

RESEARCH ARTICLE

A Histopathological Feature of *EGFR*-Mutated Lung Adenocarcinomas with Highly Malignant Potential – An Implication of Micropapillary Element -

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Abstract

The purpose of this study was to define histological features determining the malignant potential of *EGFR*-mutated lung adenocarcinoma (LADC). Surgically resected tumors (*EGFR*-mutated LADCs with (21) and without (79) lymph node metastasis and *EGFR* wild-type LADCs with (26) and without (108) lymph node metastasis) and biopsy samples from inoperably advanced tumors (*EGFR*-mutated LADCs (78) and *EGFR* wild-type LADCs (99)) were examined. In surgically resected tumors, the *EGFR*-mutated LADCs with lymph node metastasis had the micropapillary element in a significantly greater proportion than others (Mann-Whitney tests $P \leq 0.026$). The proportion of micropapillary element was higher in the *EGFR*-mutated LADC at the advanced stage (stage II, III, or IV) than in the tumor at the early stage (stage I) (Mann-Whitney test, $P < 0.0001$). In the biopsy samples from inoperably advanced LADCs (177), *EGFR*-mutated tumors also had micropapillary element at a higher frequency than *EGFR*-wild type tumors (53/78 (68%), versus 30/99 (30%), Pearson χ^2 test, $P < 0.0001$). In stage I *EGFR*-mutated LADCs (84), the tumors with the micropapillary element (34) exhibited a significantly higher recurrence rate than tumors without micropapillary element (50) (5-year Recurrence-free survival 64.4% versus 93.3%, log-rank test $P = 0.028$). The micropapillary element may be an exclusive determinant of malignant potential in *EGFR*-mutated LADC. It is suggested that *EGFR*-mutated LADC may develop through a distinct histogenesis, in which the micropapillary element is important for promoting progression.

Introduction

Lung cancer is the leading cause of cancer-related death in the developed world, and lung adenocarcinoma (LADC) is the most common histological type of the disease. Recent research in

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molecular oncology has revealed that oncogenic mutations are required to promote tumor expansion, namely driver mutations, in LADC. These driver oncogenes include the *EGFR*, *KRAS*, *ALK*, *RET*, and *ROS* genes, mutations of which are mutually exclusive, and are crucial determinants indicating a favorable response to different molecular targeting agents [1] [2] [3] [4] [5] [6].

EGFR is the most common driver oncogene in LADCs, and mutations in this gene are seen in 20 to 50% of LADCs in Asians and 5 to 10% LADCs in Westerners [7] [8] [9]. *EGFR*-mutated LADCs have several unique features. They predominantly occur in females and non-smokers, and most cases are of the lepidic element-predominant histological subtype [7] [10] [11] [12] [13]. The lepidic element is a low-grade malignancy and is associated with a favorable outcome [14] [15] [16]. On the other hand, *EGFR*-mutated LADCs also include highly malignant tumors that are inoperably advanced. It remains unclear whether resectable tumors progress to become inoperable tumors or whether inoperable tumors develop independently through an exclusive carcinogenetic pathway. This is an important matter to be solved for better understanding of pathologic basis of *EGFR*-mutated LADC.

This study examined surgically resected tumors and biopsy samples from inoperably advanced tumors, and also defined the histopathological features associated with malignant potential in *EGFR*-mutated LADCs.

Materials and Methods

Patients

Three hundred and thirty-six LADCs that had been surgically resected (clinicopathological characteristics are presented in Table 1) and 177 LADC biopsy samples from inoperably

Table 1. Clinicopathological characteristics of surgically resected lung adenocarcinomas.

	<i>EGFR</i>		<i>P</i> -value
	Mutation (n = 142)	Wild-type (n = 194)	
Age (y/o)			0.001*
Median	70.5	67	
Range	38–86	36–84	
Gender			<0.0001*
Male	47	135	
Female	95	59	
Smoking status			<0.0001*
Never smoked	89	48	
Smoker	53	146	
Tumor size (mm)			0.628
≤30 mm	100	134	
>30 mm	42	60	
Stage			0.003*
I	103	106	
II	9	32	
III	29	51	
IV	1	5	

EGFR, *EGFR* mutation; y/o, years old; n, number of cases; *P*-values were calculated using the Mann-Whitney test (Age) and the Fisher’s exact test (other subjects); Asterisk(*), statistically significant

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Table 2. Clinical characteristics of inoperable lung adenocarcinomas.

	<i>EGFR</i>		<i>P</i> -value
	Mutation (n = 78)	Wild-type (n = 99)	
Age (y/o)			0.003*
Median	66	71	
Range	37–86	32–87	
Gender			<0.0001*
Male	25	74	
Female	53	25	
Smoking status			0.0002*
Never smoked	32	18	
Smoker	19	46	
Unknown	27	35	

EGFR, *EGFR* mutation; y/o, years old; n, number of cases; *P*-values were calculated using the Mann-Whitney test (Age) and the Pearson χ^2 test (other subjects); Asterisk(*), statistically significant

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advanced tumors (Table 2) were examined. These tumors were resected or biopsied between January 1997 and December 2013. Informed consent for the use of these samples for research purposes was obtained in writing. The ethics committees of Kanagawa Prefectural Cardiovascular and Respiratory Center and Yokohama City University approved the research plan.

Histopathological examination

Hematoxylin and eosin-stained sections were subjected to histological examination.

Mutational analysis of the *EGFR* gene

The *EGFR* mutations (in exons 18, 19, 20, and 21) in surgically resected tumors were analyzed using previously described methods [17] [18]. The Scorpion amplification refractory mutation system method was used to search for mutations in the biopsy samples [19] [20].

Statistical analysis

Pearson's χ^2 test or Fisher's exact test were used in combination with the Mann-Whitney test to analyze categorical and continuous variables, respectively. Recurrence curves were plotted using the Kaplan-Meier method and the absolute risk of recurrence at five years was estimated. Differences in the recurrence-free survival (RFS) were analyzed using the log-rank test. The Fleiss kappa statistic was used to measure interobserver agreement [21]. *P*-values of <0.05 were considered to be significant. All analyses were performed using JMP 9.0.2 (SAS Institute, Cary, NC, USA), SPSS version 21 (SPSS, Chicago, IL, USA), or the statistical software R (R Development Core Team 2014).

Results

Histological element that associates with malignant potential in *EGFR*-mutated LADCs

The study groups were assigned according to a flowchart described in figure 1 (Fig 1). Proportions of the histological elements (lepidic, acinar, papillary, micropapillary (mPAP), and solid elements) were described in 5% increments according to the World Health Organization

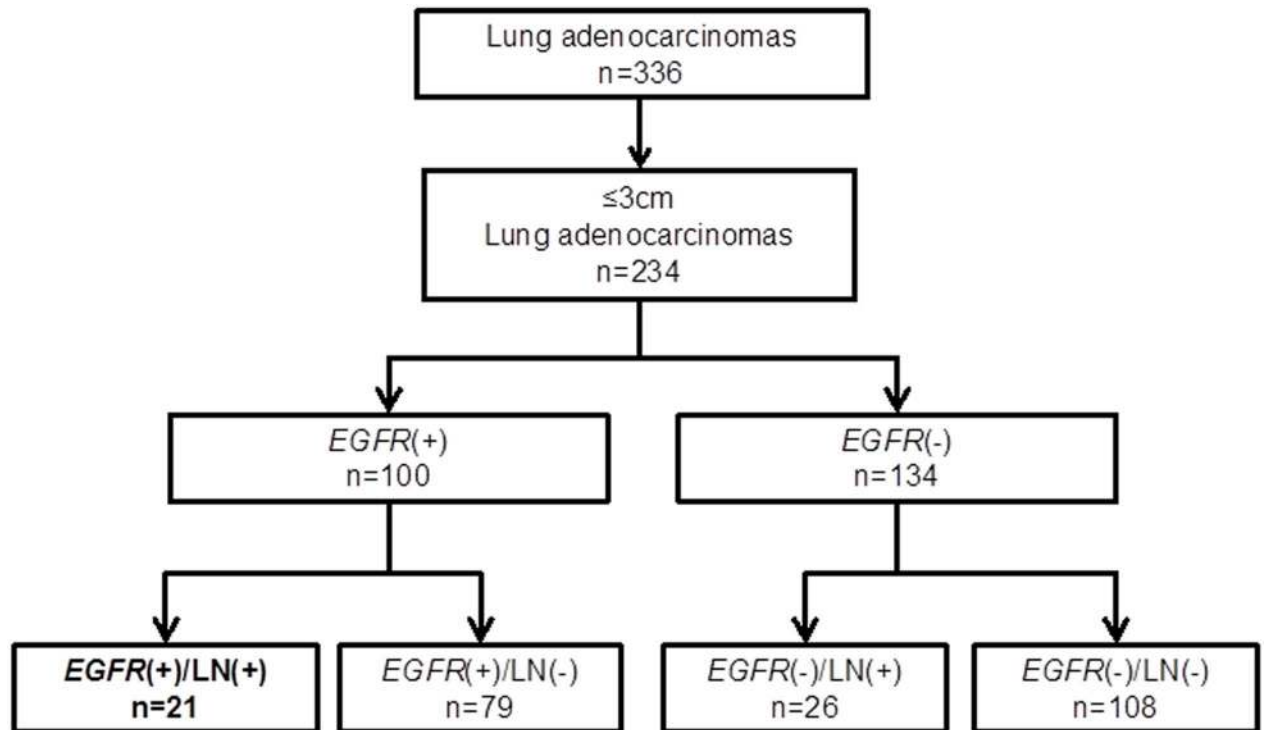


Fig 1. The flowchart used to assign the tumors to the four groups. n, number of tumors; *EGFR*, *EGFR* mutation; LN, lymph node metastasis; +, positive; -, negative.

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(WHO) classification [22] [23]. The proportions in the *EGFR*-mutated LADCs with lymph node metastasis were compared with those in the other three groups. The proportion of mPAP element was consistently and significantly greater in *EGFR*-mutated LADCs with lymph node metastasis than in any of the other groups (Table 3). Differences in proportions of the other elements were not consistent in comparisons between *EGFR*-mutated LADCs with lymph node metastasis and the other groups (Table 3). Representative appearances of the elements are shown in figure 2 (Fig 2).

The mPAP element and disease stage

In *EGFR*-mutated LADCs, the proportion of mPAP element in the tumor at the advanced stage (stage II, III, or IV) was significantly higher than that in the tumor at the early stage (stage I) (Mann-Whitney test, $P < 0.0001$; Fig 3A). In *EGFR* wild-type LADCs, the proportion of mPAP element showed no significant differences between the early stage tumors and the advanced stage tumors (Mann-Whitney test, $P = 0.085$; Fig 3B). These results suggested that the mPAP element may participate exclusively in the progression of *EGFR*-mutated LADC.

The mPAP element in inoperably advanced LADCs

Biopsy samples from inoperably advanced LADCs were also examined. Representative histological appearances of the biopsy specimens are shown in figure 4 (Fig 4). The mPAP element was detected at a significantly higher frequency in *EGFR*-mutated LADCs than in the *EGFR* wild-type LADCs (53/78 (68%), versus (vs) 30/99 (30%), Pearson χ^2 test, $P < 0.0001$). This

Table 3. Differences in the histological elements between the *EGFR*(+)/LN(+) group and the other groups.

	<i>EGFR</i> (+)/LN(+)	<i>EGFR</i> (+)/LN(-)	<i>P</i> -value
LEP	30 (0–95)	70 (5–100)	0.0008*
ACI	30 (5–80)	10 (0–75)	0.023*
PAP	5 (0–60)	0 (0–80)	0.321
mPAP	5 (0–40)	0 (0–80)	0.025*
SOL	0 (0–70)	0 (0–30)	0.217
	<i>EGFR</i> (+)/LN(+)	<i>EGFR</i> (-)/LN(+)	<i>P</i> -value
LEP	30 (0–95)	7.5 (0–80)	0.044*
ACI	30 (5–80)	32.5 (0–100)	0.554
PAP	5 (0–60)	0 (0–50)	0.009*
mPAP	5 (0–40)	0 (0–30)	0.026*
SOL	0 (0–70)	10 (0–100)	0.019*
	<i>EGFR</i> (+)/LN(+)	<i>EGFR</i> (-)/LN(-)	<i>P</i> -value
Lepidic	30 (0–95)	80 (0–100)	0.013*
ACI	30 (5–80)	10 (0–100)	0.031*
PAP	5 (0–60)	0 (0–95)	<0.0001*
mPAP	5 (0–40)	0 (0–15)	<0.0001*
SOL	0 (0–70)	0 (0–95)	0.702

P-values were calculated using the Mann-Whitney test. Asterisk(*), statistically significant; *EGFR*, *EGFR* mutation; LN, lymph node metastasis; +, positive; -, negative; LEP, lepidic; ACI, acinar; PAP, papillary; mPAP, micropapillary; SOL, solid subtype

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result supports the idea that the mPAP element may participate exclusively in the progression of *EGFR*-mutated LADC.

The mPAP element and postoperative recurrence

The association between the proportion of mPAP element and postoperative recurrence was analyzed in surgically resected stage I *EGFR*-mutated LADCs. The median follow-up period was 57 months (range: 1–159 months). Seventeen patients had recurrent disease and 15 patients died during follow-up. The recurrence-free survival (RFS) of *EGFR*-mutated LADCs that contained the mPAP element was worse than that of the *EGFR*-mutated LADCs that did not contain the mPAP element (Fig 5A). The difference was statistically significant when the mPAP element proportion cut-off value was set at 5% (5-year RFS 64.4% vs 93.3%, *P* = 0.028) or 10% (5-year RFS 57.1% vs 87.6%, *P* = 0.005) (Fig 5A and 5B), although no significant difference was found when the cut-off value was set at 20% (5-year RFS 40.0% vs 84.0%, *P* = 0.102) (Fig 5C). Number of tumors with mPAP element proportions of ≥20% may be too small for analysis. It was confirmed that the mPAP element could be a determinant of the malignant potential in *EGFR*-mutated LADCs.

The potential prognostic impact of mPAP element for *EGFR*-mutated LADCs

We additionally evaluated a prognostic impact of mPAP element for *EGFR*-mutated LADCs, as we considered an absolute volume of mPAP element may be more closely correlated with the malignant potential of the tumor than mPAP proportion. We defined the mPAP estimated

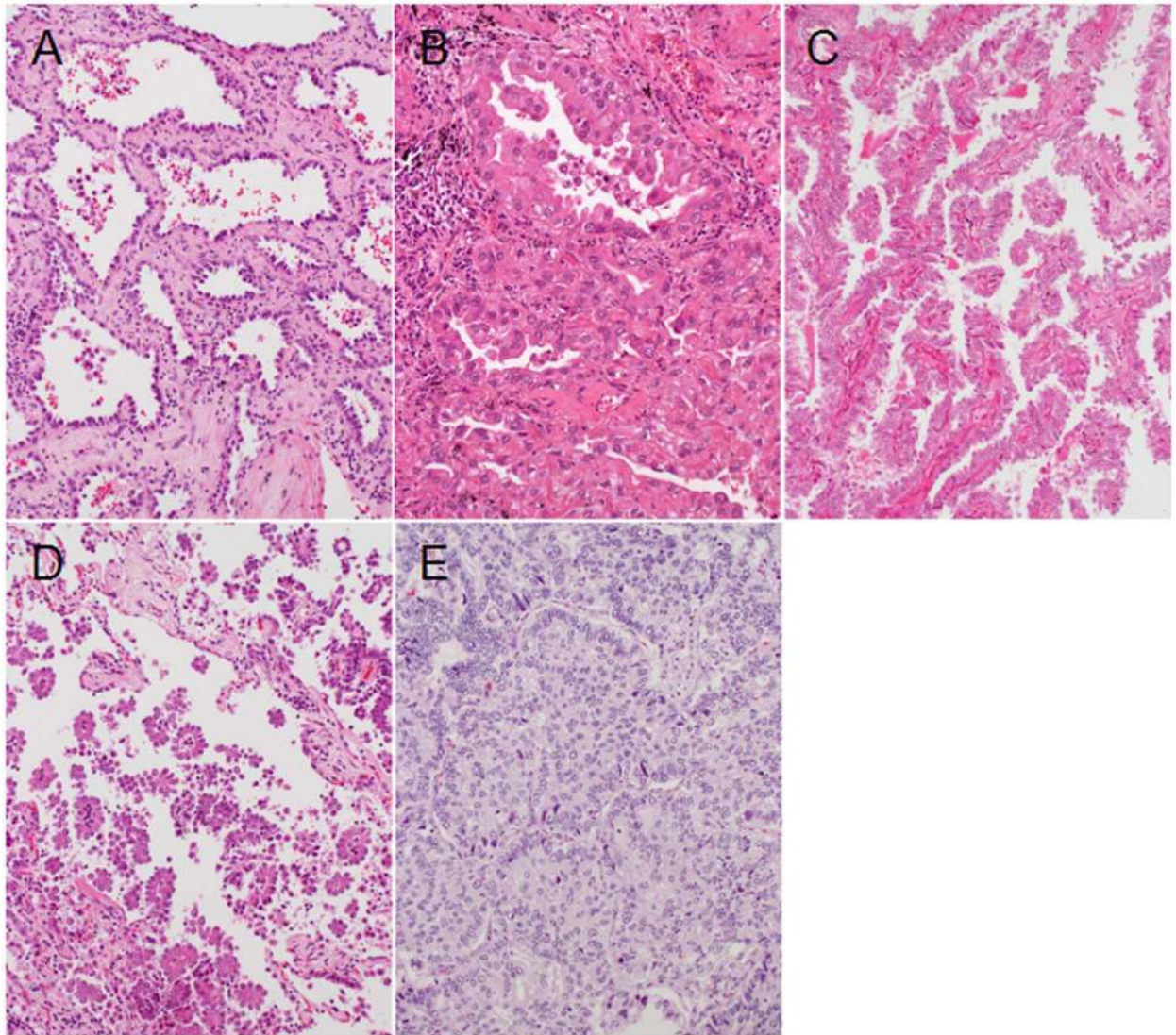


Fig 2. Representative appearances of the major histological subtypes of lung adenocarcinoma (hematoxylin and eosin stain, $\times 200$). A, The lepidic subtype is characterized by the extension of neoplastic cells along the surface of the alveolar walls; B, The acinar subtype is characterized by tubular or glandular structures invading a fibrous stroma; C, The papillary subtype is characterized by the extension of neoplastic cells on the surfaces of fibrovascular cores; D, The micropapillary subtype is characterized by the formation of tufted papillary structures that lack a central fibrovascular core and float in the alveolar space; E, The solid subtype is characterized by the formation of solid nests consisting of neoplastic cells.

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volume (EV) as the percentage of the mPAP element multiplied by the square of the tumor's largest radius [mPAP EV = (the tumor's largest radius [mm])² × (percentage of the mPAP element [%])/100]. The mPAP EV was found to be significantly correlated with RFS (Fig 6A, 6B and 6C). The lowest *p*-value ($P < 0.0001$) was obtained when the mPAP EV cut-off value was set at 15 (5-year RFS 42.3% vs 89.9%; Fig 6B). Table 4 summarizes the univariate association between clinicopathological factors and RFS. Lymphatic canal invasion ($P < 0.001$), vascular invasion ($P = 0.011$) and mPAP EV (cut-off value: 15, $P < 0.001$) were associated with worse RFS. Multivariate analysis revealed that the mPAP EV ($P = 0.004$) and lymphatic canal invasion ($P = 0.009$) were independent predictors of disease recurrence (Table 5). These results

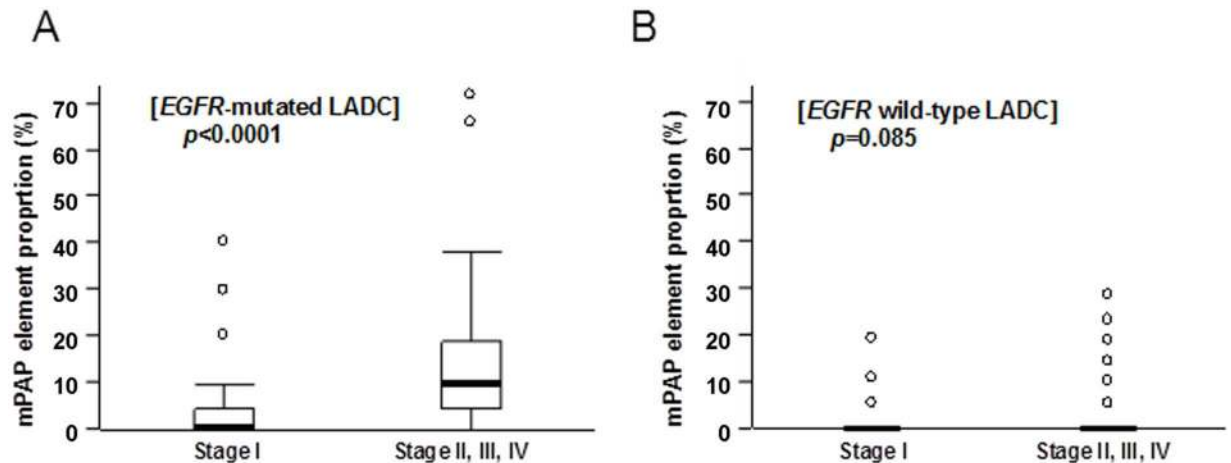


Fig 3. Proportions of the micropapillary (mPAP) element in different stages of surgically resected lung adenocarcinomas (LADCs). A, stage I *EGFR*-mutated LADCs (n = 103) versus (vs) stage II-IV *EGFR*-mutated LADCs (n = 39); B, stage I *EGFR* wild-type LADCs (n = 106) vs stage II-IV *EGFR* wild-type LADCs (n = 88); n, number of tumors examined mPAP element proportions are displayed as a box-and-whiskers plot with median (thick line), 25th to 75th percentile (box) and 10th to 90th percentile (whiskers) and outliers (circles). *P*-values were calculated using the Mann-Whitney test.

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confirmed again that the mPAP element may be an important determinant of the malignant grade in *EGFR*-mutated LADCs. The mPAP EV also has a prognostic impact for predicting the postoperative recurrence of *EGFR*-mutated LADCs, which may be superior to the mPAP proportion (EV vs proportion, sensitivity 39% vs 33%; specificity 90% vs 86%; significance level <0.0001 vs 0.005 , Figs 6B vs 5B). A Fleiss kappa statics from the mPAP EV (cut-off value: 15) judged by five pathologists supported good diagnostic concordance (Fleiss kappa value 0.689 , $P < 0.001$). The mPAP EV may be fit for clinical use.

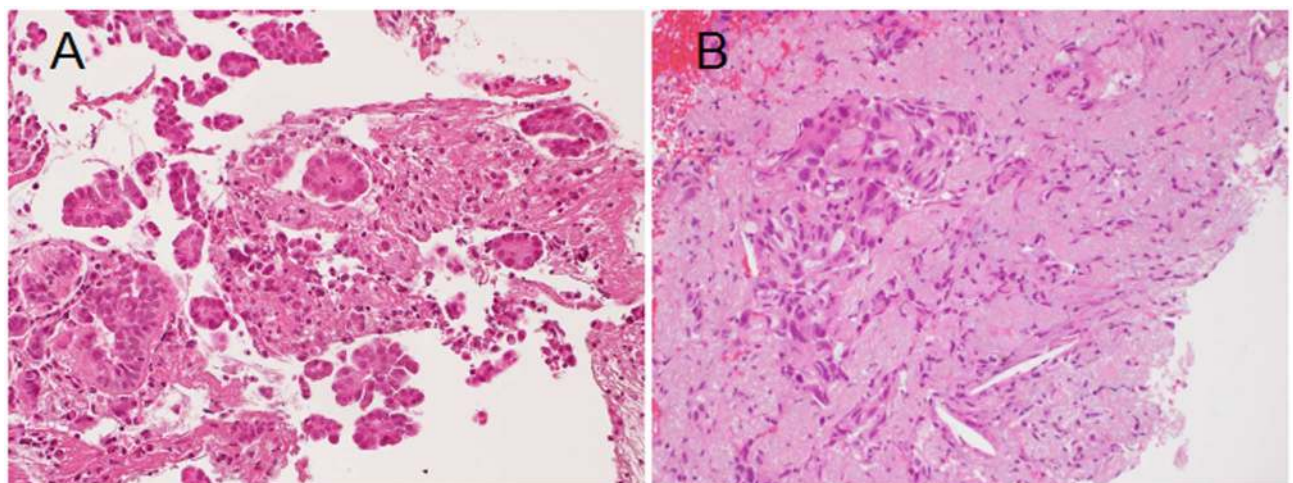


Fig 4. Representative histological appearances of the biopsy specimens (A, *EGFR*-mutated lung adenocarcinoma (LADC); B, *EGFR* wild-type LADC). The micropapillary element, which is composed of papillary structures lacking fibrovascular cores, floats in alveolar spaces (A, hematoxylin and eosin (HE) stain, $\times 200$). The acinar element (and some crush artifacts) grows in collapse fibrosis (B, HE stain, $\times 200$).

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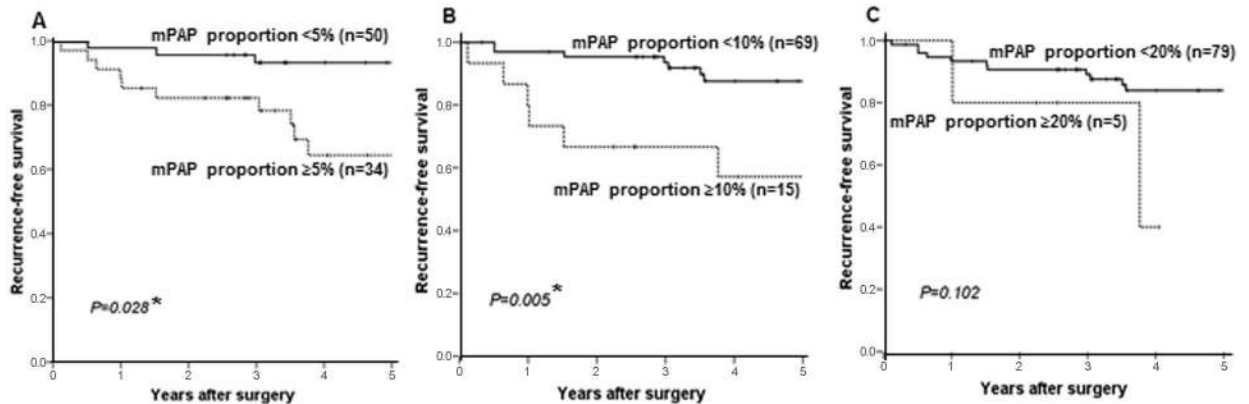


Fig 5. Kaplan-Meier recurrence-free survival curves of the association between the proportion of micropapillary (mPAP) element and disease recurrence in patients with stage I *EGFR*-mutated lung adenocarcinomas. A, tumors in which the mPAP element accounted for $\geq 5\%$ of the tumor versus (vs) those in which the mPAP element accounted for $< 5\%$ of the tumor ($P = 0.028$ in the Log-rank test); B, tumors in which the mPAP element accounted for $\geq 10\%$ of the tumor vs those in which the mPAP element accounted for $< 10\%$ of the tumor ($P = 0.005$ in the Log-rank test); C, tumors in which the mPAP element accounted for $\geq 20\%$ of the tumor vs those in which the mPAP element accounted for $< 20\%$ of the tumor ($P = 0.102$ in the Log-rank test); n, number of tumors examined; asterisk(*), statistically significant.

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The mPAP element and types of *EGFR* mutations

No significant difference in types of *EGFR* mutations (major or minor mutations) between tumors with mPAP and those without mPAP was found (Table 6).

Discussion

The histopathological features of *EGFR*-mutated LADC have been extensively investigated [12] [13]. However, most studies examined only surgically resected tumors. The histological features of inoperably advanced *EGFR*-mutated LADC, which are really indicative for *EGFR* tyrosine kinase inhibitor (*EGFR*-TKI) treatment [22] [24], have not been defined. Thus, it is

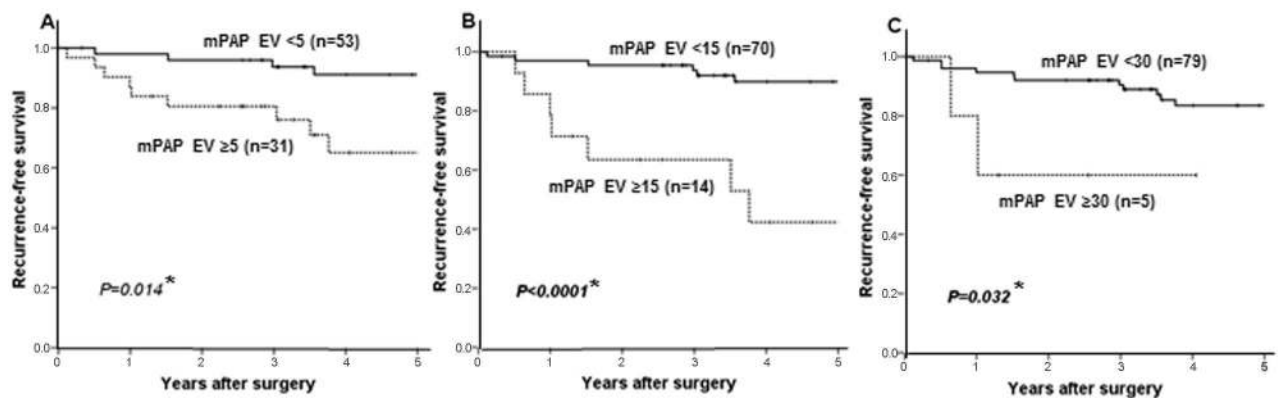


Fig 6. Kaplan-Meier recurrence-free survival curves of the association between the micropapillary (mPAP) estimated volume (EV) and disease recurrence in patients with stage I *EGFR*-mutated lung adenocarcinomas. A, tumors with mPAP EV of ≥ 5 versus (vs) those with mPAP EV of < 5 ($P = 0.014$ in the Log-rank test); B, tumors with mPAP EV of ≥ 15 vs those with mPAP EV of < 15 ($P < 0.0001$ in the Log-rank test); C, tumors with mPAP EV of ≥ 30 vs those with mPAP EV of < 30 ($P = 0.032$ in the Log-rank test); n, number of tumors examined; asterisk(*), statistically significant.

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Table 4. Clinicopathological characteristics and disease recurrence in patients with stage I EGFR-mutated lung adenocarcinomas (univariate analyses).

	n	%	5-year RFS (%)	P-value
Sex				0.683
Male	24	28.6	83.3	
Female	60	71.4	81.1	
Age (y/o)				0.656
≤65	29	34.5	72.6	
≥66	55	65.5	87.1	
Smoking status				0.55
Never	56	66.7	82.1	
Former & current	28	33.3	80.9	
Surgical procedure				0.176
Lobectomy	65	77.4	78	
Segmentectomy	7	8.3	85.7	
Partial resection	12	14.3	100	
Tumor size (mm)				0.152
≤30 mm	61	72.6	84.7	
>30 mm	23	27.4	73.9	
Stage				0.098
IA	57	67.9	86.4	
IB	27	32.1	70.8	
Adjuvant chemotherapy				0.099
No	77	91.7	82.9	
Yes	7	8.3	71.4	
Lymphatic canal invasion				<0.001*
Present	4	4.8	75	
Absent	80	95	84.7	
Vascular invasion				0.011*
Present	19	22.6	59.6	
Absent	65	77.4	88.5	
Pleural invasion				0.252
Present	5	6	53.3	
Absent	79	94	83.9	
EGFR mutations				0.611
Major mutation (exon 19, 21)	75	89.3	81.2	
Minor mutation (exon 18, 20)	9	10.7	87.5	
mPAP estimated volume				<0.001*
<15	70	83.3	89.9	
≥15	14	16.7	42.3	

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Table 5. Multivariate analysis performed using the Cox proportional hazards model.

	HR	95% CI	P-value
mPAP estimated volume (cut-off: 15)	6.274	1.78–22.17	0.004*
Lymphatic canal invasion	8.8	1.71–45.20	0.009*
Vascular invasion	0.949	0.238–3.78	0.940

HR, hazard ratio; CI, confidence interval; mPAP, micropapillary; Asterisk(*), statistically significant

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Table 6. Difference in types of *EGFR* mutations between tumors with mPAP and without mPAP element.

	tumors with mPAP element	tumors without mPAP element
Major mutation (exon 19, 21)	63 [52]	65 [24]
Minor mutation (exon 18, 20)	8 [1]	6 [1]

EGFR, *EGFR* mutation; mPAP, micropapillary;

The numbers of surgically resected tumors and [inoperably advanced tumors] are shown.

P-values were calculated using the Fisher's exact test.

P-values were 0.779 (surgically resected tumors) and 0.541 (inoperably advanced tumors).

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unclear whether resectable tumors progress to become inoperable tumors or whether inoperable tumors develop independently de novo. In this study, we examined both surgically resected tumors and biopsy samples from inoperable tumors and defined histological features determining the malignant potential of *EGFR*-mutated LADCs. The mPAP element preferentially arose in *EGFR*-mutated LADCs and was more common in advanced tumors. Previous studies have demonstrated that the mPAP element is associated with lymphatic canal involvement, leading to lymph node metastasis, which results in unfavorable LADC outcomes [25] [26] [27] [28]. Chao et al. recently reported that the mPAP element is associated with worse outcomes in patients with *EGFR*-mutated LADC, supporting our findings [29]. Taken together with these findings, *EGFR*-mutated LADC may develop through a unique carcinogenetic pathway in which the low-grade lepidic subtype progresses to the high-grade mPAP subtype (Schema shows the virtual carcinogenetic pathways of the *EGFR*-mutated and the *EGFR* wild-type LADCs; Fig 7).

On the other hand, it is noteworthy that the papillary element as well as mPAP element was also detected at a higher frequency in *EGFR*-mutated LADCs. This finding agrees with the notion that the papillary element may be a precursor for the mPAP element [30]. Papillary and mPAP are also occasionally found in *EGFR* wild-type LADCs, although these elements were rarely detected and their association with the malignancy grade was not statistically significant. Undefined mutations having potential biological activity equivalent to that of *EGFR* mutations (mutations of *EGFR* family members) may occur in *EGFR* wild-type LADCs with mPAP elements [31].

The present study also proposed that the mPAP EV may be a useful prognostic marker for predicting the recurrence of *EGFR*-mutated LADCs. Although patients with *EGFR*-mutated LADC generally exhibit favorable postoperative outcomes, a considerable proportion still dies of recurrent disease [12] [32]. Clinical trials of postoperative adjuvant *EGFR*-TKI therapy for patients with *EGFR*-mutated LADCs are currently in progress (WJOG6410L study, CTONG1104 study, ALCHEMIST study) [33] [34] [35]. The identification of tumors that are at high risk of recurrence and the adjuvant use of appropriate molecular targeting agents may be one way of improving postoperative survival. The mPAP EV parameter proposed here can be used to aid the identification of tumors that are at high risk of recurrence.

In summary, *EGFR*-mutated LADC may develop through a distinct carcinogenetic pathway, in which the mPAP element may play an important role in promoting progression. The mPAP element also has prognostic value. We hope that our efforts will increase current knowledge about the carcinogenesis of *EGFR*-mutated LADC and lead to improvements in the therapeutic strategies for such tumors.

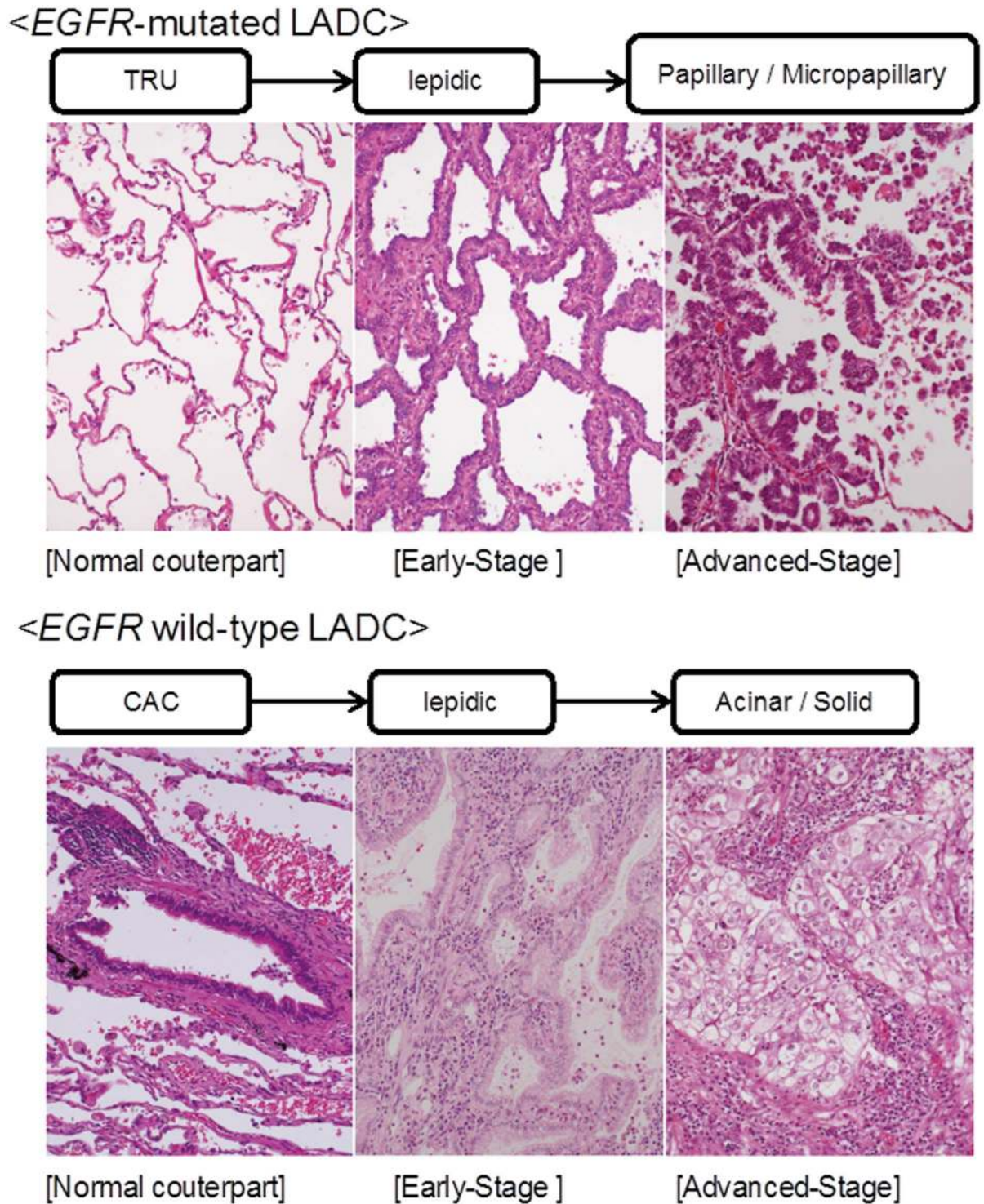


Fig 7. Hypothetical schema for histogenesis of the *EGFR*-mutated and the *EGFR* wild-type lung adenocarcinomas (LADCs). In early stages, *EGFR*-mutated LADC, which may develop from terminal respiratory units (TRU) [22], exhibits lepidic patterns consisting of neoplastic cells with hobnail or spheroid morphology. In advanced stages, they progress to form papillary and micropapillary patterns (upper panel). *EGFR* wild-type LADC, which may develop from the central airway compartment (CAC) [22], exhibits a lepidic pattern consisting of neoplastic cells with columnar morphology, and progresses to form acinar and solid patterns (lower panel). Magnification of all photographs is $\times 200$.

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Author Contributions

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Data curation: MM YK.

Formal analysis: MM SU TW.

Funding acquisition: KOk KOH.

Investigation: MM YK.

Methodology: MM KOk.

Project administration: KOk KOH.

Resources: AS HA MT.

Supervision: KOk KOH.

Validation: MM KOk SU YT KOH.

Writing – original draft: MM.

Writing – review & editing: KOk KOH.

References

1. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, et al. (2007) Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 448: 561–566. doi: [10.1038/nature05945](https://doi.org/10.1038/nature05945) PMID: [17625570](https://pubmed.ncbi.nlm.nih.gov/17625570/)
2. Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, et al. (2012) RET, ROS1 and ALK fusions in lung cancer. *Nat Med* 18: 378–381. doi: [10.1038/nm.2658](https://doi.org/10.1038/nm.2658) PMID: [22327623](https://pubmed.ncbi.nlm.nih.gov/22327623/)
3. Gainor JF, Shaw AT (2013) Novel targets in non-small cell lung cancer: ROS1 and RET fusions. *Oncologist* 18: 865–875. doi: [10.1634/theoncologist.2013-0095](https://doi.org/10.1634/theoncologist.2013-0095) PMID: [23814043](https://pubmed.ncbi.nlm.nih.gov/23814043/)
4. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, et al. (2004) EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 304: 1497–1500. doi: [10.1126/science.1099314](https://doi.org/10.1126/science.1099314) PMID: [15118125](https://pubmed.ncbi.nlm.nih.gov/15118125/)
5. Ou SH (2011) Crizotinib: a novel and first-in-class multitargeted tyrosine kinase inhibitor for the treatment of anaplastic lymphoma kinase rearranged non-small cell lung cancer and beyond. *Drug Des Devel Ther* 5: 471–485. doi: [10.2147/DDDT.S19045](https://doi.org/10.2147/DDDT.S19045) PMID: [22162641](https://pubmed.ncbi.nlm.nih.gov/22162641/)
6. Gainor JF, Varghese AM, Ou SH, Kabraji S, Awad MM, et al. (2013) ALK rearrangements are mutually exclusive with mutations in EGFR or KRAS: an analysis of 1,683 patients with non-small cell lung cancer. *Clin Cancer Res* 19: 4273–4281. doi: [10.1158/1078-0432.CCR-13-0318](https://doi.org/10.1158/1078-0432.CCR-13-0318) PMID: [23729361](https://pubmed.ncbi.nlm.nih.gov/23729361/)
7. Kosaka T, Yatabe Y, Endoh H, Kuwano H, Takahashi T, et al. (2004) Mutations of the epidermal growth factor receptor gene in lung cancer: biological and clinical implications. *Cancer Res* 64: 8919–8923. doi: [10.1158/0008-5472.CAN-04-2818](https://doi.org/10.1158/0008-5472.CAN-04-2818) PMID: [15604253](https://pubmed.ncbi.nlm.nih.gov/15604253/)
8. Shigematsu H, Lin L, Takahashi T, Nomura M, Suzuki M, et al. (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 97: 339–346. doi: [10.1093/jnci/dji055](https://doi.org/10.1093/jnci/dji055) PMID: [15741570](https://pubmed.ncbi.nlm.nih.gov/15741570/)
9. Shi Y, Au JS, Thongprasert S, Srinivasan S, Tsai CM, et al. (2014) A prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER). *J Thorac Oncol* 9: 154–162. doi: [10.1097/JTO.000000000000033](https://doi.org/10.1097/JTO.000000000000033) PMID: [24419411](https://pubmed.ncbi.nlm.nih.gov/24419411/)

10. Haneda H, Sasaki H, Lindeman N, Kawano O, Endo K, et al. (2006) A correlation between EGFR gene mutation status and bronchioloalveolar carcinoma features in Japanese patients with adenocarcinoma. *Jpn J Clin Oncol* 36: 69–75. doi: [10.1093/jco/hyi228](https://doi.org/10.1093/jco/hyi228) PMID: [16449241](https://pubmed.ncbi.nlm.nih.gov/16449241/)
11. Okudela K, Woo T, Mitsui H, Yazawa T, Shimoyamada H, et al. (2010) Morphometric profiling of lung cancers-its association with clinicopathologic, biologic, and molecular genetic features. *Am J Surg Pathol* 34: 243–255. doi: [10.1097/PAS.0b013e3181c79a6f](https://doi.org/10.1097/PAS.0b013e3181c79a6f) PMID: [20061937](https://pubmed.ncbi.nlm.nih.gov/20061937/)
12. Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Fujimoto M, et al. (2013) Validation of the IASLC/ATS/ERS lung adenocarcinoma classification for prognosis and association with EGFR and KRAS gene mutations: analysis of 440 Japanese patients. *J Thorac Oncol* 8: 52–61. doi: [10.1097/JTO.0b013e3182769aa8](https://doi.org/10.1097/JTO.0b013e3182769aa8) PMID: [23242438](https://pubmed.ncbi.nlm.nih.gov/23242438/)
13. Villa C, Cagle PT, Johnson M, Patel JD, Yeldandi AV, et al. (2014) Correlation of EGFR mutation status with predominant histologic subtype of adenocarcinoma according to the new lung adenocarcinoma classification of the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society. *Arch Pathol Lab Med* 138: 1353–1357. doi: [10.5858/arpa.2013-0376-OA](https://doi.org/10.5858/arpa.2013-0376-OA) PMID: [24571650](https://pubmed.ncbi.nlm.nih.gov/24571650/)
14. Yokose T, Suzuki K, Nagai K, Nishiwaki Y, Sasaki S, et al. (2000) Favorable and unfavorable morphological prognostic factors in peripheral adenocarcinoma of the lung 3 cm or less in diameter. *Lung Cancer* 29: 179–188. PMID: [10996420](https://pubmed.ncbi.nlm.nih.gov/10996420/)
15. Warth A, Muley T, Meister M, Stenzinger A, Thomas M, et al. (2012) The novel histologic International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification system of lung adenocarcinoma is a stage-independent predictor of survival. *J Clin Oncol* 30: 1438–1446. doi: [10.1200/JCO.2011.37.2185](https://doi.org/10.1200/JCO.2011.37.2185) PMID: [22393100](https://pubmed.ncbi.nlm.nih.gov/22393100/)
16. Kadota K, Villena-Vargas J, Yoshizawa A, Motoi N, Sima CS, et al. (2014) Prognostic significance of adenocarcinoma in situ, minimally invasive adenocarcinoma, and nonmucinous lepidic predominant invasive adenocarcinoma of the lung in patients with stage I disease. *Am J Surg Pathol* 38: 448–460. doi: [10.1097/PAS.000000000000134](https://doi.org/10.1097/PAS.000000000000134) PMID: [24472852](https://pubmed.ncbi.nlm.nih.gov/24472852/)
17. Okudela K, Woo T, Yazawa T, Ogawa N, Tajiri M, et al. (2009) Significant association between EGFR-mutated lung adenocarcinoma and past illness from gastric cancer or uterine myoma: its implication in carcinogenesis. *Lung Cancer* 66: 287–291. doi: [10.1016/j.lungcan.2009.02.025](https://doi.org/10.1016/j.lungcan.2009.02.025) PMID: [19362747](https://pubmed.ncbi.nlm.nih.gov/19362747/)
18. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, et al. (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350: 2129–2139. doi: [10.1056/NEJMoa040938](https://doi.org/10.1056/NEJMoa040938) PMID: [15118073](https://pubmed.ncbi.nlm.nih.gov/15118073/)
19. Kimura H, Kasahara K, Kawaishi M, Kunitoh H, Tamura T, et al. (2006) Detection of epidermal growth factor receptor mutations in serum as a predictor of the response to gefitinib in patients with non-small-cell lung cancer. *Clin Cancer Res* 12: 3915–3921. doi: [10.1158/1078-0432.CCR-05-2324](https://doi.org/10.1158/1078-0432.CCR-05-2324) PMID: [16818687](https://pubmed.ncbi.nlm.nih.gov/16818687/)
20. Goto K, Satouchi M, Ishii G, Nishio K, Hagiwara K, et al. (2012) An evaluation study of EGFR mutation tests utilized for non-small-cell lung cancer in the diagnostic setting. *Ann Oncol* 23: 2914–2919. doi: [10.1093/annonc/mds121](https://doi.org/10.1093/annonc/mds121) PMID: [22776705](https://pubmed.ncbi.nlm.nih.gov/22776705/)
21. Fleiss JL (1971) Measuring nominal scale agreement among many raters. *Psychological bulletin* 76: 378.
22. Travis WD, Noguchi M, Yatabe Y, Brambilla E, Nicholson AG, et al. (2015) Adenocarcinoma. In: Travis WD, Brambilla E, Burke AP, Marx A, Nicholson AG, editors. *WHO Classification of Tumours of the Lung, Pleura, Thymus and Heart*. Lyon: IARC Press.
23. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, et al. (2011) International association for the study of lung cancer/american thoracic society/european respiratory society international multi-disciplinary classification of lung adenocarcinoma. *J Thorac Oncol* 6: 244–285. doi: [10.1097/JTO.0b013e318206a221](https://doi.org/10.1097/JTO.0b013e318206a221) PMID: [21252716](https://pubmed.ncbi.nlm.nih.gov/21252716/)
24. Goldstraw P, Ball D, Jett JR, Le Chevalier T, Lim E, et al. (2011) Non-small-cell lung cancer. *Lancet* 378: 1727–1740. doi: [10.1016/S0140-6736\(10\)62101-0](https://doi.org/10.1016/S0140-6736(10)62101-0) PMID: [21565398](https://pubmed.ncbi.nlm.nih.gov/21565398/)
25. Amin MB, Tamboli P, Merchant SH, Ordonez NG, Ro J, et al. (2002) Micropapillary component in lung adenocarcinoma: a distinctive histologic feature with possible prognostic significance. *Am J Surg Pathol* 26: 358–364. PMID: [11859208](https://pubmed.ncbi.nlm.nih.gov/11859208/)
26. Miyoshi T, Satoh Y, Okumura S, Nakagawa K, Shirakusa T, et al. (2003) Early-stage lung adenocarcinomas with a micropapillary pattern, a distinct pathologic marker for a significantly poor prognosis. *Am J Surg Pathol* 27: 101–109. PMID: [12502932](https://pubmed.ncbi.nlm.nih.gov/12502932/)
27. Makimoto Y, Nabeshima K, Iwasaki H, Miyoshi T, Enatsu S, et al. (2005) Micropapillary pattern: a distinct pathological marker to subclassify tumours with a significantly poor prognosis within small peripheral lung adenocarcinoma ($\leq 20\text{ mm}$) with mixed bronchioloalveolar and invasive subtypes

- (Noguchi's type C tumours). *Histopathology* 46: 677–684. doi: [10.1111/j.1365-2559.2005.02126.x](https://doi.org/10.1111/j.1365-2559.2005.02126.x) PMID: [15910599](https://pubmed.ncbi.nlm.nih.gov/15910599/)
28. Kamiya K, Hayashi Y, Douguchi J, Hashiguchi A, Yamada T, et al. (2008) Histopathological features and prognostic significance of the micropapillary pattern in lung adenocarcinoma. *Mod Pathol* 21: 992–1001. doi: [10.1038/modpathol.2008.79](https://doi.org/10.1038/modpathol.2008.79) PMID: [18516041](https://pubmed.ncbi.nlm.nih.gov/18516041/)
 29. Chao L, Yi-Sheng H, Yu C, Li-Xu Y, Xin-Lan L, et al. (2014) Relevance of EGFR mutation with micropapillary pattern according to the novel IASLC/ATS/ERS lung adenocarcinoma classification and correlation with prognosis in Chinese patients. *Lung Cancer* 86: 164–169. doi: [10.1016/j.lungcan.2014.08.018](https://doi.org/10.1016/j.lungcan.2014.08.018) PMID: [25236981](https://pubmed.ncbi.nlm.nih.gov/25236981/)
 30. Fukutomi T, Hayashi Y, Emoto K, Kamiya K, Kohno M, et al. (2013) Low papillary structure in lepidic growth component of lung adenocarcinoma: a unique histologic hallmark of aggressive behavior. *Hum Pathol* 44: 1849–1858. doi: [10.1016/j.humpath.2013.02.008](https://doi.org/10.1016/j.humpath.2013.02.008) PMID: [23648380](https://pubmed.ncbi.nlm.nih.gov/23648380/)
 31. Sharma SV, Settleman J (2009) ErbBs in lung cancer. *Exp Cell Res* 315: 557–571. doi: [10.1016/j.yexcr.2008.07.026](https://doi.org/10.1016/j.yexcr.2008.07.026) PMID: [18721806](https://pubmed.ncbi.nlm.nih.gov/18721806/)
 32. Johnson ML, Sima CS, Chaft J, Paik PK, Pao W, et al. (2013) Association of KRAS and EGFR mutations with survival in patients with advanced lung adenocarcinomas. *Cancer* 119: 356–362. doi: [10.1002/cncr.27730](https://doi.org/10.1002/cncr.27730) PMID: [22810899](https://pubmed.ncbi.nlm.nih.gov/22810899/)
 33. Janjigian YY, Park BJ, Zakowski MF, Ladanyi M, Pao W, et al. (2011) Impact on disease-free survival of adjuvant erlotinib or gefitinib in patients with resected lung adenocarcinomas that harbor EGFR mutations. *J Thorac Oncol* 6: 569–575. doi: [10.1097/JTO.0b013e318202bffe](https://doi.org/10.1097/JTO.0b013e318202bffe) PMID: [21150674](https://pubmed.ncbi.nlm.nih.gov/21150674/)
 34. Kelly K, Altorki NK, Eberhardt WE, O'Brien ME, Spigel DR, et al. (2015) Adjuvant Erlotinib Versus Placebo in Patients With Stage IB-III A Non-Small-Cell Lung Cancer (RADIANT): A Randomized, Double-Blind, Phase III Trial. *J Clin Oncol* 33: 4007–4014. doi: [10.1200/JCO.2015.61.8918](https://doi.org/10.1200/JCO.2015.61.8918) PMID: [26324372](https://pubmed.ncbi.nlm.nih.gov/26324372/)
 35. Abrams J, Conley B, Mooney M, Zwiebel J, Chen A, et al. (2014) National Cancer Institute's Precision Medicine Initiatives for the new National Clinical Trials Network. *Am Soc Clin Oncol Educ Book*: 71–76. doi: [10.14694/EdBook_AM.2014.34.71](https://doi.org/10.14694/EdBook_AM.2014.34.71) PMID: [24857062](https://pubmed.ncbi.nlm.nih.gov/24857062/)