CANCER BIOLOGY & THERAPY
2016, VOL. 17, NO. 3, 237–245
http://dx.doi.org/10.1080/15384047.2016.1139235

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RESEARCH PAPER

Compound *EGFR* mutation is frequently detected with co-mutations of actionable genes and associated with poor clinical outcome in lung adenocarcinoma

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Compound *EGFR* mutations, defined as double or multiple mutations in the *EGFR* tyrosine kinase domain, are frequently detected with advances in sequencing technology but its clinical significance is unclear. This study analyzed 61 cases of *EGFR* mutation positive lung adenocarcinoma using next-generation sequencing (NGS) based repeated deep sequencing panel of 16 genes that contain actionable mutations and investigated clinical implication of compound *EGFR* mutations. Compound *EGFR* mutation was detected in 15 (24.6%) of 61 cases of *EGFR* mutation-positive lung adenocarcinoma. The majority (12/15) of compound mutations are combination of the atypical mutation and typical mutations such as exon19 deletion, L858R or G719X substitutions, or exon 20 insertion whereas 3 were combinations of rare atypical mutations. The patients with compound mutation showed shorter overall survival than those with simple mutations (83.7 *vs*. 72.8 mo; $P = 0.020$, Breslow test). Among the 115 missense mutations discovered in the tested genes, a few number of actionable mutations were detected irrelevant to the subtype of *EGFR* mutations, including *ALK rearrangement*, *BCL2L11* intron 2 deletion, *KRAS* c.35G>A, *PIK3CA* c.1633G>A which are possible target of crizotinib, BH3 mimetics, *MEK* inhibitors, and *PI3K-tyrosine kinase inhibitors*, respectively. 31 missense mutations were detected in the cases with simple mutations whereas 84 in those with compound mutation, showing that the cases with compound missense mutation have higher burden of missense mutations ($P = 0.001$, independent sample *t*-test). Compound *EGFR* mutations are detected at a high frequency using NGS-based repeated deep sequencing. Because patients with compound *EGFR* mutations showed poor clinical outcomes, they should be closely monitored during follow-up.

Abbreviations: DFS, disease-free survival; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; OS, overall survival; PFS, progression-free survival; TKD, tyrosine kinase domain; TKI, tyrosine kinase inhibitors.

ARTICLE HISTORY
Received 10 August 2015
Revised 14 November 2015
Accepted 1 January 2016

Keywords
Compound *EGFR* mutation; co-mutation; *EGFR*; lung adenocarcinoma; NGS; repeated deep sequencing; simple *EGFR* mutation

Introduction

Despite relentless efforts to decrease the mortality of lung cancer, it remains a common and leading cause of cancer-related death worldwide. In the year 2012, 1,824,701 new cases were identified and 1,590,000 patients died of lung cancer worldwide (WHO annual report). During the same period, 21,753 new Korean cases were diagnosed and 16,654 Korean patients died of this devastating disease.[1]

Oncogenic driver mutations include multiple types of genomic changes that are critical for cancer development and maintenance. The identification of actionable oncogenic driver mutations that guide selection of appropriate target agents has improved clinical outcomes of lung cancer patients by incorporating tumor genotyping into therapeutic decision making.[2]

Activating *EGFR* mutations are more frequently identified in lung adenocarcinoma in East Asian patients than in other populations, and advances in tumor genotyping facilitate discovery of such mutations in small population samples.[3-6] The most common type of *EGFR* mutation is in-frame deletion of exon 19 (E19del) around the LREA motif (amino acid residues 747 to 750; ~45% of *EGFR* mutations), followed by L858R point mutation of exon 21 (~40% of *EGFR* mutations).[7-9] Tumors with these activating *EGFR* mutations or less frequent mutations, such as point mutations in exon 18 at position G719 (~3% of *EGFR* mutations) and the exon 21 L861Q mutant (~2% of *EGFR* mutations), show sensitivity to EGFR-tyrosine kinase inhibitors (TKIs).[10-12] On the other hand, in-frame insertion mutations within exon 20 of *EGFR*, which account for 4~10% of all *EGFR* mutations, and other rare mutations including L747S, D761Y, T790M, and T854A confer resistance to EGFR-TKIs.[11,13-15]

With the clinical application of more sensitive and precise tumor genotyping systems, rare *EGFR* mutations of unknown biological and clinical significance are frequently encountered in routine clinical practice.[14,15] Different responses to EGFR-TKI are reported even for mutations at the same approximate location within the genomic DNA.

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For example, among the in-frame insertions within *EGFR* exon 20, which were originally considered EGFR-TKI resistance mutations with a low response rate (<5%) and short interval of disease control, A763_Y764insFQEA is now reported to be a sensitizing mutation to EGFR-TKI.[14,15] These findings indicate that more attention and collaborative efforts are required to elucidate the biological and clinical significance of these rare compound mutations.

Compound *EGFR* mutations are defined as double or multiple independent mutations of the EGFR tyrosine kinase domain (TKD), in which an EGFR-TKI-sensitizing or other mutation is identified together with a mutation of unclarified clinical significance.[16] Recent advances in tumor genotyping techniques provide not only accurate data, but also a higher probability of identifying atypical and multiple mutations in the EGFR-TKD in a single sample. Kobayashi et al. reported compound *EGFR* mutations in which an EGFR-TKI-sensitizing mutation (such as G719X, E19del, L858R, or L861Q) coexists with uncommon mutations involving other residues of the *EGFR*-TKD and show some sensitivity to EGFR-TKI. In *EGFR* mutant non-small cell lung cancer (NSCLC), double mutations in *EGFR* were detected in 14∼18% of cases using Sanger method based sequencing techniques, but their biologic behavior and clinical significance have not been well characterized.[16,17]

In this study, we identified *EGFR* compound mutations in lung adenocarcinomas from patients who underwent surgical curative resection using next-generation sequencing (NGS)-based repeated deep sequencing of *EGFR* together with 15 other genes containing actionable oncogenic mutations. This study shows that the compound *EGFR* mutation is common in lung adenocarcinoma and imparts a new meaning of compound *EGFR* mutation.

Materials and methods

Patient characteristics and tumor DNA samples

A total of 143 patients with a pathologically confirmed diagnosis of pStage IB∼IIIA lung adenocarcinoma who underwent curative surgical resection and platinum-based adjuvant chemotherapy and provided informed consent for tissue collection were randomly selected from tissue archives of affiliated hospitals of Yonsei University Medical Center. Among them, 61 patients with *EGFR* mutations who had not received EGFR-TKI before tumor genotyping were enrolled in this study. All paraffin-embedded samples were loaded onto silanated slides as 4-μm-thick sections. One slide of every block was stained with H&E and re-examined for the presence of cancer cells. The enriched area was marked by an independent lung pathologist to validate the presence of tumor cells. These cancer cell-enriched areas were microdissected, and DNA was extracted using a QIAamp DNA FFPE Tissue Kit (Qiagen, Valencia, CA, USA). Institutional Review Board (IRB) approval was obtained for this study (IRB #3-2013-0298).

Library preparation, NGS with IonTorrent, and variant calling

Ten micrograms of genomic DNA were amplified by the Ion AmpliSeq™Custom Panel (Life Technologies, Carlsbad, CA). This panel contains 16 genes that contain actionable mutations; *AKT1, ALK, BCL2L11, BRAF, DDR2, EGFR, ERBB2, FGFR1, KRAS, MAP2K1, MET, NRAS, PIK3CA, PTEN, ROS1*, and *RET*. *ALK* fusion was detected by FISH using Abbott Vysis *ALK* break apart FISH probe kit (Abbott, Abbott Park, Il). Multiplex pools were purified with Agencourt AMPure XP beads (Beckman Coulter Inc.) and ligated with Ion Xpress barcode adapters (Life Technologies). The fragment size and quantity of each library were analyzed by a BioAnalyzer using a High Sensitivity Chip (Agilent, Santa Clara, CA). The library was diluted, and emulsion PCR was performed with the Onetouch™ reagent kit (Life Technologies). The emulsion PCR product was enriched using Dynabeads® MyOne™ Streptavidin C1 beads (Life Technologies). The final enriched ion spheres were mixed with a sequencing primer and polymerase and loaded onto 5 318v2 chips. The libraries were sequenced with the Ion Torrent PGM sequencer at deep coverage (aiming for 1,000×) using the Ion OneTouch 200 Template Kit v2 DL and Ion PGM Sequencing 200 Kit v2 with the 318 v2 chip kits (all from Life Technologies). The sequencing reads were aligned to the human reference GRCh37 genome, and base calling was performed using the Ion Torrent Suite V3.4.2 using tmap-f3 on the Ion Torrent server. The Ion Torrent Variant Caller (ITVC) v3.4 was used for the detection of mutations, requiring a frequency greater than 5% for a variant to be called. Bam (Binary sequence Alignment/Map format) and FASTQ files (alignment) were generated based on the base calling results and were used to report the variant calling, including single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs).

Statistical analysis

Categorical variables are expressed as percentages and compared using χ^2-tests. Differences in distribution of continuous variables between 2 independent samples were assessed by Mann–Whitney U test, and the Kaplan–Meier estimator was used for survival analysis. All analyses were performed with IBM SPSS Statistics version 20 (IBM Corp). All statistical tests were 2-sided, and a P value <0.05 was considered to indicate statistical significance.

Results

Demographic characteristics of the study population

The 61 patients with mutations in *EGFR*-TKD had a mean age of 59 ± 9.9 years (range; 34∼78 years); 17 (27.9%) were male and 44 (72.1%) were female. The difference in age at the time of diagnosis between male and female patients was not significant. The majority of patients (50; 82%) did not have a smoking history, 6 (9.8%) were current smokers, and 5 (8.2%) were ex-smokers; the ever-smokers had a pack-year average of 43 ± 48.2 years. These demographic characteristics are comparable to previous findings of *EGFR* mutation-positive Korean patients with lung adenocarcinoma.[3,4,18]

Compound EGFR mutations

Determination of the entire sequence of *EGFR* exons 18∼21 constituting EGFR-TKD revealed that simple mutations were the more frequent (46 of 61, 75.4%). These were predominantly E19del (24 of 61, 39.3%), followed by L858R point mutation (17 of 61, 27.9%), and *EGFR* exon 20 insertion mutations (2 out of 61, 3.2%). Point mutations involving exon 20, exon 19 insertions, and L861R were less frequent (Table 1). The remaining 15 cases (24.6%) had compound *EGFR* mutations, which is composed of double or multiple independent mutations in the *EGFR*-TKD (Table 1). Most of the compound mutations, (10 of 15, 66.7%) were composed of a rare atypical mutation with EGFR-TKI sensitizing mutations such as G719X ($n = 3$), L858R ($n = 6$), and E19del ($n = 1$). Interestingly, one case had a compound mutation composed of L858R and E19del. Two compound mutations involved exon 20 insertion plus H773Y and rare cases of E749Q plus A750P, L688F plus G824S, and multiple point mutations scattered throughout exon 20 and exon 21 were also detected. The partner mutations were atypical mutations in exon 18 (V689L, I706T, and E709K), those in exon 20 (H773Y and R776H), or those in exon 21 (L833V, H870R, and A871G). Table 1 summarizes the combinations of specific mutations detected in this study. Taken together, *EGFR* compound mutations are common in *EGFR* mutation-positive lung adenocarcinoma.

Clinical characteristics of cases with compound EGFR mutation

Next, we questioned whether the cases with compound *EGFR* mutation showed discernible clinical and pathologic characteristics (Table 2). There was no difference in age or gender distribution between patients with simple mutation and those with compound mutation. Smoking status and pStage at the time of diagnosis were not associated with the type of *EGFR* mutation. We also investigated whether the histologic subtype of adenocarcinoma was different according to the type of mutation. Compound *EGFR* mutation was not detected in the lepidic predominant types. The subtypes that are associated with poor clinical outcomes, such as papillary/micropapillary predominant types and solid with mucin production type, were more frequently detected in cases with compound mutations (21.7% *vs*. 33.3%) but this did not reach clinical significance. The diameter of the tumor mass at the time of operation was larger in the tumors with compound mutation but also did not reach statistical significance (2.9 ± 0.96 *vs*. 3.4 ± 1.01 cm).

Lung adenocarcinoma with compound EGFR mutation shows poor clinical outcome

Because the cases with compound *EGFR* mutation had properties which might be related to poor clinical outcome, we compared the disease-free survival (DFS) and overall survival (OS) of cases with simple and compound mutations (Fig. 1). The median follow-up duration of the study population was 81.9 months (95% confidence interval (CI): 65.7∼98.1 months). Of 61 patients, 33 (54.1%) experienced recurrence of the disease and 15 (24.6%) died of same disease during follow-up period. There was no difference in DFS between the groups, but OS was significantly poorer in the cases with compound mutation (simple mutation, 83.7 months *vs*. compound mutation, 72.8 months, $P = 0.020$, Breslow test) (Fig. 1A). A multivariate analysis including age, smoking status, *EGFR* mutation subtypes, stage, and histologic subtypes revealed that smoking history (HR, 11.47; 95% CI, 2.510∼54.404; $P = 0.002$), compound *EGFR* mutation (HR, 4.030; 95% CI, 1.305∼12.446; $P = 0.015$) were significantly associated with a shorter OS (Table 3). Based on these findings, we hypothesized that cases with compound mutation have a poor response to EGFR-TKI. Among 33

Table 1. Various types of *EGFR* mutations in exons 18–21 detected by NGS-based repeated deep sequencing.

EGFR mutation type		No.	% of total
Simple mutations			
Exon 19 deletions		24	39.3
Exon 19 insertions	V738_K739insKIPVAI	1	1.6
Exon 20 insertions			
	M766_A767insASV	1	1.6
	D770_N771insG+N771T	1	1.6
Exon 20 mutations			
	N771F	1	1.6
Exon 21 mutations			
	L858R	17	27.9
	L861R	1	1.6
Compound mutations			
	L858R + V689L	1	1.6
	L858R + L833V	1	1.6
	L858R + H870R	1	1.6
	L858R + A871G	1	1.6
	L858R + R776H	1	1.6
	L858R + E19del	1	1.6
	G719A + I706T	1	1.6
	G719S + E709K	1	1.6
	G719S + R776H	1	1.6
	E19del + I706T	1	1.6
	D770_N771insNPY +H773Y	2	3.3
	L688F + G824S	1	1.6
	E749Q + A750P	1	1.6
	T785I + Y813H + V845M + V851I + G857R	1	1.6
Total		61	100

Table 2. Clinical and pathologic characteristics of the study cases according to subtype of *EGFR* mutation.

		Simple mutation (n = 46)	Compound mutation (n = 15)	P-value
Age (mean ± SD); yrs		59.6 ± 10.52	58.9 ± 7.93	0.778*
Gender				
	Male	10	7	0.061**
	Female	36	8	
Smoking status				
	Non-smoker	39	11	0.488**
	Current smoker	4	2	
	Ex-smoker	3	2	
Stage				
	IB	4	1	0.970**
	IIA	16	5	
	IIB	2	1	
	IIIA	24	8	
Maximum tumor diameter		2.9 ± 0.96	3.4 ± 1.01	0.075*
Histologic subtype				
	Lepidic predominant	3	0	0.732**
	Acinar predominant	31	9	
	Papillary and micropapillary predominant	7	4	
	Solid with mucin production	3	1	
	Others†	2	1	

*P-value was obtained from *t*-test
**P-value was obtained from Pearson's Chi-square test
†Includes invasive mucinous adenocarcinoma and adenosquamous carcinoma

patients that experienced recurrence of lung cancer after curative resection, 24 had taken EGFR-TKI for management of the recurrence. However, when the duration of disease control with EGFR-TKI was analyzed, there was no difference between groups with compound or simple mutations (data not shown).

To further investigate the reason for the poor clinical outcome in the cases with compound mutation, we examined co-mutations in the *AKT1, BRAF, DDR2, ERBB2, FGFR, KRAS, MAPK2K1, MET1, NRAS, PIK3CA, PTEN, RET*, and *ROS1* genes, *ALK* gene rearrangement, and *BCL2L11* intron 2 deletion. A total 115 missense mutations were discovered in the tested genes (Table 4). 31 missense mutations were discovered in the cases with simple *EGFR* mutations whereas 84 were discovered in those with compound *EGFR* mutation, showing that the cases with compound *EGFR* mutation have higher chance of harboring multiple missense mutations in the clinically important genes (Table 7) (0.66 mutations / case *vs*. 6.0 mutations / case, P = 0.001, independent sample *t*-test). Similarity the cases with compound *EGFR* mutations have higher chance of co-alteration in the other genes than those with simple *EGFR* mutations (0.61 *vs*. 2.2 genes/case). Interestingly, there are a few number of actionable mutations irrelevant to the subtype of *EGFR* mutations, including *ALK* rearrangement, *BCL2L11* intron 2 deletion, *KRAS* c.35G>A, *PIK3CA* c.1633G>A which is possible target mutation of

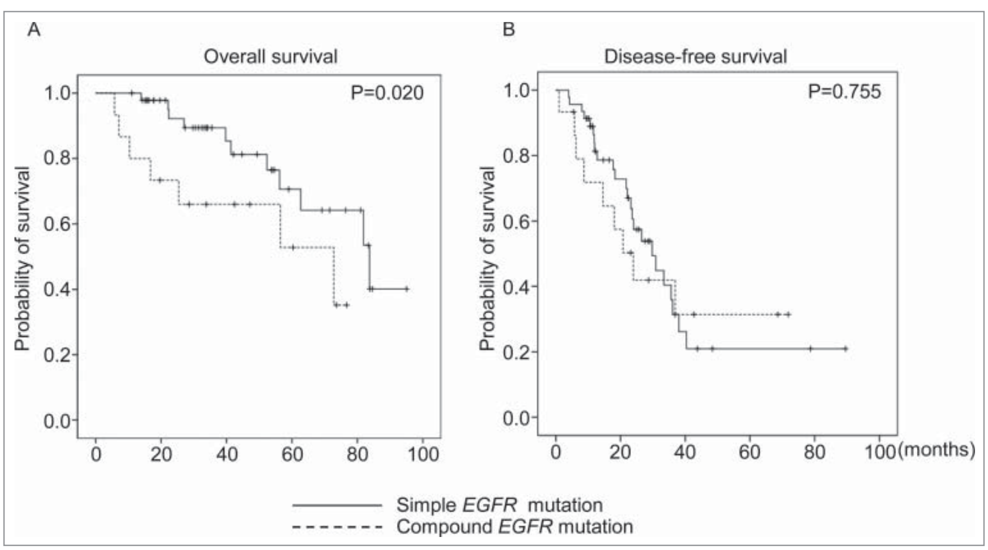

Figure 1. Comparison of overall survival and disease-free survival of patients with lung adenocarcinoma after curative resection according to *EGFR* mutation type. Kaplan–Meier estimation was used to compare overall survival (A) and disease-free survival (B) of patients with *EGFR* mutation-positive lung adenocarcinoma according to *EGFR* mutation subtype. Significant difference in OS were observed between simple and compound *EGFR* mutation (simple mutation 83.7 months *vs*. compound mutation 72.8 months, P = 0.020). P-value was obtained by Breslow test.

Table 3. Univariate and multivariate analyses for overall survival.

Variables		Univariate analysis			Multivariate analysis		
		HR	95% CI	P-value	HR	95% CI	P-value
Age	< 65	1	reference	–	1	reference	–
	≥ 65	0.777	0.482-1.251	0.299	1.824	0.628-15.299	0.269
Smoking status	None	1	reference	–	1	reference	–
	Current and ex-smoker	3.151	1.087-9.135	0.035	11.47	2.510-52.404	0.002
EGFR subtypes	Simple	1	reference	–	1	reference	–
	Compound	2.489	0.925-6.695	0.071	4.030	1.305-12.446	0.015
Stage	IB	1	reference	–	1	reference	–
	IIA-IIB	1.717	0.211-13.988	0.614	3.985	0.313-50.713	0.287
	IIIA	2.300	0.287-18.41	0.433	9.078	0.743-110.883	0.084
Histologic subtypes	Acinar	1	reference	–	1	reference	–
	Papillary and micropapillary	1.229	0.387-3.898	0.726	0.590	0.175-1.985	0.394
	Lepidic	1.575	0.357-6.943	0.548	0.890	0.161-4.928	0.894
	Solid	0.256	0.012-5.344	0.380	0	–	0.981
	Others	0.591	0.028-12.390	0.735	0	–	0.988

crizotinib, BH3 mimetics, *MEK* inhibitors, and PI3K-TKIs, respectively (Tables 5 and 6).[19,20] Taken together, the cases with compound *EGFR* mutation shows poor OS, which may attribute to the higher burden of missense mutations in the clinically important genes.

Discussion

The definition of a compound *EGFR* mutation remains as ambiguous as its clinical significance. A compound mutation is defined as a combination of 2 or more independent mutations in EGFR-TKD. In the case of E19del, approximately half of the mutations are accompanied by a continuous, in-frame point mutation or insertion around the deleted motif. In this study, these cases were considered simple mutations.

The detection rate of compound *EGFR* mutations has gradually increased from 4% in 2004 to 14% in 2013.[16,17,21] In a report from the early era of *EGFR* sequencing, cDNA of *EGFR* exon 18∼21 was generated by RT-PCR and used as a template for sequencing. In that study of Japanese cohorts, 111 of 277 lung adenocarcinomas showed *EGFR* mutations, and 4 of 111 *EGFR* mutation-positive cases (4%) were compound *EGFR* mutations.[21] A study that applied the direct sequencing of gDNA showed that the frequency of compound mutation in 443 *EGFR* mutation positive NSCLC is 4.97%.[22] Another *EGFR* study of a large East Asian cohort sequenced 2 types of specimen, gDNA from paraffin blocks and total RNA from frozen tissues. Those studies revealed that, among 627 *EGFR* mutation-positive cases, 78 (12.4%) were uncommon *EGFR* mutations and approximately half of these, 32 cases, were compound *EGFR* mutations.[17] A report that adapted bidirectional direct DNA sequencing showed that the detection rate of compound *EGFR* mutation was 14% of total *EGFR* mutations.[16] These differences in the frequency of compound *EGFR* mutations may be attributed to the progress of sequencing technology and the source of sequencing templates. Recent extensive clinical application of PNA clamping-based *EGFR* mutation detection techniques that focus on detection of the G719X, E19del, T790M, S768I, E20ins3dup, E20ins3, and L858R, or L861Q mutations showed an increased detection rate of *EGFR* mutations. However, compound *EGFR* mutations were very rarely encountered in daily practice. This study adopted NGS-based repeated deep sequencing at exon 18∼21 of *EGFR*, and the detection rate of compound *EGFR* mutations was 24.6%. These technical advances in sequencing provide a higher probability of encountering *EGFR* compound mutations.

Table 4. Comparisons of nucleotide substitution between *EGFR* mutation subtypes in the lung adenocarcinoma.

Substitution	Simple *EGFR* mutation (n = 46)	Compound *EGFR* mutation (n = 15)
C>T	6	29
A>G	3	0
G>A	17	51
C>G	1	0
G>C	4	2
A>T	0	1
A>C	0	1
Total	31	84

The majority of compound *EGFR* mutations are composed of one typical *EGFR* mutation and an atypical partner mutation. Point mutations have a higher chance of harboring an atypical partner mutation. This may be related to the definition of a compound *EGFR* mutation, in which consecutive mutation around the E19del is defined as a simple mutation. The atypical partner mutations are quite heterogeneous with respect to location in the *EGFR* gene, and it is difficult to generalize their effects on EGFR-TKI. A report by Peng et al. showed that among the 22 cases of the multiple *EGFR* mutation 20 (90.1%) had L858R or exon 19 in-frame deletion *EGFR* mutation.[23] The type of compound *EGFR* mutation is more homogenous than our findings, which showed 7 (46.7%) out of 15 cases accompanied with L858R or exon 19 in frame deletion. In a report by Kosaka et al., one tumor with a mutation at codon 719 and 3 tumors with mutations at codon 858 contained another mutation at E709H, S768I, R776C, or T790M, respectively.[21] This finding is similar to that of Wu et al., who showed that all multiple mutations contained one sensitizing mutation such as G719X, L858R, L861Q, or E19del and one or more rare atypical partner mutations. However, the findings of Kobayashi et al. and the current study indicate that 20∼27% of compound *EGFR* mutations consist of rare atypical mutations.[16]

The concept that one cancer has single driver mutation is being challenged by the advancement of techniques which

Table 5. Mutations detected in the lung adenocarcinoma with simple EGFR mutation.

Rand No.	*ALK*	*BCL2L11*	*BRAF*	*FGFR1*	*KRAS*	*MET*	*NRAS*	*PIK3CA*	*ROS1*	*RET*
E0006										
E0010										
E0016						c.2143A>G				
E0017	Rearrangement*					c.3437G>A				c.2071G>A
E0019										
E0023										c.2071G>A
E0024										
E0033									c.5326G>C	c.2071G>A
E0043		Int 2 del**								
E0051										
E0059		Int 2 del**								
E0081										
E0098										c.2071G>A
E0108	Rearrangement*	Int 2 del**								
E0110		Int 2 del**				c.3637C>T				
E0116										
E0120										
E0123		Int 2 del**	c.1750C>T	c.1456C>T	c.109G>A	c.2379G>A	c.91G>A	c.1633G>A*		c.2071G>A
E0124									c.5326G>C	
E0126										
E0130										
E0138		Int 2 del**							c.5326G>C	
E0149										
E0152										c.2071G>A
E0157										
E0168										
E0174					c.35G>A*					
E0182										
E0187										
E0191										
E0195										
E0197						c.3496C>T, c.3503A>G				
E0201										
E0203			c.1766C>T			c.3503A>G				
E0210						c.3836C>T				c.2071G>A
E0222										
E0224										
E0226										
E0233										c.2071G>A
E0242										
E0250						c.1255G>A				
E0252						c.2208C>G				
E0256		Int 2 del**							c.5704G>A, c.5326G>C	
E0260										c.2071G>A
E0269										
E0272										

*Actionable mutations (19).
**BCL2L11 intron 2 deletion mutant (20).
†No mutation was detected in *AKT1*, *DDR2*, *ERBB2*, *MAP2K*1, and *PTEN*.

are capable of sequencing multiple genes at a time. When the frequency of the co-alteration of *EGFR* and *ALK* rearrangement was evaluated by *EGFR* direct sequencing and *ALK* FISH, it is 0.27%.[24] When the *EGFR* mutations status was re-inspected in *ALK* rearrangement positive and *EGFR* mutation negative cases with the mutant enriched NGS, the co-mutation rate was increased up to 15.4%.[24] Another study that investigated mutation of *PIK3CA* exon 9 and 20 in 1,117 NSCLC showed that it was detected in 3.9% of squamous cell cancer and 2.7% of adenocarcinoma.[25] Among 34 NSCLC cases that have *PIK3CA* mutation, 17 cases had co-mutation in the *EGFR* exon 18~21 and 4 cases in the *KRAS* exon 2~3 showing *PIK3CA* mutation is frequently accompanied with *EGFR/KRAS* mutation.[25] In our study, *ALK* rearrangement and *PIK3CA* was observed in 3 cases respectively, suggesting that the representative driver mutations are not completely mutually exclusive and can occasionally be found at lower frequently. It is worthy of notice that *MET* had highest mutational burden among the genes tested in this panel. However, no mutation was detected in the exon14 and exon skipping could not be detected by the applied technique.[26,27] Mutations in the *MET* kinase domain (c.3166-c.4068; Exon 15~21) were detected in the 8 cases, but their biologic significance is not confirmed yet.

A few papers have reported that there are differences in the responses to the EGFR-TKIs among compound *EGFR* mutations. Peng et al. revealed that when the clinical outcome between NSCLC patients with L858 single mutation and those with L858 and other co-mutation in *EGFR* exon

Table 6. Mutations detected in the lung adenocarcinoma with compound EGFR mutation.

Rand No.	*ALK*	*BCL2L11*	*BRAF*	*ERBB2*	*FGFR1*	*KRAS*	*MET*	*NRAS*	*PIK3CA*	*PTEN*	*ROS1*	*RET*
E0001												c.2071G>A
E0012												
E0048												
E0092											c.5704G>A	
E0113	Rearrangement , c.3755C>T		c.1760A>T		c.2275G>A, c.1417C>T, c.1391C>T, c.1382C>T,		c.2610G>A, c.2612G>A, c.3670G>A	c.235C>T, c.38G>A, c.31G>A, c.29G>A, c.28G>A,			c.5770G>A, c.5741C>T, c.5587A>C, c.5572C>T	
E0140			c.1766C>T		c.2293C>T, c.1490C>T, c.487G>A	c.85G>A	c.2327G>A, c.2389G>A	c.203G>A, c.38G>A				c.2071G>A
E0154											c.5704G>A	
E0170												
E0176												
E0214		Int 2 del**										
E0217	c.3808G>A, c.3781G>A		c.1822C>T, c.1793C>T		c.2209G>C, c.1505G>A, c.1495G>A, c.1489C>T, c.1426C>T, c.1346C>T	c.160G>A, c.91G>A, c.35G>A*	c.1231G>A, c.1268C>T, c.1492C>T, c.2119C>T, c.2161G>A, c.2336C>T, c.2395G>A, c.3512C>T, c.3584C>T, c.3683G>A, c.3745G>A	c.201G>A, c.169G>A, c.50G>A, c.25G>A	c.1656G>A	c.724G>A, c.754G>A	c.5572C>T, c.5291G>A	c.2143C>T
E0228												
E0231												
E0235	c.3821C>T,		c.1798G>A, c.1753C>T	c.2499G>A			c.2191C>T, c.2324G>A, c.2339G>A, c.3322G>A, c.3395G>A, c.3687G>A	c.239G>A, c.176C>T, c.32C>T	c.3104C>T	c.653G>A	c.5770G>A, c.5602G>A, c.5333G>A, c.5326G>C	
E0254												

*Actionable mutations (19).
**BCL2L11 intron 2 deletion mutant (20).
†No mutation was detected in *AKT1*, *DDR2*, and *MAP2K1*.

Table 7. The number of co-mutations in the other genes tested by study panel.

Type of *EGFR* mutation		Average of co-mutations in other genes
Simple mutation	E19del (n = 24)	0.7
	V738_K739insKIPVAI (n = 1)	0
	M766_A767insASV (n = 1)	0
	D770_N771insG+N771T (n = 1)	1
	N771F (n = 1)	0
	L858R (n = 17)	0.5
	L861R (n = 1)	0
Compound mutation	L858R + V689L (n = 1)	1
	L858R + L833V (n = 1)	0
	L858R + H870R (n = 1)	0
	L858R + A871G (n = 1)	10
	L858R+ R776H (n = 1)	19
	L858R + E19del (n = 1)	0
	G719A + I706T (n = 1)	0
	G719S + E709K (n = 1)	0
	G719S + R776H (n = 1)	0
	E19del + I706T (n = 1)	0
	D770_N771insNPY + H773Y (n = 2)	1
	L688F + G824S (n = 1)	34
	E749Q + A750P (n = 1)	0
	T785I + Y813H + V845M + V851I + G857R (n = 1)	19

18∼21 was compared, there was no significant differences in OS and PFS.[22] Another study addressed the clinical significance of compound *EGFR* mutations, showing a poorer outcome for patients with rare atypical mutations combined with E19del or L858R (progression-free survival (PFS) 5.3 months, OS 18.8 months) compared with those with single classic mutations (PFS 8.5, OS 19.6 months).[17] Compound mutations that contain sensitizing mutations such as G719X or L858R seem to have good responses to EGFR-TKIs. On the other hands, those comprised of rare atypical mutations have poor response to EGFR-TKI.[17,28] In our study, a homogenous cohort was selected to identify the clinical meaning of compound mutations, and we found that patients with compound *EGFR* mutations had poorer OS than those with simple *EGFR* mutations. It is of note that there was no difference in the DFS. These findings suggest that mutation status may be related to the response to drug administered after confirmation of recurrence. The unproved supposition that tumors with a compound *EGFR* mutation do not respond to EGFR-TKI might cause clinicians to hesitate in positioning EGFR-TKI at the early line of therapy, which may have complicated evaluation of the response to EGFR-TKI in this study cohort. Several other factors such as male predominance, larger tumor size at the time of detection, and aggressive histologic subtype might have acted in combination to influence the poor OS of patients with the compound *EGFR* mutation.

The biologic significance of co-alteration of *EGFR* and other genes need to be investigated. In a study that evaluated the response to TKIs in the 14 NSCLC which had *EGFR* and *ALK* co-alteration, 3 treated with EGFR-TKI showed poor responses to gefitinib but 8 treated with *ALK* inhibitors revealed favorable responses, suggesting that signaling from *ALK* rearrangement override *EGFR*.[24] Others addressed the importance of *PIK3CA* mutation test by showing that the patients with *PIK3CA* single mutation showed poorer prognosis than those with co-mutation of *PIK3CA* and *EGFR/KRAS*.[25]

A few mutations in the *BCL2L11, ALK, PIK3CA*, and *KRAS* are key driver mutations that can be potentially targeted, while those in the other genes need further validation. It would be interesting to see if the NSCLC patients with *EGFR* compound mutation or co-alteration with other genes may be benefit from 3rd generation EGFR-TKIs when compared to 1st and 2nd generation EGFR-TKIs.[29,30]

In conclusion, compound *EGFR* mutation is frequently detected in *EGFR*-mutant tumors and is related to poor overall survival of patients with lung adenocarcinoma. Because it is expected that such mutations may be more frequently detected with wider adoption of NGS-based tests, more dedicated efforts are needed to clarify their biologic effects on disease course and drug responsiveness.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Funding

This study was supported by an NSCR grant (HI10C2020) awarded to YS Chang.

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