Prognostic value of *KRAS*/*TP53*/*PIK3CA* in non-small cell lung cancer

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Abstract. The present study explored the association between *KRAS* proto-oncogene GTPase (*KRAS*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (*PIK3CA*) and tumor protein p53 (*TP53*) mutations, and the clinical features and survival prognosis in 50 patients with non-small cell lung cancer (NSCLC). The most common concurrent single gene mutation was *TP53*, followed by *KRAS* and *PIK3CA*. Co-existing mutations were found in 17 patients. *KRAS*, *PIK3CA* and *TP53* mutations were associated with carbohydrate antigen 19-9 expression, invasive growth, vacuolar signs and margin lobulation on chest CT. The incidence of distant metastasis (bone and adrenal) with *KRAS* and *TP53* mutations was greater than that of local metastasis (pleura). Patients with the wild-type genes experienced longer progression-free survival (PFS) times than those with *KRAS*, *TP53*, *KRAS*/*TP53* or *PIK3CA*/*TP53* mutations. Patients with *KRAS*/*TP53* or *PIK3CA*/*TP53* mutations experienced shorter PFS times than those with a single *KRAS* or *TP53* mutation. *KRAS*, *PIK3CA* and *TP53* mutations were associated with distant metastases and a poor prognosis. Patients with NSCLC should receive routine *KRAS*, *PIK3CA* and *TP53* gene sequencing to determine mutations for the analysis of clinical characteristics and prognosis.

Introduction

Lung cancer has a high mortality rate of ~27% and is becoming more prevalent in younger populations (1). Despite progress in the diagnosis and treatment of lung cancer, the 5-year survival rate is only 16% (2). Individualized therapy is a promising treatment strategy for non-small cell lung cancer (3). Mutations in epidermal growth factor receptor (*EGFR*) drive the development of lung adenocarcinoma and have altered the traditional treatment approaches. Next-generation sequencing revealed that patients with wild-type *EGFR* or *ALK* could present concurrent oncogenic mutations in *KRAS* proto-oncogene GTPase (*KRAS*) (4), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α (*PIK3CA*) (5) and tumor protein p53 (*TP53*) (6). These mutations may result in differential clinical features, treatment outcomes and survival prognoses. The association between *KRAS*, *PIK3CA* and *TP53* mutations, clinical features, and the prognosis of patients with NSCLC is unclear. The present study retrospectively analyzed 89 cases of NSCLC patients with *KRAS*, *PIK3CA* and *TP53* mutations to elucidate the association between gene mutation, clinical characteristics and survival prognosis as a basis for individualized treatment.

Patients and methods

Patient selection. A total of 122 patients accepted next-generation sequencing for advanced NSCLC at Shanghai Changhai Hospital (Shanghai, China) and were enrolled between January 2015 and December 2016. Missing information and loss to follow-up resulted in the exclusion of 33 patients. Blood samples and clinical data from 89 patients with identified genes were collected, including sex, age, smoking status, symptoms, laboratory test results, chest computed tomography (CT) results, tumor location, pathological type, Tumor-Node-Metastasis stage (7) and site of metastasis. Among the 89 samples, 50 exhibited *KRAS*, *TP53* and *PIK3CA* mutations. The Ethics Committee of Shanghai Changhai Hospital approved the present study, and written informed consent was obtained from each participant.

Gene sequencing. Circulating Single-Molecule Amplification and Resequencing Technology (cSMART; Illumina CN500; Berry Genomics Co., Ltd., Beijing, China) was used to detect *KRAS*, *PIK3CA* and *TP53* mutation in all patients with NSCLC. In brief, genomic DNA was extracted from the plasma of the patients using MagMAX Cell-Free DNA Isolation kit, (Thermo Fisher Scientific, Inc., Waltham, MA, USA; Article no. A29319) DNA was purified using a DNA purification kit (Berry Genomics Co., Ltd; Article no. R0037). The libraries were prepared from 10 ng plasma DNA by ligation of universal sequencing adaptors containing unique 6-bp barcodes. Modified DNA was denatured and single strands were circularized by Taq ligase. Bidirectional

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back-to-back primers, in either singleplex or multiplex format, were annealed close to the mutation loci. Inverse PCR was performed to replicate targeted genes. Amplified products were subjected to massive parallel sequencing on the MiSeq platform (Illumina, Inc., San Diego, CA, USA) to generate paired-end reads of 2x200 bp (8).

Treatment. All patients were administered with a first-line chemotherapy regimen of pemetrexed (500 mg/m^2)/paclitaxel (135 mg/m^2) and carboplatin (area under the curve=5). All patients provided written informed consent.

Survival analysis. Tumors were evaluated every 2 cycles during chemotherapy treatment or earlier when significant signs of progression, including aggravation of cough or hemoptysis, were present. Progression-free survival (PFS) was determined according to the Response Evaluation Criteria in Solid Tumors guidelines (version 1.1) (9). The PFS time was defined as the time from the beginning of chemotherapy to the presence of objective evidence of progression. The final follow-up date was June 30, 2017.

Statistical analysis. Survival curves were calculated using the Kaplan-Meier method from the beginning of chemotherapy to documented progression or mortality from any cause, differences in PFS were assessed using the log-rank test. Statistical analysis was performed with SPSS version 21 (IBM, Corp., Armonk, NY, USA). The χ^2 test was used to compare the categorical variables. P<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. A total of 122 patients with NSCLC received cSMART sequencing and 33 patients were excluded due to missing information or loss to follow-up. A total of 89 patients were therefore enrolled in the present study, and the baseline demographic characteristics are shown in Table I. The study cohort consisted of 52 males and 37 females, with a median age of 61.0 years and a mean (± standard error) age of 59.4 (±12.2) years. Adenocarcinoma was histologically determined in 75 patients. There were 2 patients with adenosquamous carcinoma and 12 with squamous carcinoma. In total, 41 patients were smokers and 48 had never smoked.

Gene mutations. Oncogenic mutations were found in 50 patients, including *KRAS* (n=21, 23.6%), *PIK3CA* (n=8, 9.0%) and *TP53* (n=40, 44.9%). Among the 21 patients with *KRAS* mutations, 18 had mutations in exon 2, 3 in exon 3 and 2 in exon 4. There were 8 patients with a *PIK3CA* mutation in exon 10. A total of 17/40 patients had *TP53* mutations located in exon 5, 6 in exon 6, 10 in exon 7 and 19 in exon 8. Coexisting mutations were identified in 17 patients (19.1%), including *KRAS*/*TP53* (n=10, 11.2%), *PIK3CA*/*TP53* (n=4, 4.5%), *KRAS*/*PIK3CA* (n=1, 1.1%) and *KRAS*/*PIK3CA*/*TP53* (n=2, 2.2%). There were 32 cases with *EGFR* mutations (36.0%), 3 cases with the EMAP-like 4-ALK receptor tyrosine kinase fusion oncogene (3.4%), and 3 cases of c-MET exon 14 skipping (3.4%). The *KRAS*/*TP53*/*PIK3CA* mutations and percentage distribution of the 50 patients are shown in Figs. 1 and 2.

Table I. Baseline demographic characteristics of the 89 patients with non-small cell lung cancer.

Characteristics	n (%)
Sex	
Male	52 (58.4)
Female	37 (41.6)
Age, years	
<65	57 (64.0)
≥65	32 (36.0)
Surgical history	
Yes	21 (23.6)
No	68 (76.4)
Smoking status	
Former/current	41 (46.1)
Never	48 (53.9)
First symptom	
Yes	60 (67.4)
No	29 (32.6)
Tumor site	
Left lung	45 (50.6)
Right lung	44 (49.4)
Histology	
Adenocarcinoma	75 (84.3)
Adenosquamous carcinoma	2 (2.2)
Squamous cell carcinoma	12 (13.5)
Invasive growth	
Yes	50 (56.2)
No	39 (43.8)
TNM stage	
I	1 (1.1)
II	3 (3.4)
III	14 (15.7)
IV	71 (79.8)
Metastasis	
Yes	71 (79.8)
No	18 (20.2)
Metastatic site	
Bone	39 (43.8)
Brain	20 (22.5)
Adrenal	8 (9.0)
Liver	9 (10.1)
Pleura	27 (30.3)
Lymph nodes	22 (24.7)

Clinical characteristics. The clinical characteristics of the 89 patients in association with the gene mutations are shown in Table II. Patients with *KRAS*, *TP53*, *PIK3CA* and *KRAS*/*TP53* mutations had a higher incidence of bone metastasis than those with the wild-type gene (61.9 vs. 25.6%, P=0.006; 62.5 vs. 25.6%, P=0.024; 62.5 vs. 25.6%, P=0.042; 70.0 vs. 25.6%, P=0.009). There was also a higher incidence of adrenal

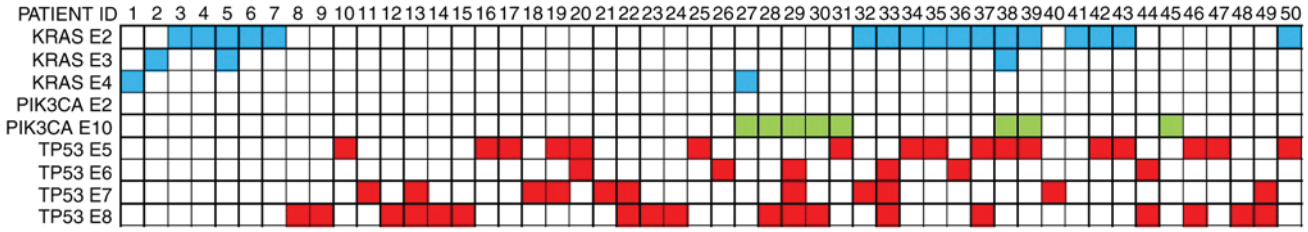

Figure 1. Mutations of *KRAS*, *PIK3CA* and *TP53* genes in 50 patients with non-small cell lung cancer. *KRAS*, *KRAS* proto-oncogene GTPase; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; *TP53*, tumor protein p53. Blue represent *KRAS* mutation; Red *TP53* mutation and Green *PIK3CA* mutation.

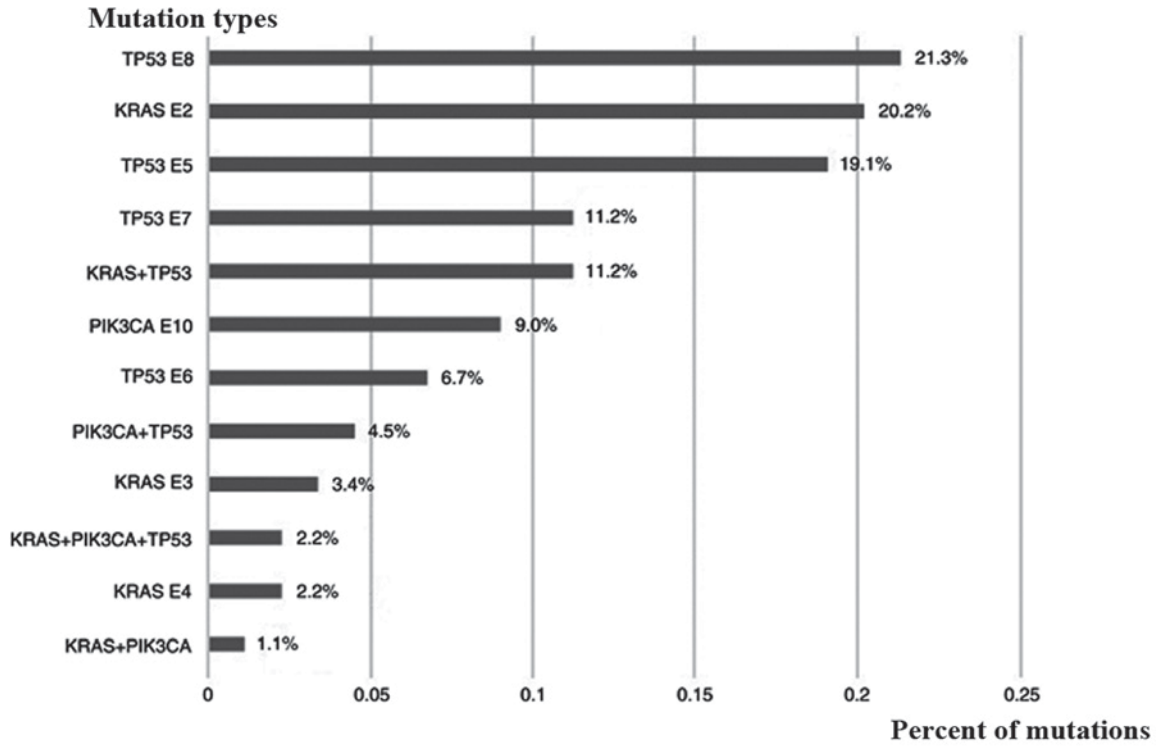

Figure 2. Percentage distributions of *KRAS*, *PIK3CA* and *TP53* gene mutations in 50 patients with non-small cell lung cancer. *KRAS*, *KRAS* proto-oncogene GTPase; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; *TP53*, tumor protein p53.

metastasis in the *TP53* mutation vs. wild-type groups (12.5 vs. 5.1%, P=0.017). Patients with *KRAS* or *KRAS*/*TP53* mutations had a lower incidence of pleural metastasis than those with the wild-type gene (14.3 vs. 43.6%, P=0.022; 0.0 vs. 43.6%, P=0.010). Infiltrative tumor growth was greater in patients with *KRAS*, *TP53* and *KRAS*/*TP53* mutations than in the wild-type group (71.4 vs. 51.3%, P=0.039; 67.5 vs. 51.3%, P=0.032; 90.0 vs. 51.3%, P=0.009).

KRAS/*TP53* mutations were associated with elevated carbohydrate antigen 19-9 (CA19-9) expression, vacuolar signs and margin lobulation in chest CT imaging in patients. Differences in *KRAS* mutation were observed in margin lobulation and invasive growth in chest CT imaging, meanwhile, first symptoms, including cough and dyspnea, indicated a statistical significance between wild-type patients and those with *PIK3CA* mutation (P=0.034).

Survival analysis. The PFS times of the *KRAS* mutation and wild-type group were 8.9±2.3 months (95% CI, 4.3-13.5) and 15.3±1.6 months (95% CI, 12.1-18.4), respectively (P=0.045). Patients with a single *TP53* mutation had a PFS time of 7.8±1.5 months (95% CI, 4.9-10.7), which was significantly shorter than that of the wild-type group (P<0.001). Patients with a *KRAS*/*TP53* coexisting mutation had a shorter PFS time of 6.6±1.6 months (95% CI, 3.5-9.7) compared with the wild-type group (P<0.001). This result was similar among *PIK3CA*/*TP53* patients (P=0.012). The difference in the PFS times was not statistically significant between the single *KRAS* and *KRAS*/*TP53* mutations, the single *TP53* and *KRAS*/*TP53* mutations or the single *TP53* and *PIK3CA*/*TP53* mutations. (Table III; Fig. 3).

Discussion

NSCLC accounts for 70-80% of lung cancer cases and 60% of patients are diagnosed at stage III or IV (10). Oncogenes such as *EGFR* and *ALK* have shifted the treatment model of lung cancer from pathology-guided to molecular-guided precision medicine

Table II. Association between gene mutation and clinical features.

Characteristics	All wt, n (%)	*KRAS* mt, n (%)	χ^2	P-value	*PIK3CA* mt, n (%)	χ^2	P-value	*TP53* mt, n (%)	χ^2	P-value	*KRAS*+ *TP53* mt, n (%)	χ^2	P-value	*PIL3CA*+ *TP53* mt, n (%)	χ^2	P-value
Sex			0.342	0.559		0.219	0.640		0.018	0.894		1.514	0.219		0.120	0.729
Male	23 (59.0)	14 (66.7)			4 (50.0)			23 (57.5)			8 (80.0)			2 (50.0)		
Female	16 (41.0)	7 (33.3)			4 (50.0)			17 (42.5)			2 (20.0)			2 (50.0)		
Age, years			0.115	0.694		0.003	0.959		0.307	0.580		0.245	0.620		0.202	0.653
<65	24 (61.5)	14 (66.7)			5 (62.5)			27 (67.5)			7 (70.0)			2 (50.0)		
≥65	15 (38.5)	7 (33.3)			3 (37.5)			13 (32.5)			3 (30.0)			2 (50.0)		
Tumor site			0.012	0.914		0.710	0.400		0.117	0.732		0.122	0.727		0.022	0.883
Left lung	21 (53.8)	11 (52.4)			3 (37.5)			20 (50.0)			6 (60.0)			2 (50.0)		
Right lung	18 (46.2)	10 (47.6)			5 (62.5)			20 (50.0)			4 (40.0)			2 (50.0)		
Smoking status			0.388	0.533		0.004	0.947		0.110	0.741		3.148	0.076		0.002	0.961
Former/current	19 (48.7)	12 (57.1)			4 (50.0)			18 (45.0)			8 (80.0)			2 (50.0)		
Never	20 (51.3)	9 (42.9)			4 (50.0)			22 (55.0)			2 (20.0)			2 (50.0)		
First symptom			0.587	0.440		4.519	0.034[a]		1.654	0.198		1.197	0.274		2.363	0.124
Yes	24 (61.5)	15 (71.4)			8 (100.0)			30 (75.0)			8 (80.0)			4 (100.0)		
No	15 (38.5)	6 (28.6)			0 (0.0)			10 (25.0)			2 (20.0)			0 (0.0)		
CA19-9			1.516	0.218		0.726	0.394		0.748	0.387		5.108	0.024[a]		1.381	0.240
Normal	30 (76.9)	13 (61.9)			3 (37.5)			27 (67.5)			6 (60.0)			2 (50.0)		
High	9 (23.1)	8 (38.1)			5 (62.5)			13 (32.5)			4 (40.0)			2 (50.0)		
Invasive growth			4.250	0.039[a]		2.621	0.105		4.575	0.032[a]		6.883	0.009[a]		1.439	0.230
No	19 (48.7)	6 (28.6)			2 (25.0)			13 (32.5)			1 (10.0)			1 (25.0)		
Yes	20 (51.3)	15 (71.4)			6 (75.0)			27 (67.5)			9 (90.0)			3 (75.0)		
Margin lobulation			4.290	0.038[a]		0.001	0.970		2.495	0.114		4.273	0.039[a]		1.336	0.248
Yes	29 (74.4)	10 (47.6)			6 (75.0)			23 (57.5)			4 (40.0)			4 (100.0)		
No	10 (25.6)	11 (52.4)			2 (25.0)			17 (42.5)			6 (60.0)			0 (0.0)		
Pleural traction			0.923	0.337		0.710	0.400		0.014	0.905		0.047	0.828		4.210	0.040[a]
Yes	18 (46.2)	7 (33.3)			5 (62.5)			19 (47.5)			5 (50.0)			4 (100.0)		
No	21 (53.8)	14 (66.7)			3 (37.5)			21(52.5)			5 (50.0)			0 (0.0)		
Vacuolar signs			1.187	0.276		0.777	0.378		0.336	0.562		3.921	0.048[a]		0.448	0.503
Yes	5 (12.8)	5 (23.8)			2 (25.0)			7 (17.5)			4 (40.0)			1 (25.0)		
No	34 (87.2)	16 (76.2)			6 (75.0)			33 (82.5)			6 (60.0)			3 (75.0)		
Site of metastasis																
Bone	10 (25.6)	13 (61.9)	7.594	0.006[a]	5 (62.5)	4.150	0.042[a]	25 (62.5)	5.081	0.024[a]	7 (70.0)	6.912	0.009[a]	2 (50.0)	1.070	0.301

Table II. Continued.

Characteristics	All wt, n (%)	*KRAS* mt, n (%)	χ^2	P-value	*PIK3CA* mt, n (%)	χ^2	P-value	*TP53* mt, n (%)	χ^2	P-value	*KRAS*+ *TP53* mt, n (%)	χ^2	P-value	*PIL3CA*+ *TP53* mt, n (%)	χ^2	P-value
Brain	6 (15.4)	7 (33.3)	2.591	0.107	3 (37.5)	2.097	0.148	10 (25.0)	1.610	0.204	2 (20.0)	0.124	0.725	1 (25.0)	0.246	0.620
Adrenal	2 (5.1)	4 (19.0)	2.939	0.086	2 (25.0)	3.367	0.067	5 (12.5)	5.648	0.017[a]	2 (20.0)	2.348	0.125	1 (25.0)	2.207	0.137
Liver	4 (10.3)	3 (14.3)	0.215	0.643	1 (12.5)	0.035	0.851	5 (12.5)	2.806	0.094	2 (20.0)	0.703	0.402	0 (0.0)	0.452	0.501
Pleura	17 (43.6)	3 (14.3)	5.275	0.022[a]	2 (25.0)	0.953	0.329	8 (20.0)	0.032	0.859	0 (0.0)	6.675	0.010[a]	0 (0.0)	2.884	0.089
Lymph nodes	6 (15.4)	6 (28.6)	1.484	0.223	3 (37.5)	2.097	0.148	14 (35.0)	1.610	0.204	4 (40.0)	2.969	0.085	1 (25.0)	0.246	0.620

[a]P<0.05. *KRAS*, KRAS proto-oncogene GTPase; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; *TP53*, tumor protein p53.

Table III. Survival prognosis in non-small cell lung cancer patients with *KRAS*, *PIK3CA* and *TP53* gene mutations.

	KRAS mt vs. all wt		*TP53* mt vs. all wt		*KRAS+TP53* mt vs. all wt		*KRAS+TP53* mt vs. *KRAS* mt		*KRAS+TP53* mt vs. *TP53* mt		*PIK3CA+TP53* mt vs. all wt		*PIK3CA+TP53* mt vs. *TP53* mt	
Variables	*KRAS* mt	All wt	*TP53* mt	All wt	*KRAS*+ *TP53* mt	All wt	*KRAS*+ *TP53* mt	*KRAS* mt	*KRAS*+ *TP53* mt	*TP53* mt	*PIK3CA*+ *TP53* mt	All wt	*PIK3CA*+ *TP53* mt	*TP53* mt
PFS time, months	8.9±2.3	15.3±1.6	7.8±1.5	15.3±1.6	6.6±1.6	15.3±1.6	6.6±1.6	8.9±1.3	6.6±1.6	7.8±1.5	7.1±1.5	15.3±1.6	7.13±11	7.8±1.5
95% CI	4.3-13.5	12.1-18.4	4.9-10.7	12.1-18.4	3.5-9.7	12.1-18.4	3.5-9.7	4.3-13.5	3.5-9.7	4.9-10.7	3.1-11.2	12.1-18.4	3.1-11.2	4.9-10.7
P-value		0.045		<0.001		<0.001		0.398		0.873		0.012		0.986

KRAS, *KRAS* proto-oncogene GTPase; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; *TP53*, tumor protein p53; wt, wild-type; mt, mutant; CI, confidence interval.

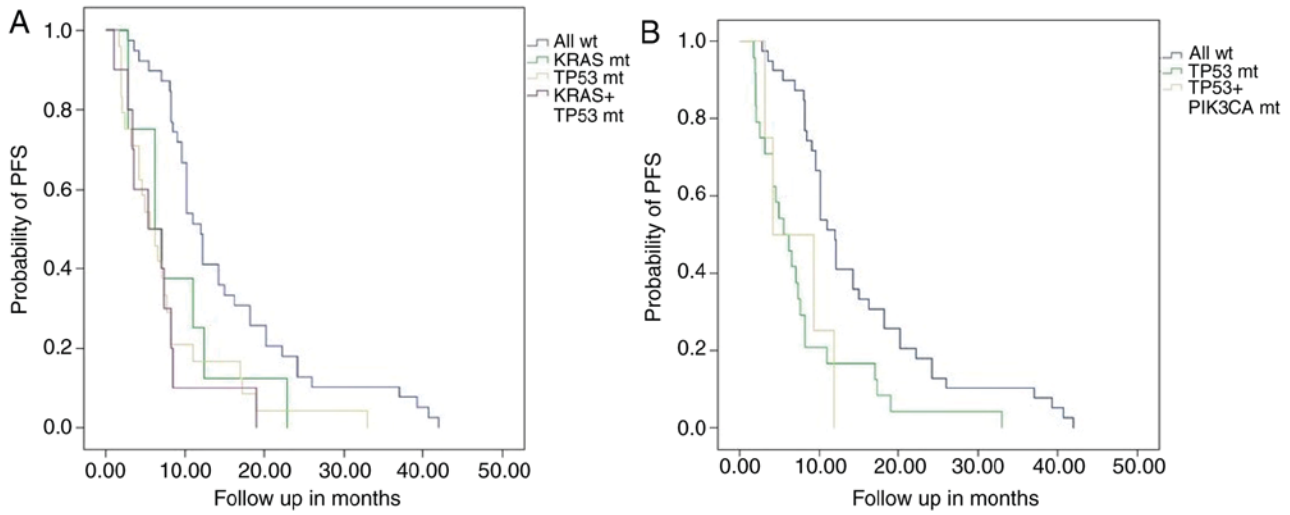

Figure 3. PFS Kaplan-Meier curves between different gene mutations group and wild-type group. (A) PFS in patients with non-small cell lung cancer was compared between *KRAS* mutant and all wild-type groups (8.9±2.3 vs. 15.3±1.6 months; P=0.045). PFS was compared between *TP53* mutant and all wild-type groups (7.8±1.5 vs. 15.3±1.6 months; P<0.001), and between *KRAS+TP53* mutant and all wild-type groups (6.6±1.6 vs. 15.3±1.6 months; P<0.001). Compared with the *KRAS* mutant and *TP53* mutant, respectively, the PFS time of patients with the *KRAS+TP53* mutant was shorter, but not statistically different. (B) PFS was also compared between patients with the *PIK3CA+TP53* mutant and all wild-type groups (7.1±2.1 vs. 15.3±1.6 months; P=0.012). The PFS time of the patients with the *PIK3CA+TP53* mutant was shorter than that of patients with the *TP53* mutant, but was not statistically different. PFS, progression-free survival; mt, mutant; wt, wild-type; *KRAS*, *KRAS* proto-oncogene GTPase; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α; *TP53*, tumor protein p53.

with targeted therapy (11). With the improvement in examination technology and the increase in available treatment methods, the genetic and clinical characteristics of NSCLC-related genes, including *KRAS, PIK3CA* and *TP53*, are highly informative.

The present study evaluated 89 cases of NSCLC patients with *KRAS, PIK3CA* and *TP53* mutations. *KRAS* mutations were found in 21 cases within exon 2 (n=18), exon 3 (n=3) and exon 4 (n=2). The total mutation rate of *KRAS* was 23.6%, which was similar to the results of a study undertaken by Mao *et al* (12), but higher than the mutation rates of 4.4-5.3% reported by Luo *et al* (13) and Yi *et al* (14). The mutation rate of *PIK3CA* was 3% in a study undertaken by Scheffler *et al* (15), but Liang *et al* (16) reported a rate of 47.83%. The present study included 8 cases of *PIK3CA* exon 10 mutations and the total mutation rate was 9.0%. *TP53* has the highest mutation rate of all NSCLC-related genes, reported as 39-46% (15,16). The present study identified 40 cases with *TP53* mutations within exon 5 (n=17), exon 6 (n=6), exon 7 (n=10) and exon 8 (n=19). In present study the total mutation rate of *TP53* was 44.9%, which is in accordance to previous researches (17,18).

Kris *et al* (19) found that 3% of patients with NSLCL exhibited a double gene mutation. The Cancer Genome Atlas determined that the mutation rate of *KRAS/TP53* coexisting mutation could reach 20% (20). The present study identified 17 co-mutated samples with a rate of 19.1%, including 15 double-mutations of *KRAS/TP53, PIK3CA/TP53* and *KRAS/PIK3CA*, and 2 cases of *KRAS/PIK3CA/TP53* co-mutation. This difference may result from the sensitivity and sequencing depth of next-generation sequencing by cSMART. The varied sample size between studies may also contribute toward the discrepancies in gene mutation rates.

Clinical characteristics, including the baseline demographics, clinical manifestations, partial laboratory tests, partial pathological features and certain features of chest CT imaging, of patients with mutations were not significantly different from those of wild-type patients (P>0.05). This was consistent with the results of numerous previous studies (6,21-25). By contrast, *KRAS/TP53* were associated with elevated CA19-9 expression, vacuolar signs and margin lobulation in chest CT imaging. However, it is possible that the sample size of each subgroup resulted in the difference in certain clinical characteristics to some extent, and further study is required due to the limited sample size used in the present study.

Invasive growth of the tumor tissue in patients was associated with *KRAS*, *TP53* and *KRAS/TP53*, which was consistent with the clinical features observed. The incidence of distant metastasis was higher than that of local metastasis in patients with *KRAS* and *TP53* mutations. The possible mechanism of this is the activation of the *EGFR* downstream Rat sarcoma/Rapidly Accelerated Fibrosarcoma/mitogen-activated protein kinases signaling pathways by *KRAS* mutations to regulate cell differentiation and proliferation. Prolonged activation of the *KRAS* signal is hypothesized to cause tumor cell proliferation and progression (26). *TP53* gene mutations result in an oncogenic transformation of the tumor suppressor gene due to a conformational change; therefore, the regulation of cell growth, apoptosis and DNA repair is disrupted, which allows tumor cells to proliferate, grow and metastasize (27,28).

The biological significance of these mutations remains uncertain, but to some extent specific driver genes have prognostic value. Mascaux *et al* (29) first reported a poor prognosis in NSCLC patients with *KRAS* mutations, and other studies have confirmed this hypothesis (30). Recent studies have found that *TP53* gene mutations may generate the same results in patients with NSCLC (31-33). *PIK3CA* encodes the type I phosphatidylinositol-3-kinase p110α catalytic subunit (34) and is important for the development of

NSCLC. *PIK3CA* phosphorylates the EGFR bypass pathway, PI3K/AKT/mTOR, to activate downstream signaling that promotes the proliferation, survival, adhesion and differentiation of tumor cells (35). Liang *et al* (16) proposed that *PIK3CA* gene mutations are more likely to co-exist with other oncogenic mutations and that they may weakly induce independent carcinogenesis.

In the present study, patients with NSCLC who underwent first-line chemotherapy were divided into groups according to their genotype. For all patients who have *EGFR* mutation in Changhai hospital, targeted therapy is discussed and anti-*EGFR* tyrosine kinase inhibitors are recommended as the first-line treatment. The majority of these patients do receive targeted therapy. However, due to economic problems or for other reasons, certain patients cannot afford targeted therapy. For the baseline balance of the present study, the 89 patients who received first-line chemotherapy were chosen. Patients with a single *KRAS or TP53* mutation experienced shorter PFS times than the wild-type patients, which was consistent with the results of the studies by Molina-Vila *et al* (36) and Meng *et al* (37). Shepherd *et al* (6) hypothesized that a double gene mutation, such as *KRAS*/*TP53*, in NSCLC patients may indicate a poor prognosis. Patients with *KRAS*/*TP53* or *PIK3CA*/*TP53* mutations experienced a shorter PFS time than those patients with the wild-type. The PFS time of the *KRAS*/*TP53* group was shorter than that in the single *KRAS* and single *TP53* groups, as was the time in the *PIK3CA*/*TP53* group compared with the single *TP53* group (P>0.05). We hypothesized that there could be a 'gene superposition' effect in NSCLC patients with a co-mutated gene, which leads to a shortened PFS compared with a single gene mutation. However, the trend observed in the present study was not statistically significant, which was in agreement with the results of a study by Jao *et al* (38). The mean PFS time of patients with *KRAS*/*PIK3CA*/*TP53* gene co-mutations was 6.2 months, which was shorter than that of the double and single mutation groups. Only 2 patients had this co-mutation and therefore, a larger sample size is necessary for further study. Sampling error may also exist due to the next-generation sequencing technology and the limited sample size. The subgroups of gene mutations, as well as the chemotherapy regimen and doses, were not identical; therefore, further evidence should be obtained in a large clinical study.

In conclusion, the treatment strategy for NSCLC patients with *KRAS*, *PIK3CA* and *TP53* mutations has not yet been defined. The present study determined the predictive value of *KRAS*, *PIK3CA* and *TP53* mutations in patients with NSCLC. Additionally, the results of the present study suggested that patients with NSCLC should undergo routine *KRAS*, *PIK3CA* and *TP53* sequencing to determine single or multiple gene mutations for the analysis of patient clinical characteristics and prognosis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JZ, YH, RC and CB conceived and designed the study. JL analyzed the statistics. CB provided a part of patients' clinical data and monitored the whole study; JZ and RC wrote the original draft, YH and CB reviewed and edited the draft. All authors have read and approved the final version of this manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Changhai Hospital affiliated to Second Military Medical University (Shanghai, China).

Patient consent for publication

Written nformed consent and permission for publication was obtained for all patients in the present study.

Competing interests

The authors declare that they have no competing interests.

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