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Postrecurrence survival of surgically resected pulmonary adenocarcinoma patients according to *EGFR* and *KRAS* mutation status

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Abstract. The aim of this study was to investigate the prognosis of pulmonary adenocarcinoma patients following postoperative recurrence, according to epidermal growth factor receptor (*EGFR*) and Kirsten rat sarcoma 2 viral oncogene homolog (*KRAS*) gene mutation status and recurrence site. In total 58 adenocarcinoma patients with recurrence following surgical resection were retrospectively evaluated between 2002 and 2011. The patients were divided into groups according to the presence or absence of *EGFR* and *KRAS* mutations and the clinicopathological characteristics, recurrence sites and postrecurrence survival were compared. *EGFR* and *KRAS* mutations were detected in 26 (45%) and 11 patients (19%), respectively. Initial recurrence was distant in 25 (43%), local in 17 (29%) and both distant and local in 16 cases (28%). In *EGFR*-mutant (*EGFR*+) cases, bilateral/contralateral lung recurrence was a frequent finding. *EGFR*+ cases exhibited significantly better outcomes compared to *KRAS*+ and *EGFR-KRAS*- (wild-type) cases. The 2-year post-recurrence survival rates were 81, 18 and 47% in *EGFR*+, *KRAS*+ and wild-type cases, respectively. The patients with distant organ recurrence exhibited significantly worse survival compared with those without distant recurrence in wild-type, but not in the *EGFR*+ cases or the entire cohort. Multivariate analysis revealed that *EGFR* mutations and a number of recurrent lesions were the only statistically significant independent predictors of postrecurrence prognosis. Our results indicated distinct survival differences in recurrent adenocarcinoma patients according to driver mutations. Patients with *EGFR*-mutated tumors exhibited increased survival, regardless of recurrence at distant sites, whereas patients with *KRAS*-mutated adenocarcinoma exhibited poor outcome following postoperative recurrence. Therefore, the assessment of driver mutations is essential for predicting postrecurrence survival following surgical resection.

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Key words: lung cancer, postrecurrence survival, adenocarcinoma, epidermal growth factor receptor gene, Kirsten rat sarcoma 2 viral oncogene homolog gene

Introduction

Lung cancer remains the most common cause of cancer-related mortality worldwide. Non-small-cell lung carcinoma (NSCLC) accounts for ~85% of lung cancers and the incidence of adenocarcinomas has recently increased (1,2). Lung adenocarcinoma has been found to harbor several kinds of driver mutations and mutational analyses are required for the development of novel targeted chemotherapies, particularly in unresectable or recurrent lung adenocarcinoma. Epidermal growth factor receptor (*EGFR*) and Kirsten rat sarcoma 2 viral oncogene homolog (*KRAS*) are two proto-oncogenes that are frequently mutated in primary lung adenocarcinoma and the prognostic effect of their mutation status in advanced lung adenocarcinoma has been widely investigated (3-5).

The post-recurrence survival of surgically resected NSCLC patients was previously reported (6-10). However, those studies investigated NSCLC patient cohorts, whereas the number of available studies on adenocarcinoma patients is limited (5,11-14). Furthermore, there have been no studies on the effect of *EGFR* and *KRAS* mutations on postrecurrence survival following surgical resection, or the association of driver mutations with relapse sites in patients with recurrent lung adenocarcinoma.

The aim of this retrospective study was to investigate the prognosis of lung adenocarcinoma patients following postoperative recurrence, according to *EGFR* and *KRAS* mutation status and the relapse site.

Table I. Patient characteristics.

Characteristics	Recurrence (+) (n=58)	%	Recurrence (-) (n=239)	%	P-value
Age at surgery, years median (range)	69 (39-80)	-	69 (36-87)	-	0.222[a]
Gender					
Male	25	43.1	112	46.9	0.661[b]
Female	33	56.9	127	53.1	
Smoking history					
Never	28	48.2	123	51.5	0.770[b]
Ever	30	51.8	116	48.5	
Pathological stage					
I	21	36.2	200	83.7	<0.001[b]
II	9	15.5	19	8.0	
III	26	44.8	18	7.5	
IV	2	3.5	2	0.8	
Driver mutation					
EGFR mutant	26	44.8	104	43.5	0.262[b]
Exon 21 L858R	18	31.0	46	19.2	
Exon 19 deletion	8	13.8	57	23.9	
Other	0		1	0.4	
KRAS mutant (codon 12)	11	19.0	28	11.7	-
Wild-type	21	36.2	107	44.8	-

[a]Independent samples t-test. [b]Pearson's Chi-square test or Fisher's exact test. *EGFR*, epidermal growth factor receptor gene; *KRAS*, Kirsten rat sarcoma 2 viral oncogene homolog gene.

Table II. Characteristics of patients with recurrence.

Characteristics	Patients (n=58) No.	%
Recurrence site		
Local	17	29.3
Distant	25	43.1
Local + distant	16	27.6
Adjuvant chemotherapy (+)/(-)	23/35	39.7/60.3
Platinum-based	5	8.6
S-1	11	19.0
UFT	7	12.1
Chemotherapy for recurrence (+)/(-)	37/21	63.8/36.2
Platinum-based	21	36.2
Non-platinum-based	5	8.6
EGFR-TKI	19	32.8
Radiation therapy (+)/(-)	23/35	39.7/60.3
Metastasectomy (+)/(-)	2/56	3.4/96.6

UFT, tegafur-uracil; *EGFR*, epidermal growth factor receptor gene; TKI, tyrosine kinase inhibitor.

Patients and methods

Patient selection and follow-up. Between July, 2002 and December, 2011, a total of 297 consecutive patients underwent complete surgical resectioning of primary pulmonary adenocarcinomas at the Department of Thoracic and Visceral Organ Surgery, Gunma University Graduate School of Medicine, Gunma, Japan. Two patients with resected stage IV disease were also included: one with a single brain metastasis treated with CyberKnife therapy and one with a single adrenal metastasis resected simultaneously with the primary lesion. Following surgical resection, a portion of each sample was immediately frozen and stored at -80˚C until DNA extraction. All patients provided Institutional Review Board-approved informed consent. Of the 297 patients, 58 (18.7%) developed recurrence and were retrospectively reviewed in this study. Adjuvant chemotherapy was administered to cases with pathological stages >IB, according to postoperative performance status and age. Whenever possible, platinum-based chemotherapy was administered to stage II or III cases.

The patients were followed up at 3-month intervals for the first 2 years and at 6-month intervals thereafter, on an outpatient basis. The follow-up evaluation included a physical examination, chest radiography and blood analysis, including analysis of pertinent tumor markers. Computed tomography (CT) scans of the chest and abdomen or positron emission tomography and CT (PET-CT) were performed annually. Whenever

Table III. Patient characteristics according to *EGFR* and *KRAS* mutation status.

Characteristics	*EGFR* mutant (n=26)	*KRAS* mutant (n=11)	Wild-type (n=21)	P-value
Age at recurrence, years median (range)	67 (42-77)	71 (57-82)	71 (48-80)	0.124[a]
Gender				
Male	6	6	13	0.020[b]
Female	20	5	8	
Smoking history				
Never	16	3	8	0.101[b]
Ever	10	8	13	
Pathological stage				
I/II	9/5	4/1	8/3	0.970[b,c]
III/IV	11/1	6/0	9/1	
Recurrence site				
Local	4	5	8	0.155[b]
Distant	15	2	8	
Local + distant	7	4	5	
Number of recurrent lesions				
One	9	4	8	0.970[b]
Multiple	17	7	13	
Chemotherapy				
Platinum-based	12	3	9	-
Non-platinum-based	0	4	1	
EGFR-TKI	12	1	6	
Radiation therapy (+)/(-)	9/17	5/6	9/12	0.771[b]
Recurrence-free survival time, months median (range)	16.4 (0-56.6)	14.7 (1.0-54.7)	10.1 (3.3-56.6)	0.920[a]
2-year post-recurrence survival rate, % (median, months)	81.0 (38.0)	18.2 (3.3)	46.5 (23.3)	<0.001[d]

[a]One-way analysis of variance. [b]Pearson's Chi-square test. [c]Stage I vs. stage II-IV. [d]Log-rank test. *EGFR*, epidermal growth factor receptor gene; TKI, tyrosine kinase inhibitor; *KRAS*, Kirsten rat sarcoma 2 viral oncogene homolog gene.

symptoms or signs of recurrence were detected, further evaluations, including PET-CT, brain magnetic resonance imaging and bone scintigraphy, were performed. Recurrence was diagnosed on the basis of compatible physical examination and diagnostic imaging findings and the diagnosis was histologically confirmed when clinically feasible. Local recurrence was defined as tumor reappearance at a local site, including regional hilar and mediastinal lymph nodes, surgical margins and ipsilateral hemithorax. Distant recurrence was defined as tumor recurrence in the lung or outside the hemithorax.

DNA extraction and mutation analysis. All the surgical specimens were fixed with 10% formalin and embedded in paraffin. Representative sections were stained with hematoxylin and eosin and were reviewed by an experienced pathologist. Genomic DNA was extracted from a 3-5 mm cube of tumor tissue using a DNA Mini kit (Qiagen, Hilden, Germany) and subsequently diluted to a concentration of 20 ng/μl. *KRAS* and *EGFR* mutations in lung cancer tissue were analyzed by peptide nucleic acid-enriched sequencing, as previously described (15-17).

Statistical analysis. The patients were divided into three groups: those with *EGFR* mutations (*EGFR* mutant), those with *KRAS* mutations (*KRAS* mutant) and those negative for those types of mutation (wild-type). The correlations between the groups were evaluated using the Chi-square or Fisher's exact tests, as appropriate. The means were compared by one-way analysis of variance. All pairs of groups were compared using the Bonferroni test. Post-recurrence survival was defined as the time interval between the date recurrence was confirmed and the date of death from any cause or the last follow-up appointment. For univariate analyses, postrecurrence survival rates were estimated by the Kaplan-Meier method and differences in survival between the subgroups were compared by the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model. Forward and backward stepwise procedures were performed to determine the combination of prognostic factors.

Table IV. Comparison of recurrent organs according to *EGFR* and *KRAS* mutation status.

Recurrence site	*EGFR* mutant (n=26)	*KRAS* mutant (n=11)	Wild-type (n=21)	P-value[a]
Local				
Regional lymph nodes				
+ (n=23)	9	4	10	0.643
- (n=35)	17	7	11	
Ipsilateral hemothorax (effusion or dissemination)				
+ (n=11)	3	4	4	0.212
- (n=47)	23	7	17	
Distant				
Lung				
+ (n=23)	14	3	6	0.137
- (n=35)	12	8	15	
Ipsilateral				
+ (n=6)	2	0	4	-
- (n=52)	24	11	17	
Bilateral/contralateral				
+ (n=17)	12	3	2	0.023
- (n=41)	14	8	19	
Brain				
+ (n=10)	6	1	3	0.532
- (n=48)	20	10	18	
Bone				
+ (n=12)	5	4	3	0.332
- (n=46)	21	7	18	
Liver				
+ (n=4)	3	0	1	-
- (n=54)	23	11	20	

[a]Pearson's Chi-square test. *EGFR*, epidermal growth factor receptor gene; *KRAS*, Kirsten rat sarcoma 2 viral oncogene homolog gene.

All reported P-values are two-sided and P<0.05 was considered to indicate a statistically significant difference. The analyses were performed with SPSS 11.0 software (Dr. SPSS II for Windows, standard version 11.0; SPSS, Inc., Chicago, IL, USA).

Results

Patient characteristics and post-recurrence therapy. The characteristics of the 297 patients according to postoperative recurrence are listed in Table I. The proportion of patients with pathologically advanced disease was significantly higher in the recurrence (+) compared to that in the recurrence (-) group. There were no significant differences in gender, smoking history, or driver mutations. In the recurrence (+) group, *EGFR* mutations were detected in 26 patients (44.8%). Of the *EGFR* mutations detected, the L858R point mutation in exon 21 (observed in 18 cases) was the most frequent, followed by a deletion in exon 19 (8 cases). No alterations were detected in exons 18 and 20. *KRAS* alterations were detected in 11 patients (19.0%) and all cases were a single amino acid substitution in codon 12.

Table II shows the characteristics of patients who developed recurrence. Recurrence was initially detected in local sites in 17 (29.3%), in distant sites in 25 (43.1%) and in both local and distant sites in 16 patients (27.6%). A total of 19 patients received EGFR-tyrosine kinase inhibitor (TKI) treatment following recurrence: gefitinib, 15; erlotinib, 2; and both gefitinib and erlotinib, 2 patients. Platinum-based chemotherapies, with cisplatin or carboplatin, were administered to 21 patients and non-platinum-based chemotherapies, such as pemetrexed, S-1, docetaxel, gemcitabine and tegafur-uracil (UFT), were administered to 5 patients. Metastectomy was performed in only 2 patients (3.4%).

Correlation of EGFR and KRAS mutation status with characteristics and recurrence sites. The patient characteristics according to mutation status are presented in Table III. Gender was the only variable exhibiting a significant difference according to driver mutations, with female gender being correlated with *EGFR* mutations. Other characteristics, including smoking history, pathological stage, recurrence site and number of recurrent lesions, were not statistically significant. Of the 26 *EGFR*-mutant cases, 12 (46%) received EGFR-TKI treatment.

A comparison of initial sites of recurrence according to driver mutation is presented in Table IV. The patients with *EGFR*-mutated tumors exhibited significantly more bilateral

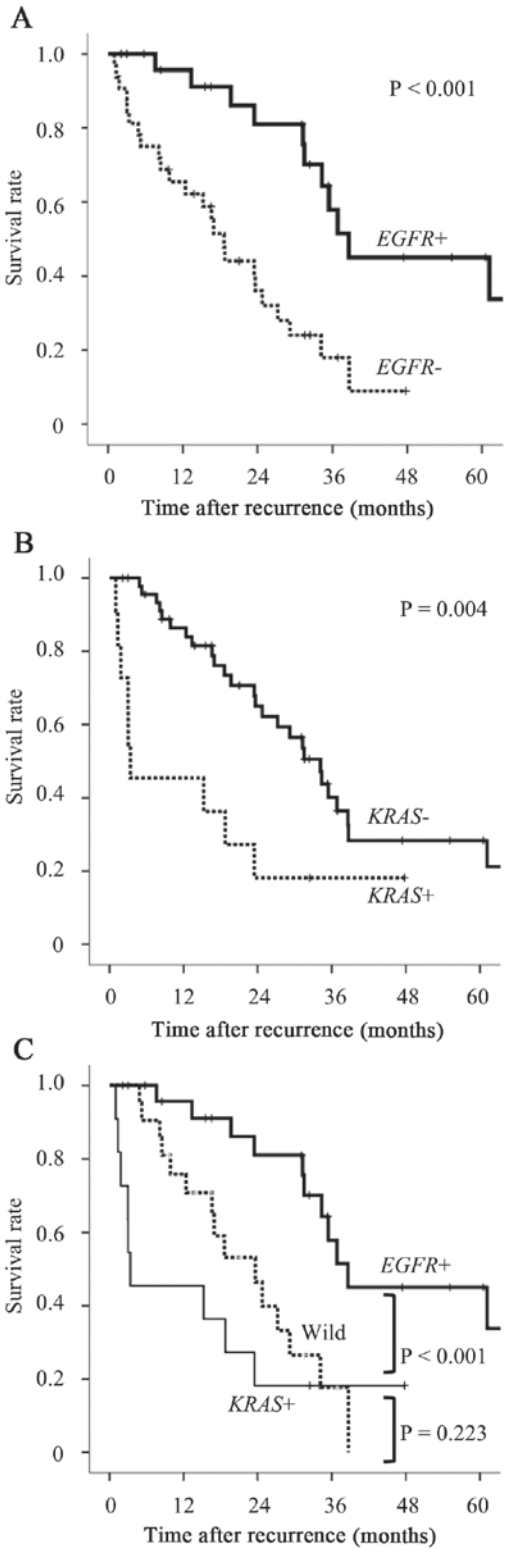


Figure 1. Postrecurrence survival curves according to driver mutations. (A) The survival of *EGFR*+ cases (n=26) was significantly longer compared to that of *EGFR*- cases (n=32) (P<0.001). (B) *KRAS*+ cases (n=11) exhibited significantly worse outcomes compared to *KRAS*- cases (n=47) (P=0.004). (C) *EGFR*+ cases (n=26) exhibited significantly better outcomes compared to *KRAS*+ (n=11) and wild-type cases (n=21) (P<0.001). There were no significant differences between *KRAS*+ and wild-type cases (P=0.223).

or contralateral lung recurrences compared to the other groups (P=0.023). No differences were observed in first recurrence sites other than the bilateral or contralateral lung.

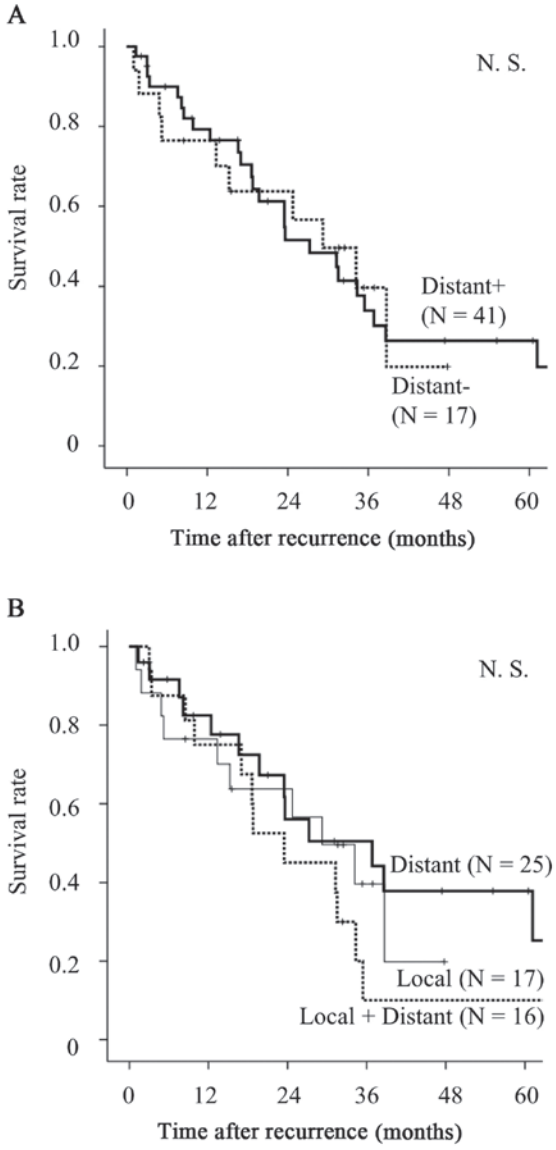
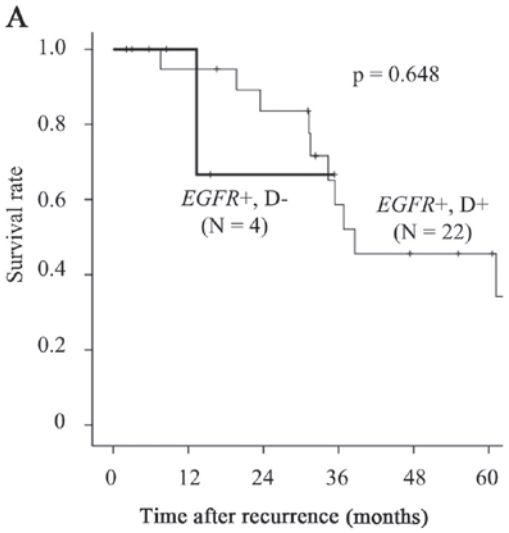
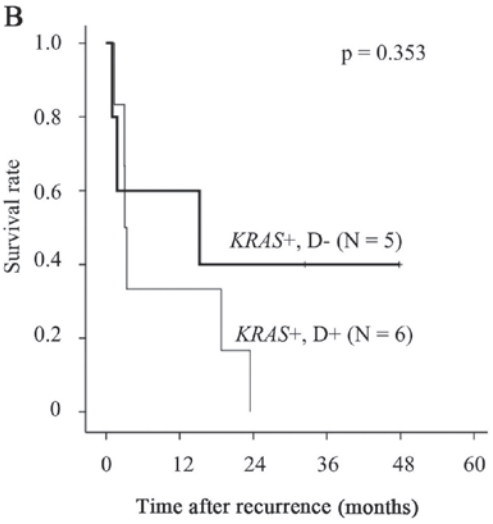
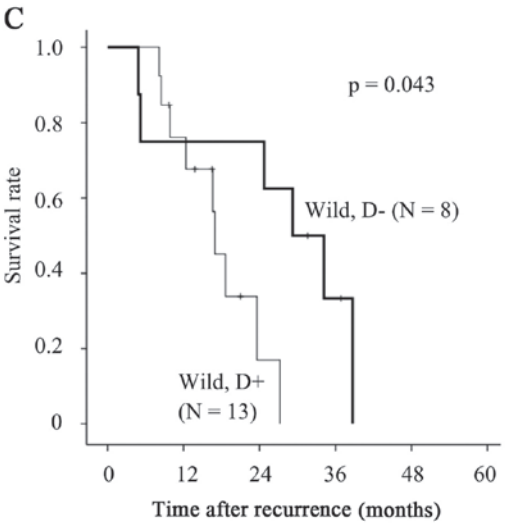


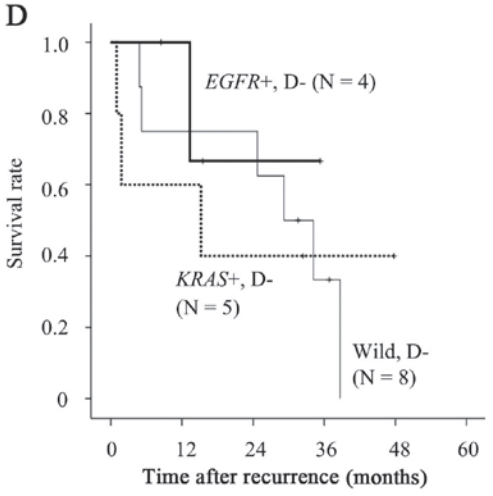
Figure 2. Survival according to initial recurrence site. (A) There was no statistically significant difference in survival according to the presence or absence of distant organ metastases (P=0.735). (B) No significant differences were observed among the distant, local and distant plus local site recurrence groups. N. S., non-significant.

Correlation between EGFR and KRAS mutation status and postrecurrence survival and prognostic factors for postrecurrence survival. Postrecurrence survival curves according to driver mutations were drawn by the Kaplan-Meier method (Fig. 1). Statistical significance was assessed using the log-rank test. The survival of *EGFR*-mutated (*EGFR*+) cases was significantly longer compared to that of *EGFR* wild-type (*EGFR*-) cases (P<0.001, Fig. 1A). By contrast, *KRAS*-mutated (*KRAS*+) cases exhibited significantly worse outcomes compared wiht those of *KRAS* wild-type (*KRAS*-) cases (P=0.004, Fig. 1B). The patients with *EGFR*+ tumors exhibited significantly better outcomes compared to those with *KRAS*+ tumors and those with wild-type tumors (P<0.001, Fig. 1C). The post-recurrence survival curves according to initial recurrence sites are shown in Fig. 2A and B. No significant differences were observed in the entire study cohort, regardless of the presence or absence of distant organ metastases.





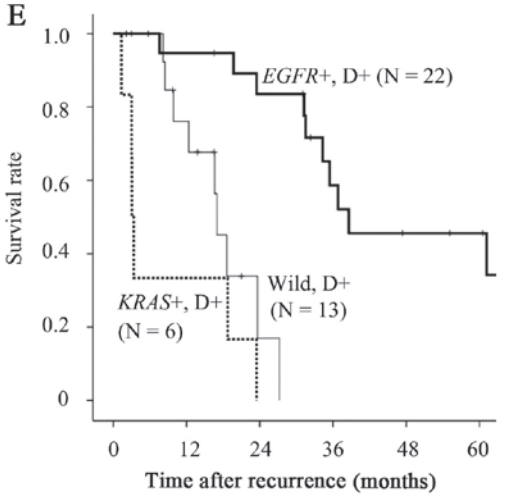




P-values in survival difference

		P-value*
EGFR+, D- vs	*KRAS*+, D-	0.395
	Wild, D-	0.578
KRAS+, D- vs	Wild, D-	0.948

*by log-rank test.



P-values in survival difference

		P-value*
EGFR+, D+ vs	*KRAS*+, D+	< 0.001
	Wild, D+	< 0.001
KRAS+, D+ vs	Wild, D+	0.072

*by log-rank test.

Figure 3. (A-C) Survival for each driver mutation according to the presence of distant site recurrence. (A) There were no differences in survival between patients with *EGFR*-mutant tumors with (D+) and without (D-) distant recurrence (P=0.648). (B) Although in *KRAS*+ cases there was no difference in survival between the D+ and D- groups, D- patients tended to have better prognoses compared to D+ patients. (C) In wild-type cases, D- patients exhibited significantly better prognoses compared to D+ patients. (D) Overall survival in in the all D- cases according to driver mutation status (n=17). In the D- cohort, there were no significant differences in survival among the groups. In the D- cohort, the difference in survival between the *KRAS*+ and wild-type groups was not statistically significant. (E) Overall survival in all the D+ cases according to driver mutation status (n=41). In the D+ cohort, the *EGFR*+ had significantly better prognoses compared with the *KRAS*+ and wild-type groups (P<0.001 and <0.001, respectively). In the D+ cohort, the *KRAS*+ tended to have worse prognoses compared with the wild-type group.

Survival according to driver mutation status and distant sites of recurrence is shown in Fig. 3. Fig. 3A-C shows the survival for each driver mutation according to the presence

Table V. Univariate and multivariate analyses of prognostic factors for postrecurrence survival.

Characteristics	n=58	2-year OS rate (%)	Univariate analysis P-value[a]	Multivariate analysis HR (95% CI)	Multivariate analysis P-value[b]
Age at recurrence (years)					
<70	27	61.9	0.579	-	-
≥70	31	50.2		-	
Gender					
Male	25	41.7	0.137	-	-
Female	33	65.3		-	
Smoking history					
Never	28	73.0	0.147	-	-
Ever	30	36.8		-	
Pathological stage					
I/II	30	69.4	0.119	-	-
III/IV	28	43.4		-	
Recurrence site					
Local	17	63.7	0.413	-	-
Distant	25	56.1		-	
Local + distant	16	45.0		-	
Recurrent lesions					
One	21	75.4	0.082	1.00	0.013
Multiple	37	45.6		2.80 (1.24-6.34)	
Adjuvant chemotherapy					
(+)	23	54.9	0.675	-	-
(-)	35	55.5		-	
EGFR-TKI					
(+)	19	57.9	0.636	-	-
(-)	39	56.9		-	
Radiation therapy					
(+)	23	45.4	0.518	-	-
(-)	35	61.8		-	
EGFR mutation					
(+)	26	81.0	< 0.001	1.00	0.002
(-)	32	36.0		3.69 (1.60-8.54)	
KRAS mutation					
(+)	11	18.2		1.89 (0.81-4.42)	0.140
(-)	47	65.0	0.004	1.00	

[a]Log-rank test. [b]Cox's proportional hazards model. OS, overall survival; HR, hazard ratio; CI, confidence interval; *EGFR*, epidermal growth factor receptor gene; TKI, tyrosine kinase inhibitor; *KRAS*, Kirsten rat sarcoma 2 viral oncogene homolog gene.

of distant site recurrence. In *EGFR*+ cases, there were no survival differences between patients with (D+) and without (D-) distant recurrence, although D- patients tended to have an improved prognosis compared with that of D+ patients (Fig. 3A). In *KRAS*+ cases, although D- patients tended to have an improved prognosis compared with that of D+ patients, there were no survival differences between the two groups (Fig. 3B). In wild-type cases, D- patients exhibited a significantly better prognosis compared with that of D+ patients (Fig. 3C). Fig. 3D shows the overall survival curves for all D- cases (n=17) according to driver mutation status. In the D- cohort, the *EGFR*+ cases exhibited an improved prognosis compared with the other groups, although the differences were not statistically significant. Fig. 3E shows the overall survival curves for all the D+ cases (n=41) according to driver mutation status. In the D+ cohort, the *EGFR*+ cases exhibited a significantly improved prognosis compared with that of the *KRAS*+ and wild-type groups (P<0.001 and <0.001, respectively). In the D- cohort, survival did not differ significantly between the *KRAS*+ and wild-type groups (Fig. 3D). However, the *KRAS*+

Table VI. Characteristics of patients who survived for >5 years following recurrence.

Case	Age, gender	Pathological stage	Adjuvant treatment	First recurrence site and organ	*EGFR* mutation	Treatment for recurrence	Survival time (years)	Outcome
1	67, M	T2aN0M0 stage IB	UFT	Distant: lung (Bi), bone	Exon 21 L858R	RT, gefitinib, CBDCA+PTX+Bev	7.2	Alive
2	71, F	T2aN0M1b (brain[a]) stage IV	Gefitinib	Distant: bone (brain[a])	Exon 19 del	Gefitinib (RT[a])	7.2	Alive
3	76, F	T2aN0M0 stage IB	None	Distant: brain	Exon 19 del	RT	5.8	Alive
4	64, F	T2aN0M0 stage IB	S-1	Distant: lung (Bi)	Exon 19 del	CDDP+GEM, gefitinib, surgery	5.6	Alive
5	57, M	T2aN2M0 stage IIIA	None	Local + distant: lung (Bi), brain, mediastinal LN	Exon 19 del	CDDP+DOC, gefitinib, RT, surgery	5.3	Alive
6	77, F	T1aN2M0 stage IIIA	None	Distant: subclavian LN	Exon 21 L858R	RT	5.0	Dead

[a]A single brain metastasis was detected at surgical resection and radiotherapy was performed after surgery. Recurrence was initially detected as multiple bone metastases. M, male; F, female; UFT, tegafur-uracil; Bi, bilateral; RT, radiotherapy; CBDCA, carboplatin; PTX, paclitaxel; Bev, bevacizumab; CDDP, cisplatin; GEM, gemcitabine; DOC, docetaxel; LN, lymph node.

cases tended to have a worse prognosis compared with that of the wild-type group in the D+ cohort (Fig. 3E).

The results of the univariate analyses of all patients for postrecurrence survival are shown in Table V. There were significant survival differences according to *EGFR* and *KRAS* mutations (P<0.001 and =0.004, respectively). The multivariate analysis revealed that multiple recurrences and the *EGFR* wild-type status were statistically significant predictors of a worse postrecurrence prognosis [multiple recurrences: hazard ratio (HR)=2.80; 95% confidence interval (CI): 1.24-6.34; P=0.013; *EGFR* wild-type: HR=3.69; 95% CI: 1.60-8.54; P=0.002]. The *KRAS* mutation status also exhibited a tendency to affect survival, albeit not statistically significantly (P=0.140).

Characteristics of patients who survived for >5 years. The characteristics of the patients who survived for >5 years following recurrence are listed in Table VI. *EGFR* mutations were detected in all primary tumors (exon 19 deletion, 4 patients; exon 21 L858R mutation, 2 patients). Relapse at distant sites was also detected in all cases. Gefitinib was administered to 4 patients, whereas the 2 remaining patients received radiotherapy alone for localized distant metastases, without additional EGFR-TKI administration.

Discussion

In this study, we investigated the correlations between *EGFR* and *KRAS* mutations, relapse site and prognosis in lung adenocarcinoma patients with postoperative recurrence.

Although several previous studies reported the postrecurrence survival of NSCLC patients (7-10,18), only a few reported survival and the effect of *EGFR* and *KRAS* mutation status on postrecurrence survival following surgical resection or investigated the association of driver mutations with relapse site in lung adenocarcinoma patients with recurrence. Johnson *et al* (19) reported that *KRAS* mutations may be predictors of shorter survival and that *EGFR* mutations were associated with longer overall survival in patients with stage IV lung adenocarcinoma. In this study, we demonstrated that the survival of patients with recurrent lung adenocarcinoma was also associated with driver mutations, similar to advanced, inoperable cases.

Although Endo *et al* (20) reported that distant or extrathoracic recurrence was an unfavorable factor following recurrence, other studies, including ours, demonstrated that it was not significant (7-9). In this study, the *EGFR*+ cases exhibited a significantly improved prognosis compared with that of the *KRAS*+ and wild-type groups, particularly the D+ patients, whereas the D+ patients had a significantly higher proportion of *EGFR*-mutant cases compared with the D- group (54 vs. 24%). These findings may explain the lack of a significant difference between the D+ and D- groups.

Despite the high frequency of distant organ recurrence in the *EGFR*-mutant cases, the patients with *EGFR*-mutated tumors exhibited significantly more favorable outcomes compared to those with *EGFR* wild-type adenocarcinomas. In *EGFR*-mutant cases, EGFR-TKIs would be expected to be effective and long-term survival could be expected with local treatment of cases of localized recurrence, such as cases 3 and 6 in Table VI.

Our study demonstrated that bilateral/contralateral lung recurrence was significantly more frequent among *EGFR*+ cases. In those cases, long-term survival may be achieved

with combination therapy, consisting of EGFR-TKI treatment, cytotoxic chemotherapy and local treatment, for each lesion (21-24). In general, long post-recurrence survival may be expected in patients with slow-growing tumors or long recurrence-free survival; however, no association between post-recurrence survival time and recurrence-free survival time according to *EGFR* mutations was observed in this study.

By contrast, *KRAS* mutations were found to be predictors of worse prognosis following postoperative recurrence, although the association was not significant. Notably, no patients with *KRAS*-mutated tumors with distant recurrence survived for >2 years after the recurrence, except 1 patient with pure invasive mucinous adenocarcinoma. It was demonstrated that *KRAS*-mutated adenocarcinomas may be divided into two groups according to lepidic histological growth pattern, with those patients without a lepidic component exhibiting a poor prognosis (17). In this study, 6 adenocarcinoma patients with no lepidic component were included, all of whom succumbed to the disease within 2 years of recurrence, whereas patients with tumors with a lepidic component also exhibited poor postrecurrence prognosis. The *KRAS* mutation was previously reported to be a predictor of poor prognosis, with a worse overall survival of *KRAS*-mutated patients (25,26). The poor postrecurrence survival may explain the poor prognosis.

The limitations of this study included the limited patient sample. Notably, there was no significant difference in survival with EGFR-TKI treatment in either the entire patient cohort or the *EGFR*-mutant cases (P=0.21 and 0.35, respectively, data not shown). This may be due to the small sample size. Other driver mutations, such as *ALK*, *BRAF* and *HER2* mutations, were not analyzed in this study; however, their involvement should be investigated in future studies. Mutational analyses were not conducted for metastatic sites in this study. Munfus-McCray *et al* (5) demonstrated acquisition of *KRAS* mutations and loss of *EGFR* mutations at metastatic sites. Therefore, driver mutations must also be confirmed in metastatic lesions.

In conclusion, we demonstrated distinct survival differences in recurrent pulmonary adenocarcinoma patients according to the presence of driver mutations. Notably, the patients with *EGFR*-mutated tumors may achieve long survival, regardless of recurrence at distant sites. By contrast, patients with *KRAS*-mutated adenocarcinoma exhibited poor outcomes following postoperative recurrence. Therefore, it is considered essential for the prediction of postrecurrence survival to consider the driver mutation status, as well as the site of recurrence.

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References

1. Jemal A, Siegel R, Xu J and Ward E: Cancer statistics, 2010. CA Cancer J Clin 60: 277-300, 2010.
2. Asamura H, Goya T, Koshiishi Y, *et al*; Japanese Joint Committee of Lung Cancer Registry: A Japanese Lung Cancer Registry study: prognosis of 13,010 resected lung cancers. J Thorac Oncol 3: 46-52, 2008.
3. Paik PK, Johnson ML, D'Angelo SP, Sima CS, Ang D, Dogan S, Miller VA, Ladanyi M, Kris MG and Riely GJ: Driver mutations determine survival in smokers and never-smokers with stage IIIB/IV lung adenocarcinomas. Cancer 118: 5840-5847, 2012.
4. Suda K, Tomizawa K and Mitsudomi T: Biological and clinical significance of KRAS mutations in lung cancer: an oncogenic driver that contrasts with EGFR mutation. Cancer Metastasis Rev 29: 49-60, 2010.
5. Munfus-McCray D, Harada S, Adams C, Askin F, Clark D, Gabrielson E and Li QK: EGFR and KRAS mutations in metastatic lung adenocarcinomas. Hum Pathol 42: 1447-1453, 2011.
6. Hung JJ, Jeng WJ, Hsu WH, Wu KJ, Chou TY, Hsieh CC, Huang MH, Liu JS and Wu YC: Prognostic factors of postrecurrence survival in completely resected stage I non-small cell lung cancer with distant metastasis. Thorax 65: 241-245, 2010.
7. Nakagawa T, Okumura N, Ohata K, Igai H, Matsuoka T and Kameyama K: Postrecurrence survival in patients with stage I non-small cell lung cancer. Eur J Cardiothorac Surg 34: 499-504, 2008.
8. Sugimura H, Nichols FC, Yang P, Allen MS, Cassivi SD, Deschamps C, Williams BA and Pairolero PC: Survival after recurrent nonsmall-cell lung cancer after complete pulmonary resection. Ann Thorac Surg 83: 409-418, 2007.
9. Williams BA, Sugimura H, Endo C, Nichols FC, Cassivi SD, Allen MS, Pairolero PC, Deschamps C and Yang P: Predicting postrecurrence survival among completely resected nonsmall-cell lung cancer patients. Ann Thorac Surg 81: 1021-1027, 2006.
10. Hung JJ, Hsu WH, Hsieh CC, Huang BS, Huang MH, Liu JS and Wu YC: Post-recurrence survival in completely resected stage I non-small cell lung cancer with local recurrence. Thorax 64: 192-196, 2009.
11. Kosaka T, Yatabe Y, Onozato R, Kuwano H and Mitsudomi T: Prognostic implication of EGFR, KRAS, and TP53 gene mutations in a large cohort of Japanese patients with surgically treated lung adenocarcinoma. J Thorac Oncol 4: 22-29, 2009.
12. Katayama T, Matsuo K, Kosaka T, Sueda T, Yatabe Y and Mitsudomi T: Effect of gefitinib on the survival of patients with recurrence of lung adenocarcinoma after surgery: a retrospective case-matching cohort study. Surg Oncol 19: e144-e149, 2010.
13. Liu HP, Isaac Wu HD, Chang JW, Wu YC, Yang HY, Chen YT, Hsieh WY, Chen YT, Chen YR and Huang SF: Prognostic implications of epidermal growth factor receptor and KRAS gene mutations and epidermal growth factor receptor gene copy numbers in patients with surgically resectable non-small cell lung cancer in Taiwan. J Thorac Oncol 5: 1175-1184, 2010.
14. Kim YT, Kim TY, Lee DS, Park SJ, Park JY, Seo SJ, Choi HS, Kang HJ, Hahn S, Kang CH, *et al*: Molecular changes of epidermal growth factor receptor (EGFR) and KRAS and their impact on the clinical outcomes in surgically resected adenocarcinoma of the lung. Lung Cancer 59: 111-118, 2008.
15. Miyamae Y, Shimizu K, Mitani Y, *et al*: Mutation detection of epidermal growth factor receptor and KRAS genes using the smart amplification process version 2 from formalin-fixed, paraffin-embedded lung cancer tissue. J Mol Diagn 12: 257-264, 2010.
16. Araki T, Shimizu K, Nakamura K, *et al*: Usefulness of peptide nucleic acid (PNA)-clamp smart amplification process version 2 (SmartAmp2) for clinical diagnosis of KRAS codon 12 mutations in lung adenocarcinoma: comparison of PNA-clamp SmartAmp2 and PCR-related methods. J Mol Diagn 12: 118-124, 2010.
17. Kakegawa S, Shimizu K, Sugano M, Miyamae Y, Kaira K, Araki T, Nakano T, Kamiyoshihara M, Kawashima O and Takeyoshi I: Clinicopathological features of lung adenocarcinoma with KRAS mutations. Cancer 117: 4257-4266, 2011.
18. Hung JJ, Jeng WJ, Hsu WH, Huang BS and Wu YC: Time trends of overall survival and survival after recurrence in completely resected stage I non-small cell lung cancer. J Thorac Oncol 7: 397-405, 2012.
19. Johnson ML, Sima CS, Chaft J, Paik PK, Pao W, Kris MG, Ladanyi M and Riely GJ: Association of KRAS and EGFR mutations with survival in patients with advanced lung adenocarcinomas. Cancer 119: 356-362, 2013.
20. Endo C, Sakurada A, Notsuda H, Noda M, Hoshikawa Y, Okada Y and Kondo T: Results of long-term follow-up of patients with completely resected non-small cell lung cancer. Ann Thorac Surg 93: 1061-1068, 2012.
21. Hotta K, Kiura K, Toyooka S, Takigawa N, Soh J, Fujiwara Y, Tabata M, Date H and Tanimoto M: Clinical significance of epidermal growth factor receptor gene mutations on treatment outcome after first-line cytotoxic chemotherapy in Japanese patients with non-small cell lung cancer. J Thorac Oncol 2: 632-637, 2007.

22. Kalikaki A, Koutsopoulos A, Hatzidaki D, Trypaki M, Kontopodis E, Stathopoulos E, Mavroudis D, Georgoulias V and Voutsina A: Clinical outcome of patients with non-small cell lung cancer receiving front-line chemotherapy according to EGFR and K-RAS mutation status. Lung Cancer 69: 110-115, 2010.
23. Takano T, Fukui T, Ohe Y, Tsuta K, Yamamoto S, Nokihara H, Yamamoto N, Sekine I, Kunitoh H, Furuta K and Tamura T: EGFR mutations predict survival benefit from gefitinib in patients with advanced lung adenocarcinoma: a historical comparison of patients treated before and after gefitinib approval in Japan. J Clin Oncol 26: 5589-5595, 2008.
24. Toyooka S, Takano T, Kosaka T, *et al*: Epidermal growth factor receptor mutation, but not sex and smoking, is independently associated with favorable prognosis of gefitinib-treated patients with lung adenocarcinoma. Cancer Sci 99: 303-308, 2008.
25. Sonobe M, Kobayashi M, Ishikawa M, *et al*: Impact of KRAS and EGFR gene mutations on recurrence and survival in patients with surgically resected lung adenocarcinomas. Ann Surg Oncol 19 (Suppl 3): S347-S354, 2012.
26. Guan JL, Zhong WZ, An SJ, *et al*: KRAS mutation in patients with lung cancer: a predictor for poor prognosis but not for EGFR-TKIs or chemotherapy. Ann Surg Oncol 20: 1381-1388, 2013.