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Article

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Hwa Kyung Park [1,2], Yoo-Duk Choi [1,3], Ju-Sik Yun [1,4], Sang-Yun Song [1,4], Kook-Joo Na [1,4], Joon Young Yoon [1,2], Chang-Seok Yoon [1,5], Hyung-Joo Oh [1,2], Young-Chul Kim [1,2] and In-Jae Oh [1,2] *

[1] Lung Cancer Center, Chonnam National University Hwasun Hospital, Hwasun, Jeollanam-do 58128, Republic of Korea
[2] Department of Internal Medicine, Chonnam National University Medical School, Gwangju 61469, Republic of Korea
[3] Department of Pathology, Chonnam National University Hospital, Gwangju 61469, Republic of Korea
[4] Department of Thoracic and Cardiovascular Surgery, Chonnam National University Hwasun Hospital, Hwasun, Jeollanam-do 58128, Republic of Korea
[5] Department of Internal Medicine, Chonnam National University Hospital, Gwangju 61469, Republic of Korea
* Correspondence: droij@chonnam.ac.kr; Tel.: +82-61-379-7617.

Simple Summary: The study of genetic alterations, including epidermal growth factor receptor (EGFR) mutations, which has already been well established in the advanced stage non-small cell lung cancer (NSCLC), is not yet sufficient in the early stage. The results showed that the prevalence of EGFR mutation, ALK rearrangement, and ROS1 fusion was 43.0%, 5.7%, and 1.6%. EGFR mutation was an independent risk factor for recurrence, and it was associated with CNS recurrence.

Abstract: Definitive surgical resection is the preferred treatment for early-stage non-small-cell lung cancer (NSCLC). Research into genetic alterations, including epidermal growth factor receptor (EGFR) mutation, in early stage NSCLC remains insufficient. Here, we investigated the prevalence of genetic alterations in early-stage NSCLC and the association between EGFR mutation and recurrence after complete resection. Between January 2019 and December 2021, 659 patients with NSCLC who underwent curative surgical resection at a single regional cancer center were recruited. We compared the clinical and pathological data between the recurrence and non-recurrence groups. Multivariate logistic regression was used to predict the risk factors for recurrence. Among the 659 enrolled cases, the most common histology was adenocarcinoma (74.5%), followed by squamous cell carcinoma (21.7%). The prevalence of EGFR mutation was 43% (194/451). Among them, L858R point mutation and exon 19 deletion was 52.3% and 42%, respectively. ALK rearrangement was found at 5.7% (26/453), and ROS1 fusion was found at 1.6% (7/441). The recurrence rate of the entire population was 19.7%. In multivariate analysis, the presence of EGFR mutation, stage II or III (vs. stage I), and pathologic subtype (presence of solid type) were associated with recurrence. Among the recurred group, 86.5% of the patients with EGFR mutation experienced distant recurrence compared to only 66.7% of wild-type ($p = 0.016$), with no significant difference in median disease-free survival ($p = 0.983$). In conclusion, the prevalence of EGFR mutation, ALK rearrangement, and ROS1 fusion was 43.0%, 5.7%, and 1.6%, respectively in patients with early-stage NSCLC who underwent curative resection. Along with stage II/III and solid pathologic subtype, EGFR mutation was an independent risk factor for recurrence. In the recurrence group, the rate of distant metastasis was higher in patients with EGFR mutation than in those with wild-type.

Keywords: Lung neoplasm; early stage; epidermal growth factor receptor

Introduction

In 2020, lung cancer was reported as the leading cause of death worldwide and the second most commonly diagnosed cancer.[1] Approximately 48% of lung cancer cases in the U.S. are diagnosed

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in the early stage.[2,3] Patients with early-stage lung cancer have a better 5-year survival rate compared to those with advanced stage (62.8% vs. 8.2%); therefore, it is crucial to diagnose lung cancer early to ensure the best chance of preventing recurrence.[4]

Definitive surgical resection is the preferred treatment for early stage non-small-cell lung cancer (NSCLC), followed by adjuvant chemotherapy if necessary, depending on the pathological stage.[5] A retrospective analysis revealed that patients with stage I NSCLC who underwent surgical resection had a 5-year survival rate ranging from 40% to 97%.[6] In the real world, it has been reported that 23% of patients with early stage NSCLC who underwent surgical resection experienced disease recurrence after 1 year. Furthermore, the 5-year disease-free survival (DFS) rate was 29.3%, which declined as the disease progressed.[7]

Epidermal growth factor receptor (EGFR) mutation is the most common driver mutation in advanced NSCLC in East Asia, with a prevalence of approximately 20%–64%.[8] In a large-scale data analysis conducted in China, the EGFR mutation rate in the early stage was 53.6%, which was comparable to the rate observed in the advanced stage (51.4%, $p = 0.379$). The proportion of EGFR mutations was higher in stage IA than in stages IIB and IIIA. Most EGFR mutations were exon 19 deletion (Ex19del) and exon 21 codon p.Leu858Arg (L858R).[9] However, studies on the prevalence of EGFR mutations in patients with early-stage NSCLC are limited. In a retrospective multi-center analysis, the DFS of patients with early stage NSCLC who had EGFR mutation was shorter than that of patients without EGFR mutation. In addition, the presence of EGFR mutation was significantly related to disease recurrence, along with lymphovascular invasion, intrapulmonary metastasis, and lymph node metastasis.[10] Another study showed that the presence of EGFR mutation was a risk factor for distant metastasis in early-stage lung cancer.[11] Many studies on EGFR tyrosine kinase inhibitors (EGFR-TKIs) as adjuvant therapy have been conducted to lower recurrence and improve prognosis in early-stage NSCLC.

Recently, compared to chemotherapy, adjuvant EGFR-TKIs have demonstrated improved DFS in patients with EGFR-mutated NSCLC.[12,13] Osimertinib, a third-generation EGFR-TKI, demonstrated a significant improvement in DFS compared to placebo in patients with stage IB–IIIA EGFR-mutated NSCLC who underwent complete resection.[14] According to the updated follow-up data of the ADAURA study, the 4-year DFS rate was 73% in the osimertinib group and 38% in the placebo group (overall hazard ratio [HR] for DFS: 0.23; 95% confidence interval [CI]: 0.18 to 0.30).[15] Based on these successful results, osimertinib is recommended as an adjuvant treatment for resected EGFR-mutant stage IB–IIIA NSCLC.[16,17]

In this study, we investigated the prevalence of genetic alteration in patients with early-stage NSCLC who underwent definitive surgery in a single center, as well as the association between EGFR mutation and recurrence after curative surgery.

Materials and Methods

2.Data Collection

We recruited 1,095 patients who underwent surgical resection at the Lung Cancer Center, Chonnam National University Hwasun Hospital (CNUHH) from January 1, 2019 to December 31, 2021 (Fig. 1). First, 205 patients were excluded for the following reasons: 34 patients received the second operation after their first operation before 2019; two patients were diagnosed with benign disease; 101 patients underwent diagnostic surgical resection in stage IV or stage III, which was indicated for chemotherapy or concurrent chemoradiotherapy; one patient underwent an open and closure operation; one patient underwent their first operation at another hospital; 11 patients were diagnosed with small cell lung cancer; 24 patients did not undergo positron emission computed tomography (PET-CT), so distant metastasis was not evaluated; 13 patients were diagnosed with a disease other than lung cancer; 3 patients were diagnosed with double primary malignancy; and 15 patients received incomplete resection. We also excluded 227 patients who did not undergo mediastinal lymph node dissection. Among patients who underwent definitive surgical resection

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with mediastinal lymph node dissection, we excluded four patients who were diagnosed with stage 0.

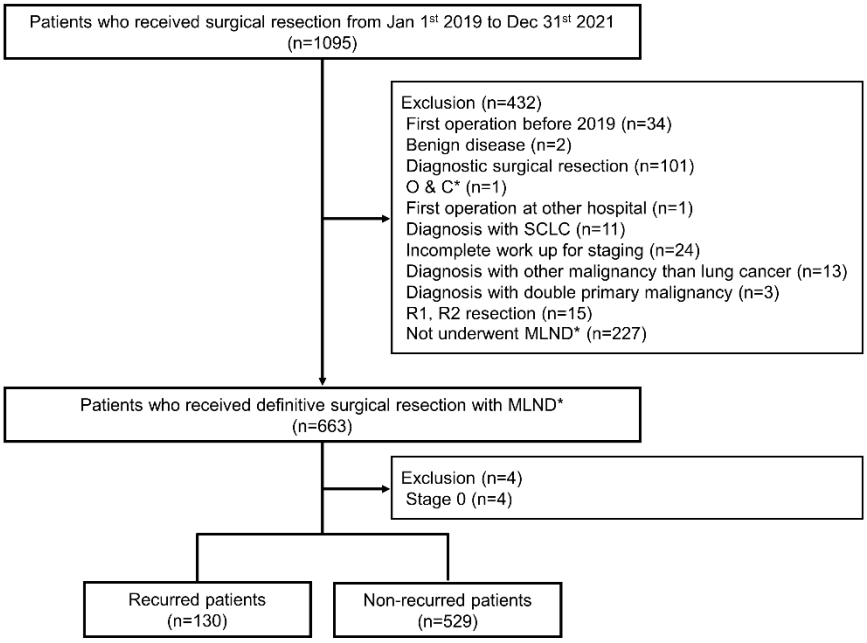

Figure 1. Flow chart of patient enrollment. *O & C: open and closure, MLND: mediastinal lymph node dissection.

We collected clinical and pathological data from 659 patients. The collected data included sex, age, smoking history, second-hand smoking, comorbidities, family history of lung cancer, Eastern Cooperative Oncology Group Performance Score (ECOG PS), parameters of pulmonary function test (PFT), serum carcinoembryonic antigen (CEA), serum pro-gastrin releasing peptide (proGRP), serum Cyfra21-1, histologic type, pathologic stage, initial therapy, operation type, approach type, tumor location, adjuvant therapy, recurrence state, date of recurrence, survival state, and date of last follow-up. The recurrence date was set the date on which the treatment for recurrence began.

In addition, we retrospectively collected pathological data from surgical tissues and tissues obtained from biopsies performed before surgery. From these specimens, we assessed the presence of visceral pleural invasion (VPI), lymphatic invasion, vascular invasion, and neural invasion, EGFR mutation, anaplastic lymphoma kinase (ALK) rearrangement, ROS proto-oncogene 1(ROS1) fusion, and programmed death-ligand 1 (PD-L1) expression.

This study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the Institutional Review Board of Chonnam National University Hwasun Hospital (CNUHH-2023-164). The requirement for patient consent was waived due to the retrospective nature of the study and the use of anonymized clinical data for analysis.

2.Detection of EGFR Mutation

DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tumor tissue using a Gene All Tissue DNA Purification Kit (General Biosystems, Seoul, Korea) according to the manufacturer's protocol. The obtained DNA was eluted in 50 μL of elution buffer, and the concentration and purity of the extracted DNA were assessed by spectroscopy using a NanoDrop spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). We then detected EGFR gene mutations by real-time polymerase chain reaction (PCR) using the PNA Clamp Mutation Detection Kit (Panagene Inc., Daejeon, Korea). All reactions were conducted in 20 μL volumes using template DNA, primer, PNA probe set, and fluorescence PCR master mix. Real-time PCR was performed using a DFX 96 (BioRad Laboratories Inc., Hercules, CA, USA). PCR cycling conditions were set as follows: a 5-min

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hold at 94°C for 40 cycles, at 94°C for 30 s, 70°C for 20 s, 63°C for 30 s, and 72°C for 30 s. The pooled sensitivity and specificity of the PNA clamp methods were 93% and 100%, respectively.[18–20]

2.Detection of ALK Rearrangement

We used an ALK break-apart probe (Abbott Vysis ALK Break Apart FISH Probe Kit; Abbott Molecular, Abbott Park, IL, USA) to investigate ALK rearrangement in FFPE operative tissue. These probes have a dual-color system, which realizes the ALK breakpoint cluster with red and green signals. Detection of ALK rearrangement was performed according to the method reported in a previous study. In FFPE tissue with maintained ALK gene, the probes are visualized by the point of fusion of red and green signals. However, in FFPE tissue with a disrupted ALK locus, the red and green signals split and consequently showed a split pattern. We defined ALK-positive tissues as those in which more than 15% of cancer cells showed split or deleted split patterns.[21]

2.Detection of ROS1 Fusion

ROS1 fusion was detected by PCR using the ROS1 fusion gene detection kit (Amoy Diagnostics Co., Ltd, Xiamen, China). Total RNA isolated from FFPE tissue from each specimen was used to detect ROS1 fusion. Reverse transcription was performed using total RNAs and the conditions were as follows: 42°C for 1 h; 95°C for 5 min. The resulting complementary DNA (cDNA) solutions were used for multiplex qRT–PCR and the ROS1 fusion gene mRNA was detected by qRT–PCR. The PCR procedure was as follows: initial denaturation at 95°C for 5 min, ensuring specificity at 95°C for 25 s, 64°C for 20 s, and 72°C for 20 s, and data collection for 31 cycles of 93°C for 25 s, 60°C for 35 s, and 72°C for 20 s. The fusion patterns were investigated according to a previous study.[22] Quantitative analysis was based on fusion fluorescence signals. Test responses that achieved a Ct value of less than 30 cycles were defined as ROS1 fusion positive.

2.Statistical Analysis

Overall survival (OS) was defined as the period from the date of operation to death, and DFS was defined as the period from the date of operation to recurrence. Genetic alterations were defined as cases in which preoperative or surgical tissue showed EGFR mutation, ALK rearrangement, or ROS1 fusion. If both preoperative and surgical tissues were available, PD-L1 expression was determined based on the surgical tissue using SP263 antibody. We defined the pathologic stage according to the 8th edition of the AJCC Cancer Staging Manual. The data cut-off date was July 12, 2023.

We compared the clinical and pathological backgrounds of patients with and without recurrence, and compared the genetic alterations between locoregional recurrence and distant recurrence in patients who experienced recurrence. We used the Mann–Whitney U test to analyze continuous variables and the chi-square test or Fisher's exact test was used for categorical variables. To predict risk factors for recurrence after surgical resection, variables with a p-value < 0.25 in univariate logistic regression were analyzed using multivariate logistic regression. Kaplan–Meier analysis and Cox proportional hazard modeling were used to investigate the correlation between EGFR mutation and DFS or OS. All statistical analyses were performed using SPSS Statistics for Windows, version 27 (IBM Corp., Armonk, NY, USA). A p-value < 0.05 was considered statistically significant.

Results

3.Baseline Characteristics of the Overall Patients

We included 659 patients with NSCLC who underwent complete resection with lymph node dissection from January 2019 to December 2021 (Table 1). The majority of patients (87.6%) underwent lobectomy. Overall, 421 patients (63.9%) had stage I, 127 patients (19.3%) had stage II, 111 patients (16.8%) had stage III, and none had stage IIIC. Out of 451 patients, 194 (43%) had EGFR mutations,

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26 out of 453 patients (5.7%) had ALK rearrangement, and 7 out of 441 patients (1.6%) had ROS1 fusion. Of the 479 patients, 295 (61.6%) had PD-L1 expression < 1%, 113 patients (23.6%) had PD-L1 expression 1%–49%, and 71 (14.8%) had PD-L1 expression ≥ 50%. The most commonly diagnosed histological type was adenocarcinoma (73.7%), followed by squamous cell carcinoma (22.2%). Overall, 243 patients (36.9%) received adjuvant therapy after surgical resection. Among them, 236 patients (97.1%) received platinum doublet chemotherapy, 1 patient (4.1%) received target therapy, and 6 patients (2.5%) received concurrent chemoradiotherapy.

Table 1. Baseline characteristics.

Characteristic	Total (N=659)	Non-recurrence (N=529)	Recurrence (N=130)	*p*-value
Age	65.86 [65.14-66.57]	65.61 [64.82-66.41]	66.86 [65.22-68.50]	0.126
Sex				0.039
Female	260 (39.5)	219 (41.4)	41 (31.5)	
Male	399 (60.5)	310 (58.6)	89 (68.5)	
Smoking				0.054
Never smoker	327 (49.6)	272 (51.4)	55 (42.3)	
Current smoker	153 (23.2)	124 (23.4)	29 (22.3)	
Ex-smoker	179 (27.2)	133 (25.2)	46 (35.4)	
Second-hand smoking	21 (3.2)	17 (3.2)	4 (3.1)	>0.999
Comorbidity				
HTN	264 (40.1)	209 (39.5)	55 (42.3)	0.560
DM	147 (22.3)	112 (21.2)	35 (26.9)	0.158
Coronary disease	51 (7.7)	37 (7.0)	14 (10.8)	0.149
Vascular disease	2 (0.3)	1 (0.2)	1 (0.8)	0.353
Liver disease				
History of pulmonary tuberculosis	33 (5.0)	26 (4.9)	7 (5.4)	0.826
ILD	14 (2.1)	8 (1.5)	6 (4.6)	0.040
COPD	78 (11.)	60 (11.3)	18 (13.8)	0.428
Other malignancy	103 (15.6)	80 (15.1)	23 (17.7)	0.470
Family history	8 (1.2)	8 (1.5)	0 (0.0)	0.223
ECOG PS score				0.007
0	500 (75.9)	414 (78.3)	86 (66.2)	
1	155 (23.5)	113 (21.4)	42 (32.3)	
2	4 (0.6)	2 (0.3	2 (1.5)	
Pulmonary function (N=650)				
FEV1, L	2.41 [2.37-2.45]	2.42 [2.36-2.47]	2.39 [2.30-2.47]	0.825
FVC, L	3.25 [3.14-3.36]	3.26 [3.12-3.39]	3.23 [3.11-3.36]	0.611
DLCO, mL/mmHg/min	17.97 [17.29-18.66]	17.65 [17.27-18.03]	19.29 [16.20-22.37]	0.779
DLCO, %	94.16 [90.54-97.78]	93.18 [89.57-96.78]	98.13 [86.99-109.26]	0.608
Serum CEA (N=229)	5.87 [4.39-7.35]	5.59 [4.08-7.10]	6.69 [2.79-10.58]	0.001
Serum proGRP (N=229)	49.79 [45.15-54.42]	50.19 [44.11-56.27]	48.58 [44.60-52.57]	0.137
Serum Cyfra21-1 (N=229)	3.50 [2.66-4.34]	3.45 [2.43-4.47]	3.63 [2.16-5.11]	0.009
Histology				0.243
Adenocarcinoma	491 (74.5)	394 (74.5)	97 (74.6)	
Squamous cell carcinoma	143 (21.7)	118 (22.3)	25 (19.2)	
Other non-small cell carcinoma	25 (3.8)	17 (3.2)	8 (6.2)	
Histologic subtype (N=477)				
Acinar type	335 (70.2)	275 (57.7)	60 (12.6)	0.084
Papillary type	310 (65.0)	242 (50.7)	68 (14.3)	0.060
Micropapillary type	92 (19.3)	59 (12.4)	33 (6.9)	<0.001
Lepidic type	136 (28.5)	126 (26.4)	10 (2.1)	<0.001

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Solid type	112 (23.5)	68 (14.3)	44 (9.2)	<0.001
Cribriform type	5 (1.0)	4 (0.8)	1 (0.2)	0.668
Mucinous type	36 (7.5)	33 (6.9)	3 (0.6)	0.050
Others	10 (2.1)	9 (1.9)	1 (0.2)	0.695
Pathologic stage (TNM 8th)				<0.001
Stage I	421 (63.9)	377 (71.3)	44 (33.8)	
Stage II	127 (19.3)	100 (18.9)	27 (20.8)	
Stage III	111 (16.8)	52 (9.8)	59 (45.4)	
Driver mutation				
EGFR (*n*=451)	194 (43.0)	142 (31.5)	52 (11.5)	0.151
ALK (*n*=453)	26 (5.7)	15 (3.3)	11 (2.4)	0.016
ROS1 (*n*=441)	7 (1.6)	4 (1.0)	3 (0.7)	0.204
PD-L1 (SP263) (*n*=479)				0.734
TPS <1%	295 (61.6)	221 (46.1)	74 (15.4)	
TPS ≥1%, <50%	113 (23.6)	87 (18.2)	26 (5.4)	
TPS ≥50%	71 (14.8)	51 (10.7)	20 (4.2)	
Initial therapy				0.121
Operation	645 (97.8)	520 (98.3)	125 (96.1)	
Chemotherapy	5 (0.8)	4 (0.8)	1 (0.8)	
Radiotherapy	1 (0.2)	0 (0.0)	1 (0.8)	
Concurrent chemoradiotherapy	8 (1.2)	5 (0.9)	3 (2.3)	
Operation type				0.954
Wedge resection	13 (2.0)	10 (1.9)	3 (2.3)	
Segmentectomy	37 (5.6)	31 (5.9)	6 (4.6)	
Lobectomy	577 (87.6)	461 (87.1)	116 (89.2)	
Bilobectomy	26 (3.9)	22 (4.2)	4 (3.1)	
Pneumonectomy	6 (0.9)	5 (0.9)	1 (0.8)	
Approach				<0.001
VATS	502 (76.2)	423 (80.0)	79 (60.8)	
Non-VATS	157 (23.8)	106 (20.0)	51 (39.2)	
Tumor location				0.879
Right upper lobe	160 (24.3)	129 (24.4)	31 (23.8)	
Right middle lobe	48 (7.3)	37 (7.0)	11 (8.5)	
Right lower lobe	152 (23.0)	123 (23.3)	29 (22.3)	
Left upper lobe	164 (24.9)	129 (24.4)	35 (26.9)	
Left lower lobe	131 (19.9)	107 (20.2)	24 (18.5)	
Other	4 (0.6)	4 (0.7)	0 (0.0)	
Visceral pleural invasion (N=658)				0.013
Yes	140 (21.3)	102 (19.3)	38 (29.2)	
No	518 (78.7)	426 (80.7)	92 (70.8)	
Lymphatic invasion (N=658)				<0.001
Yes	91 (13.8)	49 (7.4)	42 (6.4)	
No	567 (86.2)	479 (72.8)	88 (13.4)	
Vascular invasion (N=658)				<0.001
Yes	46 (7.0)	24 (3.7)	22 (3.3)	
No	612 (93.0)	504 (76.6)	108 (16.4)	
Neural invasion (N=658)				<0.001
Yes	16 (2.4)	6 (1.0)	10 (1.5)	
No	642 (97.6)	522 (79.3)	120 (18.2)	
Adjuvant therapy (N=243)				0.117
Platinum base chemotherapy	236 (97.1)	162 (98.2)	74 (94.9)	
Target therapy	1 (0.4)	1 (0.6)	0 (0.0)	

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Concurrent chemoradiotherapy	6 (2.5)	2 (1.2)	4 (5.1)	
Survival				<0.001
Alive	572 (86.8)	479 (90.5)	93 (71.5)	
Death	27 (4.1)	10 (1.9)	17 (13.1)	
Censored	60 (9.1)	40 (7.6)	20 (15.4)	

ILD, interstitial lung disease; COPD, chronic obstructive pulmonary disease; ECOG PS score, Eastern Cooperative Oncology Group Performance Score; CEA, carcinoembryonic antigen; proGRP, pro-gastrin releasing peptide; EGFR, epidermal growth factor receptor; ALK, anaplastic lymphoma kinase; ROS1, ROS proto-oncogene 1; PD-L1, programmed death-ligand 1; TPS, tumor proportion score; VATS, Video-Assisted Thoracic Surgery. *p-value by chi-square test, Fisher's exact test, or Mann-Whitney U test, as appropriately.

One hundred and thirty patients (19.7%) experienced disease recurrence. We divided the patients into two groups: the non-recurred group and the recurred group. In the non-recurred group, 377 patients (71.3%) had stage I, 100 patients (18.9%) had stage II, and 52 patients (9.8%) had stage III disease. In the recurred group, 44 patients (33.8%) had stage I, 27 patients (20.8%) had stage II, and 59 patients (45.4%) had stage III disease ($p < 0.001$). The proportion of EGFR mutations was similar in the non-recurred and recurred groups (41.2% vs. 49.1%, $p = 0.151$). In both groups, most patients underwent lobectomy, with 87.1% in the non-recurrence group and 89.2% in the recurrence group.

3.Prevalence of Genetic Alterations

In patients with EGFR-mutated NSCLC, the majority had stage I (64.4% with stage I compared to 17% with stage II and 18.6% with stage III, $p = 0.047$). In patients with ALK-positive NSCLC, most patients had stages I or III (46.2% with stage I or III compared to 7.7% with stage II, $p = 0.002$). The same trend was observed in ROS1 fusion, although it did not reach statistical significance (42.9% with stage I or III compared to 14.3% with stage II, $p = 0.283$) (Fig. 2a). Among the EGFR-mutant patients, the most frequently observed mutation was L858R, noted in 101 patients (52.3%), followed by Ex19del, observed in 79 patients (40.9%). Moreover, 2 patients (1.0%) had both L858R and Ex19del. Other rare mutations were noted in 11 patients (5.7%) (Table 2). The higher the expression of PD-L1, the higher the proportion of higher stage, but there was no statistical significance ($p = 0.063$) (Fig. 2b).

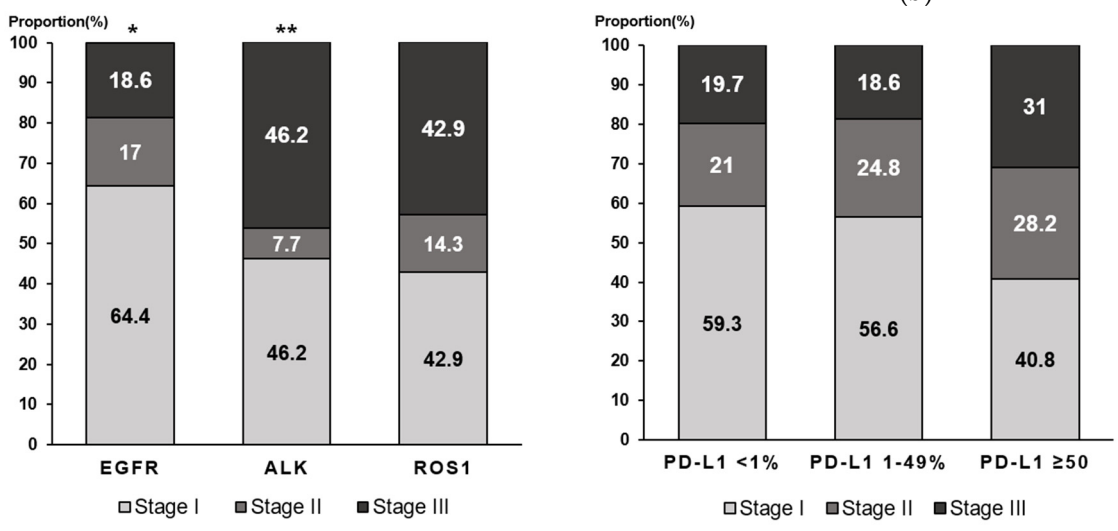

Figure 2. Proportion of genetic alteration. (a) The bar graph showed the percentage of genetic alteration by stage in the overall patients. Among patients with EGFR-mutation, the majority had stage I. In patients with ALK-positive, the majority had stage I and III. (b) The bar graph showed that the higher expression the PD-L1 expression, the higher the proportion of high stage, but it was not statistically significant ($p = 0.063$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.$EGFR, epidermal growth factor receptor, ALK, anaplastic lymphoma kinase; PD-L1, programmed death-ligand 1.

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Table 2. Prevalence of EGFR subtypes.

EGFR Mutations	Total (N=193)	Non-recurred (N=142)	Recurred (N=51)	*p*-value
L858R	101 (52.4)	75 (52.8)	26 (50.9)	
L858R only	97 (50.3)	72 (50.7)	25 (49.0)	
L858R + T790M	4 (2.1)	3 (2.1)	1 (1.9)	
Ex19del	79 (40.9)	58 (40.8)	21 (41.2)	
Ex19del only	76 (39.4)	56 (39.4)	20 (39.2)	0.896
Ex19del + T790M	2 (1.0)	1 (0.7)	1 (2.0)	
Ex19del + G719C	1 (0.5)	1 (0.7)	0	
L858R + Ex19del	2 (1.0)	1 (0.7)	1 (2.0)	
Others*	11 (5.7)	8 (5.6)	3 (5.9)	

* Others include 7 patients with E20ins (6 patients in non-recurred group and 1 patient in recurred group), 2 patients with L861Q (1 patient in recurred group and 1 patient in recurred group), 1 patient with G719C in non-recurred group, and 1 patient with G719S in recurred group. L858R, exon 21 codon p.Leu858Arg; T790M, Thr790Met; Ex19del, exon 19 deletion; G719C, exon 18 p.Gly719Cys; E20ins, exon 20 insertions; L861Q, exon 21 L861Q.

According to the prevalence based on the day of surgery, only 42 patients (6.4%) were found to have genetic alterations before surgery. Preoperative biopsy sites were the primary lung mass (41 cases) or metastatic lymph node (1 case). EGFR mutations were found in 14 patients (33.3%) including 6 cases of L858R, 6 cases of Ex19del, 1 case of G719S, and 1 case of exon 20 insertion. There was one case each of ALK rearrangement and ROS1 fusion.

3.Prognostic Factors Associated with Disease Recurrence

The median follow-up duration for all patients was 31.54 months (95% CI: 30.10–32.98). Death, hopeless discharge, or loss of follow-up occurred in 87 patients (13.2%), and the median DFS was 30.52 months (95% CI: 29.21–31.83). Recurrence after definitive surgery occurred in 130 patients (19.7%). Out of the 106 patients investigated for the presence of EGFR mutation among those with recurrence, 52 patients (49.1%) had EGFR mutation.

First, we analyzed the risk factors for recurrence after definitive surgery in all patients. In univariate analysis, being male (vs. female), ECOG PS of 1 (vs. PS 0), stage II or III (vs. stage I), presence of ALK rearrangement, VPI, lymphatic invasion, or vascular invasion, and pathologic subtype (presence of micropapillary type, lepidic type or solid type) were associated with disease recurrence. In multivariate analysis, stage II or III (vs. stage I), presence of EGFR mutation, and pathologic subtype (presence of solid type) were associated with disease recurrence (Fig. 3a). However, there was no significant difference in DFS between the EGFR-wild-type group and the mutant group (Fig. 3b).

In stage IB–IIIA, recurrence occurred in 109 patients (27.7%). In univariate analysis, stage III (vs. stage I), presence of EGFR mutation, ALK rearrangement, lymphatic or vascular invasion, and pathologic subtype (presence of micropapillary type, lepidic type, or solid type) were associated with recurrence. In multivariate analysis, stage III (vs. stage I) and pathologic subtype (presence of acinar type or mucinous type) were associated with recurrence (Fig. 3c). However, there was no significant difference in DFS between the EGFR wild-type group and the mutant group (Fig. 3d).

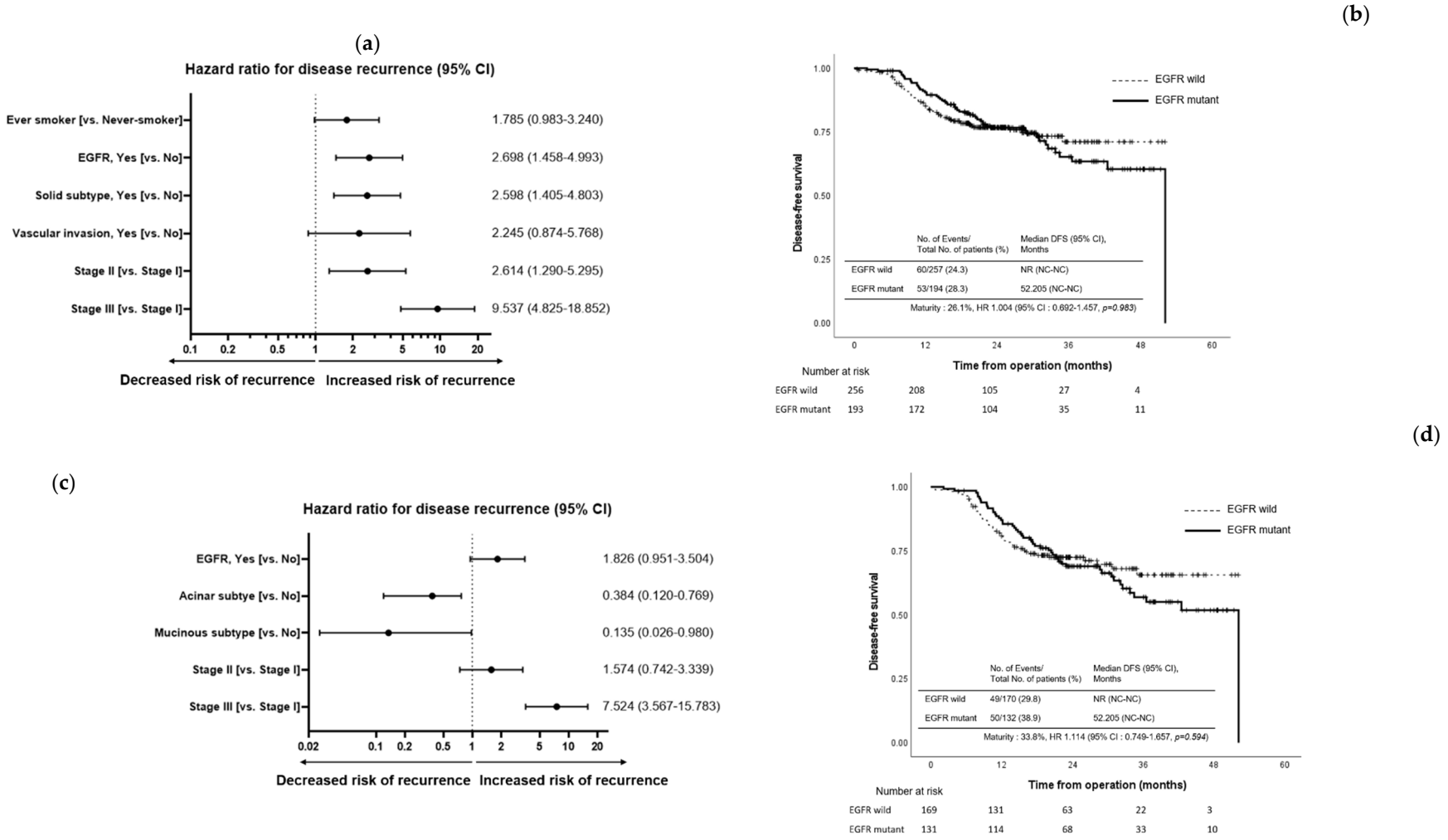

Figure 3. Risk factor for disease recurrence (a) Forest plots for risk factors of disease recurrence in overall patients. (b) Kaplan–Meier survival curve for DFS according to presence of EGFR mutation in overall patients. (c) Forest plots for risk factors of disease recurrence in stage IB–IIIA. (d) Kaplan–Meier survival curve for DFS according to the presence of EGFR mutation in stage IB–IIIA. NR, not reached; NC, not checked; HR, hazard ratio; CI, confidence interval; DFS, disease-free survival; EGFR, epidermal growth factor receptor.

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3.Association between EGFR Mutation and Type of Recurrence

In patients who experienced recurrence, locoregional recurrence occurred in 37 patients (28.5%), whereas distant recurrence occurred in 93 patients (71.5%). The most commonly recurred extra-thoracic location was the bone (30 patients, 23.1%), followed by the central nervous system (CNS) (23 patients, 17.7%). The CNS was the most common recurrence location for patients with EGFR-mutated NSCLC, and the presence of EGFR mutation was a risk factor for CNS recurrence (Table 3). Among the overall patients, 86.5% of EGFR-mutant patients experienced distant recurrence, which was higher than the rate observed in EGFR-wild patients (66.7%, $p = 0.016$). Additionally, in patients with stage III disease, a higher proportion of those with EGFR mutations experienced distant recurrence compared to those without EGFR mutations (92% vs. 68%, $p = 0.034$). Moreover, there was no significant difference in distant recurrence between EGFR-mutant patients and EGFR-wild patients with stage I (86.7% vs. 68.4%, $p = 0.257$) and stage II (75.0% vs. 60.0%, $p = 0.652$) disease (Fig. 4a). Although the proportion of distant metastases was higher in the EGFR-mutant group than in the wild group in the overall patients, there was no significant difference in OS (Fig. 4b).

Table 3. Prevalence of recurred location.

EGFR status	Wild (n=54)	Mutant (n=52)	Unknown (n=51)	HR [95% CI]
Lung or Regional lymph node	34 (63.0)	26 (50.0)	17 (70.8)	0.588 [0.271-1.277]
Central nervous system	5 (9.3)	14 (26.9)	4 (16.7)	3.611 [1.195-10.907]
Bone	17 (31.5)	12 (23.1)	1 (4.2)	0.653 [0.275-1.549]
Extrathoracic visceral pleura	4 (7.4)	2 (3.8)	1 (4.2)	0.500 [0.088-2.855]
Pleura	8 (14.8)	14 (26.9)	1 (4.2)	2.118 [0.804-5.583]
Peritoneum	1 (1.9)	2 (3.8)	0 (0.0)	2.121 [0.186-24.114]
Head and neck	1 (1.9)	0 (0.0)	0 (0.0)	
Others	5 (9.3)	3 (5.8)	3 (12.5)	0.600 [0.136-2.649]

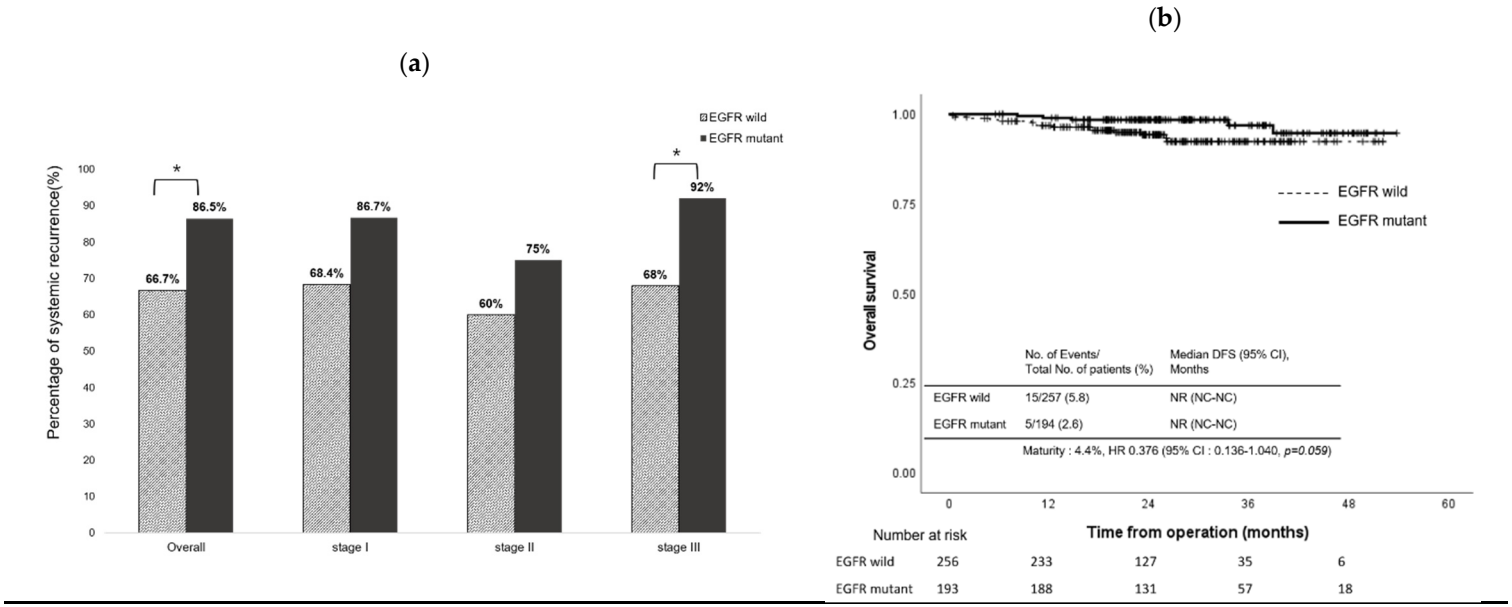

Figure 4. Type of recurrence and overall survival (a) Bar graph showing the proportion of distant metastasis among overall patients (p = 0.016), stage I (p = 0.257), stage II (p = 0.652), and stage III (p = 0.034). (b) Kaplan–Meier survival curve for OS according to the presence of EGFR mutation among the overall patients. NR, not reached; NC, not checked; HR, hazard ratio; CI, confidence interval; OS, overall survival; EGFR, epidermal growth factor receptor.

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Discussion

In this study, we evaluated the prevalence of genetic alterations in patients with early-stage NSCLC and identified a prognostic factor for disease recurrence. EGFR mutation is more common in Asians than in Caucasians.[9,23] It has been noted that EGFR mutations occur in approximately 30%–50% of Asian patients with advanced NSCLC. The prevalence of EGFR mutation in early-stage NSCLC was found to be similar to that in advanced stage NSCLC according to a retrospective analysis published in China.[8,9] We found that approximately two-thirds of all patients were tested for genetic alterations, and about half of them had EGFR mutations. The prevalence of EGFR mutations in this study was consistent with that in previous studies.

Various prognostic factors have been found to be associated with disease recurrence in early-stage NSCLC after complete resection, including age, histologic pattern, lymphovascular invasion, invasive size, pathologic stage, and genetic alterations.[24–27] Recently, the significance of EGFR mutation as a prognostic factor for early-stage NSCLC has been reported. Saw, Stephanie P.L. et al. reported the association of EGFR mutation with recurrence in resected NSCLC, stage IA to IIIA. The 5-year OS was better, but the recurrence rate was higher in patients with EGFR-mutated NSCLC than in those with wild-type. However, the difference in DFS between EGFR-mutant and wild-type NSCLC was not statistically significant.[27] Nishii et al. reported that EGFR mutation was a poor prognostic marker in patients with NSCLC < 80 years who underwent complete resection.[28] In our study, we found that the presence of EGFR mutation, pathologic subtype (presence of solid type), and advanced stage were associated with recurrence in all patients. In addition, advanced stage and pathologic subtype (presence of acinar type or mucinous type) were associated with recurrence in patients with stage IB–IIIA disease. We have discovered that EGFR mutation could be an impactive factor in recurrence, which is consistent with the findings of previous studies.

Although the EGFR mutation did not impact DFS and OS in this study, it was associated with the type of metastasis. Among all patients, the proportion of distant recurrence was higher in the EGFR-mutant group than in the EGFR-wild group, and the same trend was observed in patients with stage III NSCLC. In addition, the presence of EGFR mutation was a risk factor for CNS recurrence, which represents a major concern after definitive surgery in early-stage NSCLC. Among sites of distant recurrence, the brain is the most common site.[29] Approximately half of patients who underwent definitive surgery for early-stage NSCLC received adjuvant therapy, and 41% of patients with EGFR-positive NSCLC received adjuvant therapy after definitive resection. In the real world, more than 95% of patients with EGFR-positive NSCLC who received adjuvant therapy received platinum-based chemotherapy.[7,30,31] The situation was similar in our center; however, platinum-based adjuvant chemotherapy has been shown to be ineffective in preventing CNS recurrence.[32]

With many efforts to reduce recurrence and improve survival in early-stage NSCLC, in recent years, a new generation of EGFR-TKIs has opened up a new era of adjuvant therapy after definitive surgery. Icotinib, a first-generation EGFR-TKI, improved DFS compared to chemotherapy in patients with stage II–IIIA NSCLC after resection.[12] Gefitinib, another first-generation EGFR-TKI, improved DFS in patients with completely resected stage II–IIIA EGFR-mutated NSCLC.[13] According to the recently updated results of the ADAURA study, adjuvant osimertinib in EGFR-mutated NSCLC after complete resection improved not only DFS but also 5-year OS in patients with stage IB–IIIA disease. As a result of the analysis by the stage, the 5-year OS was significantly different only in stage IIIA.[33] In result, it is important to gather more individualized information about patients. The prognosis varies depending on the genetic alteration and pathologic subtype, even within the same stage. It is crucial to select appropriate high-risk patients and determine adjuvant therapy to prevent recurrence in early-stage lung cancer and improve survival. Furthermore, some promising results of neoadjuvant targeted therapy are being reported.[34] Given the high rate of distant metastasis after resection in EGFR-mutated NSCLC and the benefit of EGFR-TKI on survival, it seems important to detect genetic alterations before surgery. In this study, it was only found in 6.4% of cases before surgery, but if the genetic alterations can be found with a more sensitive method such as liquid biopsy, the prognosis of lung cancer might be improved with neoadjuvant targeted therapy.

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This study has several limitations that warrant discussion. First, this was a single-center retrospective study. Although the proportion of genetic alterations and prognosis of patients followed the same trend as that in previous studies, the characteristics of the patients in this study may not fully represent all Koreans or Asians. Second, analysis of genetic alteration was not performed in all patients. The presence of genetic alteration was mainly investigated in patients with adenocarcinoma, and in other types of NSCLC, including squamous cell carcinoma, the expression of PD-L1 was investigated. Third, other factors such as sex, age, and pathologic subtype were not adjusted when analyzing the association between metastatic organs and the presence of EGFR mutation.

Conclusions

In the present study, we showed that the prevalence of EGFR mutation, ALK rearrangement, and ROS1 fusion was 43.0%, 5.7%, and 1.6% in patients with NSCLC who underwent complete resection, respectively. EGFR mutation was an independent risk factor for recurrence along with stage II/III and solid pathologic subtype. Among patients with recurrent malignancy, the rate of distant metastasis was higher in patients with EGFR mutations than in patients without EGFR mutations, and EGFR mutations were associated with CNS recurrence. With regard to the promising results of EGFR-TKI as adjuvant therapy, it is advisable to identify the histological characteristics of patients with NSCLC who underwent curative surgery and actively consider adjuvant targeted therapy.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

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