pISSN 1598-2998, eISSN 2005-9256

https://doi.org/10.4143/crt.2023.728 Cancer Res Treat. 2024;56(1):104-114

Original Article

Clinical Impact of Genomic and Pathway Alterations in Stage I *EGFR*-Mutant Lung Adenocarcinoma

Jae Seok Lee[1], **Eun Kyung Kim**[2], **Kyung A Kim**[3], **Hyo Sup Shim**[3]

[1]*Department of Pathology, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon,* [2]*Department of Pathology, National Health Insurance Service Ilsan Hospital, Goyang,* [3]*Department of Pathology, Yonsei University College of Medicine, Seoul, Korea*

Purpose We investigated the clinical impact of genomic and pathway alterations in stage I epidermal growth factor receptor (*EGFR*)–mutant lung adenocarcinomas, which have a high recurrence rate despite complete surgical resection.

Materials and Methods Out of the initial cohort of 257 patients with completely resected stage I *EGFR*-mutant lung adenocarcinoma, tumor samples from 105 patients were subjected to analysis using large-panel next-generation sequencing. We analyzed 11 canonical oncogenic pathways and determined the number of pathway alterations (NPA). Survival analyses were performed based on co-occurring alterations and NPA in three patient groups: all patients, patients with International Association for the Study of Lung Cancer (IASLC) pathology grade 2, and patients with recurrent tumors treated with EGFR–tyrosine kinase inhibitor (TKI).

Results In the univariate analysis, pathological stage, IASLC grade, *TP53* mutation, NPA, phosphoinositide 3-kinase pathway, p53 pathway, and cell cycle pathway exhibited significant associations with worse recurrence-free survival (RFS). Moreover, *RPS6KB1* or *EGFR* amplifications were linked to a poorer RFS. Multivariate analysis revealed that pathologic stage, IASLC grade, and cell cycle pathway alteration were independent poor prognostic factors for RFS (p=0.002, p < 0.001, and p=0.006, respectively). In the grade 2 subgroup, higher NPA was independently associated with worse RFS (p=0.003). Additionally, in patients with recurrence treated with EGFR-TKIs, co-occurring *TP53* mutations were linked to shorter progression-free survival (p=0.025).

Conclusion Genomic and pathway alterations, particularly cell cycle alterations, high NPA, and *TP53* mutations, were associated with worse clinical outcomes in stage I *EGFR*-mutant lung adenocarcinoma. These findings may have implications for risk stratification and the development of new therapeutic strategies in early-stage *EGFR*-mutant lung cancer patients.

Key words Adenocarcinoma of lung, *EGFR* mutation, Early stage, Recurrence, Genomics

Introduction

Complete surgical resection with mediastinal lymph node dissection remains the gold standard treatment for patients with early-stage non–small cell lung carcinoma (NSCLC) [1]. However, 20%-50% of patients experience recurrence and eventually die of recurrent lung cancer [2]. Adjuvant chemotherapy is recommended for patients with stage II and III lung cancer to prevent recurrence; however, it is not recommended for patients with stage IA of the disease. Further, even though the use of adjuvant chemotherapy for patients with stage IB lung cancer is controversial, it is recommended for patients with high-risk clinicopathological factors, such as lymphovascular invasion, visceral pleural invasion, and poor differentiation. However, using only clinicopathological factors to stratify the risk of tumor recurrence in patients with early-stage NSCLC to determine the course of adjuvant therapy is inadequate. To fully clarify the potential benefits of adjuvant chemotherapy or targeted therapy in patients with early-stage lung cancer, it is necessary to predict the risk of tumor recurrence using additional genomic alterations.

Epidermal growth factor receptor (*EGFR*) mutations are the most common driver mutations in East Asian patients with lung adenocarcinoma (40%-60%), and several potent *EGFR*–tyrosine kinase inhibitors (TKIs) have increased the median overall survival of patients with advanced-stage disease to longer than 2.5 years. However, not all patients benefit equally from TKI treatment owing to clinical and biological heterogeneity. Recently, the ADAURA clinical trial revealed that adjuvant treatment of resected stage IB-IIIA *EGFR*-mutant NSCLCs with third-generation EGFR-TKI osimertinib shows significantly better outcomes than placebo [3]. However, considering the high cost, adverse effects, and long treatment period associated with EGFR-TKIs, whether all patients with stage I NSCLC should be treated via this approach is still controversial. Thus, a strategy for the selec-

Correspondence: Hyo Sup Shim
Department of Pathology, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
Tel: 82-2-2228-1760 Fax: 82-2-362-0860 E-mail: shimhs@yuhs.ac
Received June 6, 2023 **Accepted** July 21, 2023 **Published Online** July 24, 2023
*Jae Seok Lee and Eun Kyung Kim contributed equally to this work.

tion of patients at high risk of recurrence is necessary when considering adjuvant TKI therapy. Studies on predictive genomic markers of the benefits of adjuvant TKI therapy in patients with early-stage *EGFR*-mutant lung adenocarcinoma are limited [4]. Recent studies have focused on the risk stratification of advanced-stage *EGFR*-mutant NSCLC using next-generation sequencing (NGS) [5]. Further, several studies have been conducted to discover novel predictive biomarkers for the recurrence of resected early-stage NSCLC using various methods, such as gene expression profiling, quantitative reverse transcriptase–polymerase chain reaction, microRNA assays, and mass spectrometry [6-8]. Despite these efforts, the genomic biomarkers and mechanisms associated with recurrence of resected early-stage NSCLC are still unclear.

NGS allows the simultaneous evaluation of multiple genes and has recently been used in clinical practice to elucidate tumor biology and select targeted therapies to facilitate precision medicine strategies for patients with lung cancer [9]. Compared to traditional single-gene assays, NGS is a feasible and cost-effective method for diagnostic genomic profiling. However, it is unclear how the high volume of genomic alterations identified via NGS interact and how they can be used alongside *EGFR* mutation status and clinicopathological predictors to stratify the risk of tumor recurrence. To address this issue, we used large-panel NGS to analyze stage I *EGFR*-mutant lung adenocarcinoma specimens to investigate genomic alterations and oncogenic pathway co-alterations as well as their association with clinical outcomes.

Materials and Methods

1. Patients and samples

Patients with lung cancer who underwent complete tumor resection and were histologically confirmed to have stage I lung adenocarcinoma between 2005 and 2013 were screened for inclusion in this study. Of these patients, those with *EGFR*-mutant stage I adenocarcinoma were selected based on a review of electronic pathological reports and additional confirmation of *EGFR* mutation status using the PNAClamp *EGFR* Mutation Detection Kit (Panagene Inc., Daejeon, Korea) or the PANAMutyper *EGFR* Kit (Panagene Inc.), according to the manufacturer's instructions. Electronic medical records were reviewed, and age, sex, smoking history, pathologic stage, lymphovascular invasion, visceral pleural invasion, and dates of tumor recurrence and death/last follow-up were collected. Patients with non-adenocarcinoma histology, insufficient tumor specimens for complete molecular analysis, and those who received neoadjuvant treatment were excluded. Pathological stages were reviewed and assigned according to the 8th American Joint Committee on Cancer (AJCC) criteria. Further, the International Association for the Study of Lung Cancer (IASLC) grades were determined according to previously described criteria [10]. This retrospective study was approved by the institutional review board (approval No. 4-2021-1181).

2. Targeted NGS and interpretation

DNA was extracted from formalin-fixed, paraffin-embedded whole tissue sections with more than 30% tumor cellularity using a QIAamp DNA kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's protocol. A TrueSight Oncology 500 panel (Illumina, San Diego, CA) was used to target 523 cancer-related genes. Further, libraries were sequenced on the NextSeq 550Dx platform (Illumina). Raw sequencing data were processed, and variants were identified using the Illumina bioinformatics pipeline (Illumina). Somatic mutations, including single-nucleotide variations, small insertions and deletions (indels), and copy number variations (CNVs) were also identified. Pathogenic and likely pathogenic somatic mutations with variant allele frequencies (VAFs) > 5% were regarded as significant and used for analysis. Fold changes above 1.5 or below 0.5 were regarded as CNVs. Furthermore, variant interpretation was based on recommendations from the Association for Molecular Pathology, the American Society of Clinical Oncology, and the College of American Pathologists [11].

The tumor mutation burden (TMB) scores (total variant number/megabase) were calculated according to the TrueSight Oncology 500 Local App Manual (Illumina). We also included single-nucleotide variants, insertions, and deletions in the coding region with a VAF between 5% and 90% and a read depth of 50×. However, we excluded certain variants that were annotated with ≥ 50 counts in the COSMIC database, ≥ 10 counts in normal population databases (gnomAD exome/genome, 1000 Genomes), or were present in the internal germline variant database. For the classification of samples based on TMB, we categorized samples with TMB scores below the median as 'low TMB,' while those with TMB scores equal to or above the median were classified as 'high TMB.'

3. Number of pathway alterations

We evaluated 11 recognized signaling pathways using templates provided by The Cancer Genome Atlas PanCancer Atlas project [12]. The analyzed pathways included the receptor tyrosine kinase (RTK/RAS), Hippo, phosphoinositide 3-kinase (PI3K), transforming growth factor β (TGFβ), β-catenin/Wnt, Myc, p53, oxidative stress response/Nrf2, Notch, cell cycle, and DNA damage pathways. A tumor sample was considered 'altered' with respect to a specific pathway when one or more genes in the pathway con-

Table 1. Patient characteristics based on recurrence status (n=105)

Characteristic	Total (n=105)	Recurrence (n=50)	No recurrence (n=55)	p-value
Age at diagnosis (yr), mean (range)	63.8 (26-87)	63 (26-77)	64.6 (42-87)	0.477
Sex				
Male	46 (43.8)	22 (44.0)	24 (43.6)	0.970
Female	59 (56.2)	28 (56.0)	31 (56.4)	
Smoking status				
Ever	31 (29.5)	15 (30.0)	16 (29.1)	0.919
Never	74 (70.5)	35 (70.0)	39 (70.9)	
Pack-years, mean (range)	8.2 (0-110)	9.1 (0-110)	7.3 (0-60)	0.625
Pathologic stage				
IA1	8 (7.6)	0	8 (14.5)	< 0.001
IA2	20 (19.0)	2 (4.0)	18 (32.7)	
IA3	35 (33.3)	15 (30.0)	20 (36.4)	
IB	42 (40.0)	33 (66.0)	9 (16.4)	
IASLC grade				
1	20 (19.0)	0	20 (36.4)	< 0.001
2	60 (57.1)	27 (54.0)	33 (60.0)	
3	25 (23.8)	23 (46.0)	2 (3.6)	
Lymphovascular invasion				
Present	8 (7.6)	7 (14.0)	1 (1.8)	0.019
Absent	97 (92.4)	43 (86.0)	54 (98.2)	
Visceral pleural invasion				
Present	34 (32.4)	26 (52.0)	8 (14.5)	< 0.001
Absent	71 (67.6)	24 (48.0)	47 (85.5)	
***EGFR* mutation**				
E19del	40 (38.1)	22 (44.0)	17 (30.9)	< 0.001
L858R	56 (53.3)	19 (38.0)	38 (69.1)	
Others	9 (8.6)	9 (18.0)	0	
***TP53* mutation**				
Mutant	17 (16.2)	11 (22.0)	6 (10.9)	0.123
Wild type	88 (83.8)	39 (78.0)	49 (89.1)	
NPA				
1	40 (38.1)	10 (20.0)	30 (54.5)	< 0.001
2	32 (30.5)	14 (28.0)	18 (32.7)	
3	24 (22.9)	17 (34.0)	7 (12.7)	
4	9 (8.6)	9 (18.0)	0	
TMB				
Low (< 4.7)	49 (46.7)	15 (30.0)	34 (61.8)	0.001
High (≥ 4.7)	56 (53.3)	35 (70.0)	21 (38.2)	

Values are presented as number (%). *EGFR*, epidermal growth factor receptor; E19del, exon 19 deletion; IASLC, International Association for the Study of Lung Cancer; NPA, number of pathway alterations; TMB, tumor mutational burden.

tained a recurrent or known driver alteration. Additionally, the status of specific pathways was determined to be either altered or wild-type for each patient, and the number of pathway alterations (NPA) in each sample was calculated as the total number of altered pathways [13]. We classified samples with one or two pathway alterations as 'NPA low,' and those with three or more pathway alterations as 'NPA high,' and grouped them accordingly.

4. Statistical analysis

Clinical and pathological parameters were evaluated by performing the chi-square test or Fisher's exact test for categorical variables and the Spearman rank correlation test for continuous variables. To assess prognostic values, we performed survival analyses. Recurrence-free survival (RFS) is the period from complete surgical resection to the time of the first documentation of recurrence or last follow-up, and

Fig. 1. Genomic landscape of stage I *EGFR*-mutant lung adenocarcinoma according to recurrence status. *EGFR*, epidermal growth factor receptor; NR, not recurred; PY, pack-years; R, recurred.

progression-free survival (PFS) is the period from EGFR-TKI treatment to the time of progression or last follow-up, were analyzed using the Kaplan-Meier method, and differences were tested using the log-rank test via univariate analysis. Additionally, the Cox proportional hazards model was used for multivariate survival analysis. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using the SPSS software ver. 26 (IBM Corp., Armonk, NY).

Results

1. Clinicopathological characteristics

Of 412 patients with stage I lung adenocarcinoma identified, 257 (62%) harbored *EGFR* mutations, and of these, 50 (19.5%) experience disease recurrence. All recurrent tumors (n=50) as well as randomly selected non-recurrent tumors (n=55) were further analyzed via NGS. The distributions of age, sex, smoking history, pathologic stage, and follow-

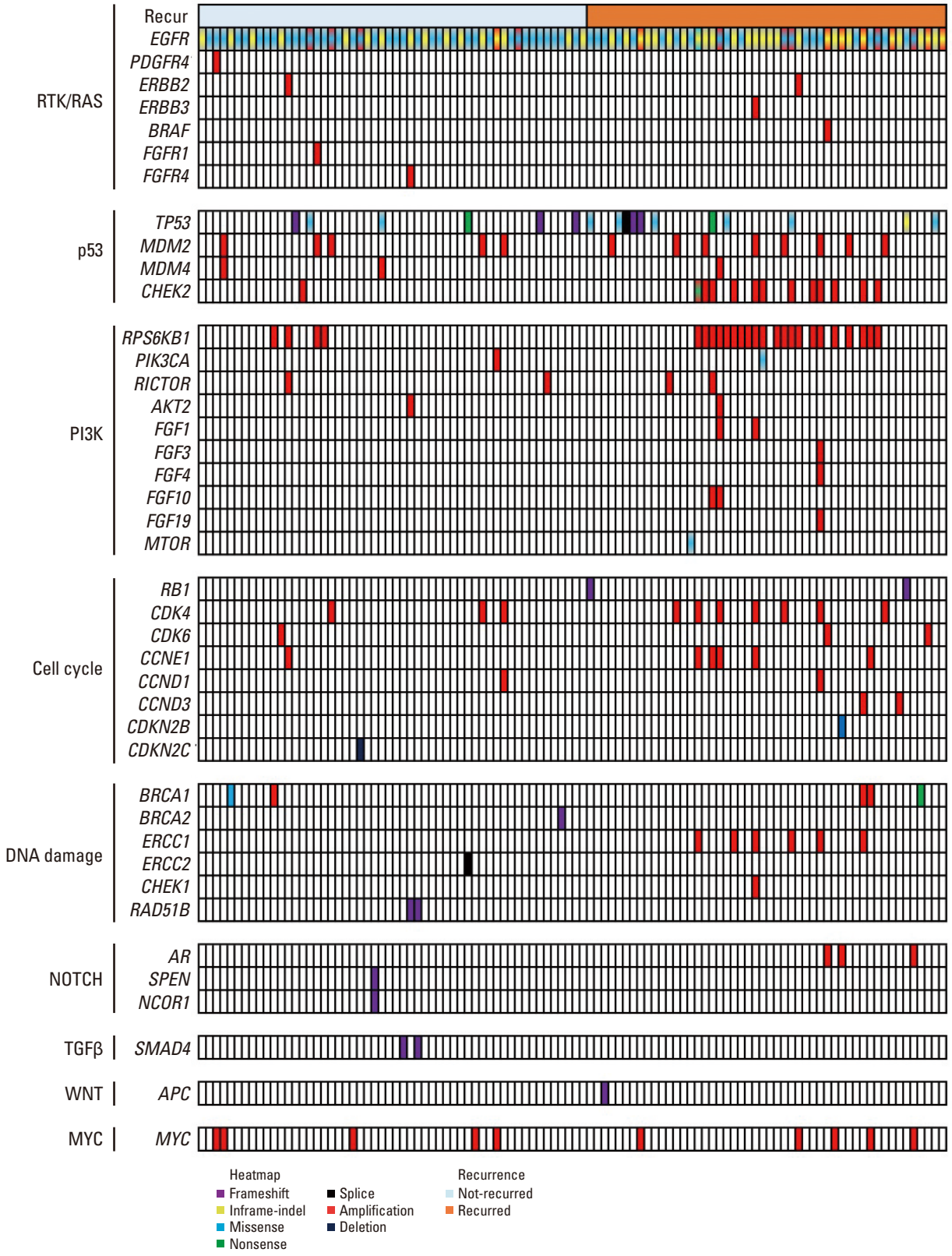

Fig. 2. List of altered genes and distribution of pathway alterations.

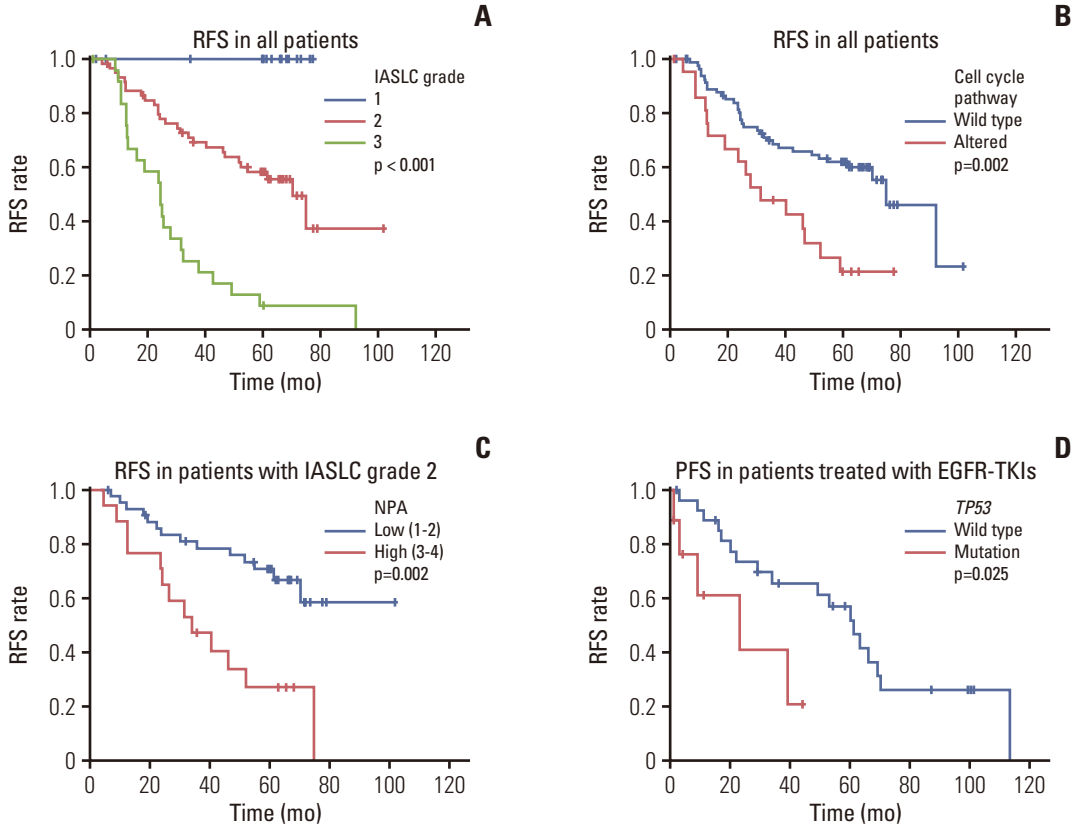

Fig. 3. Survival curves based on IASLC grades and genomic alterations. (A) RFS of all patients based on IASLC grades. (B) RFS for all patients based on cell cycle pathway alterations. (C) RFS for patients with IASLC grade 2 *EGFR*-mutant lung adenocarcinoma based on NPA. (D) PFS for patients treated with EGFR-TKI after recurrence based on *TP53* mutations. *EGFR*, epidermal growth factor receptor; IASLC, International Association for the Study of Lung Cancer; NPA, number of pathway alterations; PFS, progression-free survival; RFS, recurrence-free survival; TKI, tyrosine kinase inhibitor.

up time of the 55 patients in the non-recurrent group were similar to those of the remaining 152 unselected patients (S1 Table). The median follow-up duration was 65 months (range, 7 to 126 months). The patient characteristics are summarized in Table 1, from which it is evident that the mean patient age was 63.8 years (range, 26 to 87 years). Further, 59 patients (56.2%) were female and 74 (70.5%) had never smoked. The mean number of pack-years smoked was 8.2 (range, 0 to 110), and the baseline pathological stage distribution was as follows: stage IA1, 8 (7.6%); stage IA2, 20 (19.0%); stage IA3, 35 (33.3%); and stage IB, 42 (40.0%). Furthermore, IASLC grades 1, 2, and 3 of the disease were observed in 20 (19.0 %), 60 (57.1%), and 25 (23.8%) patients, respectively.

2. Genomic characteristics

The L858R mutation in exon 21 was the most frequently observed *EGFR* alteration (53.3%), followed by exon 19 deletions (E19del) (38.1%) and other alteration types (8.6%). The co-occurring genetic alterations in all the 105 patients included are shown in Fig. 1 and S2 Table. Among the co-occurring mutations, *TP53* mutations were the most frequently observed (n=17, 16.2%), followed by *RBM10* mutations (n=9, 8.6%). Further, the most frequent co-occurring CNVs were: *RPS6KB1* amplification, *EGFR* amplification, *MDM2* amplification, *CDK4* amplification, and *MYC* amplification observed in 25 (23.8%), 22 (21.0%), 12 (11.4%), 10 (9.5%), and 10 (9.5%) tumors, respectively.

The distribution of pathway alterations is shown in Fig. 2. From this figure, the p53 pathway was identified as the most frequently altered pathway (n=39, 37.1%), followed by the PI3K pathway (n=29, 27.6%), cell cycle (n=22, 20.9%), and DNA damage (n=15, 14.2%). The NPA distributions were as follows: NPA1, 40 (38.1%); NPA2, 32 (30.5%); NPA3, 24 (22.9%); and NPA4, 9 (8.5%). Additionally, the median NPAs were 2 (range, 1 to 4) for all patients, 2.5 (range, 1 to 4) for the recurrence group, and 1.5 (range, 1 to 3) for the non-recurrence group.

The median TMB was 4.7 per megabase considering all the

Table 2. Prognostic factors for recurrence-free survival according to univariate and multivariate analyses involving all patients (n=105)

Category	Variable	Univariate analysis			Multivariate analysis		
		HR	95% CI	p-value	HR	95% CI	p-value
Pathologic stage	IB vs. IA[a]	3.687	2.034-6.685	< 0.001	2.665	1.448-4.904	0.002[b]
IASLC grade	Grade 3 vs. Grade 2 or 1[a]	5.028	2.815-8.978	< 0.001	4.008	2.221-7.231	< 0.001[b]
Lymphovascular invasion	Present vs. Absent[a]	3.035	1.351-6.818	0.005	-	-	-
Visceral pleural invasion	Present vs. Absent[a]	2.842	1.610-5.017	< 0.001	-	-	-
TP53 mutation	*TP53* mutation vs. Wild type[a]	1.966	1.002-3.857	0.049	-	-	-
NPA	High (3-4) vs. Low (1-2)[a]	3.241	1.849-5.681	< 0.001	-	-	-
PI3K pathway	Altered vs. Wild type[a]	2.853	1.625-5.009	< 0.001	-	-	-
p53 pathway	Altered vs. Wild type[a]	2.405	1.376-4.202	0.002	-	-	-
Cell cycle pathway	Altered vs. Wild type[a]	2.536	1.390-4.626	0.002	2.325	1.267-4.265	0.006[b]
RPS6KB1 Amp	Amp vs. Wild type[a]	3.119	1.764-5.513	< 0.001	-	-	-
EGFR Amp	Amp vs. Wild type[a]	2.110	1.091-4.082	0.026	-	-	-
TMB	High vs. Low[a]	2.419	1.315-4.449	0.004	-	-	-

Amp, amplification; CI, confidence interval; *EGFR*, epidermal growth factor receptor; HR, hazard ratio; IASLC, International Association for the Study of Lung Cancer; NPA, number of pathway alterations; PI3K, phosphoinositide 3-kinase; TMB, tumor mutational burden. [a]Reference variable, [b]p-values calculated via multivariate stepwise Cox regression analysis.

Table 3. Prognostic factors for recurrence-free survival according to univariate and multivariate analysis in patients with IASLC grade 2 (n=60)

Category	Variable	Univariate analysis			Multivariate analysis		
		HR	95% CI	p-value	HR	95% CI	p-value
Pathologic stage	IB vs. IA[a]	3.349	1.495-7.505	0.003	3.327	1.465-7.557	0.004[b]
Visceral pleural invasion	Present vs. Absent[a]	2.360	1.102-5.056	0.023	-	-	-
TP53 mutation	*TP53* mutation vs. Wild type[a]	1.335	0.458-3.891	0.597	-	-	-
NPA	High (3-4) vs. Low (1-2)[a]	3.303	1.536-7.102	0.002	3.271	1.497-7.147	0.003[b]
PI3K pathway	Altered vs. Wild type[a]	3.048	1.402-6.624	0.005	-	-	-
p53 pathway	Altered vs. Wild type[a]	1.934	0.904-4.138	0.089	-	-	-
Cell cycle pathway	Altered vs. Wild type[a]	2.203	1.002-4.843	0.049	-	-	-
RPS6KB1 Amp	Amp vs. Wild type[a]	3.047	1.360-6.827	0.007	-	-	-
EGFR Amp	Amp vs. Wild type[a]	1.501	0.563-4.006	0.414	-	-	-

Amp, amplification; CI, confidence interval; *EGFR*, epidermal growth factor receptor; HR, hazard ratio; IASLC, International Association for the Study of Lung Cancer; NPA, number of pathway alterations; PI3K, phosphoinositide 3-kinase. [a]Reference variable, [b]p-values calculated via multivariate stepwise Cox regression analysis.

patients. We also observed a high TMB (≥ 4.7) in 56 patients (53.3%). The median TMB was 5.7 (range, 0.8 to 40.1) in the recurrent group, and 3.9 (range, 0.8 to 49.8) in the non-recurrent group.

3. Prognostic factors of RFS in all patients

In the univariate analysis, pathological stage, IASLC grade (Fig. 3A), lymphovascular invasion, visceral pleural invasion, *TP53* mutation, NPA, PI3K pathway, p53 pathway, and cell cycle pathway (Fig. 3B) were significantly associated with a worse RFS. The hazard ratio (HR), 95% confidence interval (CI), and p-values are shown in Table 2. Additionally, *RPS6KB1* or *EGFR* amplifications were associated with a worse RFS (Table 2). A high TMB was also associated with poor RFS (p=0.004).

Multivariate Cox regression analysis revealed that independent risk factors for RFS included a higher pathologic stage (HR, 2.665; 95% CI, 1.448 to 4.904; p=0.002), higher IASLC grade (HR, 4.008; 95% CI, 2.221 to 7.231; p < 0.001), and an altered cell cycle pathway (HR, 2.325; 95% CI, 1.267 to 4.265; p=0.006) (Table 2).

4. Prognostic factors of RFS in patients with IASLC grade 2

No disease recurrence was observed in patients with grade 1 of the disease, and as expected, patients with grade 2 disease (n=60) showed an intermediate prognosis among the three patient groups according to the IASLC grading system (Fig. 3A). Additionally, univariate analysis showed that pathological stage, visceral pleural invasion, NPA (Fig. 3C), PI3K pathway, cell cycle pathway, and *RPS6KB1* amplification were significantly associated with a worse RFS. The HR, 95% CI, and p-values are shown in Table 3.

Multivariate Cox regression analysis further revealed that independent risk factors for RFS included a higher pathological stage (HR, 3.327; 95% CI, 1.465 to 7.557; p=0.004) and a higher NPA (HR, 3.271; 95% CI, 1.497 to 7.147; p=0.003) (Table 3).

5. Prognostic factors of PFS in patients treated with EGFR-TKI after recurrence

Among patients with recurrence who underwent EGFR-TKI treatment (n=37), those with co-occurring *TP53* mutations showed shorter PFS periods (p=0.025) (Fig. 3D). Other factors did not show statistically significant associations between PFS and the EGFR-TKI treatment.

Discussion

Here, we evaluated the prognostic implications of co-occurring genetic and pathway alterations in patients with surgically resected stage I *EGFR*-mutant lung adenocarcinoma. Thus, we observed that: (1) cell cycle pathway alteration and IASLC grade were independent poor prognostic factors in stage I *EGFR*-mutant adenocarcinoma, (2) a higher NPA was an independent prognostic factor in IASLC grade 2 *EGFR*-mutant adenocarcinoma, and (3) *TP53* mutation was associated with a poor response to EGFR-TKI therapy after recurrence in completely resected stage I *EGFR*-mutant adenocarcinoma.

The prognostic factors associated with disease recurrence in early-stage lung adenocarcinomas after complete resection have been investigated in previous studies. Among these, it has been consistently reported that a histologically high-grade pattern is associated with a poor prognosis [14-16]. As an extension of these findings, the IASLC pathology committee proposed a grading system for invasive non-mucinous lung adenocarcinoma [10], and this grading system has been validated in another cohort [17-19]. Thus, as expected, IASLC grade was identified as an independent prognostic factor for RFS in patients with stage I *EGFR*-mutant cancers.

The use of NGS panel testing has become increasingly common in clinical practice, particularly for patients with advanced lung cancer. This test primarily provides information on targetable genetic alterations [9]. In addition to targetable drivers, the presence of co-occurring genetic alterations can affect cancer biology, microenvironmental interactions, and therapeutic outcomes [20]. Thus, comprehensive genomic profiling of cancer-related genes has become important, and large-panel NGS, which offers the possibility to detect mutations in tumor suppressor genes and CNVs, is frequently used in clinical settings. In this study, mutations or amplifications in the genes, *TP53*, or *RPS6KB1* were associated with a worse RFS. These findings confirmed the effect of co-occurring genetic alterations on clinical outcomes, even in stage I lung cancers, including *EGFR* oncogene-driven adenocarcinomas.

In addition to the gene-centric approach described above, the pathway-centric approach can also provide additional clinical information [13]. In this study, we investigated 11 oncogenic pathway alterations as well as NPA and observed that certain co-occurring pathway alterations (PI3K, p53, and cell cycle) and a high NPA (≥ 3) were associated with a worse prognosis. Via multivariate analysis of the study population, we also observed that an altered cell cycle pathway and a high NPA were independent prognostic markers associated with poor RFS. This possibly indicated that the pathway-centric approach offers the possibility to more effectively identify patients at high risk of recurrence as well as candidates for adjuvant therapy following lung adenocarcinoma resection. Similar findings using the pathway-centric approach were obtained in a study on resected lung adenocarcinoma [13]. In this previous study, NPA was identified as an independent risk factor for a poor RFS and cell cycle, Hippo, TGFβ, and p53 pathway alterations were also found to be associated with a poor RFS [13]. Another recent study found that higher NPA, TMB, and CNVs are linked to high-grade histologic patterns and worse clinical outcomes in stages I-III lung adenocarcinoma [21].

In this study, *TP53* mutations, which were the most frequently observed co-mutations, were associated with a worse RFS. Previous studies have suggested that *TP53* alterations may be associated with treatment resistance and shorter survival in patients with *EGFR*-mutant lung cancer [22-24]. Further, it was recently suggested that *TP53* mutations influence clinical outcomes by facilitating genomic instability and the acquisition of additional co-occurring driver events in early-stage *EGFR*-mutant lung cancer [25]. Furthermore, *TP53* mutations are associated with faster resistance evolution independent of the resistance mechanism after TKI treatment in advanced-stage *EGFR*-mutant lung cancer [26]. In this study, *TP53* mutations showed an association with a poor PFS following EGFR-TKI treatment in patients with recurrent disease after complete surgical

resection of stage I disease.

Our results also indicated that *RPS6KB1* and *EGFR* amplifications were significantly associated with a worse RFS in stage I *EGFR*-mutant lung adenocarcinoma. Among these, *RPS6KB1* amplification was the most frequently observed (in 23.8% of cases). Reportedly, the *RPS6KB1* gene encodes a member of the ribosomal S6 family of serine and threonine kinases. The encoded proteins then respond to mTOR signaling to promote protein synthesis, cell growth, and proliferation. Additionally, *RPS6KB1* amplification has been reported in approximately 10% of breast cancers and is associated with poor clinical outcomes [27]. Hyperphosphorylation of *RPS6KB1* has recently been correlated with poor clinical outcomes in patients with NSCLC after surgical excision [28]. Notwithstanding, further studies on *RPS6KB1* amplification in a larger cohort with functional validation are needed to confirm its biological and clinical significance.

Surgically resected lung adenocarcinoma is graded according to the IASLC-proposed grading system [10]. Real-world data show that grade 2 tumors constitute the largest proportion (> 50%) of lung adenocarcinomas [19]. Further, grade 2 tumors, formerly intermediate-grade adenocarcinoma, can be heterogeneous tumors with various clinical outcomes [29]. In the present study, we examined the significance of co-occurring genetic and pathway alterations in grade 2 tumors. In particular, NPA was identified as an independent prognostic factor for a worse RFS. This indicated that genomic analysis of tumors with an intermediate prognosis could provide additional information for predicting clinical behavior in lung cancer.

Systemic targeted or immunotherapies have been increasingly used in adjuvant or neoadjuvant settings for patients with resectable early-stage lung cancer [30]. Accordingly, the early determination of genomic and immunological information for early-stage lung cancer is becoming increasingly important [31]. NGS, which is intensively performed for advanced-stage cancers, is expected to expand the indications for early-stage cancers. We believe that understanding the detailed genetic and pathway alterations in early-stage lung cancer may facilitate the development of new therapeutic strategies to improve patient care, such as risk stratification and the development of novel candidates for combination therapy, even in patients with stage I *EGFR*-mutant lung adenocarcinoma.

Our study had several limitations. First, this was a retrospective study; therefore, the possibility of selection bias cannot be ruled out. Second, we used only DNA sequencing data. Genes that were epigenetically silenced were excluded from the analysis; transcriptome analysis can provide additional information on high-risk factors [24]. Third, we sequenced only a portion (27%) of the non-recurrent tumors owing to resource limitations. Although the selected non-recurrent group showed a similar distribution of clinical characteristics as the remaining unselected patients, there could be a selection bias. The non-recurrent group was predominantly composed of patients with grades 1 and 2 tumors. Additionally, in this study, we mainly focused on recurrence-associated genomic factors. Due to the limited number of cases in our study, we did not perform detailed analyses regarding grade-associated genomic alterations. It is worth noting that previous reports have described associations between high-grade tumors and their genomic findings [21,32].

In conclusion, the results of this study suggested that large-panel NGS with genomic and pathway-centric analysis strategies may provide valuable information for the risk stratification of patients with resected stage I *EGFR*-mutant lung adenocarcinoma. In particular, cell cycle alterations and high NPA were independently associated with a worse RFS, and *TP53* mutations were associated with poor response to EGFR-TKIs after recurrence. These results may facilitate risk stratification and the development of new therapeutic strategies for patients with early-stage *EGFR*-mutant lung cancer.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (https://www.e-crt.org).

Ethical Statement

This study was approved by the Institutional Review Board (local IRB number: 4-2021-1181). As this was a retrospective study, the IRB waived the requirement for informed consent due to the study design.

Author Contributions

Conceived and designed the analysis: Lee JS, Kim EK, Kim KA, Shim HS.
Collected the data: Lee JS, Kim EK, Kim KA, Shim HS.
Contributed data or analysis tools: Lee JS, Kim EK, Kim KA, Shim HS.
Performed the analysis: Lee JS, Kim EK, Kim KA, Shim HS.
Wrote the paper: Lee JS, Kim EK, Kim KA, Shim HS.

ORCID iDs

Jae Seok Lee : https://orcid.org/0000-0002-4712-9368
Eun Kyung Kim : https://orcid.org/0000-0002-9439-0446
Hyo Sup Shim : https://orcid.org/0000-0002-5718-3624

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

Acknowledgments

This research was funded by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant number NRF-2018R1D-1A1B07047811) and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (grant number 2022R1A2C1009364).

References

1. Chansky K, Detterbeck FC, Nicholson AG, Rusch VW, Vallieres E, Groome P, et al. The IASLC Lung Cancer Staging Project: external validation of the revision of the TNM stage groupings in the eighth edition of the TNM classification of lung cancer. J Thorac Oncol. 2017;12:1109-21.
2. Jeon DS, Kim HC, Kim SH, Kim TJ, Kim HK, Moon MH, et al. Five-year overall survival and prognostic factors in patients with lung cancer: results from the Korean Association of Lung Cancer Registry (KALC-R) 2015. Cancer Res Treat. 2023;55:103-11.
3. Wu YL, Tsuboi M, He J, John T, Grohe C, Majem M, et al. Osimertinib in resected EGFR-mutated non-small-cell lung cancer. N Engl J Med. 2020;383:1711-23.
4. Liu SY, Bao H, Wang Q, Mao WM, Chen Y, Tong X, et al. Genomic signatures define three subtypes of EGFR-mutant stage II-III non-small-cell lung cancer with distinct adjuvant therapy outcomes. Nat Commun. 2021;12:6450.
5. Christopoulos P, Kirchner M, Roeper J, Saalfeld F, Janning M, Bozorgmehr F, et al. Risk stratification of EGFR(+) lung cancer diagnosed with panel-based next-generation sequencing. Lung Cancer. 2020;148:105-12.
6. Shukla S, Evans JR, Malik R, Feng FY, Dhanasekaran SM, Cao X, et al. Development of a RNA-Seq based prognostic signature in lung adenocarcinoma. J Natl Cancer Inst. 2017;109: djw200.
7. Patnaik SK, Kannisto E, Knudsen S, Yendamuri S. Evaluation of microRNA expression profiles that may predict recurrence of localized stage I non-small cell lung cancer after surgical resection. Cancer Res. 2010;70:36-45.
8. Soltis AR, Bateman NW, Liu J, Nguyen T, Franks TJ, Zhang X, et al. Proteogenomic analysis of lung adenocarcinoma reveals tumor heterogeneity, survival determinants, and therapeutically relevant pathways. Cell Rep Med. 2022;3:100819.
9. Park E, Shim HS. Detection of targetable genetic alterations in Korean lung cancer patients: a comparison study of single-gene assays and targeted next-generation sequencing. Cancer Res Treat. 2020;52:543-51.
10. Moreira AL, Ocampo PS, Xia Y, Zhong H, Russell PA, Minami Y, et al. A grading system for invasive pulmonary sdenocarcinoma: a proposal from the International Association for the Study of Lung Cancer Pathology Committee. J Thorac Oncol. 2020;15:1599-610.
11. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: a joint consensus recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn. 2017;19:4-23.
12. Sanchez-Vega F, Mina M, Armenia J, Chatila WK, Luna A, La KC, et al. Oncogenic signaling pathways in The Cancer Genome Atlas. Cell. 2018;173:321-37.
13. Zhou J, Sanchez-Vega F, Caso R, Tan KS, Brandt WS, Jones GD, et al. Analysis of tumor genomic pathway alterations using broad-panel next-generation sequencing in surgically resected lung adenocarcinoma. Clin Cancer Res. 2019;25:7475-84.
14. Kadota K, Kushida Y, Kagawa S, Ishikawa R, Ibuki E, Inoue K, et al. Cribriform subtype is an independent predictor of recurrence and survival after adjustment for the eighth edition of TNM staging system in patients with resected lung adenocarcinoma. J Thorac Oncol. 2019;14:245-54.
15. Ujiie H, Kadota K, Chaft JE, Buitrago D, Sima CS, Lee MC, et al. Solid predominant histologic subtype in resected stage I lung adenocarcinoma is an independent predictor of early, extrathoracic, multisite recurrence and of poor postrecurrence survival. J Clin Oncol. 2015;33:2877-84.
16. Nitadori J, Bograd AJ, Kadota K, Sima CS, Rizk NP, Morales EA, et al. Impact of micropapillary histologic subtype in selecting limited resection vs lobectomy for lung adenocarcinoma of 2cm or smaller. J Natl Cancer Inst. 2013;105:1212-20.
17. Deng C, Zheng Q, Zhang Y, Jin Y, Shen X, Nie X, et al. Validation of the novel International Association for the Study of Lung Cancer grading system for invasive pulmonary adenocarcinoma and association with common driver mutations. J Thorac Oncol. 2021;16:1684-93.
18. Rokutan-Kurata M, Yoshizawa A, Ueno K, Nakajima N, Terada K, Hamaji M, et al. Validation study of the International Association for the Study of Lung Cancer histologic grading system of invasive lung adenocarcinoma. J Thorac Oncol. 2021;16:1753-8.
19. Park BJ, Woo W, Cha YJ, Shim HS, Yang YH, Moon DH, et al. Proposal of a revised International Association for the Study of Lung Cancer grading system in pulmonary non-mucinous adenocarcinoma: the importance of the lepidic proportion. Lung Cancer. 2023;175:1-8.
20. Skoulidis F, Heymach JV. Co-occurring genomic alterations in non-small-cell lung cancer biology and therapy. Nat Rev Cancer. 2019;19:495-509.
21. Caso R, Sanchez-Vega F, Tan KS, Mastrogiacomo B, Zhou J, Jones GD, et al. The underlying tumor genomics of predominant histologic subtypes in lung adenocarcinoma. J Thorac Oncol. 2020;15:1844-56.
22. Canale M, Petracci E, Delmonte A, Chiadini E, Dazzi C, Papi

M, et al. Impact of TP53 mutations on outcome in EGFR-mutated patients treated with first-line tyrosine kinase inhibitors. Clin Cancer Res. 2017;23:2195-202.
23. Kim IA, Hur JY, Kim HJ, Lee SA, Hwang JJ, Kim WS, et al. Targeted next-generation sequencing analysis predicts the recurrence in resected lung adenocarcinoma harboring EGFR mutations. Cancers (Basel). 2021;13:3632.
24. Jung HA, Lim J, Choi YL, Lee SH, Joung JG, Jeon YJ, et al. Clinical, pathologic, and molecular prognostic factors in patients with early-stage EGFR-mutant NSCLC. Clin Cancer Res. 2022;28:4312-21.
25. Nahar R, Zhai W, Zhang T, Takano A, Khng AJ, Lee YY, et al. Elucidating the genomic architecture of Asian EGFR-mutant lung adenocarcinoma through multi-region exome sequencing. Nat Commun. 2018;9:216.
26. Vokes NI, Chambers E, Nguyen T, Coolidge A, Lydon CA, Le X, et al. Concurrent TP53 mutations facilitate resistance evolution in EGFR-mutant lung adenocarcinoma. J Thorac Oncol. 2022;17:779-92.
27. Perez-Tenorio G, Karlsson E, Waltersson MA, Olsson B, Holmlund B, Nordenskjold B, et al. Clinical potential of the mTOR targets S6K1 and S6K2 in breast cancer. Breast Cancer Res Treat. 2011;128:713-23.
28. Chen B, Yang L, Zhang R, Gan Y, Zhang W, Liu D, et al. Hyperphosphorylation of RPS6KB1, rather than overexpression, predicts worse prognosis in non-small cell lung cancer pati-ents. PLoS One. 2017;12:e0182891.
29. Kim M, Chung YS, Kim KA, Shim HS. Prognostic factors of acinar- or papillary-predominant adenocarcinoma of the lung. Lung Cancer. 2019;137:129-35.
30. Chaft JE, Rimner A, Weder W, Azzoli CG, Kris MG, Cascone T. Evolution of systemic therapy for stages I-III non-metastatic non-small-cell lung cancer. Nat Rev Clin Oncol. 2021;18:547-57.
31. Lengel HB, Connolly JG, Jones GD, Caso R, Zhou J, Sanchez-Vega F, et al. The emerging importance of tumor genomics in operable non-small cell lung cancer. Cancers (Basel). 2021;13:3656.
32. Ahn B, Yoon S, Kim D, Chun SM, Lee G, Kim HR, et al. Clinicopathologic and genomic features of high-grade pattern and their subclasses in lung adenocarcinoma. Lung Cancer. 2022;170:176-84.