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ORIGINAL RESEARCH

The Prevalence and Concurrent Pathogenic Mutations of *KRAS*^{G12C} in Northeast Chinese Non-small-cell Lung Cancer Patients

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Objective: *KRAS* mutation is one of important driver genes in non-small-cell lung cancer (NSCLC) and the patients with *KRAS*^{G12C} mutations benefit from the inhibitor AMG510. However, the frequency, concurrent pathogenic mutations, and clinical characteristic of *KRAS*^{G12C} is unknown in the NSCLC population of Northeast China.

Methods: The retrospective analysis was derived from 431 NSCLC patients in Jilin Cancer Hospital between January 2018 and June 2019. The mutation frequency and concurrent mutations of *KRAS*^{G12C} in tumor or peripheral blood was detected by next-generation sequencing (NGS).

Results: The *RAS* mutant rate was observed in 10.7% (46/431) of this cohort. All *RAS*-driver cancers are caused by mutations in the *KRAS* isoform, while the *NRAS* and *HRAS* isoforms were not detected. Among *KRAS*-mutant patients, 42 (91.3%) showed exon 2 mutation in 12 codon and 13 codon. *KRAS*^{G12C} showed a 4.6% (20/431) mutation rate in this cohort and the highest frequency (43.5%, 20/46) in *KRAS*-mutant-positive patients. There was no difference between tumor tissue and plasma in terms of either *KRAS* or *KRAS*^{G12C} mutation. The most frequent co-occurrence mutations with *KRAS*^{G12C} were *TP53*, followed by *PTEN*. Furthermore, *KRAS*^{G12C} was exclusive with *STK11* mutation. *KRAS*^{G12C} mutation was associated with age, disease stage, and smoking status (P=0.024; P=0.02; P=0.006), smoking remained an independent factor for *KRAS*^{G12C} mutation (P=0.037), and higher mutation frequency in patients older than 60, stage I–III, or smoking in NSCLC (P=0.0151, P=0.0343, P=0.0046, respectively).

Conclusion: *KRAS* mutation was the only isoforms of *RAS* family, of these 43.5% harbored the *KRAS*^{G12C} subtype in northeastern Chinese NSCLC patients. *KRAS*^{G12C} is associated with age, pathological stage and smoking status, more commonly harbored *TP53*/*PTEN* mutations, and providing more genome profile for targeted therapy in local clinical practice.

Keywords: next-generation sequencing, non-small-cell lung cancer, *KRAS*^{G12C}, tissue, plasma, mutations

Introduction

Non-small-cell lung cancer (NSCLC) is the most common histological type of lung cancer, accounting for 80–85% of lung cancers and has become the most fatal cancer in the world.[1] Recently, targeted therapy based on various driver oncogene variants (*EGFR*, *ALK* and *ROS1*, *KRAS*, *MET*, *PIK3CA*, *RET*, *BRAF*) has shown great antitumor activity; unfortunately, *KRAS* mutations had a more complicated mechanism in comparison with other driver genes such as *EGFR*, with poor prognosis and high risk of tumor recurrence.[2] Although prevalent, no specific treatment has been successfully developed for these NSCLCs.

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KRAS mutations are some of the most prevalent alterations, approximately 10% of Asian NSCLC patients and 7.5% of Chinese NSCLC patients harbor the *KRAS* mutation, with codon 12 and 13 mutations being the most frequent and the most common subtypes are *G12C*, *G12V* and *G12D*.[3,4] $KRAS^{G12C}$ is a mutant type of *KRAS* guanosine triphosphatase (GTPase), and an inhibitor targeting $KRAS^{G12C}$ is a promising novel tumor-specific therapy for tumors driven by mutant proteins.[5] Current studies on $KRAS^{G12C}$ inhibitors and the mechanism of drug resistance have confirmed that patients with $KRAS^{G12C}$ mutations benefit from the inhibitor AMG510,[6] which has also been approved by the FDA as an orphan drug for NSCLC and colon cancer with $KRAS^{G12C}$ mutation. $KRAS^{G12C}$ can induce allosteric switch II pocket (s-iip) and take cys-12 as the specific covalent target of alleles, which were considered as potential drug targets.[2] Now, $KRAS^{G12C}$ mutation was verified by the NGS, various clinical parameters and genetic mutation have been proposed to predict the relevance with $KRAS^{G12C}$ (such as sex, age, smoking, co-mutation gene). In the current study we aim to discover a more precise delineation of candidate target populations and distinctive $KRAS^{G12C}$ co-mutation subtypes in the northeast Chinese population. We retrospectively investigated and evaluated the $KRAS^{G12C}$ mutation in northeast Chinese NSCLC, and the association between clinical factors and $KRAS^{G12C}$ mutation status.

Materials and Methods

Patients and Samples

Four hundred and thirty-one samples were collected from Jilin Cancer Hospital between January 2018 and June 2019, 268 cases were tested through eight gene panel, 81 cases by 168 gene panel and 82 matched cases using 520 gene panel, respectively (Figure 1). Clinic pathological data were collected from the electronic medical records in Jilin Cancer Hospital, and the factors included age, sex, and clinical stage, smoking history, brain metastasis, PS score and histology. All participants signed the informed consent agreement before participating in the study, the data were anonymized, the study was approved by the Clinical Research Ethics Committee of Jilin Cancer Hospital and was conducted in accordance with the Declaration of Helsinki.

DNA Extraction

DNA was extracted by DNA FFPE tissue kit (AmoyDx, China) and ctDNA extraction kit (QIAGEN, Germany) according to the manufacturer's instructions. DNA concentration was quantified by Nanodrop 3000C and Qubit 4.0 (Thermo Fisher Scientific, Waltham, MA, USA).

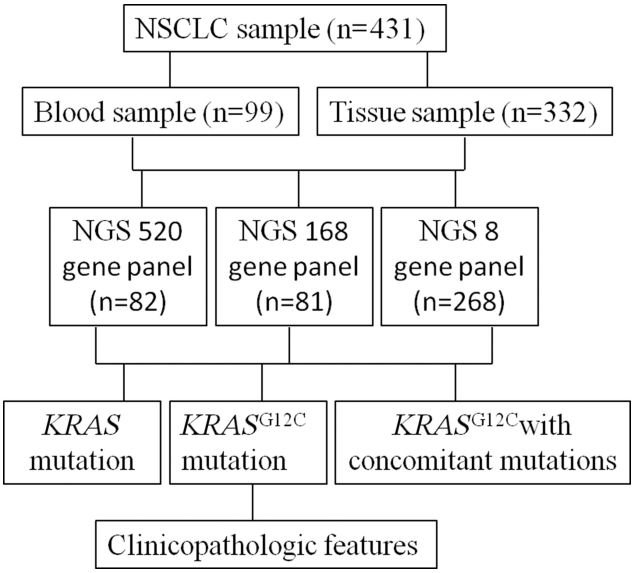

Figure 1 Study flowchart.
Abbreviations: NSCLC, non-small-cell lung cancer; NGS, next-generation sequencing.

Next-generation Sequencing Analysis

Library preparation was performed following manufacturer's protocol (Burning Rock Biotech, Guangzhou, China). DNA Fragments (range: 200–400 bp) were purified by AMPure beads (Beckman Coulter, CA, USA), and captured with probe baits, hybrid selection with magnetic beads by RT-PCR amplification. Subsequently, DNA quality and size were assessed by high-sensitivity DNA assay. Indexed samples were sequenced on a MiSeq system (Beckman Coulter) with paired-end reads. The input of extracted DNA should be in the range of (30−200 ng). Sequencing platform was used by Illumina NextSeq 500 Sequencing Platform with tissue DNA (1000X) and cfDNA (20000X). All samples were analyzed by NGS targeted panel (Burning Rock Dx, China), which eight-gene panel covers well-known lung adenocarcinoma driver genes, 168 genes covers known lung cancer-related genes and 520 genes covers solid tumor-related genes. (Supplemental Table 1).

Statistical Analysis

All data was performed by SPSS Statistics 19.0 software (IBM Corporation, Armonk, NY, USA). Fisher's exact test was used to evaluate mutation differences and clinical factor between $KRAS^{G12C}$ and $KRAS^{wt}$. Logistic regression analysis was used

to identify as independent factors for *KRAS*^{G12C} mutations. A *P*-value of <0.05 was considered statistically significant.

Results

Patient Population

Among 431 samples were those from tumor tissue 332 (77.04%), 99 (22.96%) plasma; 198 women (54.07%) and 233 men (45.93%), with a median age of 63 years (range: 34–86 years), respectively. Of the 431 patients, 263 (61.02%) were smokers, and 168 were nonsmokers. The histological characterization of tumors revealed that 370 samples were adenocarcinoma (85.85%), 61 were squamous cell carcinoma (14.15%). Of the 431 patients, characterization of the pathological stage showed 115 samples in stage I–III (26.68%), and 316 samples in stage IV (73.32%) (Table 1).

KRAS^{G12C} is the Most Common Mutation Type of *KRAS* in NSCLC

The *RAS* mutation rate was 10.7% (46/431), and *KRAS* was the only mutation subtype of *RAS* (*NRAS*, *KRAS*, *HRAS*). 42 (91.3%) indicated *KRAS* gene exon 2 mutation, 12 and 13 codon of *KRAS* gene mutations were detected, and *KRAS*^{G12C} showed the highest frequency, the total mutation rate of *KRAS*^{G12C} in NSCLC was 4.6% (20/431) and 43.5% (20/46) of *KRAS* mutant subtypes, followed by 17.4% (8/46) of *KRAS*^{G12D}, 8.7% (4/46) of *KRAS*^{G12V}, and 8.7% (4/46) of *KRAS*^{G12A}. The mutation frequency of other *KRAS* types was lower (Figure 2).

Table 1 Patient Characteristics

Characteristics	n (%)
Age (years)	63 (34–86)
Sex	
Male	198 (45.93)
Female	233 (54.07)
Stage	
I–III	115 (26.68)
IV	316 (73.32)
Smoking history	
Yes	168 (38.98)
No	263 (61.02)
Brain metastasis	
Yes	106 (24.59)
No	325 (75.41)
PS score	
0–1	365 (84.68)
2–3	66 (15.32)
Histology	
Adenocarcinoma	370 (85.85)
Squamous cell carcinoma	61 (14.15)

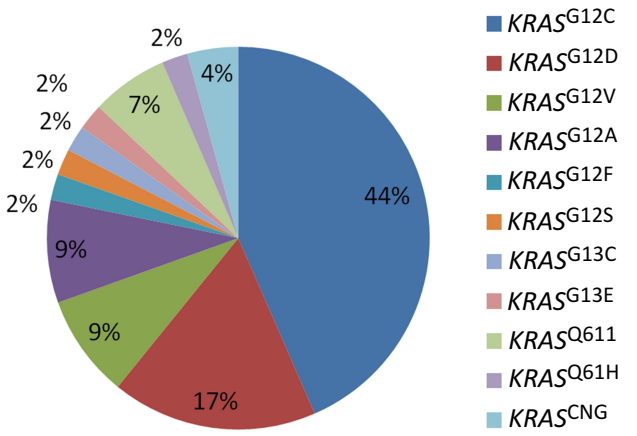

Figure 2 Mutation frequencies of *KRAS* subtypes.

KRAS^{G12C} Mutation Between Tumor Tissue and Plasma

We compared the *KRAS* mutation spectrums between tumor tissue and ctDNA derived from peripheral blood in this study. Collectively, 37 (11.14%) and 16 (4.81%) patients had *KRAS* and *KRAS*^{G12C} mutation spectrum in tumor tissue, nine (9.09%) and four (4.04%) patients in ctDNA, but no significant difference was found in the two sample types (*P*=0.711, *P*=1.000, Table 2), respectively.

Co-occurring Genomic Alterations Between *KRAS*^{G12C} and Lung Cancer Pathogenic Gene

Lung cancer driver genes (include *EGFR*, *RAS*, *ALK*, *ROS1*, *MET*, *RET BRAF,* and *HER-2*) mutation samples were observed in 332 (77.3%) of 431 patients. Eight (40%) of 20 patients harbored only *KRAS*^{G12C}

Table 2 Mutation Frequencies of *KRAS* Subtypes Between Tumor Tissue and Plasma

Sample Type	*KRAS*		*P*	*KRAS*^{G12C}		*P*
	mut	wt		mut	wt	
Tumor tissue	37	295	0.711	16	316	1.000
Plasma	9	90		4	95	
Total	46	385		20	411	

mutations, and 12 (60%) had multiple $KRAS^{G12C}$ mutations, including eight (40%) $KRAS^{G12C}$ patients had co-occurring driver oncogenes, was higher trend than $KRAS^{other}$ with driver oncogenes mutations (6/26,23%), but no statistical significance (P=0.33), the most commonly co-occurring genomic alterations with $KRAS^{G12C}$ were *EGFR* (10%, 2/20), *ROS1* (10%, 2/20), *MET* (10%), *HER2* (5%, 1/20), *ALK* (5%, 1/20), *BRAF* (5%, 1/20), and *RET* (0%), respectively (Figure 3, Supplemental Table 2). One hundred and sixty-three patients from 168 gene panel or 520 gene panel found that the $KRAS^{G12C}$ gene is often accompanied by *TP53* and *PTEN* mutation, the mutation rates were 50% (3/6) and 16.7% (1/6), respectively, but *STK11* (0.0%, 0/6).

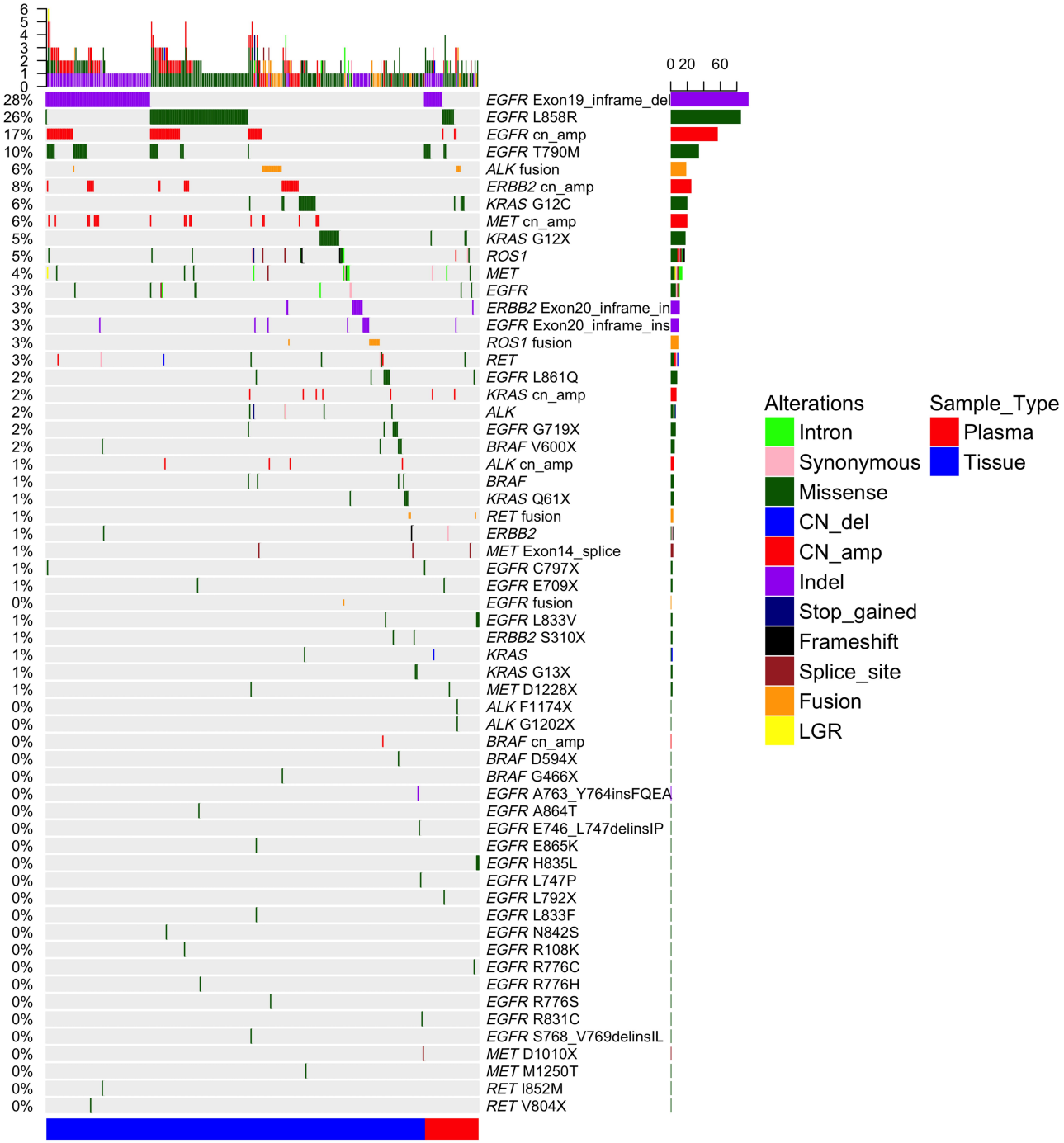

Figure 3 Driver genetic mutations spectrums identified by next-generation sequencing of 332 patients with NSCLC tumor tissue and plasma. Side bar represents the percentage of patients with driver gene mutation. Top bar represents the number of mutations per patient. Different types of mutations are denoted in different colors.

Age, Smoking History and Pathological Stage Associated with $KRAS^{G12C}$ Mutation

The mutation rate of $KRAS^{G12C}$ gene in smokers was higher than that in nonsmokers, 8.33% (14/168) vs 2.28% (6/263), P=0.0046). $KRAS^{G12C}$ has a higher mutation rate in age (≥60 years) 15.2% (18/274) vs 1.27% (2/157); P=0.0151). $KRAS^{G12C}$ mutation was associated with the pathological staging of the patients, 8.69% (10/115) vs 3.16% (10/316), P=0.0343), but was not associated with gender, brain metastasis, PS score, and histology (P=0.2515, P=0.4282, P=0.5266 and P=0.7526) (Table 3), to further identify the values of clinical factor on $KRAS^{G12C}$ mutations, logistic regression analysis was included. In the univariate logistic analysis, age, smoker, clinical stage were identified as independent factors for $KRAS^{G12C}$ mutations (OR=0.551, P=0.024; OR=5.449, P=0.02; OR=0.343, P=0.006). In the multivariate logistic model, smoker (OR=0.306, P=0.037) remained independent factors for $KRAS^{G12C}$ (Table 4).

Table 3 431 Correlation Analysis Between $KRAS^{G12C}$ and Clinic Pathological Factors in Patients

	$KRAS^{G12C}$-mut n=20	$KRAS^{G12C}$-wt n=411	*P*-value
Sex			
Male	12	186	0.2515
Female	8	225	
Age			
<60 year	2	155	0.0151*
≥60 year	18	256	
Stage			
I–III	10	105	0.0343*
IV	10	306	
Smoking history			
Yes	14	154	0.0046**
No	6	257	
Brain metastasis			
Yes	3	103	0.4282
No	17	308	
PS score			
0–1	16	349	0.5266
2–3	4	62	
Histology			
Adenocarcinoma	18	352	0.7526
Squamous cell carcinoma	2	59	

Notes: *P-value <0.05; **P-value <0.01.
Abbreviations: mut, mutation; wt, wild type.

Furthermore, we found that $KRAS^{G12C}$ was dominant in male smokers (100%, 4/4)

Discussion

Previously reported *RAS* was detected in about 25–30% of tumors, several studies consistently reported that Westerners have a higher mutation rate than Asians (26% vs 11%).[7] Another report similarly indicated 30% of *RAS* mutations in Western patients and 5–15% in the Asian population,[8] which accounts for about 86% *KRAS*, 11% *NRAS* and 3% *HRAS* mutation of *RAS*-induced NSCLC, *KRAS* accounts for 90% of *RAS* gene mutations in lung adenocarcinoma and is the most common oncogene in NSCLC.[9] Our data are consistent with recent studies, our results might indicate the current view that *KRAS* was the only *RAS*-mutant isoform, the mutation rate was 10.7% in 431 NSCLC patients, similar to the rates reported by Jia's group and Liu's group.[10,11] Further studies showed that the $KRAS^{G12C}$ mutation rate is 4.6% in lung cancer, and 43.5% in *KRAS* mutation for our study. It was similar to several studies in that the $KRAS^{G12C}$ mutation frequency range is from 35% to 45% followed by $KRAS^{G12V}$ and $KRAS^{G12D}$ in *KRAS* mutant lung cancer, but a lower frequency reported by Liu's group.[9,11–15] One key finding of our study was that *KRAS*, including $KRAS^{G12C}$ mutation of NSCLC reflected no difference in tissue and blood. Furthermore, this study also reveals the widespread existence of concomitant mutations in patients with $KRAS^{G12C}$ mutant advanced NSCLC, especially driver gene mutations. The three predominant *KRAS* co-mutations were detected including *EGFR*-$KRAS^{G12C}$ (10%), equal to *ROS1*-$KRAS^{G12C}$ (10%) and *MET*-$KRAS^{G12C}$ (10%). We found the four cases with *EGFR-KRAS* concomitant mutations in our cohort were all tested before *EGFR*-TKI treatment, thus partly ruling out the possibility that *EGFR-KRAS* co-mutation was related to *EGFR*-TKI resistance.[16] Unfortunately, neither were the four cases derived from two separate tumor tissue. The incidence rate of *EGFR-KRAS* in the Chinese cohort might be likely ethnic-unique, based on the knowledge that the prevalence of *EGFR* mutation is higher in the Asian population.[17] The co-occurrence of *EGFR* and *KRAS* was 0.92% (4/431) in our study, which was supported by Scheffler et al[13] (1.2%). The four concomitant mutations were $KRAS^{G12C}$ (n=2) co-occurr ing with either *EGFR V1097I* (n=1) or *EGFR* amplification (n=1) and $KRAS^{other}$ (n=2) co-occurring with *EGFR* 19del (n=2). Although previous studies had reported that *KRAS* are mutually exclusive with mutations in *EGFR* and *ALK* in NSCLC,[18,19] but coexisting *EGFR* and *KRAS* mutations have also been reported..[20,21] (Zhu et al reported that three

Table 4 Univariate and Multivariate Analysis of $KRAS^{G12C}$ and Clinical Factor

		Univariate Analysis			Multivariate Analysis		
		OR	**95%CI**	***P*-value**	**OR**	**95%CI**	***P*-value**
Sex	Male	1		0.202	1		0.936
	Female	0.551	0.221–1.377		1.044	0.363–3.001	
Age	<60	1		0.024	1		0.076
	≥60	5.449	1.247–23.805		3.932	0.868–17.823	
Stage	I–III	1		0.02	1		0.082
	IV	0.343	0.139–0.847		0.415	0.154–1.118	
Smoking history	Yes	1		0.006	1		0.037
	No	0.257	0.097–0.682		0.306	0.101–0.929	
Brain metastasis	Yes	1		0.315	1		0.871
	No	1.895	0.544–6.598		0.892	0.226–3.516	
PS score	0–1	1		0.553	1		0.704
	2–3	1.407	0.455–4.350		1.256	0.388–4.066	
Histology	Adenocarcinoma	1		0.588	1		0.617
	Squamous cell carcinoma	0.663	0.15–2.932		0.677	0.147–3.116	

patients with coexisting *EGFR* and *KRAS* mutations were found in 206 patients (1.4%).[22] We infer that genetic mutation status could be related with different races, sample numbers, as well as test methodology. Nevertheless, current data about *KRAS* co-occurring mutations in lung cancer is insufficient. Co-occurrence with *TP53* or *STK11* mutations is common in *KRAS* mutations.[23,24] *KRAS* and *TP53* co-mutations indicated that tumors harboring those mutations couldbe more responsive to immune checkpoint inhibition in lung cancer.[25] Conversely, tumors harboring concurrent *KRAS* and *STK11* mutations could be associated with an immunosuppressive microenvironment.[26,27] Furthermore, the absence of *PTEN* promotes resistance to T cell-mediated immunotherapy.[28] So we evaluated the mutation status of *TP53*, *STK11* or *PTEN* in $KRAS^{G12C}$ mutant patients, and it indicated that in the landscape of concurrent genetic alterations in patients with $KRAS^{G12C}$, the co-mutation rates were 50% and 16.7%, but $KRAS^{G12C}$ was exclusive with *STK11* mutation.

$KRAS^{G12C}$ (c.34G>T) alteration is a transversion and *KRAS* transversion mutations (G→T or G→C) were more common in smokers, in contrast, transition mutations (G→A) were more common in never-smokers in lung adenocarcinomas (n=500).[29] Our data showed that smokers more commonly harbored $KRAS^{G12C}$ mutations than $KRAS^{wt}$ (70% vs 37.5%), which is consistent with reports by Liu et al and Dogan et al.[11,30] Data showed that *KRAS*-mutant NSCLC is genetically complex, with a higher frequency of co-occurring mutations with *TP53*, *STK11*, *MET* and *ERBB2* amplifications,[29] however, no conclusions implied that the co-occurrence mutations were related to the transversion. In comparison to $KRAS^{other}$, $KRAS^{G12C}$ showed higher mutation frequency in patients older than 60 years, and stage I–III. Our findings were supported by other studies.[11,13,31]

In summary, our study indicated that $KRAS^{G12C}$ mutations were the most frequent mutant subtype of *KRAS* in northeast Chinese NSCLC patients and might be involved in the smoking, age, and clinical stage, especially we demonstrated a high frequency of $KRAS^{G12C}$ concomitant *TP53*/*PTEN*/*EGFR*. In addition, no difference was observed between tissue and plasma in the $KRAS^{G12C}$

subgroup of the northeast Chinese NSCLC patients. Our findings might contribute to distinct therapeutic guidance in NSCLC. More data should be collected and explored to address predictive and prognostic value of $KRAS^{G12C}$ in future studies.

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Disclosure

Qiang Zhang is an employee of Burning Rock Biotech. The authors report no other potential conflicts of interests in this work.

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