IVYSPRING INTERNATIONAL PUBLISHER

Journal of Cancer

2018; 9(16): 2930-2937. doi: 10.7150/jca.24425

Research Paper

Clinicopathologic characteristics and survival outcome in patients with advanced lung adenocarcinoma and *KRAS* mutation

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Received: 2017.12.18; Accepted: 2018.06.06; Published: 2018.07.30

Abstract

Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mutations are one of the most common observed genetic events in lung adenocarcinoma. The present study aimed to characterize treatment patterns and to estimate survival for patients in China with advanced lung adenocarcinoma and *KRAS* mutation. We identified *KRAS*-mutant lung adenocarcinoma between February 2013 and June 2017 in Zhejiang Cancer Hospital. Patients' characteristics and treatment outcomes were analyzed. A total of 159 lung adenocarcinoma were included, and 26 (16.4%) patients harbored *KRAS* mutations. Compared to *KRAS*-wild patients, patients with *KRAS*-mutant tumors were more likely to be smokers (76.9% vs. 51.9%, $P = 0.029$). Median tumor mutation burden (TMB) was significantly higher in the *KRAS*-mutant cohort than in the *KRAS*-wild cohort (5.4 vs. 4.2 mutations/megabases; $P=0.041$). Of the 93 patients receiving first-line chemotherapy, the median progression-free survival (PFS) in the *KRAS*-mutant group was significantly shorter than in the *KRAS*-wild group (1.5 vs. 7.2 months; $P<0.001$). The median overall survival (OS) in the *KRAS*-mutant group was also significantly shorter than in the *KRAS*-wild group (hazard ratio for progression or death for patients with *KRAS* mutation, 3.260; 95% CI, 1.516 to 7.013; $P=0.001$). In summary, our findings have several important implications for the molecular characterization and therapeutic outcome of lung adenocarcinoma initiated by oncogenic *KRAS*. Since the number of *KRAS*-mutant lung cancer is considerable, it should be taken seriously in clinical diagnosis and treatment. *KRAS*-mutant lung adenocarcinoma was not sensitive to chemotherapy, new and effective drugs targeting the *KRAS* pathway are in urgent need.

Key words: *KRAS* mutation; lung adenocarcinoma; clinicopathologic characteristics; survival outcome.

Introduction

Lung cancer is the leading cause of cancer death in the People's Republic of China as well as worldwide [1]. Approximately 80% of lung cancers are non-small-cell lung carcinoma (NSCLC), the overall 5-year relative survival rate for this cohort was less than 20%, patients with advanced NSCLC has an extremely high mortality rate [2]. In the past decade, the advent of personalized medicine has seen the introduction of a number of targeted treatments for the NSCLC with druggable driver gene mutations [3]. Among these, the presence of activating mutations of the epidermal growth factor receptor (*EGFR*) and of chromosomic rearrangements in the anaplastic-lymphoma kinase (*ALK*) protooncogene, have been well characterized genetic alterations with approved inhibitors to be put into the clinical practice and resulted in improved outcomes for patients [4,5]. Beyond *EGFR* and *ALK* mutations, a series of other oncogenic drivers have been identified as novel molecular targets with potential therapeutic implications, such as mutations in the genes *KRAS, BRAF, HER2, PI3KCA, MET, RET* [6].

Kirsten rat sarcoma viral oncogene homolog (*KRAS*), one of the guanosine triphosphatases (GTPases), belongs to the RAS kinase family involved in cell survival, cell cycle progression, cell polarity and movement, actin cytoskeletal organization, as well as vesicular and nuclear transport [7,8]. When activated by mutations, *KRAS* protein activates downstream cytosolic effectors including the *RAF/MEK/ERK* pathway and the *PI3K/AKT/mTOR* pathway to promote cell growth, proliferation, and survival [9,10]. The transforming protein that results is implicated in various malignancies, including lung adenocarcinoma [11], colorectal cancer [12], pancreatic cancer [13], and leukemias [14].

KRAS is one of the most frequently mutated genes in NSCLC, which are found in approximately 30% of lung adenocarcinomas in western populations and 10% in Asian populations. Despite activating *KRAS* mutations are one of the most common observed genetic events in lung adenocarcinoma, little progress has been made during the past decades with no new agents being approved for this indication. Currently, platinum-containing chemotherapy remains the standard treatment regimen for advanced NSCLC patients with *KRAS* mutations.

In previous research, *KRAS* has been explored as a potential prognostic and predictive factor in patients with resected NSCLC receiving chemotherapy [15-17]. However, the results remain controversial. Until now, published data about treatment patterns and outcomes for advanced NSCLC with *KRAS* mutation in China have been limited. The present study aimed to characterize treatment patterns and to estimate survival for patients in China with advanced lung adenocarcinoma and *KRAS* mutation.

Methods

Patients Enrollment and Follow up procedures

Included were patients with pathologically confirmed locally advanced or metastatic lung adenocarcinoma who received targeted NGS analysis at Zhejiang Cancer Hospital during the period February 2013 and June 2017. Patients' data were collected including the following variables: gene mutation status, gender, age, smoking history, stage, involvement status, therapeutic regimens, efficacy of treatment.

Patients who received chemotherapy were evaluated for response every two treatment cycles during treatment and then every 2 months after treatment. Patients received targeted therapy were evaluated for response one month after the initial treatment and then every 2 months during treatment. The response evaluation of the tumor to therapy was based on computed tomography (CT) or magnetic resonance imaging (MRI) scan. The short-term efficacy was defined based on version 1.1 of the Response Evaluation Criteria in Solid Tumors (RECIST) [18]. The long-term efficacy was evaluated according to the progression-free survival (PFS) and overall survival (OS). PFS was defined as the time from the initiation of treatment to the radiological evidence of PD. OS was calculated from initiation of treatment to death. Patients who were still alive till the last follow-up were recorded as censored. Patients who did not relapse or metastases were censored on the day of last follow-up.

Molecular Analysis

All mutational analyses were conducted at the core facility of Nanjing Shihe Jiyin Biotechnology Inc. (Nanjing, China). 5-8 of 10 μm tissue sections from tumor FFPE samples were used for genomic DNA extraction with QIAamp DNA FFPE Tissue Kit (QIAGEN) following the manufacturer's instructions. Extracted tumor genomic DNA was fragmented into 300~350bp using Covaris M220 instrument (Covaris). Sequencing libraries were prepared with KAPA Hyper Prep kit (KAPA Biosystems) with optimized protocols. Libraries were then subjected to PCR amplification and purification before targeted enrichment.

DNA libraries from different samples were marked with unique indices during library preparation and up to 2 μg of different libraries were pooled together for targeted enrichment. Human cot-1 DNA (Life Technologies) and xGen Universal blocking oligos (Integrated DNA Technologies) were added to block nonspecific binding of library DNA to targeted probes. Customized xGen lockdown probes panel (Integrated DNA Technologies) were used to targeted enrich for 416 predefined genes. The hybridization reaction was performed by using NimbleGen SeqCap EZ Hybridization and Wash Kit (Roche). Dynabeads M-270 (Life Technologies) was used to capture probe-bind fragments, followed by library amplification with Illumina p5 (5′ AAT GAT ACG GCG ACC ACC GA 3′) and p7 primers (5′ CAA GCA GAA GAC GGC ATA CGA GAT 3′) in KAPA HiFi HotStart ReadyMix (KAPA Biosystems), and purification by Agencourt AMPure XP beads. Library quantification was analyzed by KAPA Library Quantification kit (KAPA Biosystems). The size distribution of libraries was measured by Agilent Technologies 2100 Bioanalyzer (Agilent Technologies). The enriched libraries were sequenced on Hiseq 4000 NGS platforms (Illumina) to coverage depths of at least 300x after removing PCR duplicates for FFPE.

Statistical Analysis

Statistical analysis was performed using SPSS22.0 package. Statistical significance was defined as when $P<0.05$. The categorical variables were analyzed by chi-square tests, or Fisher's exact tests when needed. The continuous variable was compared using ANOVA and Tukey's multiple comparison tests. Survivals were analyzed using the Kaplan –Meier method and were compared using the log-rank test.

Results

An overall characterization of cancer-related mutations identified in all patients

A total of 159 advanced lung adenocarcinoma patients treated in Zhejiang Cancer Hospital who received targeted NGS analysis (416 genes) between February 2013 and June 2017 were enrolled in this study. Patients' characteristics are described in Table 1. The median age of diagnosis was 59.0 years (ranging from 21 to 86 years), and more than half of the patients were male (66.0%). Nineteen (11.9%) patients were diagnosed as stage IIIB, and others were stage IV.

Table 1. Characteristics between patients with *KRAS* mutations and without *KRAS* mutations.

Characteristic	All patients	*KRAS* mutation	Non-*KRAS* mutation	*P* value
Gender	159	26	133	
Female	54	5	49	
Male	105	21	84	0.113
Age				
<60 years	82	10	72	
≥60 years	77	16	61	0.198
Stage				
IIIB	19	1	18	
IV	140	25	115	0.317
Smoking history				
Yes	89	20	69	
No	70	6	64	0.029*
bone metastasis				
Yes	70	11	59	
No	89	15	74	0.512
Intracranial metastasis				
Yes	20	4	16	
No	139	22	117	0.746
Liver metastasis				
Yes	19	5	14	
No	140	21	119	0.202
Pleural effusion				
Yes	27	5	22	
No	132	21	111	0.776

A total of 407 cancer-related genetic mutations were detected in these patients with a median of 2.6 mutations per patient and a range of 1-9 mutations per patient (Figure 1A). The most common driver genetic alterations were *EGFR* mutations in 72 (45.3%) patients, followed by *ALK* mutations in 16 (10.1%), *KRAS* mutations in 26 (16.4%), *ROS1* mutations in 7 (4.4%), *BRAF* mutations in 6 (3.8%), *RET* mutations in 5 (3.1%), *MET* mutations in 5 (3.1%), *HER2* mutations in 5 (3.1%), *NF1* mutations in 4 (2.5%), *PIK3CA* mutations in 3 (1.9%), *STK11* mutations in 2 (1.3%), *FGFR* mutations in 2 (1.3%) and others in 6 (3.8%) (Figure 1B).

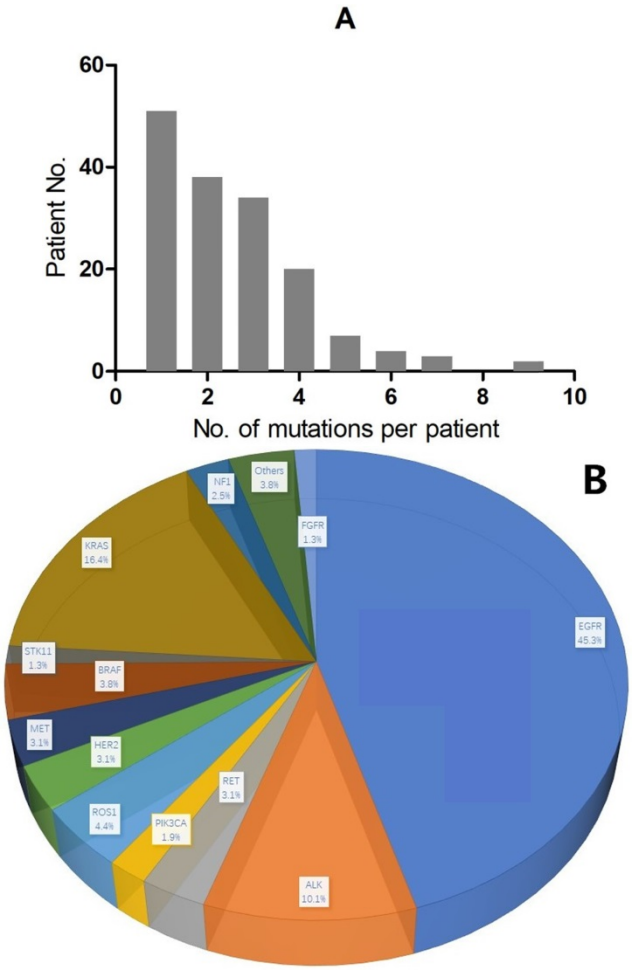

Fig. 1: mutation types and mutation number identified in 159 patients. A. The number of mutations identified in each patient was plotted to a histogram. **B.** Total mutations detected in 159 patients were classified according to the mutation genes.

Clinicopathologic characteristics associated with KRAS-mutant NSCLC

KRAS mutations were present in 5 female and 21 male with an average age of 60 years (range, 48-73 years). Six patients (23.1%) were never smokers. Histopathologic stage varied and included IIIB (n = 1) and IV (n = 25). Compared to patients with non-*KRAS* mutation, patients with *KRAS*-mutant tumors were more likely to be smokers (76.9% vs. 51.9%, $P = 0.029$). There were no significant differences in the gender, age, stage, metastatic sites between patients with *KRAS*-mutant and *KRAS* wild-type tumors (Table 1). More details of the 26 patients' *KRAS* mutations are listed in Table 2.

Tumor mutation burden between KRAS-mutant patients and KRAS-wild patients

Of the 159 patients, the median tumor mutation burden (TMB) was 3.7 (range from 1.1 to 16.7) mutations/megabases. The median TMB was significantly higher in the *KRAS*-mutant group (5.4 mutations/megabases; range from 1.1 to 16.7) than in the *KRAS*-wild group (4.2 mutations/megabases; range from 1.1 to 11.0) (P=0.041). In addition, 13 of the 26 *KRAS* mutant patients have co-existence *TP53* mutations. The median TMB was 5.9 (range from 2.2 to 16.7) mutations/megabases in this cohort.

Treatment efficacy and survival between KRAS-mutant and KRAS-wild patients

Efficacy of the first-line therapy for all 159 patients were listed in Table 3. In the *KRAS*-mutant group, all the 26 patients received chemotherapy as the first-line treatment. Among them, 13 patients received pemetrexed+cisplatin/carboplatin regimen, 4 patients received paclitaxel+cisplatin/carboplatin regimen, 4 patients received gemcitabine+cisplatin/carboplatin regimen, 1 patient received pemetrexed, 1 patient received docetaxel+carboplatin, 1 patient received docetaxel+bevacizumab, 1 patient received pemetrexed+cisplatin and bevacizumab, 1 patient was included in the clinical trial and received nivolumab+pemetrexed+carboplatin. In the *KRAS*-wild group, 66 patients received targeted therapy and 67 patients received chemotherapy as the first-line treatment. The objective response rate (ORR) (11.5% vs 43.6%, P=0.002) and the disease response rate (DCR) (50.0% vs 84.2%, P<0.001) were both significantly lower in the *KRAS*-mutant cohort than in the *KRAS*-wild cohort.

Table 2. More details of the 26 patients with *KRAS* mutations.

Patient ID.	Gene Name	Mutation	Co-mutation genes	Chemotherapy regimen
001	*KRAS*	G12V	*TP53, FLT4, PDGFRB, AKT2, CCND1, ERCC2, FGFR1, FGFR4*	Docetaxel + bevacizumab
002	*KRAS*	G12D	None	Pemetrexed + cisplatin
003	*KRAS*	G12C	*TP53, ERCC2, PIK3CA*	Paclitaxel + cisplatin
004	*KRAS*	G12A	*TP53*	Pemetrexed + carboplatin
005	*KRAS*	G12S	*TP53, IL7R, MYC, RECQL4, RICTOR, LKB1*	Pemetrexed + carboplatin
006	*KRAS*	G12C	*TP53, CDKN2A*	Pemetrexed
007	*KRAS*	G12S	*TP53, PIK3CA, CDK4, RB1, SOX2*	Pemetrexed + cisplatin
008	*KRAS*	G12C	None	Gemcitabine + carboplatin
009	*KRAS*	G13D	*CHEK1, SMARCA4*	Pemetrexed + carboplatin
010	*KRAS*	G12D	*MEK2, CCND1, LKB1*	Gemcitabine + carboplatin
011	*KRAS*	G12D	*NF2, CDKN2A, GNAS, KMT2A, MPL, SMAD4*	Pemetrexed + cisplatin + bevacizumab
012	*KRAS*	G12C	*TP53, RB1*	Pemetrexed + carboplatin
013	*KRAS*	G12C	*FGFR3, RB1, LKB1, NTRK1*	Pemetrexed + carboplatin
014	*KRAS*	G13D	*TP53, HGF*	Pemetrexed + carboplatin
015	*KRAS*	G13C	*BRAF*	Gemcitabine + carboplatin
016	*KRAS*	G12R	*CDKN2A, SMARCA4, STK11*	Pemetrexed + cisplatin
017	*KRAS*	G12C	*TP53, ERCC2, PIK3CA*	Paclitaxel + cisplatin
018	*KRAS*	G12V	*TP53, CDKN2A, GRM3*	Gemcitabine + cisplatin
019	*KRAS*	Q61H	*TP53, DDR2*	Pemetrexed + cisplatin
020	*KRAS*	G12A	*STK11*	Paclitaxel + cisplatin
021	*KRAS*	G12C	-	Pemetrexed + carboplatin
022	*KRAS*	G12C	*NF2, STK11*	Paclitaxel + carboplatin
023	*KRAS*	G12V	*TP53, CBL, IL7R, RICTOR*	Docetaxel + carboplatin
024	*KRAS*	G13C	*TP53*	Pemetrexed + carboplatin
025	*KRAS*	Q61K	-	Pemetrexed + carboplatin + Nivolumab (Clinical trial)
026	*KRAS*	G13C	*BTK, SMARCA4, STK11*	Pemetrexed + carboplatin

Table 3. Comparison of efficacy between *KRAS*-mutant patients and *KRAS*-wild patients.

Comparison of efficacy in the total 159 patients.			
	KRAS-mutant patients (n= 26)	*KRAS*-wild patients (n= 133)	*P* value
CR	0	0	
PR	3	58	
SD	10	54	
PD	13	21	
ORR	3 (11.5%)	58 (43.6%)	0.002
DCR	13 (50.0%)	112 (84.2%)	<0.001
Comparison of efficacy in the 93 patients receiving first-line chemotherapy.			
	KRAS-mutant patients (n= 26)	*KRAS*-wild patients (n= 67)	*P* value
CR	0	0	
PR	3	20	
SD	10	35	
PD	13	12	
ORR	3 (11.5%)	20 (29.9%)	0.106
DCR	13 (50.0%)	55 (82.1%)	0.003

Efficacy of the first-line chemotherapy for the 93 patients were also listed in Table 3. The DCR (50.0% vs 82.1%, *P*=0.003) was significantly lower in the *KRAS*-mutant cohort than in the *KRAS*-wild cohort. There were no significant differences in ORR between patients with *KRAS* mutation and non-*KRAS* mutation.

Until the last follow up, 25 patients in the *KRAS*-mutant group and 62 patients in the *KRAS*-wild group had disease progression. Of all the 159 patients, the median PFS was 1.5 months (95% confidence interval [CI], to 4.7) in the *KRAS*-mutant cohort, as compared with 7.6 months (95% CI, 6.5 to 8.7) among patients in the *KRAS*-wild cohort (hazard ratio for progression or death for patients with *KRAS* mutation, 3.131; 95% CI, 1.946 to 5.037; *P*<0.001) (Fig. 2A); the median OS in the *KRAS*-mutant cohort was significantly shorter than in the *KRAS*-wild cohort (hazard ratio for progression or death for patients with *KRAS* mutation, 3.182; 95% CI, 1.597 to 6.341; *P*=0.002) (Fig. 2B).

Of all the 93 patients receiving chemotherapy as the first-line treatment, the median PFS was 1.5 months (95% confidence interval [CI], 0.3 to 4.7) in the *KRAS*-mutant cohort, as compared with 7.2 months (95% CI, 5.8 to 8.7) among patients in the *KRAS*-wild cohort (hazard ratio for progression or death for patients with *KRAS* mutation, 3.042; 95% CI, 1.801 to 5.137; *P*<0.001) (Fig. 2C); the median OS in the *KRAS*-mutant cohort was significantly shorter than in the *KRAS*-wild cohort (hazard ratio for progression or death for patients with *KRAS* mutation, 3.260; 95% CI, 1.516 to 7.013; *P*=0.001) (Fig. 2D).

Dramatic response to PD_1 inhibitor in patient with advanced NSCLC and KRAS mutation

A 73-year-old woman (patient case 025) was diagnosed with lung adenocarcinoma with bilateral intrapulmonary, hilar lymph node, mediastinal lymph node, and subcutaneous metastasis (cT3N2M1b IV; AJCC 7th Edition). Expanded molecular testing revealed *KRAS* exon3 Q61K mutation. Then she was included in the clinical trial (NCT02477826) and received 4 cycles of nivolumab plus chemotherapy (pemetrexed and carboplatin) as first-line treatment from June 8, 2017 to Aug 10, 2017. After two cycles of treatment, the patient was with rapid dramatic clinical improvement, later confirmed as an excellent radiographic partial response by computed tomography scanning (Fig 3). After then, she received 5 cycles of nivolumab plus pemetrexed as maintenance therapy from Sep 1, 2017 to Nov 24, 2017. The PFS was 6.2 months, significantly better than the median PFS of 1.5 months in the *KRAS*-mutant cohort.

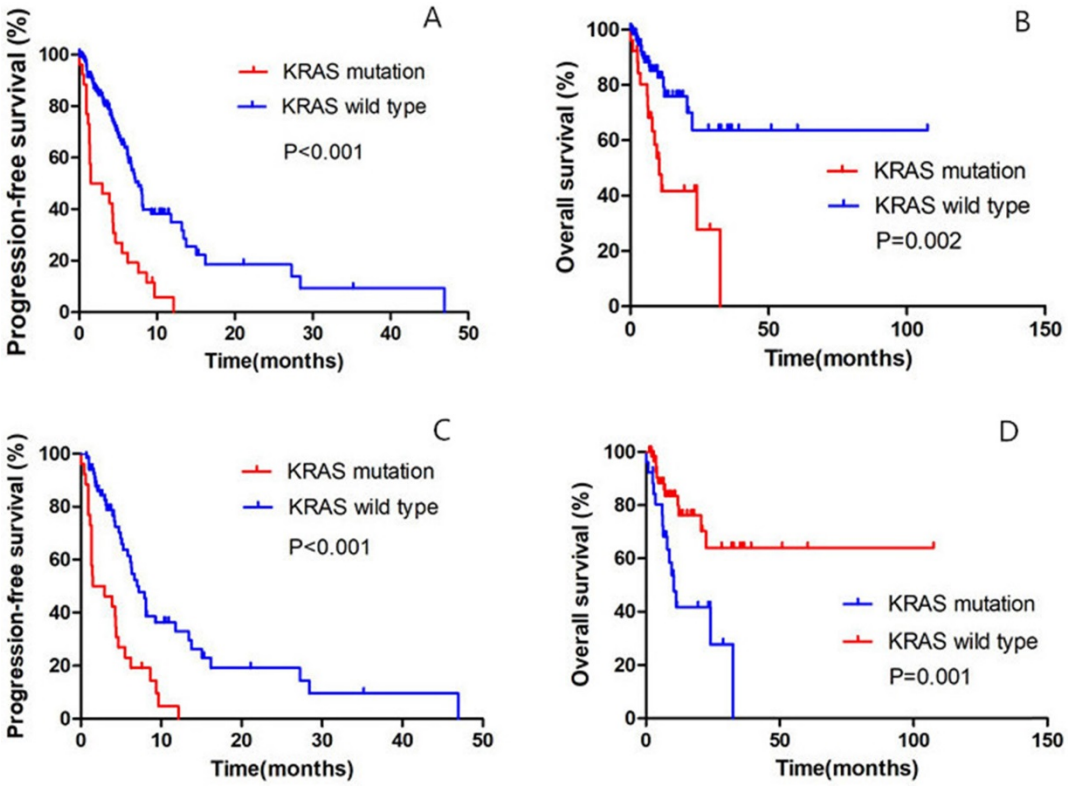

Fig. 2: Comparison of PFS and OS between *KRAS*-mutant patients and *KRAS*-wild patients. A. Of the 159 patients, Kaplan-Meier PFS curves are shown between *KRAS*-mutant patients and *KRAS*-wild patients (*P*<0.001). **B**. Of the 159 patients, Kaplan-Meier OS curves are shown between *KRAS*-mutant patients and *KRAS*-wild patients (*P*=0.002). **C**. Of all the 93 patients receiving first-line chemotherapy, Kaplan-Meier PFS curves are shown between *KRAS*-mutant patients and *KRAS*-wild patients (*P*<0.001). **D**. Of all the 93 patients receiving first-line chemotherapy, Kaplan-Meier OS curves are shown between *KRAS*-mutant patients and *KRAS*-wild patients (*P*=0.001).

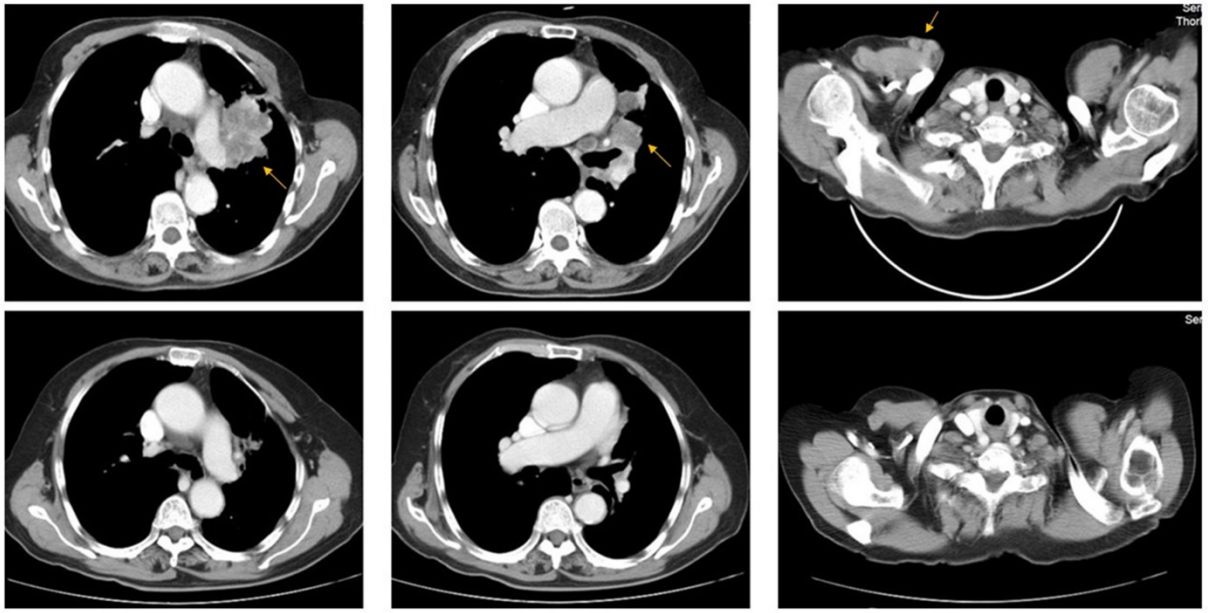

Fig. 3: Representative pre- (upper row) and post-treatment (lower row) computed tomography (CT) images in a 73-year-old woman (patient case 025) diagnosed with advanced lung adenocarcinoma with *KRAS* exon3 Q61K mutation. After 2 cycles of treatment (Nivolumab + pemetrexed + carboplatin), metastatic lesions (yellow arrow) became markedly reduced compared with those in pre-treatment CT images.

Discussion

In this study, we included 159 patients with advanced lung adenocarcinoma and 26 (16.4%) of them had *KRAS* mutations. The rate of *KRAS* mutations in our result was similar to those reported in Asian populations, but was lower than those reported in Caucasian populations [19, 20]. In our study, of the 26 *KRAS*-mutant patients, 8 was the G12C point mutation, 3 was G12V point mutation, 3 was the G12D point mutation, 3 was G13C point mutation, 2 was G13D point mutation, 2 was the G12S point mutation, 2 was the G12A point mutation, 1 was G12R point mutation, 1 was Q61H point mutation, and 1 was Q61K point mutation. Previous research also indicated that most of *KRAS* mutations in NSCLC occur at the G12C point mutation, followed by G12V and G12D [21]. In addition, our findings showed that compared to patients with non-*KRAS* mutation, patients with *KRAS*-mutant tumors were more likely to be smokers. This phenomenon was consistent with most of previous reports. In a study including 106 patients with lung adenocarcinoma, *KRAS* mutations were detected in 40 of 106 tumors (38%) and were significantly more common in smokers compared with nonsmokers (43% vs 0%; P=0.001) [22]. In a large French nationwide study only 6% of *KRAS*-mutant NSCLC patients were never smokers [23]. In another study, the frequency of *KRAS* mutation was not associated with smoking history, but the findings showed that never smokers were significantly more likely than former or current smokers to have a transition mutation (G → A) rather than the transversion mutations known to be smoking related (G→T or G→C; p<0.0001) [24]. Similar results have been reported by a research based on the cohort of lung adenocarcinomas patients in Korean [25].

Despite the fact that activating mutations of the *KRAS* gene are one of the most common recurring molecular aberrations in NSCLC, its utility as a direct treatment target remains disappointing [26]. In our study, all the 26 patients with *KRAS* mutations received chemotherapy as the first-line treatment. In the *KRAS*-wild group, 66 patients received targeted therapy and 67 patients received chemotherapy as the first-line treatment. The results demonstrated that both in the general cohort (n=159) and in the subgroup cohort (n=93) receiving first-line chemotherapy, *KRAS* mutations were associated with lower DCR, shorter PFS and OS. As far as we know, this is the first research from a single center in China, suggesting that the *KRAS* mutation is a poor predictive and prognostic indicator in advanced lung adenocarcinoma. Although the analysis of OS may be confounded by the long-periods tumor control of the *KRAS*-wild patients with actionable mutations who received targeted drugs, at least we can conclude that *KRAS* mutations have negative predictive and prognostic effect on efficacy and PFS for advanced lung adenocarcinoma receiving first-line chemotherapy.

Actually, conclusions about the prognostic value of *KRAS* mutations in NSCLC remain controversial. An initial research demonstrated that *KRAS* mutations were associated with poor disease-free survival (DFS, P=0.038) and OS (P=0.002) in resected

NSCLC [27]. However, this result has not been reproduced at the E4592 trial in which *KRAS* mutations were not found to be prognostic for OS [28]. The results of a meta-analysis of resected NSCLC receiving adjuvant chemotherapy corroborate this finding, which showed that *KRAS*-mutant patients had the same prognosis as *KRAS*-wild patients [29]. The results of research exploring the prognostic value of *KRAS* mutations in advanced NSCLC seem to be more consistent. In a meta-analysis of 43 trials that included 5216 patients, *KRAS* mutations as an adverse prognostic factor for OS in advanced lung adenocarcinoma has been confirmed [30]. A recently published meta-analysis including more than 1000 III/IV TNM stage lung adenocarcinoma has also indicated that patients with *KRAS* mutations had a significantly shorter OS and PFS compared to wild-type lung cancer patients. However, in those studies authors did not collect the data of patients' treatment and the predictive role of *KRAS* mutations in such cohort receiving chemotherapy have not been analyzed [31].

The predictive value of *KRAS* mutations in NSCLC also remain uncertain. In a study analyzing the survival outcome according to *KRAS* mutation status in newly diagnosed patients with stage IV NSCLC treated with platinum doublet chemotherapy, *KRAS* mutations are not associated with inferior PFS (6.2m vs. 7.0m, p=0.51) and OS (15.6m vs. 19.0m, p=0.34) [32]. In another study analyzing the 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, *KRAS* mutation was a negative predictive factor of PFS, and patients with *KRAS* G12V mutations exhibited the poorest PFS compared with those with other *KRAS* mutant types [33]. In a meta-analysis including 41 studies, the results demonstrated that *KRAS* mutation is a weak, but valid predictor for chemotherapy in advanced NSCLC [34].

The co-existence of *TP53* mutations was observed in 13 (50.0%) of patients with *KRAS* mutations in our results, which was consistent with previous report. Recently, lung adenocarcinoma with the co-existence of *TP53* mutations and *KRAS* mutations were shown to have higher levels of TMB and inflammation markers, which may be helpful to select patients who will benefit from immune checkpoint blockade and other novel immunotherapy approaches [35, 36]. In our study, we found the similar result: the median TMB was significantly higher in the *KRAS*-mutant group than in the *KRAS*-wild group. In addition, in our current study, one patient with *KRAS* mutation (patient case 025) was included in the clinical trial (NCT02477826) and received 4 cycles of nivolumab plus chemotherapy (pemetrexed and carboplatin) as first-line treatment and later confirmed as an excellent radiographic PR as well as a relative long PFS. This case confirms the previous finding that NSCLC patients with *KRAS* mutation may benefit from checkpoint inhibitors. In a previous research investigating the potential relevant gene expression signatures that predict efficacy of checkpoint blockade, the results demonstrated *TP53* and *KRAS* mutation had remarkable effect on increasing PD_L1 expression, facilitating T cell infiltration and augmenting tumor immunogenicity. And patients with *TP53* and/or *KRAS* mutation showed sensitivity to PD_1 blockade [37]. Due to failure of therapeutic targeting drugs and unsatisfactory response to conventional chemotherapy in *KRAS*-mutant lung adenocarcinoma, it is worth exploring the role of immune checkpoint inhibitor in such cohort. In our future studies, we plan to collect more data from *KRAS*-mutant patients to analyze the TMB information and the value of immunotherapy.

Conclusions

In summary, our findings have several important implications for the molecular characterization and therapeutic outcome of lung adenocarcinoma initiated by oncogenic *KRAS*. First, this study identified *KRAS* mutations in about 16.4% of Chinese patients with lung adenocarcinoma. Due to the large population base and the first incidence of lung cancer in China, it can be anticipated that the number of *KRAS*-mutant lung cancer is considerable, which should be taken seriously in clinical diagnosis and treatment. Second, *KRAS*-mutant patients were associated with lower DCR, shorter PFS and OS than *KRAS*-wild patients receiving first-line chemotherapy. This indicates that new and effective drugs targeting the *KRAS* pathway are in urgent need. Third, the co-existence of *TP53* mutations was observed in half of patients with *KRAS* mutations and the median TMB was significantly higher in the *KRAS*-mutant group than in the *KRAS*-wild group. Therefore, clinical trials enrolling patients with *KRAS*-mutant NSCLC should take into account the co-mutation status of individual tumors.

This emphasizes the significance of comprehensive genomic profiling in assessing patients with NSCLC.

Acknowledgements

This work was supported by National Natural Science Foundation of China (Grant No.81702248).

Competing Interests

The authors have declared that no competing interest exists.

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