

# The prevalence and prognostic significance of *KRAS* mutation subtypes in lung adenocarcinomas from Chinese populations

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**Background:** We performed this retrospective study to identify the prevalence of *KRAS* mutation in Chinese populations and make a comprehensive investigation of the clinicopathological features of *KRAS* mutation in these patients.

**Patients and methods:** Patients from 2007 to 2013 diagnosed with primary lung adenocarcinoma who received a radical resection were examined for *KRAS*, *EGFR*, *HER2*, *BRAF* mutations, and *ALK*, *RET*, and *ROS1* fusions. Clinicopathological features, including sex, age, tumor-lymph node-metastasis stage, tumor differentiation, smoking status, histological subtypes, and survival information were analyzed.

**Result:** *KRAS* mutation was detected in 113 of 1,368 patients. Nine different subtypes of *KRAS* mutation were identified in codon 12, codon 13, and codon 61. *KRAS* mutation was more frequently found in male patients and former/current smoker patients. Tumors with *KRAS* mutation had poorer differentiation. Invasive mucinous adenocarcinoma predominant and solid predominant subtypes were more frequent in *KRAS* mutant patients. No statistical significance was found in relapse-free survival or overall survival between patients with *KRAS* mutation and patients with other mutations.

**Conclusion:** In Chinese populations, we identified *KRAS* mutation in 8.3% (113/1,368) of the patients with lung adenocarcinoma. *KRAS* mutation defines a molecular subset of lung adenocarcinoma with unique clinicopathological features.

**Keywords:** *KRAS*, NSCLC, surgery, prognosis

## Introduction

Lung cancer remains the leading cause of cancer deaths worldwide.<sup>1</sup> In the past few decades, lung cancer was binary classified into small-cell lung cancer and non-small-cell lung cancer (NSCLC). Nowadays, however, the discovery of the driver mutations and related target therapies has changed the way scientists and doctors are treating this disease. The 5-year survival rate of lung cancer remains low (only 16.8%) when taking all stages into account;<sup>2</sup> some selected patients still benefit a lot from targeted therapy.<sup>3-6</sup>

*KRAS* protein functions as a GTPase that is essential for cell signaling. When extracellular stimuli, such as epidermal growth factor (EGF), activates the *KRAS* protein; the activated *KRAS* subsequently binds to a spectrum of downstream effector targets, and performs the signal transduction.<sup>7</sup> Mutant *KRAS* protein, however, is stimulus independent. It can persistently activate the downstream effectors, such as the RAF-MEK-ERK cascade and PI3K-AKT-mTOR pathway, regardless of the absence of the upstream stimuli, and ultimately impact cell proliferation, survival, and metastasis.<sup>8</sup>

In NSCLC, the prevalence of *KRAS* mutation is 15%–32% according to the latest data.<sup>9</sup> The mutation frequency varies among different ethnic populations. A relatively low frequency is identified in Asian and Latin America populations (15%–20%) compared with European population (20%–30%).<sup>10</sup> This imbalance may partially attribute to the high frequency of *EGFR* mutation in Asian and Latin America populations, which are mutually exclusive with *KRAS* mutation, or other associated risk factors, such as tobacco use. Mutant *KRAS* gene is predominantly found in adenocarcinoma histology. In squamous histology tumors, it is a rare event. Many studies suggest that *KRAS* mutation is closely associated with cigarette smoking.<sup>11</sup> Current or former smokers have a higher frequency of *KRAS* mutations than never smokers.<sup>11</sup> Further analysis reveals that different smoking status leads to a different *KRAS* point mutation profile. 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;  $P<0.0001$ ).<sup>12</sup>

In 1990, Slebos et al first reported that *KRAS* codon 12 point mutation was a strong and unfavorable prognostic factor based on an analysis of 69 lung adenocarcinoma patients.<sup>13</sup> Since then, controversy remains on its prognostic significance for predicting the survival time in lung cancer. In 2005, a systematic review and meta-analysis revealed that *KRAS* mutation was of poor prognostic significance for survival in patients with NSCLC. Recently, a meta-analysis of 12 randomized trials confirmed that *KRAS* mutation was associated with poor prognosis for NSCLC, particularly for those with advanced stage disease or who received second or later line therapy or treated with EGFR TKIs.<sup>14</sup>

Although many studies analyzed the prevalence, clinicopathological features, and prognosis of *KRAS* mutation, contradictory conclusions have appeared and controversy remains.<sup>9–11</sup> Besides, data from Asian population were insufficient. Therefore, here, we performed the largest retrospective study of *KRAS* mutation in patients with lung adenocarcinoma in an Asian ethnic group – to identify the prevalence of *KRAS* mutation in Chinese population, describe the clinicopathological features of these patients, and analyze their survival with other types of gene mutations.

## Methods

### Patients and samples

Patients were selected retrospectively at the Department of Thoracic Surgery, Fudan University Shanghai Cancer Center from 2007 to 2013. Patients who received neoadjuvant chemotherapy were excluded. All the qualified patients

had primary lung cancer and received a radical resection. (Patients with stage I to stage III underwent complete surgical excision. As for stage IV patients, all of them had solitary or surgically resectable metastasis. Both the primary lesion and the metastasis were surgically removed through one or two surgeries). Two independent pathologists (Yuan Li and Xuxia Shen) pathologically confirmed their tumor samples as lung adenocarcinoma according to the new WHO classification of lung tumors. Tumor tissues and normal para-carcinoma tissues were sampled just after the surgical resection and immediately stored in liquid nitrogen.

This study was conducted in line with the Declaration of Helsinki and approved by the Institutional Review Board of the Fudan University Shanghai Cancer Center. Written informed consent was obtained from all patients to allow their biological samples to be genetically analyzed. The experimental protocol of this study was performed strictly in accordance to the guidelines.

### RNA extraction

Total RNA was extracted from the tumor and normal para-carcinoma tissue as per standard protocols (RNeasy Mini Kit, Qiagen, Hilden, Germany), respectively. Total RNA samples were reverse transcribed into complementary DNA using a Revert Aid First Strand cDNA Synthesis Kit (Fermentas, St Leon-Rot, Germany).

### Mutation analysis

*KRAS* (exons 2–3), *EGFR* (exons 18–22), *HER2* (exons 18–21), and *BRAF* (exons 11–15) were amplified by polymerase chain reaction (PCR) using KOD-plus DNA polymerase and cDNAs.<sup>15</sup> (The primers are listed in Table S1). Direct dideoxynucleotide method sequencing was performed to analyze the gene mutations. A combined strategy of reverse transcriptase PCR and quantitative real-time PCR was performed to detect *ALK*, *RET*, and *ROS1* fusions. Fluorescent in situ hybridization was used as a validation for these fusion genes.<sup>16–18</sup>

### Clinicopathological features

Clinical information including sex, age at diagnosis, pathologic tumor–lymph node–metastasis stage (according to the seventh edition of the lung cancer staging classification system) tumor differentiation, and smoking status was collected. Histologic subtypes of adenocarcinoma were confirmed based on the newest International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society multidisciplinary classification of lung adenocarcinoma. Survival and tumor relapse information was collected every 3 or 6 months after surgery in the clinic or by telephone.

## Statistical analysis

Pearson's chi-squared test or Fisher's exact test (when the count in any cell of a contingency table was less than required) was used on categorical variables. The Kaplan–Meier method was performed to estimate the survival curve. Log-rank test was performed to compare the survival data. Multivariate analysis (Cox regression analysis) was used to assess the effect of covariates on relapse-free survival (RFS) and overall survival. We used SPSS for Windows (version 19.0) (IBM Corporation, Armonk, NY, USA) to analyze data. All tests were two-tailed and a *P*-value <0.05 indicated statistical significance.

## Results

### Clinicopathological features

A total of 1,368 patients from 2007 to 2013 were qualified in this retrospective study cohort. The average age was 59.7 years, ranging from 22.4 to 84.1 years. There were

623 (45.5%) males and 745 (54.5%) females. Of them, 369 were current smokers (27.0%), 79 were former smokers (5.8%), and 920 were never smokers (67.2%). The numbers of patients in pathological tumor–lymph node–metastasis stages I to IV were 749 (54.8%), 167 (12.2%), 425 (31.0%), and 27 (2.0%), respectively. In adenocarcinoma subtypes, acinar predominant (43.1%) was the most frequent, followed by solid predominant (18.6%), and papillary predominant (15.6%). More detailed clinicopathological features are summarized in Table 1.

### Gene mutation spectrum

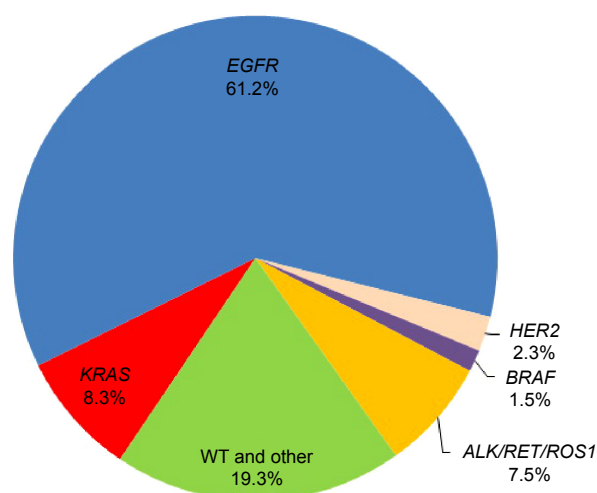
There were 837 patients who harbored *EGFR* mutation, accounting for approximately 60% of the population. *KRAS* mutation was detected in 113 (8.3%) of all the patients. Thirty-two (2.3%) patients had *HER2* mutation, 20 (1.5%) patients had *BRAF* mutation, and 102 (7.5%) patients had *ALK*, *RET*, or *ROS1* fusion genes (Figure 1). No patients

**Table 1** Clinicopathological features of 1,368 lung adenocarcinoma patients

| Variables             | KRAS       | EGFR        | HER2      | BRAF      | ALK/RET/ROS1 | WT and others |
|-----------------------|------------|-------------|-----------|-----------|--------------|---------------|
| Mutation type, n (%)  | 113 (8.26) | 837 (61.18) | 32 (2.34) | 20 (1.46) | 102 (7.46)   | 264 (19.30)   |
| Age, years            |            |             |           |           |              |               |
| <60, n (%)            | 63 (56)    | 414 (49)    | 23 (72)   | 11 (55)   | 64 (73)      | 116 (44)      |
| ≥60, n (%)            | 50 (44)    | 423 (51)    | 9 (28)    | 9 (45)    | 38 (27)      | 148 (56)      |
| Mean                  | 59.46      | 59.95       | 54.43     | 57.76     | 56.66        | 60.87         |
| Standard deviation    | 9.04       | 9.96        | 9.85      | 9.47      | 9.81         | 10.72         |
| Sex                   |            |             |           |           |              |               |
| Male, n (%)           | 90 (80)    | 303 (36)    | 2 (6)     | 12 (60)   | 39 (6)       | 177 (67)      |
| Female, n (%)         | 23 (20)    | 534 (64)    | 30 (94)   | 8 (40)    | 63 (94)      | 87 (33)       |
| Smoker                |            |             |           |           |              |               |
| Never, n (%)          | 34 (30)    | 657 (78)    | 32 (100)  | 8 (40)    | 75 (100)     | 114 (43)      |
| Current/former, n (%) | 79 (70)    | 180 (22)    | 0 (–)     | 12 (60)   | 27 (–)       | 150 (57)      |
| Differentiation       |            |             |           |           |              |               |
| Well, n (%)           | 11 (10)    | 131 (16)    | 9 (28)    | 4 (20)    | 7 (27)       | 30 (11)       |
| Moderate, n (%)       | 52 (46)    | 494 (59)    | 14 (44)   | 8 (40)    | 55 (46)      | 102 (39)      |
| Poor, n (%)           | 50 (44)    | 212 (25)    | 9 (28)    | 8 (40)    | 40 (27)      | 132 (50)      |
| Pathological stage    |            |             |           |           |              |               |
| I, n (%)              | 56 (50)    | 496 (59)    | 20 (63)   | 11 (55)   | 39 (61)      | 127 (48)      |
| II, n (%)             | 22 (19)    | 90 (11)     | 1 (3)     | 2 (10)    | 16 (3)       | 36 (14)       |
| III, n (%)            | 33 (29)    | 235 (28)    | 11 (34)   | 7 (35)    | 42 (33)      | 97 (37)       |
| IV, n (%)             | 2 (2)      | 16 (2)      | 0 (–)     | 0 (–)     | 5 (3)        | 4 (2)         |
| Pathological subtype  |            |             |           |           |              |               |
| AIS, n (%)            | 0 (–)      | 15 (1.8)    | 3 (9.4)   | 0 (–)     | 1 (1.0)      | 9 (3.4)       |
| MIA, n (%)            | 2 (1.8)    | 25 (3.0)    | 2 (6.3)   | 0 (–)     | 0 (–)        | 4 (1.5)       |
| Lepidic, n (%)        | 5 (4.4)    | 92 (11.0)   | 1 (3.1)   | 3 (15.0)  | 3 (2.9)      | 17 (6.4)      |
| Acinar, n (%)         | 34 (30.1)  | 430 (51.4)  | 13 (40.6) | 5 (25.0)  | 28 (27.5)    | 79 (30.0)     |
| Papillary, n (%)      | 14 (12.4)  | 139 (16.6)  | 4 (12.5)  | 3 (15.0)  | 18 (17.6)    | 35 (13.3)     |
| Micropapillary, n (%) | 0 (–)      | 18 (2.2)    | 0 (–)     | 0 (–)     | 1 (1.0)      | 2 (0.8)       |
| Solid, n (%)          | 34 (30.1)  | 90 (10.8)   | 4 (12.5)  | 5 (25.0)  | 30 (29.4)    | 91 (34.5)     |
| IMA, n (%)            | 23 (20.4)  | 18 (2.2)    | 5 (15.6)  | 2 (10.0)  | 19 (18.6)    | 15 (5.7)      |
| Other, n (%)          | 1 (0.9)    | 10 (1.2)    | 0 (–)     | 2 (10.0)  | 2 (2.0)      | 12 (4.5)      |

**Note:** Other includes enteric subtype and unknown.

**Abbreviations:** AIS, adenocarcinoma in situ; IMA, invasive mucinous adenocarcinoma; MIA, minimally invasive adenocarcinoma; WT, wild type.

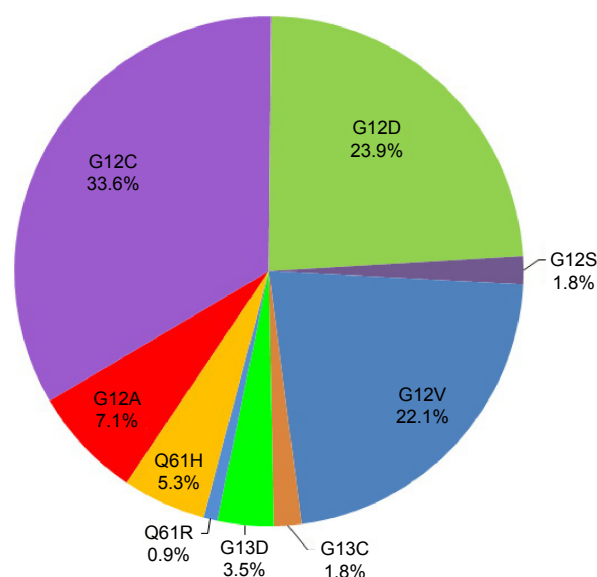


**Figure 1** Frequency of gene mutations in 1,368 patients with lung adenocarcinoma. Abbreviation: WT, wild type.

with *KRAS* mutations had a concomitant mutation in *EGFR*, *HER2*, *BRAF*, or *ALK/RET/ROS1*.

### *KRAS* mutation subtypes

Nine different subtypes of *KRAS* mutation were identified, including five types of G12\* mutations, two types of G13\* mutations, and two types of Q61\* mutations. G12C (GGT→TGT) was the most frequent amino acid substitution seen in this cohort, accounting for 33.6% of the patients followed by G12D (GGT→GAT) mutation (23.9%), G12V (GGT→GTT) mutation (22.1%), and G12A (GGT→GCT) mutation (7.1%). Seventy percent of patients (79/113) harbored transversion mutations (G12A, G12C, G12V, G13C, Q61H) (Figure 2).



**Figure 2** Frequency of *KRAS* mutation subtypes in 113 *KRAS* mutation patients.

### *KRAS* mutation and clinicopathological variables

In this study, *KRAS* mutation was more frequently found in male patients (odds ratio 5.30; 95% confidence interval (CI) 3.31–8.49;  $P<0.001$ ) and former/current smoke patients (odds ratio 5.58; 95% CI 3.67–8.49;  $P<0.001$ ). Tumors with *KRAS* mutation were poorly differentiated (odds ratio 1.69; 95% CI 1.15–2.50;  $P=0.008$ ). Among all the adenocarcinoma subtypes, invasive mucinous adenocarcinoma predominant (20.4% vs 4.7%,  $P<0.001$ ) and solid predominant (30.1% vs 17.5%,  $P=0.004$ ) were more frequent in *KRAS* mutant patients compared with *KRAS* wide-type patients (Table 2).

### Survival analysis

In all, 1,131 patients were involved in survival analysis, including 108 patients harboring *KRAS* mutation. Follow-up started from October 2007 to June 2015.

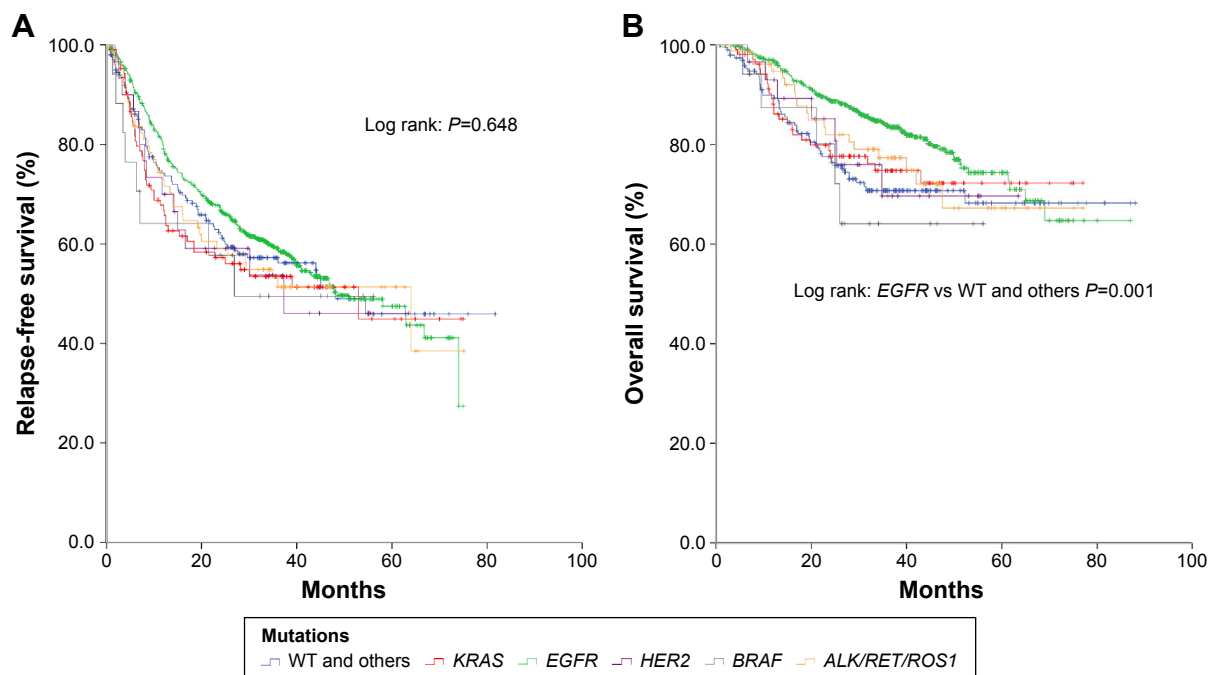
The follow-up time ranged from 1 to 88 months. The median RFS for all patients and *KRAS* mutant patients was 49 months (95% CI 40–57 months) and 53 months (95% CI 16–90 months), respectively. No statistical significance was found in RFS between patients with *KRAS* mutation

**Table 2** Features of patients with lung adenocarcinoma harboring *KRAS* mutation

|                    | <i>KRAS</i> mutation<br>(n=113) |    | <i>KRAS</i> WT<br>(n=1,255) |    | P-value     |
|--------------------|---------------------------------|----|-----------------------------|----|-------------|
|                    | n                               | %  | n                           | %  |             |
| Sex                |                                 |    |                             |    | $P<0.001^*$ |
| Male               | 90                              | 80 | 533                         | 42 |             |
| Female             | 23                              | 20 | 722                         | 58 |             |
| Age                |                                 |    |                             |    | $P=0.245$   |
| <60 years          | 63                              | 56 | 628                         | 50 |             |
| ≥60 years          | 50                              | 44 | 627                         | 50 |             |
| Smoker             |                                 |    |                             |    | $P<0.001^*$ |
| Never              | 34                              | 30 | 886                         | 71 |             |
| Former/current     | 79                              | 70 | 369                         | 29 |             |
| Differentiation    |                                 |    |                             |    | $P=0.008^*$ |
| Well/moderate      | 63                              | 56 | 854                         | 68 |             |
| Poor               | 50                              | 44 | 401                         | 32 |             |
| Pathological stage |                                 |    |                             |    | $P=0.626$   |
| I/II               | 78                              | 69 | 838                         | 67 |             |
| III/IV             | 35                              | 31 | 417                         | 33 |             |
| IMA predominant    |                                 |    |                             |    | $P<0.001^*$ |
| Yes                | 23                              | 20 | 59                          | 5  |             |
| No                 | 90                              | 80 | 1,196                       | 95 |             |
| Solid predominant  |                                 |    |                             |    | $P=0.004^*$ |
| Yes                | 34                              | 30 | 220                         | 18 |             |
| No                 | 79                              | 70 | 1,035                       | 82 |             |

**Note:** \*Indicates statistical significance.

**Abbreviations:** IMA, invasive mucinous adenocarcinoma; WT, wild type.



**Figure 3** Relapse-free survival (A) and overall survival (B) in all patients.

**Note:** Kaplan–Meier curve for relapse-free survival and overall survival in 1,131 patients with lung adenocarcinoma.

**Abbreviation:** WT, wild type.

and patients with other mutations (Figure 3A). Patients with *EGFR* mutation lived longer than mutation wide type patients ( $P=0.001$ ). No statistical significance was found between patients with *KRAS* mutation and patients with other mutations in overall survival (Figure 3B). In multivariate analysis, former/current smoke patients (hazard ratio [HR] 1.32; 95% CI 1.09–1.58;  $P=0.004$ ), advanced stage (HR 3.36; 95% CI 2.76–4.08;  $P<0.001$ ), and poor differentiation in tumor (HR 3.07; 95% CI 2.09–4.50;  $P<0.001$ ) were risk factors in recurrence. The occurrence of *KRAS* mutation in patients did not mean an unfavorable prognosis; nevertheless, male patients, patients with advanced stage, or patients with poor differentiation in tumor lived shorter than others (Table 3).

### Survival analysis of *KRAS* subtypes

In 108 qualified patients with *KRAS* mutation, the median RFS was 53 months, while the median overall survival was not reached. No significant difference was found in survival among patients with different *KRAS* point mutations. Sex (Figure 4A and B), age (Figure 4C and D), and smoking status (Figure 4E and F) did not affect survival time either. Those patients with advanced stage or whose tumor was poorly differentiated got an earlier recurrence and shorter survival (Table 3). Mutation sites in *KRAS* did not affect the prognosis (Figure 5A and B). There was no difference in survival between patients with *KRAS* transition mutations and patients with *KRAS* transversion mutations (Figure 5C and D). When the *KRAS* mutation was divided into two groups (hydrophobic

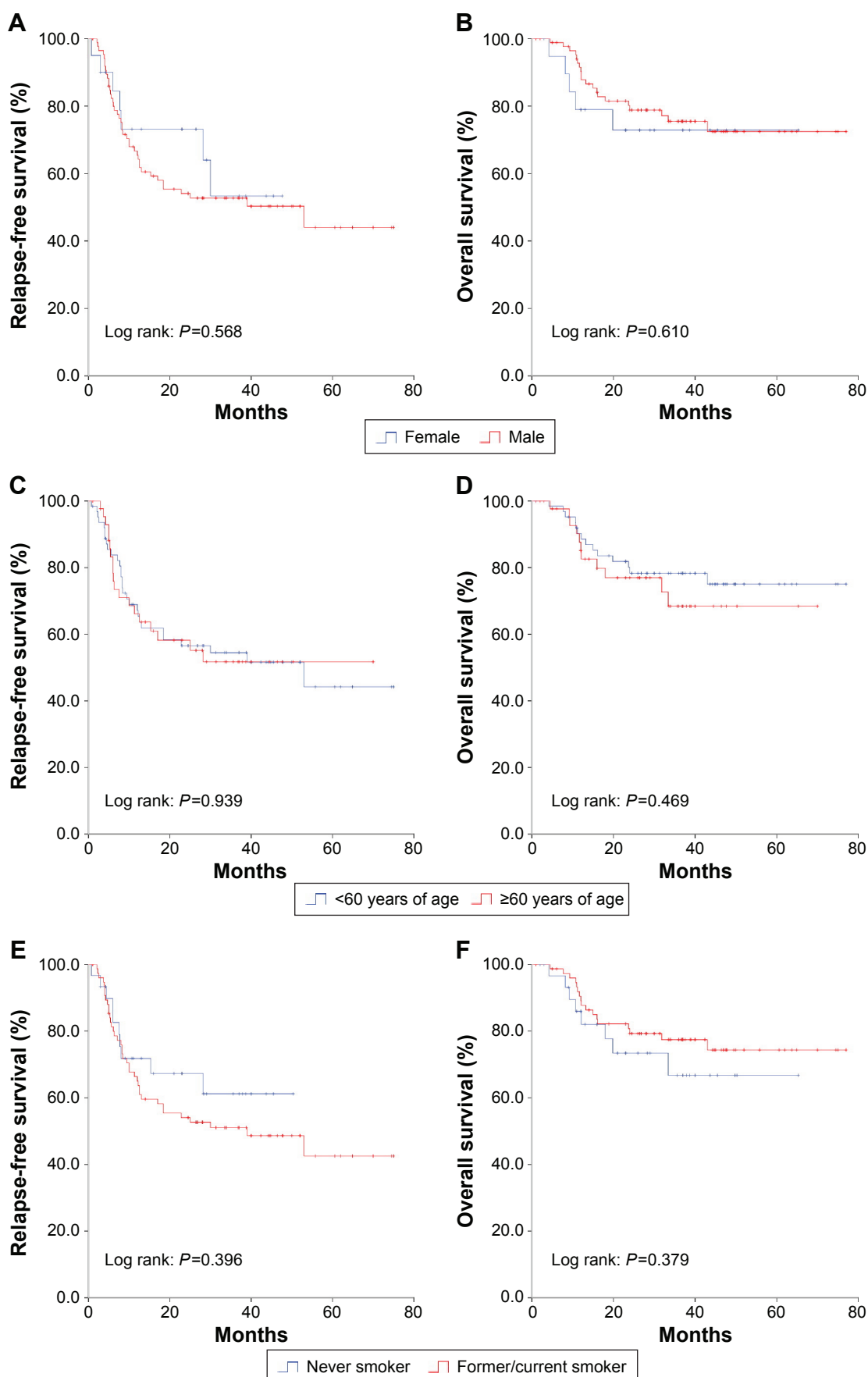
**Table 3** Univariate and multivariate survival analysis of 1,131 patients with lung adenocarcinoma

| Variable           | Category      | RFS analysis |             |         |              |             |         | OS analysis |              |         |              |              |         |
|--------------------|---------------|--------------|-------------|---------|--------------|-------------|---------|-------------|--------------|---------|--------------|--------------|---------|
|                    |               | Univariate   |             |         | Multivariate |             |         | Univariate  |              |         | Multivariate |              |         |
|                    |               | HR           | 95% CI      | P-value | HR           | 95% CI      | P-value | HR          | 95% CI       | P-value | HR           | 95% CI       | P-value |
| Age, years         | ≥60/<60       | 1.03         | (0.86–1.23) | 0.77    | NA           | NA          | NA      | 1.18        | (0.91–1.53)  | 0.219   | 1.30         | (0.99–1.69)  | 0.052   |
| Sex                | Male/female   | 1.60         | (1.33–1.91) | <0.001* | NA           | NA          | NA      | 1.77        | (1.36–2.30)  | <0.001* | 1.34         | (1.02–1.75)  | <0.033* |
| Smoker             | Yes/no        | 1.76         | (1.47–2.11) | <0.001* | 1.32         | (1.09–1.58) | 0.004*  | 1.75        | (1.34–2.27)  | <0.001* | NA           | NA           | NA      |
| Pathological stage | III, IV/I, II | 4.31         | (3.59–5.17) | <0.001* | 3.36         | (2.76–4.08) | <0.001* | 4.06        | (3.11–5.31)  | <0.001* | 2.94         | (2.21–3.92)  | <0.001* |
| Differentiation    | Moderate/well | 2.86         | (2.00–4.09) | <0.001* | 2.24         | (1.56–3.22) | <0.001* | 3.73        | (1.95–7.15)  | <0.001* | 2.78         | (1.44–5.37)  | 0.002*  |
|                    | Poor/well     | 5.91         | (4.10–8.51) | <0.001* | 3.07         | (2.09–4.50) | <0.001* | 9.94        | (5.21–18.99) | <0.001* | 5.17         | (2.64–10.12) | <0.001* |
| KRAS mutation      | Mutation/WT   | 1.18         | (0.88–1.60) | 0.266   | NA           | NA          | NA      | 1.26        | (0.83–1.92)  | 0.271   | NA           | NA           | NA      |

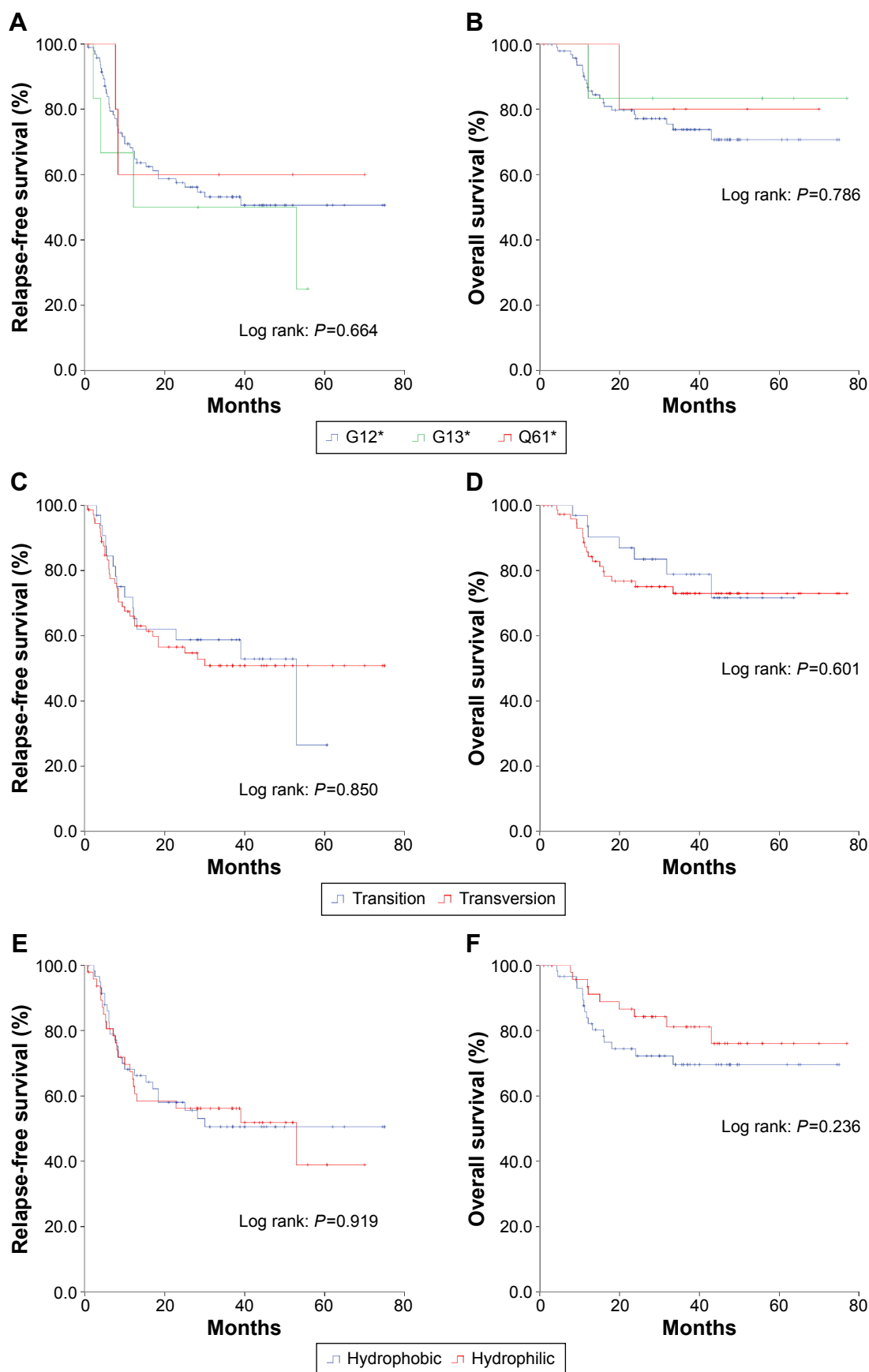
**Note:** \*Indicates statistical significance.

**Abbreviations:** CI, confidence interval; HR, hazard ratio; NA, not applicable; OS, overall survival; RFS, relapse-free survival; WT, wild type.





**Figure 4** Relapse-free survival and overall survival of sex (**A** and **B**), age (**C** and **D**), and smoking status (**E** and **F**) in KRAS mutation patients.



**Figure 5** Relapse-free survival and overall survival of codon (A and B), transition/transversion (C and D), and hydrophobic/hydrophilic (E and F) subtypes in KRAS mutation patients.

alterations [G12C and G12V] and hydrophilic alterations [such as G12D]), no significant difference was found in either RFS or overall survival (Figure 5E and F). Multivariate analysis revealed that advanced stage was the only risk factor that indicated shorter recurrence (HR 2.66; 95% CI 1.51–4.70;  $P=0.001$ ), and smoking status, sex, age, or different *KRAS* subtypes did not affect the outcome (Table 3).

## Discussion

In the present study, *KRAS* mutation was detected in 8.3% (113/1,368) of lung adenocarcinoma patients in Chinese population. This number was relatively lower compared with the data from Asian population. European population, however, had a much higher mutation rate of *KRAS* gene.<sup>9,10</sup> Besides, *KRAS* mutation was predominantly found in male patients and smokers. *KRAS* mutation and other driver mutations, such as *EGFR*, *HER2*, and *BRAF*, were mutually exclusive. Poor differentiation appeared in almost half of the tumors with *KRAS* mutation, compared with less than a third of the non-*KRAS* mutant tumors. This suggested that *KRAS* mutation probably led to an unfavorable prognosis. Meanwhile, the proportion of invasive mucinous adenocarcinoma predominant subtype and solid predominant subtype was significantly higher in *KRAS* mutation. Considering these two subtypes, especially the solid predominant subtype as indicators of poor prognosis, *KRAS* mutation might be a late event in lung cancer. Keohavong et al<sup>19</sup> reported that specific *KRAS* point mutations (such as G12V) were associated with poorer outcome. Recently, a meta-analysis which synthesized 12 randomized trials also confirmed that *KRAS* mutations were related to poor survival benefit for NSCLC.<sup>14</sup> Our data, however, did not show this pattern in survival analysis.

*KRAS* point mutation in human cancers takes place primarily at residues G12, G13, or Q61, with single amino acid substitution. In lung cancer, *KRAS* mutation occurs predominantly at residues G12 or G13.<sup>7,9,12</sup> Mutations can be further divided into transversion and transition subtypes according to its biological features and prognostic impact. Riely's discovery of different smoking status leading to a different *KRAS* point mutation profile suggested that there might be an explicit mechanism of tobacco carcinogens on *KRAS* mutation.<sup>12</sup> Conflicting data also arouse the suspicion on cigarette smoke.<sup>20</sup> In animal experiments, scientists revealed quite a similar pattern in lung tumors from mice treated with BaP, 5-methylchrysene, and benzo[b] fluoranthene, which are carcinogens commonly found in tobacco smoke.<sup>21</sup> However, detailed analysis showed that

the methylguanine pathway of nicotine-derived nitrosamine ketone, also known as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) metabolic results in a high percentage of GGT→GAT mutations (transition) in codon 12 of *KRAS*,<sup>22</sup> while pyridyloxobutylation leads to more G→T mutation (transversion) in codon 12.<sup>23</sup> The relative significance of these pathways in human lung cells remains unknown, and further investigations are needed.

Remarkably, Ihle et al<sup>24</sup> classified *KRAS* mutation into hydrophobic (G12C and G12V) and hydrophilic alteration (G12D) subgroups. In vitro experiments demonstrated different patterns of downstream signal transduction and response to targeted therapies between these two subgroups. Specifically, mutant G12C or G12V *KRAS* protein would activate Ral signaling and decrease growth factor-dependent Akt activation, while mutant G12D *KRAS* protein would predominantly activate the PI3K and MEK/ERK pathways. Such results could be partially explained by the changes in spatial conformation after mutation. Finally, tumor behavior and drug sensitivity varied in different subgroups of *KRAS* mutation.<sup>25</sup> Although in general, *KRAS* codon 12 point mutation was proven to indicate a poor prognosis, a more specific classification was required to assess its biological malignancy and therapeutic efficacy. In clinical practice, individualized treatment was recommended concerning patients harboring *KRAS* mutation.

Nowadays, scientists also focus on *KRAS* mutation co-occurring with other gene mutations. Lung adenocarcinomas harboring *STK11/LKB1*, *TP53*, or *CDKN2A/B* mutation as well as *KRAS* mutation identified distinct clinicopathological features and different therapeutic responses.<sup>26</sup> This insight would direct the treatment strategy not only pointing at a single *KRAS* gene, but a set of related genes.

There are no known direct *KRAS* targeted agents. Survival of patients harboring *KRAS* mutation is almost disappointing with or without chemotherapy, targeted therapy, or other treatment. Some researchers came to a conclusion that *KRAS* status might indicate poor response to *EGFR* TKIs.<sup>27–31</sup> This evidence should be considered with caution due to the small numbers involved in the analyses. Meanwhile, meta-analysis showed that *KRAS* mutations are highly specific negative predictors of response to single-agent *EGFR* TKIs in advanced NSCLC.<sup>32</sup> Limited success was developed on the treatment of *KRAS* mutation in the past decade; however, delightful consequences emerged.

A Phase II randomized trial showed that selumetinib plus docetaxel had promising efficacy, albeit with a higher number of adverse events than with docetaxel alone, in previously



treated advanced *KRAS*-mutant NSCLC.<sup>33</sup> Patients in the experiment group had a longer progression-free survival and 37% of them had an objective response.

By blocking one of the most important downstream pathways of *KRAS*, an MEK1/2 inhibitor (AZD6244) combined with cisplatin showed an antitumor effect in *KRAS*-dependent lung cancer cells and animal models.<sup>34</sup> Clinical studies of applications of MEK1/2 inhibitors in cisplatin-based chemotherapy for lung cancer patients harboring *KRAS* mutation are urgently required.

Ostrem et al<sup>35</sup> developed a small molecule that irreversibly bound to a common oncogenic mutant, K-Ras (G12C), while it had no effect on the wild-type protein. Once bound, the inhibitor-*KRAS* compound subverted the native nucleotide preference to favor GDP over GTP and therefore impaired binding to the downstream Raf protein. If it is possible to make this molecule available in clinics in the future, it will make *KRAS* mutation targetable and may bring benefit to patients.

Furthermore, oncogenic drivers, such as mutated *KRAS* can be targeted with synthetic lethality approaches.<sup>36</sup> Although synthetic lethality till now is a concept and has not yet been applied in clinical practice, it is a promising rationale and will ultimately become a therapeutic approach.

## Conclusion

In Chinese population, we identified *KRAS* mutation in 8.3% (113/1,368) of the patients with lung adenocarcinoma. *KRAS* mutation defines a molecular subset of lung adenocarcinoma with unique clinicopathological features. *KRAS* mutations were more frequent in male patients, former/current smokers, and patients harboring *KRAS* mutations who showed poor differentiation in tumor tissues.

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## Author contributions

Haiquan Chen is the guarantor of the manuscript and contributed to conception and study design, analysis of data, and review and revision of the manuscript. Difan Zheng, Rui Wang, and Yang Zhang contributed to conception and study design, acquisition and analysis of

data, and writing and revision of the manuscript. Yunjian Pan and Xinghua Cheng contributed to acquisition of data and writing and revision of the manuscript. Chao Cheng and Xuxia Shen contributed to analysis of data and revision of the manuscript. Shanbo Zheng, Hang Li, Ranxia Gong, and Yuan Li: contributed to acquisition of data and revision of the manuscript. Yihua Sun contributed to conception and study design, and review and revision of the manuscript. All authors gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

## Disclosure

The authors report no conflicts of interest in this work.

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## Supplementary material

**Table S1** Primers used in this study

|  |  |   |
|--|--|---|
| Primers for detecting <i>KRAS</i> mutation |  |   |
| Target<br><i>KRAS</i> exon 2–3             | Forward primer (5'→3')<br>AGGCCTGCTGAAAATGACTG   | Reverse primer (5'→3')<br>TGGTGAATATCTTCAAATGATTAGT |
| Primers for detecting <i>EGFR</i> mutation |  |   |
| Target<br><i>EGFR</i> exon 18–22           | Forward primer (5'→3')<br>TGAAGGCTGTCCAACGAATG   | Reverse primer (5'→3')<br>AGGCGTTCTCCTTTCTCCAG      |
| Primers for detecting <i>HER2</i> mutation |  |   |
| Target<br><i>HER2</i> exon 18–21           | Forward primer (5'→3')<br>CCCTCTGACGTCCATCATCT   | Reverse primer (5'→3')<br>GCAGGGTCTGGACTGAAGAA      |
| Primers for detecting <i>BRAF</i> mutation |  |   |
| Target<br><i>BRAF</i> exon 11–15           | Forward primer (5'→3')<br>TCAGAAGACAGGAATCGAATGA | Reverse primer (5'→3')<br>GATGACTTCTGGTGCCATCC      |

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