Characterization of distinct types of *KRAS* mutation and its impact on first-line platinum-based chemotherapy in Chinese patients with advanced non-small cell lung cancer

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Received April 6, 2017; Accepted September 7, 2017

DOI: 10.3892/ol.2017.7016

Abstract. We performed this retrospective study to investigate whether the *KRAS* mutation status and its subtypes could predict the effect of first-line platinum-based chemotherapy in Chinese patients with non-small cell lung cancer (NSCLC). Patients received who had KRAS mutations were enrolled. Correlations between KRAS mutations, specific mutant subtypes and responses to chemotherapy were analyzed using Kaplan-Meier and Cox proportional hazard methods. A total of 2,183 cases who received *KRAS* mutation detection were included. A total of 218 of these cases were indicated to have *KRAS* mutations. *KRAS* mutations were identified more commonly in males compared with females (P=0.035). The most common subtypes were G12C, G12D and G12V. Among 73 *KRAS* mutant patients and 100 *EGFR/ALK/KRAS* wild-type patients with advanced NSCLC, *KRAS*-mutant NSCLC patients had a significantly shorter progression-free survival (P=0.007) compared with NSCLC patients with *KRAS* wild-type. In addition, there was a shorter but marginally statistically significant progression-free survival (PFS) in *KRAS* mutant patients with adenocarcinoma compared with those with non-adenocarcinoma (P=0.051). In the *KRAS* mutant group, patients with the *KRAS* G12V mutation had the poorest PFS compared with non-G12V mutant cases (P=0.045). In conclusion, *KRAS* mutation was a negative predictive factor of PFS in Chinese patients with advanced NSCLC who received first platinum-based chemotherapy. Patients with *KRAS* G12V mutations exhibited the poorest PFS compared with those with other *KRAS* mutant types.

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Key words: KRAS, non-small cell lung cancer, chemotherapy, prediction

Introduction

Lung cancer remains one of the most common malignancies and leading causes of cancer-related mortality both in China and worldwide (1,2). Approximately 80-85% of lung cancers are non-small cell lung cancer (3). In recent years, huge progress had been made in the treatment of non-small cell lung cancer (NSCLC) patients harboring *EGFR* mutation and *ALK* rearrangement (4-7). However, effective therapy specifically targeting *KRAS* mutation, which accounts for 25-50% of NSCLC patients in white populations and 5-10% in Asian populations, has not been developed yet (8-11).

KRAS is a member of the *Ras* gene family, which encodes small G proteins with intrinsic GTPase activity, contributing to activation of downstream effectors involved in multiple pathways including apoptosis, proliferation and differentiation (8,12,13). Point mutations occurred in tumors result in the loss of intrinsic GTPase activity and consequently in the deregulation of cell proliferation signals (13,14). *KRAS* mutation occurs mainly in codon 12, 13 or 61. Most common types of *KRAS* mutation are G12C, G12V, and G12D (8,9). In addition, *in vitro* data reported by Garassino *et al* suggested that NSCLC cell lines harboring a G12C, G12V or G12D *KRAS* mutation had differential sensitivity to chemotherapeutic agents (15). It appears that various types of *KRAS* mutations differ in clinical characters and drug response (16,17).

As early as 1990, *KRAS* mutation was already described as a negative prognostic marker for both overall survival (OS) and disease-free survival in lung cancer (18). Not until the last decades, more and more attention has been paid to the clinical significance of *KRAS* mutation in NSCLC. When it comes to the first-line platinum-based chemotherapy for advanced NSCLC patients, some researchers tend to believe there is no difference between *KRAS* mutant and wild-type patients regarding therapeutic response and prognosis (14,19,20). However, there were several studies indicated that *KRAS* mutation was a predictive factor of worse progression-free survival (PFS) or OS in advanced NSCLC patients treated with platinum-based chemotherapy (21-24). Considering the

discrepant role of *KRAS* and its subtypes on effect of chemotherapy, the aim of this study was to investigate the predictive significance of *KRAS* mutation and its subtypes on clinical response and PFS in advanced NSCLC patients treated with first-line platinum-based chemotherapy.

Materials and methods

Study design. In this retrospective study, patients received *KRAS* mutation detection between August 2014 and June 2016 at Shanghai Pulmonary Hospital affiliated to Tongji University School of Medicine were included. We retrospectively reviewed patients' medical records to evaluate clinicopathological features and treatment regimens. All eligible patients' clinical data including age, sex, smoking status, histological type, TNM stage, Eastern Cooperative Oncology Group (ECOG) performance status (PS), treatment regimens, response to treatment, date of first diagnosis, date of starting chemotherapy, and date of disease progression or date of last contact. Pathological diagnosis was made by pathologists. Staging was carried out according to the staging system of the 2009 International Association for the Study of Lung Cancer (version 7) (25). Nonsmokers were defined as patients with the smoking dose of <100 cigarettes in their lifetime. Clinical response was evaluated by at least two clinicians according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (26). Inclusion criteria were: Adult (age≥18 years old) patients; recurrent or IIIB/IV NSCLC patients; patients received first-line platinum-based chemotherapy. Exclusion criteria were: Unknown mutational status; detected *EGFR* mutation or *ALK* rearrangement; no complete documentation; no response evaluation; adjuvant chemotherapy or radiochemotherapy. The study was approved by the Ethics Committees of Shanghai Pulmonary Hospital. Informed consent was obtained from all individual participants included in the present study. This study was conducted according to the Declaration of Helsinki, 7th version.

KRAS mutation analysis. Total DNA was extracted from tissue samples using AmoyDx DNA kit (Amoy Diagnostics Co., Ltd., Xiamen, China). The quality and quantity of extracted DNA were measured by NanoDrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). *KRAS* mutation was identified by an AmoyDx® Human *KRAS* gene 7 Mutations Fluorescence Polymerase Chain Reaction (PCR) Diagnostic kit (Amoy Diagnostics Co., Ltd.). The real-time PCR conditions were as previously described (27-29). β-actin was used as an internal reference gene to ensure the quality of the extracted DNA and *KRAS* mutant DNA was used as positive control.

Statistical analysis. The relation between categorical parameters was tested using Pearson's χ^2 test (Fishers exact test was used when n≤5). Kaplan-Meier curve was used to estimate the distribution of survival and log-rank test was used to analyze differences between groups. PFS was defined as the first day of treatment until either tumor progression or death. We used cox proportional hazards models for univariate and multivariate analysis to estimate clinicopathological features, *KRAS* mutation types and treatment regimens for their associations with PFS. Independent variables with P<0.10 in the univariate analysis were enrolled in multivariate analysis. P-values <0.05 were defined statistically significant. Confidence intervals were calculated at a 95% CI. Statistical tests were carried out using SPSS 20.0 software (IBM Corporation, Armonk, NY, USA).

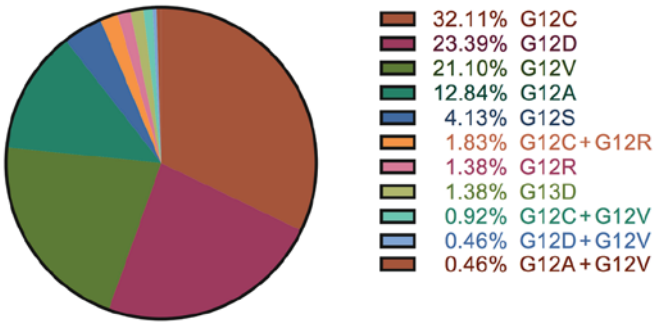

Figure 1. Distribution of KRAS mutation in whole population.

Results

Patient characteristics. In total, 2,183 patients received *KRAS* mutation detection at Shanghai Pulmonary Hospital between August 2014 and June 2016 were enrolled into this study and 218 (10.0%) cases harbored *KRAS* mutation. Distribution of different types of *KRAS* mutation found within 218 patients are listed in Fig. 1. Three most common *KRAS* mutations were G12C (32.1%), G12D (23.4%) and G12V (21.1%). Other codon 12 mutations including G12A (12.8%), G12S (4.1%) and G12R (1.4%) were found in 20% of the patients. 3 patients had codon 13 G13D mutation. Four types of double mutations were found in 8 patients: G12C + G12R (4 patients), G12C + G12V (2 patients), G12D + G12V (1 patient) and G12A + G12V (1 patient). Based on our inclusion and exclusion criteria, we further analyzed 100 EGFR/ALK/*KRAS* wild-type and 70 *KRAS* mutant patients. The median age of whole study group was 61 years old (range 28-78). In total, 84.1% of patients were stage IV disease at diagnosis, and 77.6% of patients displayed histology of adenocarcinoma. The patient characteristics were listed in Table I. The patient basic characteristics were well-matched between *KRAS* mutant and wild-type groups except for sex (P=0.035). As for the treatment regimens, 74.1% of all patients received first-line chemotherapy with carboplatin-based chemotherapy, with a higher percentage of wild-type *KRAS* patients (78.0%) receiving carboplatin-based doublet comparing with mutant *KRAS* patients (68.6%). Numerically more *KRAS* mutant patients received a cisplatin-based chemotherapy when compared with *KRAS* wild-type patients (28.6% vs. 21.0%, respectively). However, there seems to be more patients in the *KRAS* wild-type group received platinum/pemetrexed treatments (68.0% in *KRAS* wild-type group vs. 57.1% in *KRAS* mutant group). Whereas patients with wild-type *KRAS* were as likely as patients with mutant *KRAS* to receive platinum/gemcitabine chemotherapies. Of note, 6 patients within the *KRAS* mutant group received platinum/docetaxel whereas only 1 patient within the *KRAS* wild-type group received platinum/docetaxel treatments.

Table I. Patient characteristics.

	KRAS mutant (n=70)	*KRAS* wild-type (n=100)	P-value
Mean age at diagnosis, mean ± SD	61±7.34	60±9.31	0.334
Sex, n (%)			
Male	60 (85.7)	72 (72.0)	0.035
Female	10 (14.3)	28 (28.0)	
Smoking history, n (%)			0.302
Smoker	42 (60.0)	52 (52.0)	
Non-smoker	28 (40.0)	48 (48.0)	
Histology, n (%)			0.826
Adenocarcinoma	55 (78.6)	77 (77.0)	
Squamous	6 (8.6)	12 (12.0)	
Other	0 (0.0)	1 (1.0)	
NSCLC-NOS	9 (12.9)	10 (10.0)	
Stage, n (%)			
IIIB	6 (8.6)	12 (12.0)	0.475
IV	64 (91.4)	88 (88.0)	
Platinum, n (%)			
Cisplatin	20 (28.6)	21 (21.0)	0.287
Carboplatin	48 (68.6)	78 (78.0)	
Other	2 (2.9)	1 (1.0)	
Platinum doublets, n (%)			
Platinum/pemetrexed	40 (57.1)	68 (68.0)	0.029
Platinum/gemcitabine	23 (32.9)	31 (31.0)	
Platinum/docetaxel	6 (8.6)	1 (1.0)	
Other	1 (1.4)	0 (0.0)	

P-value based on Kruskal-Wallis test, otherwise P-value based on χ^2 test or Fisher's exact test. NSCLC-NOS, non-small cell lung cancer-not otherwise specified; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

Effect of KRAS mutation on response rate and PFS. None of the patients reached complete response. Partial response was similar between two groups (21.4% in *KRAS* mutant patients vs. 19.0% in *KRAS* wild-type patients). Comparatively, stable disease was observed more in wild-type *KRAS* patients than in mutant *KRAS* patients (67.0% vs. 44.3%, respectively). However, numerically more disease progressed in patients with mutant *KRAS* than wild-type *KRAS* (34.3% vs. 14.0%). There were no statistically significant differences in the objective response rate (ORR). In contrast, disease control rate (DCR) of *KRAS* wild-type patients to platinum-based chemotherapy was obviously higher than *KRAS* mutant patients (86.0% vs. 65.7%, P=0.002; Table II). In Table II, we also listed clinical outcomes of three most common *KRAS* mutation subtypes and other rare mutations. Among them, although G12V has the lowest DCR for 55.6%, response to platinum-based chemotherapy had no statistically significant differences between mutation subtypes.

A total of 140 (82.4%) patients had progressed disease during the study period, with a median PFS for all subjects of 5.9 months (95% CI, 4.9-6.9 months). In all included patients with metastatic NSCLC at diagnosis, PFS was shorter in the *KRAS* mutant group vs. wild-type group (4.2 vs. 6.3 months; P=0.007; Fig. 2A). In addition, there was a shorter but only marginally statistically significant PFS in *KRAS* mutant patients with adenocarcinoma histology patients (4.3 months vs. 6.7 months; P=0.051; Fig. 2B). It suggested that the presence of *KRAS* mutation may be associated with a worse response to first-line platinum-based chemotherapy in advanced NSCLC patients. Next, we compared PFS of wild-type *KRAS* patients with three most common *KRAS* subtypes G12V, G12C, G12D and other rare mutations. When comparing patients with G12V mutant vs. wild-type, there was a statistically significant shorter PFS (2.9 months and 6.4 months, respectively; P=0.001). While other *KRAS* subtypes had no differences in PFS compared with wild-type *KRAS* (Fig. 2C). Patients with *KRAS* G12V mutation had inferior PFS compared with patients with non-G12V mutation (median PFS, 2.9 vs. 4.7 months; P=0.045; Fig. 3B). When comparing patients with G12C vs. non-G12C mutation and patients with G12D vs. non-G12D mutation, there was no differences in PFS, 4.4 months (95% CI, 3.3-5.5) vs. 4.2 months (95% CI, 2.3-6.1; P=0.202; Fig. 3A) and 7.0 months (95% CI, 1.1-12.8) vs. 4.3 months (95% CI, 3.8-4.8; P=0.519; Fig. 3C). It suggested that response to chemotherapy is not the same among *KRAS* mutation subtypes and patients with *KRAS*

Table II. Response to first line chemotherapy in *KRAS* mutant vs. *KRAS* wild-type NSCLC patients.

	KRAS wild-type (n=100)	*KRAS* mutant (n=70)						
		Total (n=70)	G12C (n=23)	G12V (n=18)	G12D (n=9)	Rare (n=20)	P-value[a]	P-value[b]
Response								
CR	-	-	-	-	-	-		
PR	19	15	6	4	1	4		
SD	67	31	12	6	5	8		
PD	14	24	5	8	3	8		
ORR	19.0%	21.4%	26.1%	22.2%	11.1%	20.0%	0.893	0.697
DCR	86.0%	65.7%	78.3%	55.6%	66.7%	60.0%	0.442	0.002

[a]P-value was calculated among KRAS mutation subtypes. [b]P-value was calculated between *KRAS* wild-type and mutated patients. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; PFS, progression-free survival; ORR, objective response rate; DCR, disease control rate.

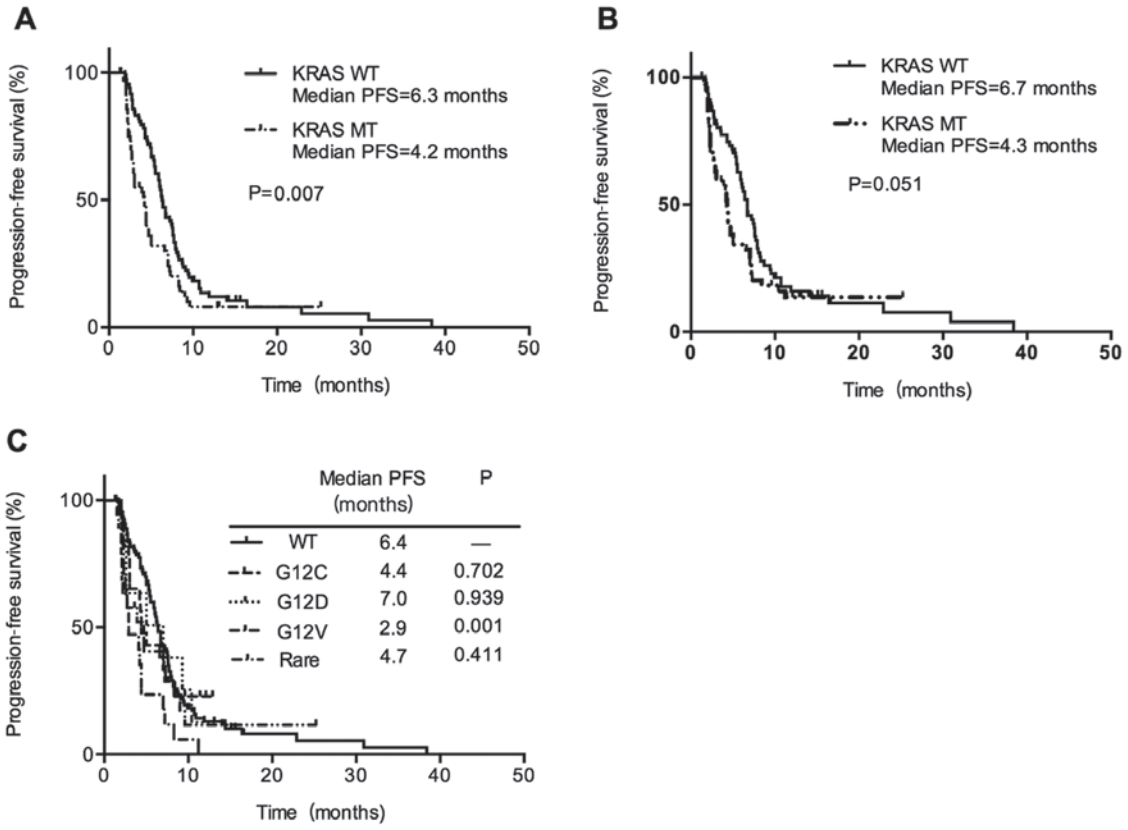

Figure 2. PFS of whole patients' cohort. Subgroup analysis of progression-free survival in KRAS mutant vs. wt patients with metastatic NSCLC at diagnosis (A). Subgroup analysis of progression-free survival in KRAS mutant vs. wild-type patients with adenocarcinoma histology (B). PFS in KRAS wild-type patients vs. three most common KRAS mutation subtypes and other rare mutations (C). PFS, progression-free survival; NSCLC, non-small cell lung cancer; wt, wild-type, mt, mutant.

G12V mutation showed the poorest PFS than those with other *KRAS* mutant types.

Univariate and multivariate analysis. In univariate analysis, sex, smoking history and *KRAS* G12V mutation were significantly associated with PFS. Women had decreased risk of progressed disease when compared with men (HR, 0.616; 95% CI, 0.405-0.937; P=0.024). Smoking history also affected PFS (never smokers vs. current/former smokers; HR,0.665; 95% CI, 0.472-0.937; P=0.020). *KRAS* G12V was associated with shorter PFS (HR, 2.342; 95% CI, 1.378-3.981; P=0.002). In multivariate analysis, only *KRAS* G12V mutation was associated with shorter PFS (HR, 2.116; 95% CI, 1.211-3.696; P=0.008; Table III).

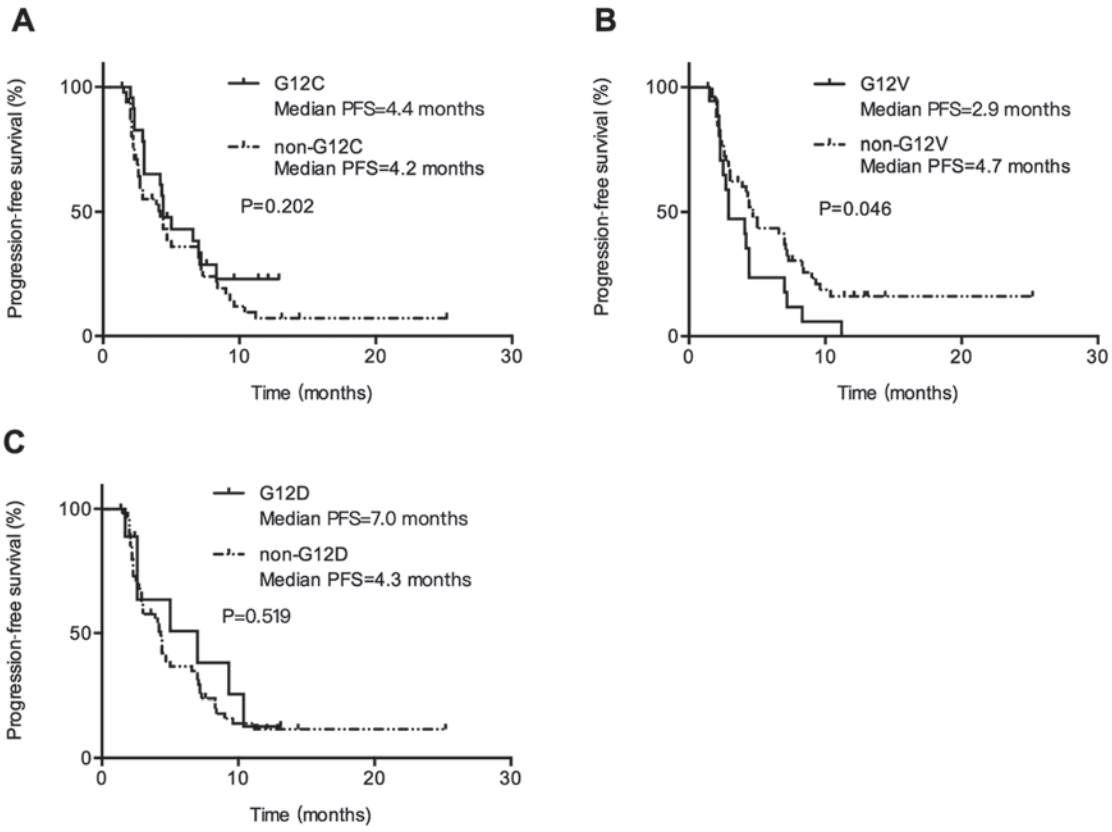

Figure 3. PFS of KRAS mutant patients' cohort. Three most common KRAS mutation subtypes G12C vs. wild-type (A), KRAS mutation subtypes G12V vs. wild-type (B) KRAS mutation subtypes G12D vs. wild-type (C). PFS, Progression-free survival.

In *KRAS* mutant group, univariate analysis showed that smoking history did not have impact on outcome for PFS (HR, 0.799; 95% CI, 0.462-1.379; P=0.420). And there was marginally statistic difference in outcome of G12V mutant patients vs. other mutant *KRAS* patients in univariate analysis (HR, 1.762; 95% CI, 0.992-3.129; P=0.053). In multivariate analysis based on age, G12V mutation status and cisplatin- or carboplatin-based chemotherapy, results showed that G12V mutant patients did have a shorter PFS than other *KRAS* mutant types (HR, 1.831; 95% CI, 1.025-3.270; P=0.041; Table III).

Discussion

Our treatment of NSCLC has been dramatically improved with the introduction of molecular markers. Targeted therapies, including tyrosine kinase inhibitors (TKIs), for *EGFR* mutation and *ALK* rearrangement improved PFS in patients bearing the relevant mutations (4,7,30). However, effective therapy specifically targeting *KRAS* mutation has not been developed yet. For patients with *KRAS* mutation, platinum-based chemotherapy remains their first choice. Nevertheless, the predictive value of KRAS mutation in NSCLC for chemotherapy also remains unclear.

In the last decades, although a large number of studies had been conducted focusing on *KRAS* mutation, the prognostic and predictive value of *KRAS* in lung cancer is still a highly debated issue. Considering the enormous discrepancy of studies in terms of races, tumor stage, histological types and various treatments, it is difficult to draw a definite conclusion. Therefore, we analyzed a well-defined Chinese patient cohort with advanced NSCLC received first-line platinum-based chemotherapy in our study. *KRAS* mutation rate in all tested population was 10.0%, which is in accordance with other studies of Asian NSCLC study cohort (10,11,29,31,32). Furthermore, we found a ratio of the major subtypes, G12C (32.1%), G12V (23.4%), G12D (21.1%), which is almost identical with the previous reports (31-35). We also identified four kinds of co-mutations in our study group: Four patients with G12C/G12R, two patients with G12C/G12V, one patient with G12D/G12V and one patient with G12A/G12V. And no significant differences in PFS between *KRAS* co-mutant and other KRAS mutant or wild-type patients were found (data not shown).

Prior findings indicated patients with *KRAS* mutation were preferably to be smokers and have histology of adenocarcinoma comparing with patients of wild-type *KRAS* (36,37). However, in the current study, we noted that there were no differences in smoking history and pathological types between two groups of patients. Nevertheless, we observed *KRAS* mutation was not exclusively found in patients with adenocarcinoma. Hence testing all patients with NSCLC for *KRAS* mutation is necessary. Although *KRAS* mutant patients and *KRAS* wild-type patients shared similar smoking habits, smokers had increased risk of shorter PFS compared with non-smoker in our univariate analysis of whole study group. There seemed to be more males in the *KRAS*-mutant group comparing with the group of patients with wild-type *KRAS*. But the significance of this finding was complicated to explain regarding clinical

Table III. Prognostic evaluation of clinical and histopathological characteristics in whole group and in *KRAS* mutant subgroup-progression free survival.

Variable	Univariate analyses 1[a] HR (95% CI) P-value	Multivariate analyses 1[a] HR (95% CI) P-value	Univariate analyses 1[b] HR (95% CI) P-value	Multivariate analyses 1[b] HR (95% CI) P-value
Age, <61 vs. ≥61 years old	1.170 (0.837-1.634) 0.359		1.622 (0.952-2.746) 0.075	- 0.121
Sex, female vs. male	0.616 (0.405-0.937) 0.024		0.844 (0.399-1.784) 0.657	
Stage, IIIB/recurrent vs. IV	0.811 (0.457-1.438) 0.473		0.781 (0.259-1.990) 0.525	
Smoking, never vs. current/former	0.665 (0.472-0.937) 0.020	- 0.126	0.799 (0.462-1.379) 0.420	
Pathology, SQC vs. ADC	1.301 (0.775-2.183) 0.319		0.779 (0.306-1.981) 0.599	
KRAS, mutant vs. wt	1.324 (0.942-1.861) 0.106		-	
G12C vs. wt	1.107 (0.654-1.873) 0.705		-	
G12V vs. wt	2.342 (1.378-3.981) 0.002	2.116 (1.211-3.696) 0.008	-	
G12D vs. wt	1.031 (0.474-2.239) 0.939		-	
G12C vs. others	-		0.697 (0.395-1.231) 0.214	
G12V vs. others	-		1.762 (0.992-3.129) 0.053	1.831 (1.025-3.270) 0.041
G12D vs. others	-		0.774 (0.350-1.714) 0.528	
Chemotherapy, cisplatin vs. carboplatin	1.296 (0.883-1.902) 0.186		1.720 (0.982-3.013) 0.058	- 0.158
Chemotherapy, gemcitabine vs. pemetrexed	1.390 (0.971-1.991) 0.072	- 0.335	1.527 (0.852-2.736) 0.155	

Independent variables with P<0.10 in the univariate analyses were included in the model; [a]Univariate and multivariate analysis for PFS in all patients after first line platinum-based chemotherapy; [b]Univariate and multivariate analysis for PFS in *KRAS* mutated patients after first line platinum-based chemotherapy. PFS, progression-free survival HR, hazard ratio; CI, confidence interval; Cox's model, multivariate analyses with forward elimination; wt, wild-type.

outcome. Although male sex was dramatically associated with worse outcomes in our univariate analysis, survival was similar in whole study group between *KRAS* mutant and wild-type groups despite the *KRAS* cohort had a higher percentage of males. The majority of patients in the study group received a cisplatin or carboplatin plus pemetrexed or gemcitabine chemotherapy. The different choice of chemotherapy regimens did not affect the PFS both in whole group and in *KRAS* mutant cohort in univariate and multivariate analysis.

There were many articles reporting inconsistent results in regards to the impact of *KRAS* mutation on survival of advanced NSCLC patients who received platinum-based chemotherapy. For example, a retrospective analysis performed by Mellema *et al* showed no significant differences in clinical response to chemotherapy or OS when compared patients with *KRAS* mutation with patients without *KRAS* mutation (19). Conversely, Metro *et al* demonstrated that patients with *KRAS* mutation had lower response rates, and shorter PFS compared with *EGFR* wild-type/*KRAS* wild-type patients (23). Besides, Hames *et al* reported that the presence of *KRAS* mutation in advanced NSCLC patients displayed a worse prognosis of platinum-based chemotherapy compared with those absence of detectable driver mutations (21). In the current analysis, our results suggested that *KRAS* mutant patients did have lower DCR compared with *KRAS* wild-type patients, but not ORR. In addition, *KRAS* mutant patients demonstrated a decrease PFS comparing with wild-type patients, which was in accordance with prior report (21) and we found more convincing results in patients with metastatic NSCLC at diagnosis, PFS was significantly shorter in the *KRAS* mutant group vs. wild-type

group (4.2 vs. 6.3 months; P=0.007). In addition, there was a shorter but only marginally statistically significant PFS in *KRAS* mutant patients with adenocarcinoma histology patients (4.3 months vs. 6.7 months; P=0.051). Based on the above results, we made the conclusion that KRAS mutation was a negative predictive factor of PFS in Chinese patients with advanced NSCLC received first platinum-based chemotherapy. Admittedly, this study was conducted at a single institution and had limited patient samples. We considered that, to make our findings more convincing, sharing of more data from multicenter studies, especially those covering various populations should be encouraged. We will also stay focuced on this issue and further exploration of the prognostic value of *KRAS* and its underlying mechanism is needed. Although recent research in colorectal cancer reported that G12V mutation demonstrated poor response to therapy and survival (38), the relevance of specific mutation subtypes in *KRAS* and clinical outcome remains controversial in NSCLC (16,39-41). In recent studies of advanced NSCLC, effects of *KRAS* G12V mutation regrading as either response to chemotherapy or OS were not obvious (40). However, in our study, patients with G12V mutant not only responded poorer to platinum-based chemotherapy, although not statisticly significant, but also had a significantly shorter PFS than those with other *KRAS* mutations. Our finding was in accordance with results carried out by Ihle *et al* (16). Downstream signaling of *RAS* differed in mutation subtypes. *KRAS* G12C/G12V preferably activated RalA/B signaling while *KRAS* G12D activated Akt pathway and the former demonstrated decreased survival (42). Taking all our presented results together, there is reason to believe that, in NSCLC, patients with different *KRAS* mutant subtypes may lead to distinct response to first-line platinum-based chemotherapy. Furthermore, subtype-specific mutation analysis is necessary in clinical practice, which may help to identify the most effective treatment regimens for each individual patient. Despite some of our results were consistent with previous publication, our study was conducted among Chinese population. Considering the differences in gene background between Caucasian and East Asian people (43,44), whether previous observation is also true among East Asian population remains uncertain. The conclusions we made in the study will provide clinicians with more comprehensive evidence when making clinical decisions for NSCLC patients with *KRAS* mutation.

There are several limitations in the present study that should be acknowledged. First of all, selection bias was inevitable due to the nature of retrospective studies. Second this study design was at a single institution. Taking the high cost of molecular detection into consideration, not all patients in our hospital received *KRAS* mutation test, therefore patients included in our study may not be representative of a general population. Sufficiency of cancer samples was also one of the limitations in this study. However, according to previous reports, in white populations KRAS accounts for 25-50% of NSCLC patients but KRAS mutations are only found in 5-10% of NSCLC patients in Asian populations (8-11). When we reviewed relative studies conducted among Caucasian populations, we found our patient number was very similar to other studies. In a retrospective analysis performed by Hames *et al* and colleagues, they compared 70 patients with pan-mutation negative and 80 patients with KRAS-mutant advanced NSCLC patients (21). On the other hand, considering the lower incidence of KRAS mutation among Asian people, we only focused on whether KRAS mutation was a negative predictive factor of PFS in Chinese patients with advanced NSCLC received first platinum-based chemotherapy. Further studies should be done aiming at the prognostic value of *KRAS* mutation on chemotherapy and also comparing responses with different cytotoxic chemotherapy regimens in patients with advanced NSCLC based on *KRAS* mutation and subtypes. Thus, considering the above limitations, multi-centered, international cooperative and larger number of NSCLC patients should be analyzed to valid our present findings.

The current study suggested that the presence of *KRAS* mutation was associated with a worse response in advanced NSCLC patients received first-line platinum-based chemotherapy. Responses to cytotoxic chemotherapy are not same among *KRAS* mutation subtypes. As the currently available literatures are still conflicting on the predictive value of *KRAS* mutation and its subtypes in advanced NSCLC, future studies should be done aiming at comparing responses with different cytotoxic chemotherapy regimens in patients with advanced NSCLC based on *KRAS* mutation and subtypes.

Acknowledgements

The present study was supported by grants from the National Natural Science Foundation of China (nos. 81372392 and 81402486) and Shanghai Committee of Science and Technology, China (no. 134119b1001).

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J: Cancer statistics in China, 2015. CA Cancer J Clin 66: 115-132, 2016.
3. Chen Z, Fillmore CM, Hammerman PS, Kim CF and Wong KK: Non-small-cell lung cancers: A heterogeneous set of diseases. Nat Rev Cancer 14: 535-546, 2014.
4. Zhou C, Wu YL, Chen G, Feng J, Liu XQ, Wang C, Zhang S, Wang J, Zhou S, Ren S, *et al*: Erlotinib versus chemotherapy as first-line treatment for patients with advanced EGFR mutation-positive non-small-cell lung cancer (OPTIMAL, CTONG-0802): A multicentre, open-label, randomised, phase 3 study. Lancet Oncol 12: 735-742, 2011.
5. Miyanaga A, Shimizu K, Noro R, Seike M, Kitamura K, Kosaihira S, Minegishi Y, Shukuya T, Yoshimura A, Kawamoto M, *et al*: Activity of EGFR-tyrosine kinase and ALK inhibitors for EML4-ALK-rearranged non-small-cell lung cancer harbored coexisting EGFR mutation. BMC Cancer 13: 262, 2013.
6. Friboulet L, Li N, Katayama R, Lee CC, Gainor JF, Crystal AS, Michellys PY, Awad MM, Yanagitani N, Kim S, *et al*: The ALK inhibitor ceritinib overcomes crizotinib resistance in non-small cell lung cancer. Cancer Discov 4: 662-673, 2014.
7. Solomon BJ, Mok T, Kim DW, Wu YL, Nakagawa K, Mekhail T, Felip E, Cappuzzo F, Paolini J, Usari T, *et al*: First-line crizotinib versus chemotherapy in ALK-positive lung cancer. N Engl J Med 371: 2167-2177, 2014.
8. Riely GJ, Marks J and Pao W: KRAS mutations in non-small cell lung cancer. Proc Am Thorac Soc 6: 201-205, 2009.
9. Piva S, Ganzinelli M, Garassino MC, Caiola E, Farina G, Broggini M and Marabese M: Across the universe of K-RAS mutations in non-small-cell-lung cancer. Curr Pharm Des 20: 3933-3943, 2014.
10. Xia N, An J, Jiang QQ, Li M, Tan J and Hu CP: Analysis of EGFR, EML4-ALK, KRAS, and c-MET mutations in Chinese lung adenocarcinoma patients. Exp Lung Res 39: 328-335, 2013.

11. Gao B, Sun Y, Zhang J, Ren Y, Fang R, Han X, Shen L, Liu XY, Pao W, Chen H and Ji H: Spectrum of LKB1, EGFR, and KRAS mutations in chinese lung adenocarcinomas. J Thorac Oncol 5: 1130-1135, 2010.
12. Malumbres M and Barbacid M: RAS oncogenes: The first 30 years. Nat Rev Cancer 3: 459-465, 2003.
13. Pylayeva-Gupta Y, Grabocka E and Bar-Sagi D: RAS oncogenes: Weaving a tumorigenic web. Nat Rev Cancer 11: 761-774, 2011.
14. Brady AK, McNeill JD, Judy B, Bauml J, Evans TL, Cohen RB, Langer C, Vachani A and Aggarwal C: Survival outcome according to KRAS mutation status in newly diagnosed patients with stage IV non-small cell lung cancer treated with platinum doublet chemotherapy. Oncotarget 6: 30287-30294, 2015.
15. Garassino MC, Marabese M, Rusconi P, Rulli E, Martelli O, Farina G, Scanni A and Broggini M: Different types of K-Ras mutations could affect drug sensitivity and tumour behaviour in non-small-cell lung cancer. Ann Oncol 22: 235-237, 2011.
16. Ihle NT, Byers LA, Kim ES, Saintigny P, Lee JJ, Blumenschein GR, Tsao A, Liu S, Larsen JE, Wang J, *et al*: Effect of KRAS oncogene substitutions on protein behavior: Implications for signaling and clinical outcome. J Natl Cancer Inst 104: 228-239, 2012.
17. Miller MS and Miller LD: RAS mutations and oncogenesis: Not all RAS mutations are created equally. Front Genet 2: 100, 2012.
18. Slebos RJ, Kibbelaar RE, Dalesio O, Kooistra A, Stam J, Meijer CJ, Wagenaar SS, Vanderschueren RG, van Zandwijk N, Mooi WJ, *et al*: K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. N Engl J Med 323: 561-565, 1990.
19. Mellema WW, Dingemans AM, Thunnissen E, Snijders PJ, Derks J, Heideman DA, Van Suylen R and Smit EF: KRAS mutations in advanced nonsquamous non-small-cell lung cancer patients treated with first-line platinum-based chemotherapy have no predictive value. J Thorac Oncol 8: 1190-1195, 2013.
20. Rulli E, Marabese M, Torri V, Farina G, Veronese S, Bettini A, Longo F, Moscetti L, Ganzinelli M, Lauricella C, *et al*: Value of KRAS as prognostic or predictive marker in NSCLC: Results from the TAILOR trial. Ann Oncol 26: 2079-2084, 2015.
21. Hames ML, Chen H, Iams W, Aston J, Lovly CM and Horn L: Correlation between KRAS mutation status and response to chemotherapy in patients with advanced non-small cell lung cancer. Lung Cancer 92: 29-34, 2016.
22. Campos-Parra AD, Zuloaga C, Manriquez ME, Avilés A, Borbolla-Escoboza J, Cardona A, Meneses A and Arrieta O: KRAS mutation as the biomarker of response to chemotherapy and EGFR-TKIs in patients with advanced non-small cell lung cancer: Clues for its potential use in second-line therapy decision making. Am J Clin Oncol 38: 33-40, 2015.
23. Metro G, Chiari R, Bennati C, Cenci M, Ricciuti B, Puma F, Flacco A, Rebonato A, Giannarelli D, Ludovini V, *et al*: Clinical outcome with platinum-based chemotherapy in patients with advanced nonsquamous EGFR wild-type non-small-cell lung cancer segregated according to KRAS mutation status. Clin Lung Cancer 15: 86-92, 2014.
24. Marabese M, Ganzinelli M, Garassino MC, Shepherd FA, Piva S, Caiola E, Macerelli M, Bettini A, Lauricella C, Floriani I, *et al*: KRAS mutations affect prognosis of non-small-cell lung cancer patients treated with first-line platinum containing chemotherapy. Oncotarget 6: 34014-34022, 2015.
25. Sobin LH, Gospodarowicz MK and Wittekind C: TNM Classification of Malignant Tumors. 7th edition. Wiley-Blackwell, New York, 2009
26. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1). Eur J Cancer 45: 228-247, 2009.
27. Deng LL, Deng HB, Lu CL, Guo Y, Wang D, Yan CH, Lv X and Shao YX: Mutations of EGFR or KRAS and expression of chemotherapy-related genes based on small biopsy samples in stage IIIB and IV inoperable non-small cell lung cancer. J Cancer Res Clin Oncol 140: 2097-2105, 2014.
28. Wu C, Zhao C, Yang Y, He Y, Hou L, Li X, Gao G, Shi J, Ren S, Chu H, *et al*: High discrepancy of driver mutations in patients with NSCLC and synchronous multiple lung ground-glass nodules. J Thorac Oncol 10: 778-783, 2015.
29. Ren S, Kuang P, Zheng L, Su C, Li J, Li B, Chen X, Wang Y, KimCurran V, Liu L, *et al*: Analysis of driver mutations in female non-smoker Asian patients with pulmonary adenocarcinoma. Cell Biochem Biophys 64: 155-160, 2012.
30. Costa DB, Shaw AT, Ou SH, Solomon BJ, Riely GJ, Ahn MJ, Zhou C, Shreeve SM, Selaru P, Polli A, *et al*: Clinical experience with crizotinib in patients with advanced ALK-rearranged non-small-cell lung cancer and brain metastases. J Clin Oncol 33: 1881-1888, 2015.
31. Wang Z, Wu YL, Zhang GC, Zhou Q, Xu CR and Guo AL: EGFR/KRAS mutations and gefitinib therapy in Chinese NSCLC patients. Onkologie 31: 174-178, 2008.
32. Li M, Liu L, Liu Z, Yue S, Zhou L, Zhang Q, Cheng S, Li RW, Smith PN and Lu S: The status of KRAS mutations in patients with non-small cell lung cancers from mainland China. Oncol Rep 22: 1013-1020, 2009.
33. Jia XL and Chen G: EGFR and KRAS mutations in Chinese patients with adenosquamous carcinoma of the lung. Lung Cancer 74: 396-400, 2011.
34. Kim HR, Shim HS, Chung JH, Lee YJ, Hong YK, Rha SY, Kim SH, Ha SJ, Kim SK, Chung KY, *et al*: Distinct clinical features and outcomes in never-smokers with nonsmall cell lung cancer who harbor EGFR or KRAS mutations or ALK rearrangement. Cancer 118: 729-739, 2012.
35. Sun JM, Hwang DW, Ahn JS, Ahn MJ and Park K: Prognostic and predictive value of KRAS mutations in advanced non-small cell lung cancer. PLoS One 8: e64816, 2013.
36. Ahrendt SA, Decker PA, Alawi EA, Zhu Yr YR, Sanchez-Cespedes M, Yang SC, Haasler GB, Kajdacsy-Balla A, Demeure MJ and Sidransky D: Cigarette smoking is strongly associated with mutation of the K-ras gene in patients with primary adenocarcinoma of the lung. Cancer 92: 1525-1530, 2001.
37. Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, Nafa K, Riedel ER, Hsu M, Pao W, *et al*: Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clin Cancer Res 14: 5731-5734,2008.
38. Imamura Y, Morikawa T, Liao X, Lochhead P, Kuchiba A, Yamauchi M, Qian ZR, Nishihara R, Meyerhardt JA, Haigis KM, *et al*: Specific mutations in KRAS codons 12 and 13, and patient prognosis in 1075 BRAF wild-type colorectal cancers. Clin Cancer Res 18: 4753-4763,2012.
39. Shepherd FA, Domerg C, Hainaut P, Jänne PA, Pignon JP, Graziano S, Douillard JY, Brambilla E, Le Chevalier T, Seymour L, *et al*: Pooled analysis of the prognostic and predictive effects of KRAS mutation status and KRAS mutation subtype in early-stage resected non-small-cell lung cancer in four trials of adjuvant chemotherapy. J Clin Oncol 31: 2173-2181, 2013.
40. Cserepes M, Ostoros G, Lohinai Z, Raso E, Barbai T, Timar J, Rozsas A, Moldvay J, Kovalszky I, Fabian K, *et al*: Subtype-specific KRAS mutations in advanced lung adenocarcinoma: A retrospective study of patients treated with platinum-based chemotherapy. Eur J Cancer 50: 1819-1828, 2014.
41. Izar B, Zhou H, Heist RS, Azzoli CG, Muzikansky A, Scribner EE, Bernardo LA, Dias-Santagata D, Iafrate AJ and Lanuti M: The prognostic impact of KRAS, its codon and amino acid specific mutations, on survival in resected stage I lung adenocarcinoma. J Thorac Oncol 9: 1363-1369, 2014.
42. Stolze B, Reinhart S, Bulllinger L, Fröhling S and Scholl C: Comparative analysis of KRAS codon 12, 13, 18, 61, and 117 mutations using human MCF10A isogenic cell lines. Sci Rep 5: 8535, 2015.
43. Thomas RK, Weir B and Meyerson M: Genomic approaches to lung cancer. Clin Cancer Res 12: 4384s-4391s, 2006.
44. Zhou W and Christiani DC: East meets West: Ethnic differences in epidemiology and clinical behaviors of lung cancer between East Asians and Caucasians. Chin J Cancer 30: 287-292,2011.