

ORIGINAL ARTICLE

Clinical value of the new International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification of lung adenocarcinoma

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Keywords

EGFR mutation; histologic subtypes; lung adenocarcinoma; prognosis.

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Abstract

Background: We explored correlations between the new International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification, epidermal growth factor receptor (*EGFR*) mutation status, and prognosis.

Methods: Data from 293 patients with lung adenocarcinoma were classified according to the new classification. Fisher's exact, χ^2 , and log-rank tests and Cox regression analysis were used to analyze correlations between *EGFR* mutation status, lung cancer prognosis, and the new histologic subtype. Disease-free survival and progression-free survival (PFS) were estimated using the Kaplan–Meier method.

Results: Lepidic and non-solid adenocarcinomas showed significantly elevated *EGFR* mutation rates (79.0% and 65.8%, respectively; $P < 0.05$). *EGFR* mutation status was only associated with gender ($P < 0.001$). *EGFR* mutation-positive patients who received targeted therapy had better median PFS than those who received chemotherapy as first-line treatment ($P < 0.001$). The median PFS of patients with exon 19 and exon 21 mutations who received first-line targeted therapy were 12.5 and 9.5 months, respectively ($P = 0.970$). Patients with micropapillary predominant adenocarcinomas had the shortest disease-free survival (<18 months) and PFS. Histologic subtype ($P = 0.036$), treatment type ($P = 0.031$), and *EGFR* mutation status ($P = 0.019$) might be good prognostic factors for lung adenocarcinoma.

Conclusion: Patients with exon 19 mutations obtained greater benefits from targeted therapy. In the new classification, *EGFR* mutation rates are higher in lepidic cases and in cases without the solid subtype. The micropapillary subtype of adenocarcinoma has the worst prognosis, while the lepidic subtype has the best.

Introduction

Lung cancer is currently one of the major causes of cancer-related death worldwide. It is the leading cause of cancer-related death in China, and often results in greater rates of morbidity and mortality than heart disease and cerebrovascular disorders.¹ Pulmonary adenocarcinoma is the most common histological subtype of lung cancer. Its incidence has increased over the past decades, and it occurs in about 50% of non-small cell lung cancers (NSCLCs).² In 2011, in light of improvements in our understanding of pulmonary

adenocarcinoma, a joint working group of the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS), and the European Respiratory Society (ERS) proposed a new histologic classification of lung adenocarcinoma. This classification method has been referred to as “the new IASLC/ATS/ERS lung adenocarcinoma classification” or “the new classification.”³ Using the new classification, it is possible to screen for early-stage NSCLC in patients at high risk. Furthermore, the new classification can favorably guide postoperative

adjuvant treatment, although there are some limitations for advanced lung cancer.^{4–6} Epidermal growth factor receptor-tyrosine-kinase inhibitors (EGFR-TKIs) have significant survival benefits for advanced lung cancer. In NSCLC, EGFR mutations chiefly occur in adenocarcinomas, followed by adenosquamous cell, large-cell undifferentiated, and miscellaneous carcinomas, and are least common in squamous cell carcinomas (<5%).⁷ In clinical trials of patients with advanced pulmonary adenocarcinoma and EGFR mutations, chemotherapy was inferior to EGFR-TKIs, which prolonged median survival to 3.5 years.^{8–13} Therefore, pre-treatment genetic testing is recommended by the National Comprehensive Cancer Network (NCCN), the European Society for Medical Oncology (ESMO) and the American Society of Clinical Oncology (ASCO) for cases of advanced lung cancer. In 2014 in China, however, the proportion of patients who received a genetic test was only 27% (although a higher testing rate of 51% was observed in the larger cities, Beijing, Shanghai, and Guangzhou).¹⁴ These relatively low proportions may be explained by the use of mainly exploratory techniques that are yet to be widely adopted, as well as by the presence of notable limitations to specimens. Several studies have attempted to explore the predictive value of the new classification in cases with EGFR mutation.^{15,16} At present, we are unable to determine the larger clinical implications because some outcomes associated with the EGFR mutation rate are higher in micropapillary predominant adenocarcinomas than in invasive mucinous and solid predominant adenocarcinomas, both in China and internationally.

We reviewed data from patients diagnosed with lung adenocarcinoma at Peking University Third Hospital between 1 November 2011 and 31 December 2014. Additionally, we analyzed the relationships between the new classification and outcomes during follow-up in order to clarify the implications of the new classification and EGFR mutation status for prognosis and therapeutic choices.

Methods

Patients

We analyzed medical records from a population of patients who were diagnosed with and treated for pulmonary adenocarcinoma between 2011 and 2014. The inclusion criteria were: (i) primary lung adenocarcinoma histopathologically confirmed after treatment in the hospital, (ii) stage I–II or stage III for clear resection types and stage IV for non-resected lesions, (iii) lung adenocarcinoma with determined subtypes, and (iv) Eastern

Table 1 A baseline comparison of 293 lung adenocarcinoma cases

Clinical features	Cases (n)	%
Age		
<62	142	48.5
>62	151	51.5
Age	29–84	ND
Median age	62	ND
Gender		
Female	117	40.0
Male	176	60.0
T		
T1	63	21.5
T2	113	38.6
T3	65	22.2
T4	52	17.7
N		
N0	75	25.6
N1	74	25.2
N2	86	29.4
N3	58	19.8
M		
M0	165	56.3
M1	128	43.7
TNM		
Stage I	46	15.7
Stage II	57	19.4
Stage III	62	21.2
Stage IV	128	43.7
Specimens		
Resect	132	45.0
Biopsy	112	38.3
Hydrothorax	49	16.7

ND, no data; TNM, tumor node metastasis.

Cooperative Oncology Group performance status (ECOG PS) ≤ 2 . The exclusion criteria were: (i) unclear classification, (ii) metastatic lung adenocarcinoma, and (iii) EGOG PS ≥ 2 . The cases were selected on the basis of availability of archival slides and tissues. The patients' characteristics are summarized in Table 1, including information on age, gender, clinical stage, EGFR mutation status, and treatment.

Methods

The new classification includes adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), invasive carcinoma (lepidic, acinar, papillary, solid, and micropapillary predominant), and variants of invasive adenocarcinomas (invasive mucinous adenocarcinoma [IMA], colloid, fetal, and enteric).³ A large number of pathologists decided the different subtypes of the new classification by morphology. In the new classification, evidence of a subtype present in >5% of the specimen confirms a diagnosis of that subtype, a subtype present in >40% of the specimen was

defined as predominant, and other subtypes were considered mixed. We used the American Joint Committee on Cancer tumor node metastasis (TNM) classification criteria.¹⁷ The resected tumor specimens were subjected to EGFR mutational analysis using DNA sequencing, as well as fluorescence PCR analyses of biopsy specimens. The results were regarded as reliable because biopsy specimens show only pathologic analysis and not variety.

DNA sequencing was conducted as follows.

DNA extraction: The specimens were cut serially into coronal and sagittal slices of 4 μm thickness using a microtome. The tumor cells were enriched to determine which cells contained $\geq 70\%$ evidence of cancer. DNA from paraffinized tissues samples was isolated using a QIAamp DNA FFPE Kit (Qiagen, Hilden, Germany). Nucleic acid protein concentrations were measured along with DNA purity and concentration using a Nanodrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). DNA concentration was adjusted to 20–30 $\text{ng}/\mu\text{L}$ and stored at -20°C .

Epidermal growth factor receptor amplification and purification: EGFR gene PCR amplification and sequencing primers were tested and synthesized by Yingjun Life Technologies Co. Ltd. to directly target EGFR tyrosine coding region exon ranges from 18 to 21 in order to synthesize primers. The exon 18 upstream primer was 5'-AGCATGGTGAGGGCTGAGGTGAC-3' (23 bp) and the downstream primer was 5'-ATATACAGCTTGCAAG-GACTCTGG-3' (24 bp). The exon 19 upstream primer was 5'-CCAGATCACTGBGCAGCATGTGGCACC-3' (27 bp) and the downstream primer was 5'-AGCAGGGTCTAGAGCAGAGCAGCTGCC-3' (27 bp). The exon 20 upstream primer was 5'-GATCGCATT-CATGCGTCTYCACC-3' (23 bp), and the downstream primer was 5'-TrGcTATCCCAGGAGCGCAGACC-3' (23 bp). The exon 21 upstream primer was 5'-TCA-GAGCCTGGCATGAACATGACCCTG-3' (27 bp) and the downstream primer was 5'-GGTCCCTGGTGTCAG-GAAAATGCTGG-3' (26 bp). PCR products 262, 265, 362, and 297 bp were maintained with PCR amplification according to the clarification. Five microlitres of the PCR amplification product was run on 1% agarose gel to observe PCR production and was documented by absorption analysis.

Reaction, purification, and sequencing: 0.5 μL purified PCR products, 1 μL reagents of sequencing, 2 μL sequence primers, and 2.5- U ddH_2O were maintained with sequencing. To purify the samples after the sequencing reaction was completed, 1 μL NaAc and 15 μL alcohol were added to each sample. Ten microlitres of Hi-Di was subsequently added to the samples to filter DNA alcohol volatilization. The samples were then degenerated by the PCR instrument, spotted in 96-well plates, and analyzed with

ABI-3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), using chroma or alignment for analysis.

Fluorescence PCR: Procedures were performed using an EGFR mutation kit (Qiagen) comprised of 8-PCR tube reaction strips (reagents include E18, E19, E20, and E21 mutation sites), Taq enzymes, and EGFR-positive control material, according to the manufacturer's instructions. Analysis was performed for each sample, positive control material, and a negative control (purified water) during each PCR reaction. The samples were harvested from positive control material and Taq enzyme (EGFR) and centrifuged rapidly for 15 minutes. Subsequently, 2.7 μL Taq enzyme (EGFR) was added to the 42.3 μL tested DNA samples, the 42.3 μL positive control material samples, and 42.3 μL purified water samples to admix in 15 seconds on amalgamation. The samples were then centrifuged rapidly for 15 seconds. The manufacturer recommended adding 1.5–3 $\text{ng}/\mu\text{L}$ DNA samples from paraffin sections (i.e. each reaction tube included additional DNA of 7.05–14.1 ng), which is different to adding DNA according to the sample duration of paraffin sections. It is recommended to be Analysis was performed directly after DNA extraction of blood plasma and serum, as per the manufacturer's recommendation. TE buffer (pH8.0) is recommended to dilute the DNA sample. Each 5 μL mixed DNA sample along the PCR tubal wall was added to an 8-PCR tube reaction strip. Lids were then placed and the samples were centrifuged before being added to 8-PCR tube reaction strips. Reactions were subject to placement of the 8-PCR tube reaction strips into PCR instruments, according to standard procedure.

Endpoints

Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 was used to determine clinical therapeutic effects.¹⁸ The endpoints of the study were disease-free survival (DFS) and progression-free survival (PFS) rates.

Follow-up

Patients were followed up by review, telephone, and clinic service. Loss of follow-up occurred when patient information was altered without notification or a patient refused to continue.

Statistical analyses

Epidermal growth factor receptor mutation status and histologic subtype were analyzed statistically using the χ^2 -test, and Fisher's exact test was used when the sample size was lower than five. Log rank tests and Cox regression analyses were also employed. DFS and PFS were estimated using

the Kaplan–Meier method. Statistical analyses were performed using SPSS version 20 (IBM Corp., Armonk, NY, USA).

Results

Cohort characteristics

We analyzed the medical records of patients who had been diagnosed with lung adenocarcinoma at Peking University Third Hospital between 1 November 2011 and 31 December 2014. Of the 377 selected adenocarcinoma cases, 84 cases with unclear subtypes were excluded. In total, 293 patients were included in the study: 176 women and 117 men. The age range at diagnosis was 29–84 years (mean 62). Specimens were available from surgically resected adenocarcinomas (wedge or segmental resections) in 132 patients, needle or incisional biopsy in 112 patients, and pleural cytology in 49 patients. Patient details are summarized in Table 1 and Figure 1.

The new classification, epidermal growth factor receptor (*EGFR*) mutation status and patients

The new classification and patients

Lepidic predominant disease was the most common histopathologic type, while micropapillary predominant disease was the least common according to the new classification. The details of the patient cohort are summarized in

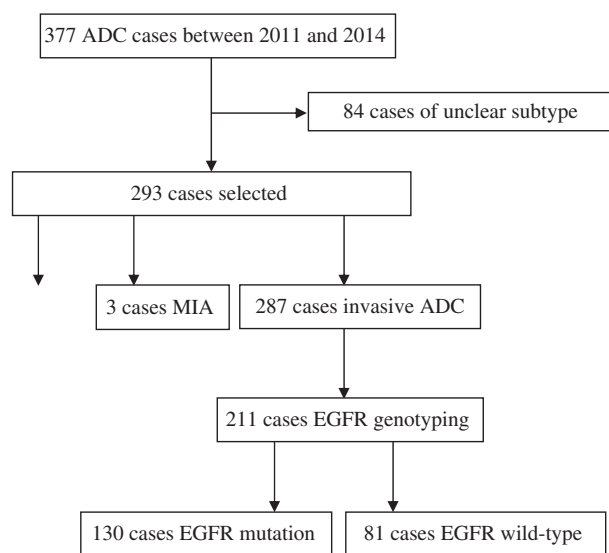


Figure 1 Screening steps for the collection of data of lung adenocarcinoma patients between 2011 and 2014. ADC, adenocarcinoma; EGFR, epidermal growth factor receptor; MIA, minimally invasive adenocarcinoma.

Table 2. There were three cases of AIS, three cases of MIA, and 287 cases of invasive adenocarcinoma; 126 were acinar predominant (43.9%), 66 were lepidic predominant (23.0%), 31 were papillary predominant (10.8%), 35 were solid predominant (12.2%), and 10 were micropapillary predominant (3.5%). Following the World Health Organization (WHO) lung adenocarcinoma classification, mixed subtypes (61.1%) and non-mixed subtypes (62.4%) were found in 173 and 120 patients, respectively.

Analysis of epidermal growth factor receptor (*EGFR*) mutation status

A total of 293 lung adenocarcinoma patients were included in this study. *EGFR* gene status was tested in 211 cases. *EGFR* mutation was found in 130 cases (130/211, 61.6%), while 81 cases showed wild-type *EGFR* (81/211, 38.4%). Among the cases of *EGFR* mutation, 54 cases were deletion mutations in exon 19 (41.3%) and 50 were L858R missense mutations in exon 21 (38.5%). *EGFR* mutation status was related to gender ($P < 0.001$), but was unrelated to tumor stage, age, or histologic subtype. Patient details are summarized in Tables 3 and 4.

Lepidic and non-solid adenocarcinoma and *EGFR* status

Epidermal growth factor receptor mutation genetic testing was not performed on the six patients with AIS or MIA as the sample size was inadequate. Correlation between the new classification and *EGFR* status was complicated. Patient details are summarized in Table 5. Lepidic adenocarcinomas and non-solid adenocarcinomas showed significantly elevated *EGFR* mutation rates (79.0% and 65.8%, respectively; $P < 0.05$). The *EGFR*-mutation positive patients who received targeted therapy had better median PFS rates than those who received chemotherapy as first-line treatment ($P < 0.001$). Lepidic containing and predominant were the most common and statistically significant (51.5%, $P = 0.004$ and 51.4%, $P = 0.008$, respectively). *EGFR* mutation rates were relatively unusual in acinar and papillary predominant and containing cases compared with lepidic adenocarcinoma, and no statistically significant differences were observed (50.0%, 51.4%, 45.2%, 53.2%; $P = 0.694, 0.215, 0.726, 0.213$, respectively). The mutation rate was lowest in solid containing cases (21.9%), and was statistically significant ($P < 0.001$). Following the WHO lung adenocarcinoma classification, mixed subtypes and non-mixed subtypes did not show significantly different *EGFR* mutation rates (61.1% and 62.4%, respectively).

Fifty-five enrolled patients had stage IV disease with *EGFR* mutations and 33 patients had stage IV disease with

Table 2 The new classification for 293 cases

Histotype	Cases (n)						%
New classification							
AIS	3						1.0
MIA	3						1.0
Invasive carcinoma	287						
	Predominant	5–10%	10–20%	20–30%	30–40%	>5%	Predominant
Lepidic	126	17	12	13	15	183	43.9
Acinar	66	11	10	10	12	109	23.0
Papillary	31	15	10	8	15	79	10.8
Solid	35	9	7	7	12	64	12.2
Micropapillary	10	11	6	4	4	35	3.5
Unclear	19						
Variants of invasive adenocarcinomas	2						0.7
IMA	2						
WHO							
Non-mixed subtypes	120						40.9
Mixed subtypes	173						59.1

AIS, adenocarcinoma in situ; IMA, invasive mucinous adenocarcinoma; MIA, minimally invasive adenocarcinoma; WHO, World Health Organization.

Table 3 Adenocarcinoma EGFR mutation and clinical characteristics for 211 cases

Pathology	Cases (n)	EGFR mutation (n)	EGFR wild (n)	Mutation rate (%)	P
Age					
<62	102	65	37	63.7	0.541
>62	109	65	44	59.6	
Gender					
Male	95	47	48	49.5	<0.001
Female	116	83	33	71.5	
T					
T1	43	27	16	62.8	0.591
T2	93	53	40	57.0	
T3	45	29	16	64.4	
T4	30	21	9	70.0	
N					
N0	65	40	25	61.5	0.738
N1	49	33	16	67.3	
N2	61	37	24	60.6	
N3	36	20	16	55.6	
M					
M0	123	75	48	60.9	0.822
M1	88	55	33	62.5	
Specimens					
Resection	95	60	35	62.1	0.857
Needle biopsy	81	48	33	59.3	
Pleural cytology	35	22	13	62.8	

EGFR, epidermal growth factor receptor.

wild-type *EGFR*. Among *EGFR* mutation cases, 13 included bone metastases and 10 included cerebral metastases. Of the wild-type *EGFR* cases, nine involved bone metastases and five involved cerebral metastases. There were 42 patients with exon 19 and exon 21 mutations. Of those with exon 19 mutations, five had bone metastases and three had cerebral metastases.

Correlation of *EGFR* status, the new classification, and progressive disease

The median PFS in stage III-IV patients with exon 19 mutations was longer than in patients with exon 21 mutations when treated with targeted first-line therapy. These 79 patients with mutations were compared with 51 patients who received chemotherapy. The median PFS

Table 4 Analysis of *EGFR* mutation status

Gene	Mutation	Cases (n)
E18	G719S	1
E19	E746-A750, L747-A750 (ins P), L747-P753 (ins S)	54
E20	Q787Q, T790M, D770-N771 (ins G)	7
E21	L858R, L861Q	50
E18, 19	G719S, E746-A750	1
E18, 20	G719A, S768I	3
E18, 21	G719A, L858R, L861Q	1
E19, 20	L747-P753 (ins S), E746-A750, L747-T751, T790M, S768I, V769L	3
E19, 21	E746-A750, L858R	3
E20, 21	T790M, Q787Q, L858R	3
E18-20	G719A, L747S, Q787Q	1

EGFR epidermal growth factor receptor.

was 13 months in the patients who received targeted therapy and 7.5 months in those who received chemotherapy ($P < 0.001$). The results indicated that median PFS was significantly prolonged in patients with *EGFR* mutations who had been selected for targeted therapy. The details are summarized in Figure 2.

Among the cases of *EGFR* mutation in metastatic tumors, 35 cases were deletion mutations in exon 19 and 32 cases were L858R missense mutations in exon 21. The median PFS rates of patients with exon 19 and exon 21 mutations who received first-line *EGFR*-targeted therapy were 12.5 and 9.5 months, respectively ($P = 0.970$). The median PFS of patients with exon 19 and exon 21 mutations who received first-line chemotherapy were six and nine months, respectively ($P = 0.341$). Patients with exon 19 mutations may have greater sensitivity to

Table 5 Adenocarcinoma *EGFR* mutation status and pathological subtypes for 293 cases

Histotype	Cases (n)	EGFR mutation	EGFR wild	EGFR unclear	Mutation rate%	P
The new classification						
Acinar predominant	126	63	37	26	63.0	0.002
Lepidic predominant	66	34	9	23	79.0	
Papillary predominant	31	14	10	7	58.3	
Solid predominant	35	7	17	11	29.1	
Micropapillary predominant	10	3	5	2	37.5	
Variants	2	0	0	2	0	
Semiquantitative						
Acinar predominant	126	63	37	26	63.0	0.694
Non-acinar predominant	167	67	44	56	60.3	
Lepidic predominant	66	34	9	23	79.0	0.008
Non-lepidic predominant	227	96	72	59	57.1	
Papillary predominant	31	14	10	7	58.3	0.726
Non-papillary predominant	262	116	71	75	64.1	
Solid predominant	35	7	17	11	29.1	0.726
Non-solid predominant	258	123	64	71	65.8	
Micropapillary predominant	10	3	5	2	37.5	0.265
Non-micropapillary predominant	283	127	76	80	62.6	
Variants of invasive	2	0	1	1	0	NS
Invasive carcinoma	287	130	81	82	61.6	
Acinar contained	183	94	52	37	64.4	0.215
Non-acinar containing	110	36	29	45	55.4	
Lepidic contained	109	56	19	34	74.7	0.004
Non-lepidic containing	184	74	62	48	54.4	
Papillary contained	79	42	20	17	67.7	0.213
Non-papillary containing	214	188	61	65	75.5	
Solid contained	64	14	32	18	30.4	<0.001
Non-solid containing	229	116	49	64	70.3	
Micropapillary containing	35	15	11	9	57.7	0.661
Non-micropapillary containing	258	115	70	73	62.1	
Variants containing	2	0	1	1	0	NS
Non-variants containing	291	130	80	81	61.9	
WHO						
Non-mixed subtypes	120	53	32	35	62.4	0.856
Mixed subtypes	173	77	49	47	61.1	

EGFR, epidermal growth factor receptor; NS, not significant; WHO, World Health Organization.

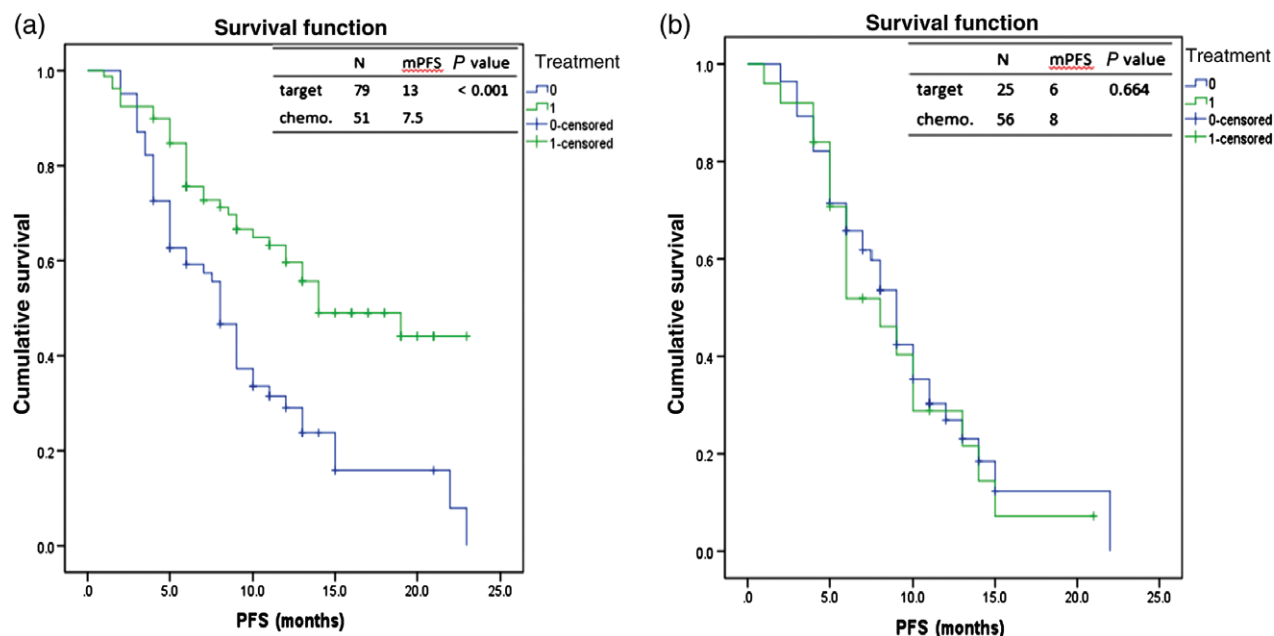


Figure 2 The relationship between epidermal growth factor receptor (*EGFR*) mutation and the choice of first-line treatment in patients with stage III–IV adenocarcinomas. **(a)** Progression-free survival (PFS) rates were longer with targeted therapy than with chemotherapy as first-line treatments in patients with *EGFR* mutations. **(b)** PFS rates were similar for targeted therapy and chemotherapy in patients with wild-type *EGFR*. (0: chemotherapy; 1-targeted therapy.) m, median.

EGFR-targeted therapy than those with exon 21 mutations. The details are summarized in Figure 3.

Different subtypes and progressive disease

Only one patient in the study had micropapillary predominant stage I–II disease, and achieved DFS of 19 months. Twelve patients had micropapillary containing disease, with a one-year DFS rate of 16.7%, the minimum adequate length for analysis. DFS rates at 12, 18, and 24 months were 62.5%, 40.0% and 22.9% in lepidic predominant cases. The details are summarized in Figure 4a and Table 6. Thus, the patients with lepidic predominant adenocarcinoma had the longest DFS of all the patients with stage I–II lung adenocarcinoma, while patients with micropapillary cancers had the shortest DFS. In patients with micropapillary predominant stage III–IV adenocarcinoma, the PFS rate at one year was 11.3%. In patients with lepidic containing disease, PFS rates at 6, 12, 18, and 24 months were 76.5%, 70.7%, 52.9%, and 3.9%, respectively. The details are summarized in Figure 4b and Table 7.

Cox regression analysis

Cox regression analysis showed that histologic subtypes ($P = 0.036$), treatment type ($P = 0.031$), and *EGFR*

mutation status ($P = 0.019$) may be good prognostic factors for lung adenocarcinoma. Details of the regression analysis are summarized in Table 8.

Discussion

As has been discussed previously by many researchers, the 2004 WHO diagnostic criteria and disease classification improved lung adenocarcinoma classification as it considered heterogeneous clinical, pathological, radiologic, and molecular criteria for this disease. Histologic subtypes and molecular typing had gradually become more important following the discovery of the target *EGFR* in 2004.¹⁹ In response to our improved understanding of the histopathology of lung adenocarcinoma, in 2011, a joint working group of IASLC/ATS/ERS proposed a new histologic classification of this cancer.³ This classification includes AIS, MIA, and invasive adenocarcinoma, but discards mixed adenocarcinoma. It further includes acinar, lepidic, papillary, solid, and micropapillary predominant disease. In the new classification, a subtype with >5% presence is considered contained, a subtype with >40% presence is defined as predominant, and other percentages are defined as mixed.²⁰ Studies from Europe and the United States reported a ratio of <20% prevalence of lepidic predominant and papillary predominant cases, 10–40% prevalence of acinar predominant cases, 20–40% prevalence of solid predominant cases,

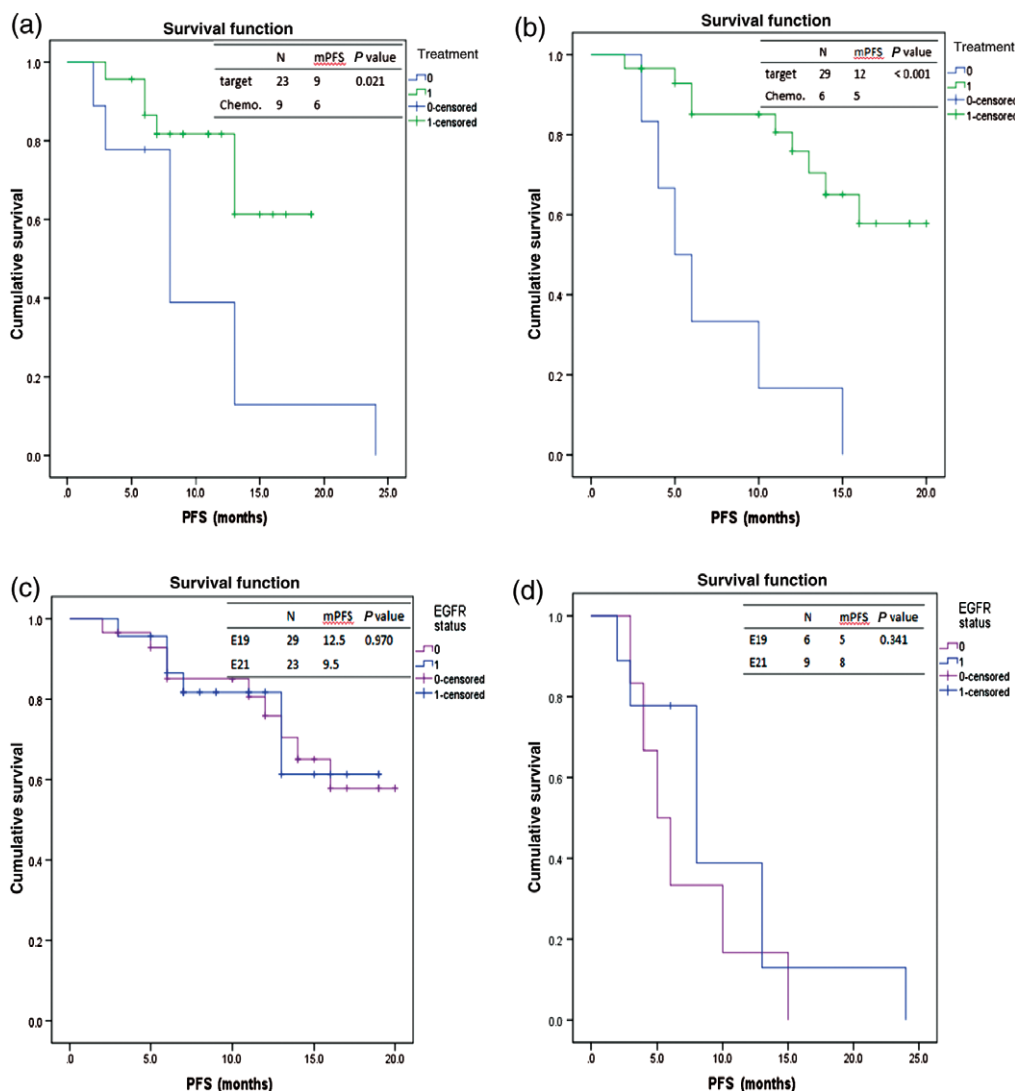


Figure 3 Correlations between treatment type and exon 19 and 21 mutations in patients with stage III–IV adenocarcinoma. Targeted therapy versus chemotherapy for (a) exon 19 mutations and (b) exon 21 mutations (0: chemotherapy; 1: targeted therapy). (c) Correlation between exon 19 and exon 21 mutation status and targeted therapy, (d) correlation between exon 19 and exon 21 mutation status and chemotherapy (0: exon 19 mutation; 1: exon 21 mutation). mPFS, median progression-free survival.

and <5% prevalence of micropapillary predominant cases.^{4,5,21,22} These studies support the suggestion that the prevalence of lepidic, papillary, micropapillary, and acinar predominant cases are elevated in Asia, and that the rate of solid cases is lower in Asia than in Europe or America.^{6,22–26} In this study, there were 126 acinar (43.9%), 66 lepidic (23.0%), 31 papillary (10.8%), 35 solid (12.2%), and 10 micropapillary predominant (3.5%) cases, similar to the results of a prior study.³ The frequency of patients with micropapillary disease in our study was low, which is possibly a result of the small sample size of the included cohort or the possible differences in race and region when compared with European populations.

Epidermal growth factor receptor mutation is reportedly most common in lepidic (40–70%), micropapillary (70–80%), papillary (60%), and acinar (50%) cases, but is less common in solid predominant (25%) cases.^{4,16,27} In the present study, *EGFR* mutation occurred in individuals with lepidic predominant (79.0%) and non-solid predominant (70.3%) disease. No *EGFR* testing was performed for the AIS and MIA cases because of the small number of relevant patients in the study. Other conclusions are similar to relevant trials. There was a statistically significant relationship between *EGFR* mutation and solid adenocarcinomas ($P < 0.001$).

In the IPASS study, the response and PFS rates in NSCLC with *EGFR* mutation were longer in the gefitinib

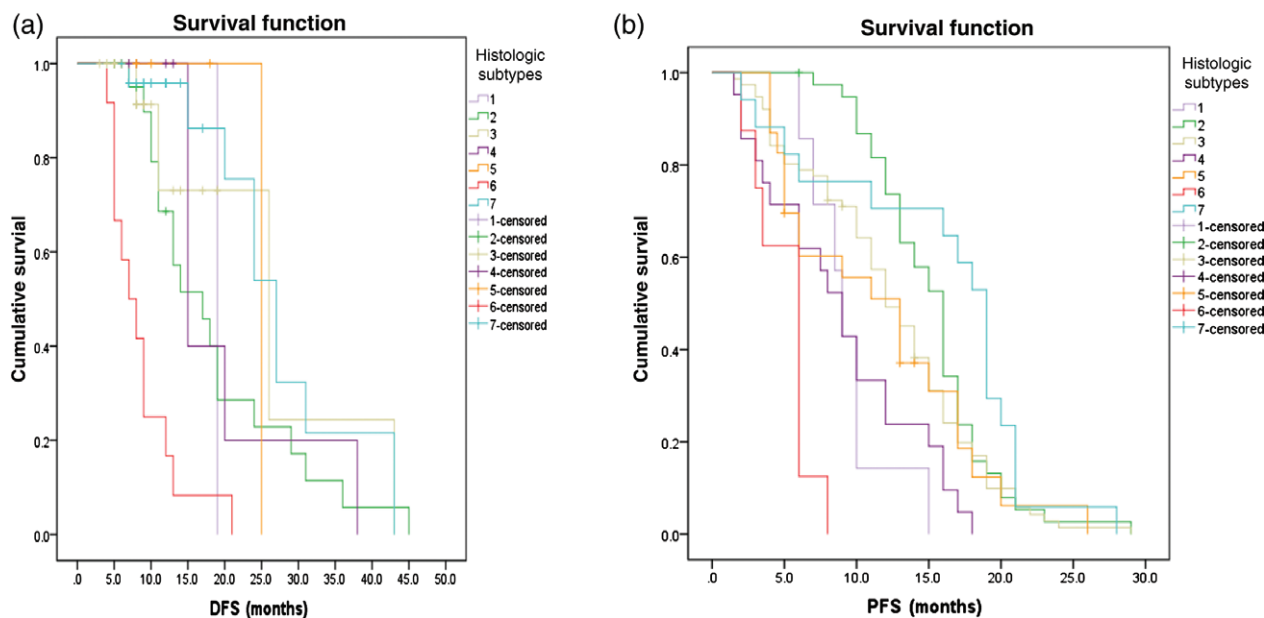


Figure 4 Correlations between histologic subtype progression and disease prognosis. **(a)** Histologic subtypes and disease recurrence in patients with stage I–II adenocarcinoma. **(b)** Histologic subtypes and disease progression in patients with stage III–IV disease (1: micropapillary predominant; 2: lepidic predominant; 3: acinar predominant; 4: solid predominant; 5: papillary predominant; 6: micropapillary containing; 7: lepidic containing). DFS, disease-free survival.

Table 6 Disease-free survival for patients with stage I–II lung cancer

Pathological subtypes(n)	DFS (months)		
	12	18	24
Micropapillary predominant (1)	100%	100%	0
Lepidic predominant (25)	62.5%	40.0%	22.9%
Acinar predominant (40)	73.0%	52.1%	24.3%
Solid predominant (12)	48.6%	37.2%	17.0%
Papillary predominant (6)	/	/	/
Micropapillary containing (12)	16.7%	0	0
Lepidic containing (25)	91.3%	80.5%	53.9%

DFS, disease-free survival.

Table 7 Progression-free survival for patients with stage III–IV lung cancer

Pathological subtypes (n)	PFS (months)			
	6	12	18	24
Micropapillary predominant (9) (%)	85.7	0	0	0
Lepidic predominant (41) (%)	97.4	81.6	15.8	2.6
Acinar predominant (86) (%)	78.9	49.2	17.0	1.4
Solid predominant (23) (%)	61.9	23.8	0	0
Papillary predominant (25) (%)	60.3	51.0	12.4	2.1
Micropapillary containing (8) (%)	12.5	0	0	0
Lepidic containing (18) (%)	76.5	70.7	52.9	3.9

PFS, progression-free survival.

than in the chemotherapy group (71.2% vs. 47.3%, $P < 0.001$; 9.5 m vs. 6.3 m, $P < 0.001$).^{8,28} The median PFS rate was 3.5 years for patients with *EGFR* mutation who received treatment with *EGFR*-TKIs. The NCCN, ESMO, and ASCO recommend performing *EGFR* gene testing before the initiation of treatment; however, some patients received chemotherapy after receiving a diagnosis and gene testing results, after receiving unclear results, or because they refused to wait for results. In our study, the median PFS rate in patients with stage III–IV disease and *EGFR* mutations who received targeted therapy was significantly longer than that for patients who received chemotherapy. Furthermore, the difference in PFS was statistically significant ($P < 0.001$), similar to the results of previous studies.^{3,8–13,28} Thus, *EGFR* status is important for therapy

selection. In 2015, ASCO noted that the median PFS in patients who received targeted therapy was longer in patients with exon 19 mutations than in patients with exon 21 mutations. In addition, patients with exon 19 mutations who received targeted therapy had longer PFS than patients with exon 21 mutations who received chemotherapy; however, the difference was not statistically significant in our study. This finding may prompt research into whether patients with exon 19 mutations can benefit from targeted therapy. No statistically significant results were observed for bone or cerebral metastases in the present study, as a result of the small sample sizes of *EGFR* mutation and wild-type *EGFR* cases; however, cerebral metastases are more common in patients with *EGFR* mutations. Sample sizes need to be increased in future research.

Table 8 Lung adenocarcinoma patients included in Cox regression analysis

	Wald	SE	P	95% CI
Gender	0.377	0.299	0.539	0.463–1.496
Age	1.056	0.013	0.304	0.961–1.013
EGFR mutation	5.539	0.195	0.019	0.431–0.926
Pathological subtypes	3.599	0.166	0.036	0.808–1.047
Therapy	4.652	0.270	0.031	1.055–3.037

CI, confidence interval; EGFR, epidermal growth factor receptor; SE, standard error.

Many studies have reported 100% five-year DFS and overall survival rates in patients with AIS and MIA.^{4–6,22–26} Our study only included six cases of AIS and MIA. The follow-up period was one to two years, and the patients showed no evidence of metastasis or relapse. In previous studies of stage I–II invasive adenocarcinoma in Asia, the five-year DFS rates were 70–95%, 60–80%, 0–50%, 55–70%, and 40–60% for lepidic, acinar, micropapillary, papillary, and solid cases, respectively.^{4–6,22–26} These findings may have been a consequence of the high rates of *EGFR* mutations in lepidic cases. Furthermore, in patients with stage I–IV invasive adenocarcinomas, overall survival rates were 70, 60, 50, 55, and 45 months for lepidic, acinar, solid, papillary, and micropapillary predominant cases, respectively.^{11,21,22} In our study, the one-year PFS rates were 11.3%, 70.7%, 49.2%, and 51.0% for micropapillary, lepidic, acinar, and papillary predominant cases. The difference between our rates and the findings of previous studies might be explained by the absence of sufficient preoperative staging, personalized therapy for surgical approaches, drugs, and duration of therapy.

The high *EGFR* mutation rates of lepidic and solid predominant cases in our study are informative for therapy selection. The findings generally support the idea that patients with exon 19 mutations can achieve greater benefits from targeted therapy; however, more clinical trials are needed on this topic. Micropapillary predominant cases had the shortest DFS and PFS and the highest degree of malignancy.

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Disclosure

No authors report any conflict of interest.

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