OncoTargets and Therapy

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ORIGINAL RESEARCH

ALK and *ROS1* concurrent with *EGFR* mutation in patients with lung adenocarcinoma

This article was published in the following Dove Press journal:
OncoTargets and Therapy
11 July 2017
Number of times this article has been viewed

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Purpose: The purpose of this study was to explore the frequencies of *ALK* and *ROS1* fusion genes in *EGFR*-mutant lung adenocarcinoma patients and examine the therapeutic efficacies of *EGFR*-tyrosine kinase inhibitors (TKIs).

Materials and methods: A total of 421 *EGFR*-mutated patients taking *EGFR*-TKIs were examined for *ALK* and *ROS1* fusion genes based on reverse transcription-polymerase chain reaction (RT-PCR). Progression-free survival (PFS) and overall survival (OS) were evaluated by the Kaplan–Meier method and compared by the log-rank test.

Results: The mutations of *ALK* rearrangement (n=10) and *ROS1* rearrangement (n=3) were detected. All the patients received *EGFR*-TKIs, and eight took subsequent *ALK/ROS1* inhibitor. PFS was longer in single *EGFR* mutants (n=408) than in *EGFR/ALK* or *EGFR/ROS1* counterparts (n=13; 10.7 vs 6.6 months, *P*=0.004). No difference in OS existed between single *EGFR* and *EGFR/ALK* or *EGFR/ROS1* mutants (21.0 vs 23.0 months, *P*=0.196). The median PFS of eight patients treated with *ALK/ROS1* inhibitor was 6.0 months.

Conclusion: Concomitant *ALK*/*ROS1* fusion genes occurred in 3.1% *EGFR*-mutated lung adenocarcinoma patients. Concomitant *ALK/ROS1–EGFR* mutations may influence the therapeutic efficacy of *EGFR*-TKIs.

Keywords: epidermal growth factor receptor, *ALK*, *ROS1*, lung adenocarcinoma, efficacy

Introduction

It is well known that the mutations of *EGFR* act as one of the most frequent driver genes in non-small cell lung cancer (NSCLC) patients, especially among East Asian female lung adenocarcinoma patients.[1,2] Previous studies have shown that *EGFR*-tyrosine kinase inhibitors (*EGFR*-TKIs) could achieve a high response rate and yield a promising efficacy for *EGFR* mutants.[3–6]

Despite a response rate of 60%–70% in *EGFR*-mutated lung cancer patients treated with *EGFR*-TKIs, 10%–20% of individuals developed primary resistance.[4–6] The mechanism is currently ill defined. Possible contributing factors included T790M mutation, *MET* amplification and *PIK3CA* mutation.[7–9] Concomitant genetic alterations were found to be associated with primary resistance in *EGFR*-mutated NSCLC patients.[10,11] However, the studies were somewhat limited, and only several case series were reported. Furthermore, the efficacies of *EGFR*-TKIs have remained elusive.

Here, *ALK* and *ROS1* fusion gene panel was used for screening the patients harboring *EGFR*-mutated samples and further detecting the therapeutic efficacies of *EGFR*-TKIs.

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Materials and methods

Patient samples

Consecutive *EGFR*-mutated patients receiving *EGFR*-TKIs for lung adenocarcinoma were screened at Hangzhou Cancer Hospital between 2009 and 2015. The inclusion criteria were as follows: 1) uses of *EGFR*-TKIs during advanced stage; 2) sufficient residual tissues for detecting *ALK* and *ROS1* fusion genes and 3) confirmed as having sensitive *EGFR* mutation. Resistant mutations such as *T790M*, *S768I* and exon 20 insertions were excluded. The study protocol was approved by the ethics committee of Hangzhou Cancer Hospital, and written informed consents were obtained from all patients.

Detection of *EGFR/ALK/ROS1* fusion gene

EGFR gene was detected by amplification refractory mutation system (ARMS)-based kit (Amoy, Xiamen, China). It was capable of detecting the following 23 mutations: three in exon 18 (G719A, G719C and G719S), 13 deletions in exon 19, two mutations in exon 20 (T790M and S768I), three insertions in exon 20 and two mutations in exon 21 (L858R and L861Q).

The *ALK* and *ROS1* fusion mRNAs were detected by polymerase chain reaction (PCR) using fusion gene detection kit (Amoy). Briefly, total RNA was extracted using Qiagen (Dusseldorf, Germany) RNeasy FFPE kit. mRNA was reverse transcribed into cDNA for 1 h at 42°C. β-actin was used as an internal control. The conditions of reverse transcription-polymerase chain reaction (RT-PCR) were as follows: initial denaturation at 95°C for 5 min, followed by 95°C for 25 s, 64°C for 20 s and 72°C for 20 s for ensuring specificity and 31 cycles at 93°C for 25 s, 60°C for 35 s and 72°C for 20 s. Data collection and sensitivity analysis were detailed previously.[12] The subtypes of *EGFR/ALK/ROS1* genes are summarized in Table S1.

Evaluations of TKI treatment

Tumors were evaluated during the treatment with *EGFR*-TKIs or *ALK/ROS1* inhibitor every 8 weeks. Objective tumor responses were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST 1.1). Objective response rate (ORR) included complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD).

Statistical analyses

The Kaplan–Meier method was used for survival analysis. Progression-free survival (PFS) of TKIs was defined as the time from initiating TKI treatment to documented progression or mortality from any cause. Statistical analysis was performed using the SPSS 18 software (SPSS Inc., Chicago, IL, USA). The median follow-up period was 28.5 months (4.5–65 months). The last follow-up time was October 31, 2016.

Results

Patient characteristics

A total of 421 patients were enrolled, and their clinical characteristics are summarized in Table 1. Among the patients, 13 were confirmed as having concomitant *ALK*/*ROS1* fusion gene. Their clinical characteristics are summarized in Table 2. There were six males and seven females with a median age of 55 years. One patient was a former smoker and 12 had never smoked. The comparisons of single *EGFR* versus concomitant gene mutations are listed in Table 1.

Gene profiles

The genetic details of 421 *EGFR* mutations are as follows: exon 19 deletions (n=217), L858R mutation in exon 21 (n=169), G719X in exon 18 (n=21) and L861Q (n=14). Concomitant gene fusions were identified in 13 patients (3.1%), including *ALK* (n=10, 2.4%) and *ROS1* (n=3, 0.7%). Their profiles are detailed in Table 2.

Table 1 Characteristics of the study population and comparison (n=421)

Characteristics	Total	Single EGFR mutation (n=408)	Concurrent gene (n=13)	*P*-value
Gender				0.37
Male	245	239	6	
Female	176	169	7	
Age, years				0.04
<60	224	213	11	
≥60	197	195	2	
Smoking status				0.20
Never	305	295	12	
Former/current	116	113	1	
Stage at *EGFR*-TKI treatment				0.83
IIIB	12	12	0	
IV	409	396	13	
EGFR mutation type				0.67
Exon 19 deletion + exon 21 L858R	386	374	12	
Other types	35	34	1	
Performance score at *EGFR*-TKI treatment				0.70
0–1	356	345	11	
2–3	65	63	2	

Abbreviation: TKI, tyrosine kinase inhibitor.

Table 2 Clinical characteristics of 13 patients with *EGFR* and *ALK*/*ROS1* concurrent genes

Case	Gender	Age (years)	Smoking history	EGFR type	EGFR-TKI PFS	Response	*ALK*/ROS1	Crizotinib PFS	Response	Post-TKI treatment	Response	OS/ month
1	Female	47	No	19del	6.6	PR	EML4–*ALK*	–	–	Chemotherapy	SD	23.0
2	Male	55	No	G719X	2.0	PD	EML4–*ALK*	11.2	PR	Supportive care	–	21.0
3	Female	57	No	19del	3.1	SD	EML4–*ALK*	6.0	PR	Supportive care	–	11.5
4	Male	59	No	L858R	4.0	SD	EML4–*ALK*	5.0	SD	Chemotherapy	PR	45.0
5	Female	65	No	19del	17.5	PR	EML4–*ALK*	–	–	Supportive care	–	23.5
6	Female	62	No	L858R	12.0	PR	EML4–*ALK*	–	–	Chemotherapy	PD	33.2
7	Male	56	Yes	19del	10.2	PR	EML4–*ALK*	–	–	Supportive care	–	24.0
8	Female	55	No	19del	1.1	PD	CD74–*ROS1*	7.0	SD	Chemotherapy	PD	15.4
9	Male	45	No	19del	2.0	PD	EZR–*ROS1*	23.0	PR	Chemotherapy	PD	35.0
10	Female	49	No	L858R	1.3	PD	EML4–*ALK*	–	–	Supportive care	–	17.6
11	Female	38	No	19del	9.0	PR	EML4–*ALK*	1.2	PD	Chemotherapy	SD	21.5
12	Male	59	No	L858R	7.6	PR	CD74–*ROS1*	2.0	PD	Chemotherapy	PD	24
13	Male	51	No	19del	8.2	SD	EML4–*ALK*	7.5	PR	Chemotherapy	SD	48+

Abbreviations: TKI, tyrosine kinase inhibitor; PFS, progression-free survival; OS, overall survival; PR, partial response; SD, stable disease; PD, progressive disease.

Efficacy of TKIs

All 421 patients received *EGFR*-TKIs. Among 13 patients with concomitant genes, eight switched to crizotinib after ineffective *EGFR*-TKIs. The agents of *EGFR*-TKIs included erlotinib (n=71), gefitinib (n=126) and icotinib (n=224). None of them received any second-generation *EGFR*-TKI since so far none have been approved in China.

The clinical efficacies of 408 single *EGFR*-mutated patients included CR (n=2, 0.5%), PR (n=249, 61.0%), SD (n=87, 21.3%) and PD (n=70, 17.2%). In concomitant *ALK*/*ROS1* mutants, the outcomes of *EGFR*-TKIs were PR (n=6), SD (n=3) and PD (n=4). The efficacy comparisons between single *EGFR* and concomitant gene mutations are shown in Table 3.

The overall value of PFS was 10.7 months (95% CI, 10.0–11.4). The median values of PFS were 10.7 and 6.6 months in mutants of single *EGFR* and concomitant *ALK*/*ROS1* fusion gene, respectively (Figure 1, *P*=0.004). For eight patients on crizotinib, the value of PFS was 6.0 months for concomitant *ALK*/*ROS1* fusion gene mutations (95% CI, 3.2–8.8).

The overall median value of OS was 21.0 months (95% CI, 18.9–23.4). No survival difference existed between single *EGFR* mutants and those with concomitant *ALK*/*ROS1* fusion gene mutations (21.0 vs 23.0 months, *P*=0.196; Figure 2).

Table 3 Clinical efficacy comparison of *EGFR*-TKI in single *EGFR* mutation and concurrent gene alterations

Best response	Single EGFR mutation (n=408)	Concurrent gene (n=13)	*P*-value
CR, n (%)	2 (0.5)	0 (0.0)	–
PR, n (%)	249 (61.0)	6 (46.2)	–
SD, n (%)	87 (21.3)	3 (23.1)	–
PD, n (%)	70 (17.2)	4 (30.8)	–
ORR (%)	61.5	46.2	0.26
DCR (%)	82.8	69.2	0.37
Median PFS	10.7	6.6	0.004
Median OS	21.0	23.0	0.196

Abbreviations: TKI, tyrosine kinase inhibitor; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; PFS, progression-free survival; OS, overall survival.

Discussion

Our results showed that 3.1% of *EGFR*-mutated lung adenocarcinoma Chinese patients harbored *ALK*/*ROS1* fusion genes. Concurrent *ALK*/*ROS1* gene decreased the therapeutic efficacy of *EGFR*-TKIs. However, it had no impact on the overall survival (OS).

Although gene alterations were presumably mutually exclusive in lung adenocarcinoma,[13] some reports

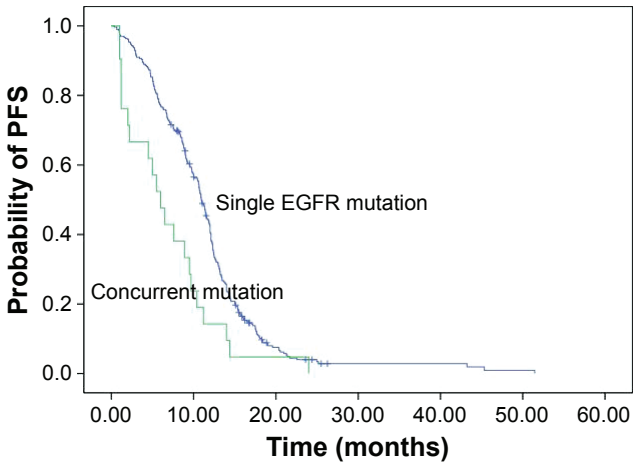

Figure 1 Comparison of PFS between single *EGFR* and concomitant *ALK*/*ROS1* gene mutants on *EGFR*-TKI (10.7 vs 6.6 months, *P*=0.004).
Abbreviations: PFS, progression-free survival; TKI, tyrosine kinase inhibitor.

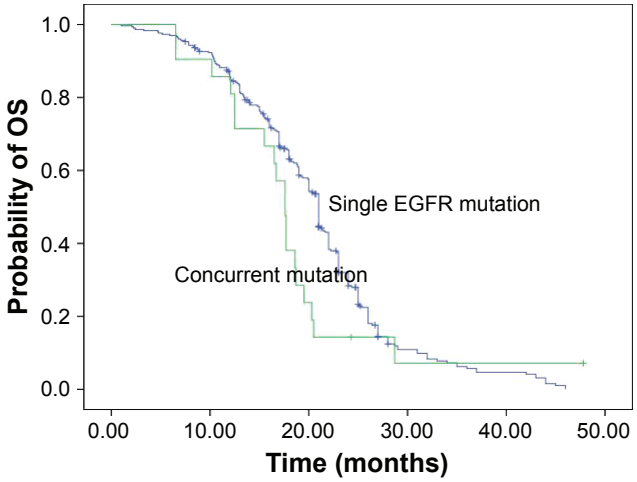

Figure 2 Comparison of OS between single *EGFR* and concomitant *ALK*/*ROS1* gene mutants on *EGFR*-TKIs (21.0 vs 23.0 months, *P*=0.196).
Abbreviations: OS, overall survival; TKI, tyrosine kinase inhibitor.

revealed that genes might occur concomitantly.[14,15] With the emerging of high-throughput sequencing, many concomitant genes have been detected. The frequency of concomitant *EGFR/ALK* or *EGFR/ROS1* mutations was 3.1% in the present study, and this figure was consistent with previous reports.[16–19]

Owing to a low frequency of concomitant genes in *EGFR*-mutated lung cancer patients, the efficacy of *EGFR*-TKIs is largely unknown. The median PFS of first-generation *EGFR*-TKIs was 11.2 months in patients harboring concomitant *EGFR/ALK* genes in one report by Yang et al.[20] For concomitant *ALK/ROS1* and *EGFR* mutants, there was a practical dilemma of therapeutic sequence of *EGFR*-TKIs and *ALK/ROS1* inhibitors. Relative levels of phospho-*EGFR* or *ALK* could predict the efficacy of targeted treatment in *EGFR*/*ALK* mutants in the study of Yang et al.[20] In the present study, all patients took *EGFR*-TKIs initially and eight took subsequent *ALK/ROS1* inhibitor. The median value of PFS was 6.6 months for 13 patients on *EGFR*-TKIs. However, among eight patients on crizotinib, six obtained disease control. Owing to efficacy difference for dosing order of targeted therapy, despite a low frequency, there is a future need of using a useful marker for selecting targeted treatment in patients with concomitant genes. Except at the level of phosphorylation, abundance of gene was identified previously as a predictor of targeted therapy;[21] the abundance level of different genes in one sample should be detected for patients with concomitant genes.

A small sample size was a major drawback of the present study. Second, not all patients received crizotinib after ineffective *EGFR*-TKIs. As a result, the clinical efficacy of further treatment could not be fully evaluated. Third, only RT-PCR was used for *ALK/ROS1* genes so that false positives might ensue. Moreover, RT-PCR could not provide convincing evidence for the dominance of expression for one oncogene aberration over another in the same samples. In addition, it was impossible to check whether individual tumor cells had these two mutations simultaneously or singly.[22] However, our findings are meaningful for clinical practice.

Conclusion

A total of 3.1% of *EGFR*-mutated patients harbored *ALK/ROS1* fusion genes. Concomitant genes of *ALK/ROS1* might decrease the therapeutic efficacy of *EGFR*-TKIs.

Disclosure

The authors report no conflicts of interest in this work.

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Supplementary material

Table S1 The subtypes of *EGFR/ALK/ROS1* genes

Gene type	Subtype of gene
ALK fusion	*EML4* exon 13; *ALK* exon 20
	EML4 exon 6 ins 33; *ALK* exon 20
	EML4 exon 20; *ALK* exon 20
	EML4 exon 18; *ALK* exon 20
	EML4 exon 2; *ALK* exon 20
ROS1 fusion	*SLC34A2* exon 4; *ROS1* exon 32
	SLC34A2 exon 14 del; *ROS1* exon 32
	CD74 exon 6; *ROS1* exon 32
	SDC4 exon 2; *ROS1* exon 32
	SDC4 exon 4; *ROS1* exon 32
	SLC34A2 exon 4; *ROS1* exon 34
	SLC34A2 exon 14 del; *ROS1* exon 34
	CD74 exon 6; *ROS1* exon 34
	SDC4 exon 4; *ROS1* exon 34
	EZR exon 10; *ROS1* exon 34
	TPM3 exon 8; *ROS1* exon 35
	LRIG3 exon 16; *ROS1* exon 35
	GOPC exon 8; *ROS1* exon 35
EGFR mutation	E746_A750del (1)
	E746_A750del (2)
	L747_P753>S
	E746_T751>I
	E746_T751del
	E746_S752>V
	L747_T751>Q
	L747_E749del
	L747_S752del
	L747_A750>P
	L747_P753>Q
	L747_T751del
	L747_T751>P
	S768I
	L858R
	G719A
	G719C
	G719S
	T790M
	L861Q
	H773_V774insH
	D770_N771insG
	V769_D770insASV