PET-CT), histological subtype, and *EGFR* mutation status]. We first evaluated the impact of *EGFR* mutation status on recurrence in pN0–1M0 adenocarcinoma without distinguishing for pStage and histological subtypes, as in previous studies. Subsequently, tumors bigger than 5 cm in pathological size were excluded in order to avoid too wide range of malignant potential. The prognostic impacts of pStage, histological subtype, and *EGFR* mutation status were analyzed definitively in pN0–1M0 adenocarcinoma cases. Informed consent was obtained from patients and this study was approved by institutional review boards at each institution (Kanagawa Cancer Center: 2012-EKI-54, Tokyo Medical University Hospital: 2017-263, Hiroshima University Hospital: E-1216).

2.2. Clinicopathological data evaluation

All analyzed cases underwent preoperative CT, PET-CT, and intraoperative lymphadenectomy. Difference in SUV among each institution due to different PET-CT devices was adjusted as previously [6]. Pathological diagnosis was performed according to 2015 WHO classification [7]. Low-frequency subtypes, that were categorized as variants of invasive adenocarcinoma [8], were designated as “variant type” in our study. The International Association for the Study of Lung Cancer (IASLC) 8th tumor-node-metastasis (TNM) staging system [9] was utilized for staging. *EGFR* mutation status was evaluated using cobas EGFR Mutation Kit v2 (518497453, Roche Diagnostics K.K., Tokyo, Japan) or as previously described [5,10,11]. *EGFR* point mutations in Ex18 (G719X) or Ex21 (L858R, L861Q), and deletion in Ex19 were regarded as positive mutation status. For the histological malignancy grading, cases were divided into low (lepidic predominant adenocarcinoma), intermediate (papillary or acinar predominant adenocarcinoma), or high malignant subtype (solid or micropapillary predominant adenocarcinoma) according to histological features as in previous studies [12–15].

2.3. Statistical analyses

Recurrence-free survival (RFS) and overall survival (OS) were calculated from the day of operation to the day of recurrence and the day of death from any cause using Kaplan-Meier method, respectively. RFS and OS were used for prognostic evaluation. The differences between RFS and OS curves were evaluated using the log-rank test. The significance of frequencies was evaluated by chi–squared test or Yates–square test. Patients’ age, SUV, and pathological tumor size were compared as continuous variables using Mann-Whitney *U* tests. The impact on recurrence of each variable was evaluated by uni- and multivariate analyses using Cox proportional hazards model with a backward stepwise procedure. A *p* value of less than 0.05 in two-tailed

Table 1
Clinicopathological characteristics of the enrolled lung adenocarcinoma cases ≤ 5 cm in pathological diameter (N = 1155).

Clinicopathological characteristic	Number of case (%)
Age, years	
Median (Interquartile range)	68 (12.0)
Sex, N (%)	
Male/Female	542 (46.9)/613 (53.1)
Smoking status, N (%)	
Ex- or current smoker	551 (47.7)
Never smoker	603 (52.2)
Unknown	1 (0.1)
SUV	
Median (interquartile range)	1.9 (3.5)
Surgical procedure, N (%)	
Pneumonectomy	1 (0.1)
Lobectomy	948 (82.1)
Segmentectomy	204 (17.7)
Wedge resection	2 (0.2)
Pathological tumor size, N (%)	
≤1.0 cm	83 (7.2)
> 1.0 and ≤2.0 cm	464 (40.2)
> 2.0 and ≤3.0 cm	368 (31.9)
> 3.0 and ≤4.0 cm	183 (15.8)
> 4.0 and ≤5.0 cm	57 (4.9)
Predominant subtype, N (%)	
AIS/MIA	75 (6.5)/82 (7.1)
Lepidic	295 (25.5)
Papillary/Acinar	446 (38.6)/143 (12.4)
Solid/Micropapillary	64 (5.5)/11 (1.0)
Variant type	39 (3.4)
IMA/H-FLAC	35 (3.0)/4 (0.3)
EGFR mutation status, N (%)	
Negative/Positive	571 (49.4)/584 (50.6)
EGFR mutant variants, N (%)	
Ex18 MUT	18 (3.1)
Ex19 DEL	227 (38.9)
Ex21 MUT	335 (57.4)
L858R/L861Q	325 (55.7)/ 10 (1.7)
Double mutation	4 (0.7)
Ex18 MUT and Ex19 DEL	2 (0.3)
Ex18 MUT and L861Q	2 (0.3)
Pleural invasion, N (%)	
Pl0	965 (83.6)
Pl1/Pl2/Pl3	119 (10.3)/44 (3.8)/27 (2.3)
Lymphovascular invasion, N (%)	
Negative/Positive	817 (70.7)/338 (29.3)
Intrapulmonary metastasis, N (%)	
Negative/Positive	1129 (97.7)/26 (2.3)
Nodal metastasis, N (%)	
N0/N1	1077 (93.2)/78 (6.8)
pStage, N (%)	
0	75 (6.5)
IA1/IA2/IA3	126 (10.9)/305 (26.4)/245 (21.2)
IB	242 (21.0)
IIA/IIB	41 (3.5)/111 (9.6)
IIIA	10 (0.9)
Recurrence, N (%)	
Negative/Positive	1071 (92.7)/84 (7.3)

Abbreviations: AIS, adenocarcinoma *in situ*; DEL, deletion; Ex, exon; H-FLAC, high-grade fetal adenocarcinoma; IMA, invasive mucinous adenocarcinoma; L, leucine; Pl, pleural inavasion; MIA, minimally invasive adenocarcinoma; MUT, mutation; Q, glutamine; R, arginine; SUV, standard uptake value.

test was regarded as significant. Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA).

3. Results

3.1. Study cohort and clinicopathological characteristics

After applying the exclusion criteria, a total of 1228 pN0–1M0 cases were evaluated for the impact of *EGFR* mutation status on recurrence without distinguishing for tumor size and histological subtypes.

Table 2
Clinicopathological characteristics of pN0-1M0 adenocarcinoma ≤ 5 cm in pathological size according to *EGFR* mutation status (N = 1155).

Clinicopathological characteristic	*EGFR* mutation status		*P* value
	Negative (N = 571)	Positive (N = 584)	
Age, years			
Median (Interquartile range)	68 (12.0)	68 (12.0)	0.507
Sex, N (%)			
Male	329 (57.6)	213 (36.5)	
Female	242 (42.4)	371 (63.5)	< 0.001*
Smoking status, N (%)			
Never smoker	228 (39.9)	375 (64.2)	< 0.001*
Ex- or current smoker	342 (59.9)	209 (35.8)	< 0.001*
Unknown	1 (0.2)	0 (0.0)	0.991
SUV			
Median (Interquartile range)	2.2 (4.6)	1.7 (2.4)	< 0.001*
Surgical procedure, N (%)			
Pneumonectomy	1 (0.2)	0 (0)	0.991
Lobectomy	472 (82.7)	476 (81.5)	0.609
Segmentectomy	97 (17.0)	107 (18.3)	0.552
Wedge resection	1 (0.2)	1 (0.2)	0.489
Pathological tumor size, cm			
Median (Interquartile range)	2.1 (1.5)	2.2 (1.4)	0.458
Predominant subtype (Histological malignant grade), N (%)			
AIS/MIA	77 (13.5)	80 (13.7)	0.916
Lepidic (Low)	119 (20.8)	176 (30.1)	< 0.001*
Papillary/Acinar (Intermediate)	277 (48.5)	312 (53.4)	0.095
Solid/Micropapillary (High)	62 (10.9)	13 (2.2)	< 0.001*
Variant	36 (6.3)	3 (0.5)	< 0.001*
Pleural invasion, N (%)			
Pl0	451 (79.0)	514 (88.0)	< 0.001*
Pl1	81 (14.2)	38 (6.5)	< 0.001*
Pl2	21 (3.7)	23 (3.9)	0.817
Pl3	18 (3.2)	9 (1.5)	0.106
Lymphovascular invasion, N (%)			
Negative	381 (66.7)	436 (74.7)	
Positive	190 (33.3)	148 (25.3)	0.003*
Intrapulmonary metastasis, N (%)			
Negative	553 (96.8)	576 (98.6)	
Positive	18 (3.2)	8 (1.4)	0.065
Nodal metastasis, N (%)			
Negative	527 (92.3)	550 (94.2)	
Positive	44 (7.7)	34 (5.8)	0.202
pStage, N (%)			
0	42 (7.4)	33 (5.7)	0.240
IA1–IB	433 (75.8)	485 (83.0)	0.002*
IIA–IIIA	96 (16.8)	66 (11.3)	0.007*
Recurrence, N (%)			
Negative	530 (92.8)	541 (92.6)	
Positive	41 (7.2)	43 (7.4)	0.905

*$P < 0.05$.
Abbreviations: AIS, adenocarcinoma *in situ*; MIA, minimally invasive adenocarcinoma; Pl, pleural invasion; SUV, standard uptake value.

Additionally, tumors bigger than 5 cm in pathological tumor size were excluded, and 1155 pN0–1M0 adenocarcinoma cases (pStage 0–IIIA) were definitively analyzed. The consort diagram of this study is shown in the Supplementary Fig. 1.

The patient characteristics for 1155 cases are shown in Table 1. The median follow-up term was 1080 days (range: 9–2785). In total, 50.6% cases harbored *EGFR* mutation. The clinicopathological features in which *EGFR* mutation was likely to be harbored were female ($p < 0.001$), never–smoker ($p < 0.001$), low SUV ($p < 0.001$), low malignant subtype ($p < 0.001$), no pleural invasion ($p < 0.001$), no lymphovascular invasion ($p = 0.003$), and pStage IA1–IB cases ($p = 0.002$) (Table 2).

3.2. Distribution of EGFR mutation according to histological subtype and pathological stage

As previous studies have suggested [5,16], *EGFR* mutations were

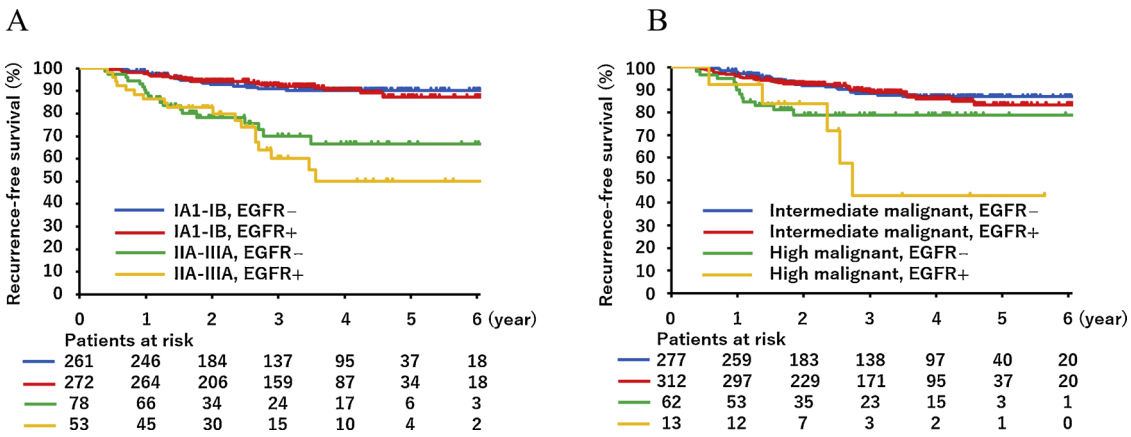

Fig. 2. RFS curves according to *EGFR* mutation status, when considering pathological stage or histological malignant subtype.(A) RFS curves according to pathological stage (IA1–IB or IIA–IIIA) and *EGFR* mutation status.(B) RFS curves according to histological malignant subtype (intermediate or high malignant subtype) and *EGFR* mutation status.

likely to be harbored in adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and low or intermediate malignant subtype, and were fewer confirmed in high malignant subtypes or variant subtypes (Fig. 1A) in the 1155 pN0–1M0 cases. Although the frequency of *EGFR* mutation was relatively high in micropapillary predominant subtype (54.5%), the frequency in high malignant subtype in total was low (17.3%). The frequencies of *EGFR* mutation in papillary predominant subtype and acinar predominant subtype were similar (54.0% and 49.7%, respectively). AIS, MIA, lepidic predominant subtype were more frequently confirmed in *EGFR*-positive cases (*EGFR*-positive vs *EGFR*-negative: 43.8% vs 34.3%, $p < 0.001$). The pStage 0 and pStage IA1–IB cases also more frequently confirmed in *EGFR*-positive cases (*EGFR*-positive vs *EGFR*-negative: 88.7% vs 83.4%, $p = 0.009$). Few variant subtype (7.7%) harbored *EGFR* mutation (Fig. 1A).

3.3. Recurrence-free survival according to histological subtype and EGFR mutation status

In 1228 pN0–1M0 cases in which histological subtype and pStage were not distinguished, positive *EGFR* mutation cases showed higher 5–year RFS than *EGFR*-negative cases (90.8% and 89.1%, respectively) (Fig. 1B). After cases were limited to ≤ 5 cm in pathological size, AIS and MIA showed no recurrence, and low malignant grade subtypes (lepidic predominant subtype) showed high 5–year RFS regardless of *EGFR* mutation status (98.7% and 98.3% in *EGFR*-positive and *EGFR*-negative cases, respectively) (Fig. 1C). Variant subtypes harboring *EGFR* mutation did not relapse. Whereas, in non-variant intermediate and high malignant grade subtypes (papillary, acinar, solid, and micropapillary predominant subtype), positive *EGFR* mutation cases showed lower 5-year RFS than *EGFR*-negative cases (82.0% and 85.4%, respectively) (Fig. 1D). Positive *EGFR* mutation cases showed higher 5–year OS partially due to the post-recurrence EGFR-TKI treatment (*EGFR*-positive vs *EGFR*-negative: 93.2% vs 90.8% in all cases, 95.8% vs 97.0% in lepidic predominant subtypes, and 91.6% vs 86.6% in non-variant intermediate and high malignant grade subtypes, respectively) (Supplementary Fig. 2A–C).

3.4. Prognostic impact of EGFR mutation in relation to pathological stage and/or histological subtype

Papillary, acinar, solid, and micropapillary predominant cases ≤ 5 cm could be stratified only by pStage or histological malignancy grading. The pStage IA1–IB cases showed worse RFS compared to pStage IIA–IIIA, and intermediate malignant subtype showed poorer RFS compared to high malignant subtype (data not shown). RFS stratified by pStage (IA1–IB or IIA–IIIA) and histological subtype (intermediate or high malignant) were further stratified by *EGFR* mutation status. Cases harboring *EGFR* mutation showed worse RFS in both pStage and histological malignant grade classification (Fig. 2A, B). Considering all three status (pStage, histological subtype, and *EGFR* mutation), some cases with different pStage, histological subtype, or *EGFR* mutation status showed similar RFS (Fig. 3A). By combining subtypes with similar prognosis, pN0–1M0 intermediate and high malignant subtypes could be more finely stratified compared to classification by single status only. Additionally, there was a significant difference in RFS between these criteria and low malignant subtype (Fig. 3B). Classification by the 3 status suggested that 5-year RFS of pStage IA1–IB/intermediate malignant subtype with or without *EGFR* mutation [IA1–IB/intermediate/*EGFR*(±)] and pStage IA1–IB/high malignant subtype without *EGFR* mutation [IA1–IB/high/*EGFR*(-)] were similar (88.4% and 91.6%, respectively). Five-year RFS of pStage IIA–IIIA/intermediate malignant subtype with *EGFR* mutation [IIA–IIIA/intermediate/*EGFR*(+)] and pStage IIA–IIIA/high malignant subtype without *EGFR* mutation [IIA–IIIA/high/*EGFR*(-)] were also similar (50.7% and 54.5%, respectively). High malignant subtypes harboring *EGFR* mutation [high/*EGFR*(+)] showed low RFS regardless of pStage. RFS was significantly different among the combined cohorts except between IIA–IIIA/intermediate/*EGFR*(+) plus IIA–IIIA/high/*EGFR*(-) cohort and high/*EGFR*(+) cohort due to the small number of cases: low malignant subtypes vs IA1–IB/intermediate/*EGFR*(±) plus IA1–IB/high/*EGFR*(-), hazard ratio (HR) = 8.15, 95% confidential index (CI) = 11.3–81.7, $p < 0.001$; IA1–IB/intermediate/*EGFR*(±) plus IA1–IB/high/*EGFR*(-) vs IIA–IIIA/intermediate/*EGFR*(-), HR = 2.78, 95% CI = 6.35–120.3, $p < 0.001$; IIA–IIIA/intermediate/*EGFR*(-) vs IIA–IIIA/intermediate/*EGFR*(+) plus IIA–IIIA/high/*EGFR*(-), HR = 2.12, 95% (CI) = 1.31–7.02, $p = 0.010$; IIA–IIIA/intermediate/*EGFR*(+) plus IIA–IIIA/high/*EGFR*(-) vs high/*EGFR*(+), HR = 1.05, 95% CI = 0.35–3.23, $p = 0.918$. By pStage or histological classification, *EGFR*-positive cases indicated higher OS in pStage IA1–IB and intermediate malignant subtypes (Supplementary Fig. 3A–B). However, OS showed similar tendency with RFS after considering 3 status. The significant difference was confirmed only between low malignant subtypes vs IA1–IB/intermediate/*EGFR*(±) plus IA1–IB/high/*EGFR*(-), HR = 2.01, 95% CI = 2.92–44.3, $p < 0.001$ (Supplementary Fig. 4A-B)

The pStage IA1–IB intermediate malignant subtypes showed high 5-year RFS both in positive and negative *EGFR* mutation cases (88.4% and 90.1%, respectively). Among pStage IA1–IB high malignant subtype and pStage IIA–IIIA cases, positive *EGFR* mutation cases showed poorer 5-year RFS than *EGFR*-negative cases (49.6% vs 75.6%, respectively; HR = 1.84; 95% CI = 1.38–7.42; $p < 0.01$) (Fig. 3C). OS was also poorer in *EGFR*-positive cases than *EGFR*-negative cases (70.0% vs

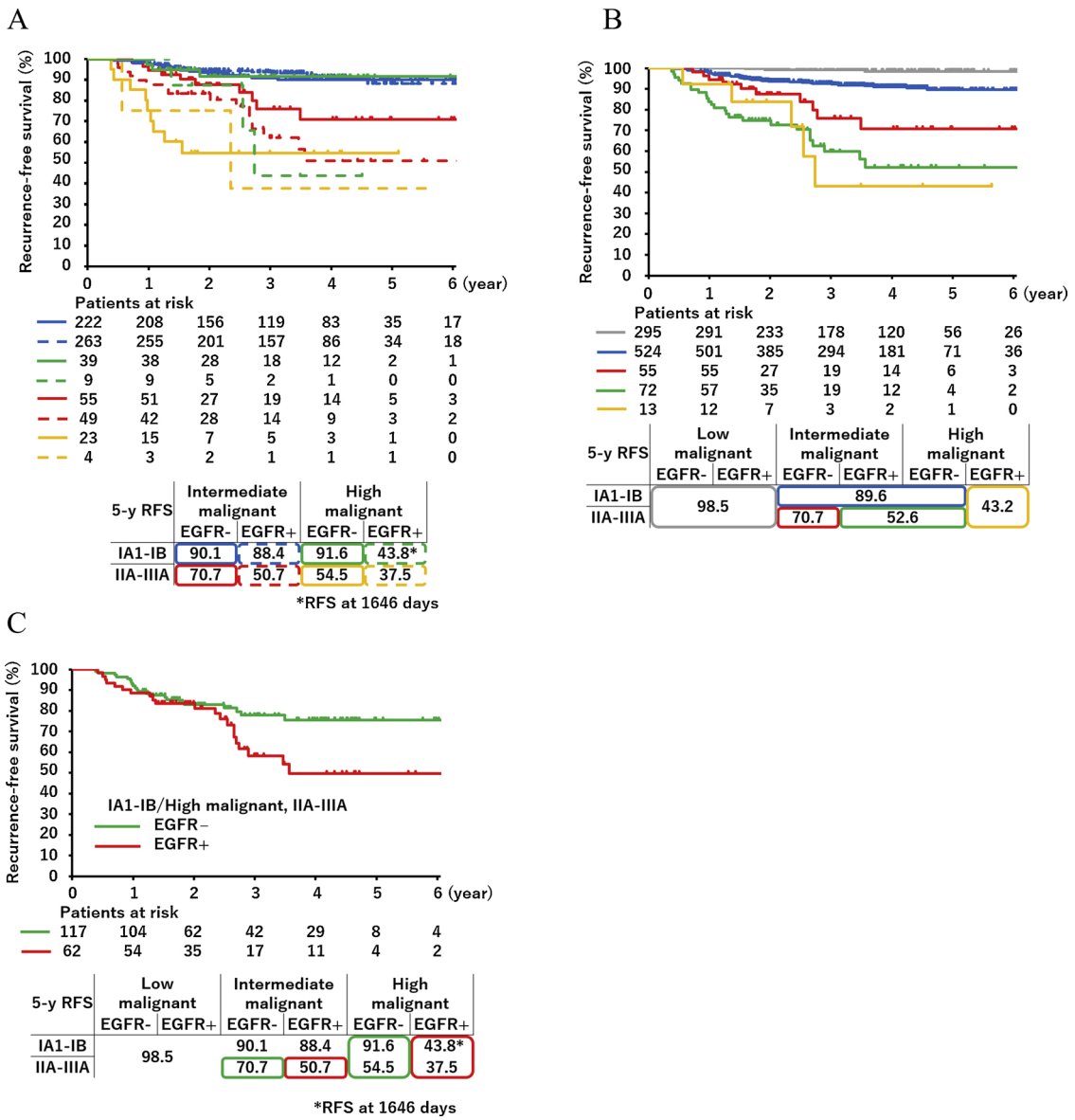

Fig. 3. RFS curves based on *EGFR* mutation status, pathological stage, and histological malignant subtype.(A) RFS curves considering *EGFR* mutation status, pathological stage, and histological malignant subtype.(B) RFS curves after unifying similar prognoses when considering *EGFR* mutation status, pathological stage, and histological malignant subtype. RFS of lepidic predominant case is also included.(C) RFS curves according to *EGFR* mutation status after excluding AIS, MIA, lepidic predominant cases, variant subtype, and pathological stage IA1–IB papillary/acinar predominant cases.

82.0%, respectively; HR = 1.62; 95% CI = 1.03–18.5; p = 0.046) (Supplementary Fig. 4C). Univariate analysis showed SUV, lymphovascular invasion, pleural invasion, intrapulmonary metastasis, LN metastasis, pStage IIA–IIIA, and positive *EGFR* mutation status were risk of recurrence. Multivariate analysis indicated positive *EGFR* mutation status, lymphovascular invasion, intrapulmonary metastasis, and lymph node metastasis were significantly related to recurrence (*EGFR* mutation: HR = 2.005, 95% CI = 1.029–3.906, p = 0.041) (Table 3).

4. Discussion

TNM staging system is the gold standard to predict recurrence risk and prognosis in cancer, and the latest TNM staging system in lung cancer has been determined based on a large worldwide database [9]. Histological classification is also useful to estimate malignant potential and has been revised in detail, especially in lung adenocarcinoma [7]. These 2 classifications were independently revised and have no corresponding categories between each other except for Tis (AIS) and T1a (mi) (MIA). In advanced stage lung adenocarcinoma, routine testing of *EGFR* mutation status is recommended [17]. However, clinical implication of routine genetic estimation in completely resected adenocarcinoma has not been studied thoroughly. Additionally, the relationship among TNM stage, histological malignant grade, and *EGFR* mutation status, and their combined impact on prognosis are unknown.

EGFR mutations drives tumorigenesis in advanced lung adenocarcinoma, therefore EGFR-tyrosine kinase inhibitor (TKI) shows therapeutic effect [18,19]. The prognostic implication of *EGFR* mutation status in resectable cases is controversial. Systematic meta-analyses showed that *EGFR* mutation status is not a prognostic factor in resected non-small cell lung cancer [20]. Some studies have assessed several histological subtypes equally regardless of the difference in frequency of *EGFR* mutation or recurrence risk, and suggested better prognostic tendency in positive *EGFR* mutation cases (Supplementary Table 5) [20–23]. This tendency was confirmed in our cohort comprising 1228 cases in which pStage and histological subtypes were not distinguished (Fig. 1B). On the other hand, we previously concluded that *EGFR*

Table 3
Uni- and multivariate analyses for recurrence in non-variant pStage IA1–IB high malignant subtype and pStage IIA–IIIA intermediate/high malignant subtype cases (N = 179).

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age	1.014 (0.982–1.047)	0.394	1.015 (0.982–1.048)	0.377
Sex (Male)	1.342 (0.700–2.574)	0.375	1.638 (0.803–3.342)	0.175
Ex– or current smoker	1.041 (0.561–1.933)	0.898	0.889 (0.375–2.108)	0.789
Procedure (Sublobar resection)	0.192 (0.026–1.396)	0.103	0.291 (0.039–2.176)	0.229
SUV	1.046 (1.005–1.088)	0.026*	1.054 (0.998–1.112)	0.058
Pathological tumor size	1.241 (0.966–1.596)	0.092	1.029 (0.761–1.390)	0.855
High malignant grade subtype	0.897 (0.487–1.654)	0.728	0.986 (0.409–2.377)	0.975
Lymphovascular invasion	3.384 (1.330–8.607)	0.010*	2.442 (0.811–7.357)	0.112
Pleural invasion	2.138 (1.146–3.990)	0.017*	1.926 (0.979–3.787)	0.058
Intrapulmonary metastasis	2.250 (1.035–4.888)	0.041*	3.636 (1.624–8.137)	0.002*
Lymph node metastasis	3.102 (1.671–5.762)	< 0.001*	2.616 (1.335–5.125)	0.005*
pStage (IIA–IIIA)	2.762 (1.165–6.548)	0.021*	1.166 (0.396–3.433)	0.781
EGFR mutation (Positive)	1.847 (1.015–3.362)	0.045*	2.005 (1.029–3.906)	0.041*

*$P < 0.05$.
Abbreviations: CI, confidential index; HR, hazard ration; SUV, standard uptake value.

mutation was a risk of recurrence in pN0 typical invasive adenocarcinoma [i.e., cases excluding AIS, MIA, and invasive mucinous adenocarcinoma (IMA)]. [5] The heterogenous distribution of *EGFR* mutations among histological subtypes has been reported [5,16]. Fundamentally, *EGFR* mutation is detected more frequently in cases accompanied by lepidic lesions (formerly known as a bronchioloalveolar carcinoma component) [24]; therefore, subtypes with low or no risk of recurrence are likely to harbor *EGFR* mutation. Comparison of *EGFR*-positive and negative cases without distinguishing histological subtypes might be akin to comparing cohort comprising a large number of little-or-no recurrence risk cases (AIS/MIA/Lepidic) to cohort including a large number of cases with higher recurrence risk (intermediate/high malignant subtype). We thus suggested that *EGFR* mutation status should be considered together with pStage and histological subtype.

TNM staging system encompasses a broad range of tumor stages, from in situ (stage 0) to distant metastatic phase (stage IVA/B). Histological subtype classification also reflects a wide range of malignant behavior, from non-/preinvasive to high grade malignant status. However, *EGFR* mutation status is usually described only as positive or negative. Because AIS and MIA never relapse after complete resection [16,25] and lepidic predominant cases seldom recur [15], there is almost no need for further categorization of these subtypes. Descriptions of AIS/MIA or pStage 0/T1a(mi) are enough to express their oncological characteristics; a simple description about *EGFR* mutation status ("positive" or "negative") is not useful to classify cases with extremely good prognosis. Although staging system is more important than *EGFR* status, intense attention considering *EGFR* status should be paid in cases with higher risk of recurrence.

In our study, lepidic predominant subtype and pStage IA1–IB intermediate malignant subtypes also showed high 5-year RFS and did not allow for further classification by *EGFR* mutation status (5-year RFS: 98.5% and 89.4% in lepidic predominant and pStage IA1–IB intermediate malignant subtypes, respectively). After excluding these cases, *EGFR* mutation positive cases showed an increased risk of recurrence (Fig. 3C). Thus, the prognostic impact of *EGFR* mutation should be considered with pStage and histological malignant subtype. Estimating the recurrent risk only by *EGFR* mutation status is misleading. In the cohort including more AIS/MIA and low recurrent risk cases, the unfavorable prognostic impact of *EGFR* mutation can be overlooked if pStage and histological malignant grade are not considered.

In addition to cases with low or no risk of recurrence, variant subtypes should be excluded in estimating the recurrence impact of *EGFR* mutations. Variant types in this study included IMA and high-grade fetal adenocarcinoma (H-FLAC). Both types include several prognosis phenotypes. Five-year OS ranges 20.0-83.3% [26,27]. Because most of the variant types are negative in *EGFR* mutation (the frequency of *EGFR* mutation is up to 0–7.1% [16 [26,27]), negative *EGFR* mutant cases show worse prognosis in variant types. These variants are quite different from typical adenocarcinoma with regard to EGFR mutation status as well as histology.

This study has some limitations. This is a retrospective study. The cohort of positive *EGFR* mutation status did not include T790 M, which is one of the targetable *EGFR* mutations for 3rd generation EGFR-TKI (osimertinib) and can be present in tumor cells before EGFR-TKI treatment [28]. We excluded cases with more than 5 cm tumor size in definitive analysis to avoid too wide a range of malignant potential. Although the number of excluded cases was relatively small, studies including a large number of resected cases with bigger tumor size might also be helpful in understanding the impact of the interaction of pStage, histological subtype, and *EGFR* mutation status on prognosis.

5. Conclusions

Positive *EGFR* mutation status is a risk of recurrence in pN0–1M0 adenocarcinoma except in cases with little or no risk of recurrence or variant type. Classification by pathological stage and histological subtype can include *EGFR* mutation status for better stratification of recurrence risk. However, estimation based solely on *EGFR* mutation can be misleading. In conclusion, *EGFR* mutation as a risk of recurrence should be considered along with pStage and histological malignant subtype in lung adenocarcinoma.

CRediT authorship contribution statement

Masaoki Ito: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft. **Yoshihiro Miyata:** Conceptualization, Formal analysis, Methodology, Supervision, Visualization, Writing - review & editing. **Yasuhiro Tsutani:** Data curation, Investigation, Methodology, Resources, Writing - review & editing. **Hiroyuki Ito:** Data curation, Investigation, Resources, Writing - review & editing. **Haruhiko Nakayama:** Resources, Writing - review & editing, Project administration. **Kentaro Imai:** Data curation, Investigation, Resources, Writing - review & editing. **Norihiko Ikeda:** Resources, Writing - review & editing, Project administration. **Morihito Okada:** Resources, Supervision, Writing - review & editing, Project administration.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

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