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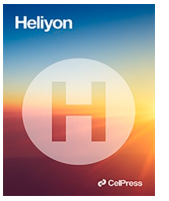

Research article

Highly consistency of *PIK3CA* mutation spectrum between circulating tumor DNA and paired tissue in lung cancer patients

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ABSTRACT

Background: Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha **(*PIK3CA*)** mutations are associated with drug resistance and prognosis in lung cancer; however, the consistency and clinical value of *PIK3CA* mutations between tissue and liquid samples are unknown.
Methods: Circulating tumor DNA (ctDNA) and matched tumor tissue samples from 405 advanced lung cancer patients were collected at Jilin Cancer Hospital between 2018 and 2022, and the *PIK3CA* mutation status was sequenced using next-generation sequencing based on a 520 gene panel. The viability of different mutant lung cancer cells was detected using MTT assay.
Results: PIK3CA mutations were detected in 46 (5.68 %) of 810 lung cancer samples, with 21 (5.19 %) of 405 plasma samples and 25 (6.17 %) of 405 matched tissues. p.Glu542Lys, p.Glu545Lys, and p.His1047Arg were the most common mutation types of *PIK3CA* in both the ctDNA and tissue samples. The concordance of *PIK3CA* mutations was 97.53 % between ctDNA and matched tissues (kappa: 0.770, $P = 0.000$), with sensitivity/true positive rate of 72.0 %, specificity/true negative rate of 99.2 %, and negative predictive value and positive predictive value of 0.982 and 0.857, respectively (AUC = 0.856, $P = 0.000$). Furthermore, the concordance of *PIK3CA* mutations was 98.26 % in lung adenocarcinoma and 96.43 % in lung squamous cell carcinoma. *TP53* and *EGFR* were the most common concomitant mutations in ctDNA and tissues. Patients with *PIK3CA* mutations showed a high tumor mutational burden (TMB) ($P < 0.001$) and a significant correlation between bTMB and tTMB (r = 0.5986, P = 0.0041). For the t*PIK3CA*mut/ctDNA *PIK3CA*mut cohort, *PI3K* pathways alteration was associated with male sex ($P = 0.022$), old age ($P = 0.007$), and smoking ($P = 0.001$); t*PIK3CA*mut/ctDNA *PIK3CA*wt patients harbored clinicopathological factors of adenocarcinoma stage IV, with low PS score (≤1) and TMB.
Conclusion: This study showed that ctDNA is highly concordant and sensitive for identifying *PIK3CA* mutations, suggesting that *PIK3CA* mutation detection in liquid samples may be an alternative clinical practice for tissues.

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1. Introduction

Lung cancer is a malignancy with a high mortality rate. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, accounting for more than 85 % of lung cancer cases [1,2]. Chemotherapy is one of the standard treatments for patients with advanced NSCLC. However, the efficacy of traditional cytotoxic drugs has plateaued recently [3]. With the rapid development of lung cancer driver genes and targeted drugs, an increasing number of tumor driver genes have been discovered, and lung cancer treatment has been transformed into a gene-oriented individualized treatment model [4,5].

Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations are present in 3%–4% of lung cancers and are one of the main cancer-causing mutations [6–8]. *PIK3CA* is a candidate driver oncogene for lung squamous cell carcinoma [9]. Overactivation of *PI3K/AKT* signaling is one of the most common driving mechanisms in many cancers, and *PIK3CA* encodes the synthesis of the p110α protein, a subunit of the *PI3K* enzyme. *PIK3CA* mutations induce persistent activation of *PI3K*, leading to uncontrolled cell proliferation and, eventually, tumor formation [10]. *PI3K* is also located at the "hub" of the intracellular signaling pathway, upstream of which *EGFR* and *HER2* are present. Patients with *PIK3CA* mutations may be resistant to *EGFR*-TKI and anti-HER2-therapy [11,12], *PIK3CA* mutations are implicated as one of the resistance mechanisms to first-line osimertinib in lung cancer patients [13], but NSCLC with concurrent *PIK3CA* and *EGFR* mutations is beneficial for third-line Anlotinib [14].

Various oncogenic driver genes are involved in NSCLC, and molecular detection of driver mutations in NSCLC tissues is a routine method. Tissue-based gene detection remains the gold standard for *PIK3CA* mutation detection [15]. However, most patients miss the opportunity for targeted therapy because of limited tissue specimens available for molecular detection [16]. Meanwhile, tissue slices for gene detection cannot accurately reflect the comprehensive characteristics of tumors owing to their heterogeneity; liquid biopsies (circulating tumor DNA [ctDNA]) may offer new opportunities for molecular diagnosis and dynamic monitoring of lung cancer [17,18]. However, it is unclear whether *PIK3CA* detection in ctDNA can replace tumor tissue genotyping. In this study, we analyzed the detection results of *PIK3CA* mutations in blood and paired tissue samples from patients with advanced NSCLC and discussed the consistency and clinical value of ctDNA and tissue samples.

2. Materials and methods

2.1. Patients and specimen collection

A retrospective study was conducted on 405 lung cancer patients with who were admitted to Jilin Cancer Hospital between January 2018 and August 2022. There were 344 - of adenocarcinoma, 28 of squamous cell carcinoma, 9 of small cell lung cancer, 7 of sarcoma, and 17 of other cancers (including lung large cell carcinoma and lung neuroendocrine carcinoma). Among them, 180 patients were aged>60 years and 225 patients were aged<60 years (19–86) years old; median age: 60 years). Samples were obtained from peripheral blood and matched paraffin-embedded tissues of 210 male and 195 female patients. The neoplastic cell percentage in paraffin sections was more than 20 %.

2.2. DNA extraction and next-generation sequencing (NGS)

DNA was extracted as previously described [19]. Briefly, tissue DNA was extracted and isolated using the nucleic acid extraction reagent (FFPE DNA) (Amoydx, China) and plasma ctDNA was extracted from 10 ml peripheral blood using a QIAamp circulating nucleic acid kit (Qiagen, Germany) according to the manufacturer's instructions. DNA concentration was measured using the Qubit dsDNA HS assay kit (Thermo Fisher Scientific) and DNA sample quality control was assessed using Agilent 2100 BioAnalyzer (Agilent). 50 ng tissue DNA and 30 ng ctDNA was inputted in library preparation.

2.3. NGS library preparation and sequencing data analysis

A library was constructed using OncoScreen™ Plus 520 gene panel (Burning Rock Biotech, Guangzhou, China; Supplementary Table 1). TG NextSeq 500/550 High Output Kit v2.5 and NextSeq 550Dx (Illumina) were used for sequencing. The target sequencing depth was set to a minimum of 1000 × for tumor tissue DNA and 10000 × for ctDNA, with a coverage uniformity of at least 90 %. The generated sequencing data were aligned to the human reference genome (hg19) using BWA sequence alignment software (version 0.7.10). abra2 (version 2.22) was used to optimize local alignment. Mutation detection was performed using VarDict software (version 1.5.1). All identified single nucleotide variants (SNVs) and insertions/deletions (INDELs) were annotated using SnpEff (version 5.0f) in conjunction with databases such as ClinVar (version 2023.09.03), ExAc (version 0.3.1), and gnomAD (release 2.0.2). After annotation, each SNV/INDEL was meticulously refined against a proprietary baseline. This refinement was complemented by using the VarScan (version 2.4.3) fpfilter to filter out spurious mutations using specified thresholds for parameters, such as max-mmqs-diff, min-mmqs-diff, max-var-mmqs, and max-ref-mmqs, set at 150, 500, 150, and 500, respectively. Finally, somatic SNVs/INDELs from tissue samples were required to meet the criteria of allelic depth (AD)≥5 and allele frequency (AF) ≥ 0.005, whereas for ctDNA samples, the SNVs/INDELs needed to satisfy AD ≥2 and AF ≥ 0.0005. The tumor mutational burden (TMB) was computed by dividing the total number of non-synonymous variants, including both SNVs and INDELs, by the coding region size of the gene panel, which was 1.003 Mb. The threshold for classifying tumors as having high TMB was set at 10 mutations per megabase.

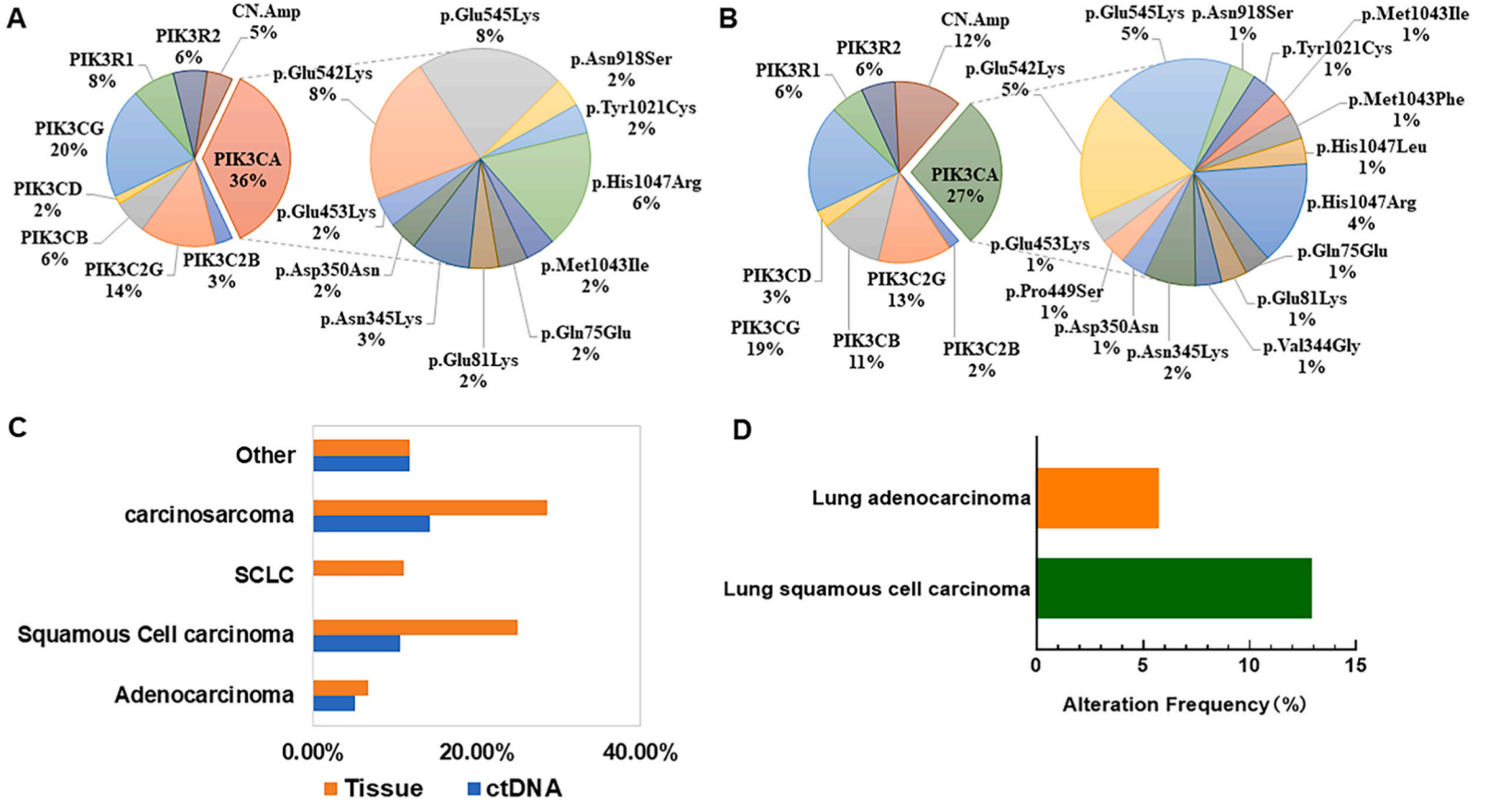

Fig. 1. Distribution of different mutations in *PI3K* family or *PIK3CA*-mutated lung cancer ctDNA and tissue. A. Distribution of *PI3K* family or *PIK3CA*-mutated lung cancer in ctDNA. B. Distribution of *PI3K* family or *PIK3CA*-mutated lung cancer in tissue. C. *PIK3CA* mutation in different histological types of lung cancer. D. *PIK3CA* mutation in the cBioPortal database. SCLC, small cell lung cancer; mut, mutation; ctDNA, circulating tumor DNA.

2.4. Database analysis

PIK3CA mutations were analyzed in 7194 samples from lung adenocarcinoma datasets (N = 14) and 1256 samples from lung squamous cell carcinoma datasets (N = 4) in the cBioPortal database; prognosis of *PIK3CA* mutation was analyzed using Kaplan-Meier Plotter database; Functional enrichment analysis was used by WebGestalt (WEB-based Gene SeT Analysis Toolkit) web.

2.5. Cell culture

Human NSCLC cell lines A549(KRAS p.Gly12Ser)、H358(KRAS p.Gly12Cys)、H2228 H2228(TP53 p.Gln331Ter)、H3255(EGFR p.Leu858Arg) and H1975(EGFR p.Thr790Met/p.Leu858Arg) were purchased from Pricella Life Science Technology Co., Ltd. (Wuhan, China). The cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10 % fetal bovine serum (Pricella).

2.6. Small interfering RNA (siRNA) transfection

siRNA-*PIK3CA* was synthesized by GeneChem Co. (Shanghai, China). A549, H358, H2228, H3255, and H1975 cells were cultured to 80%–85 % confluence, and transfection was performed using Lipofectamine™ 2000 (Invitrogen, CA) by the manufacturer's instructions. After transfection for 48 h, cells were collected and used for further assays.

2.7. Western blot analysis

Transfected cells were lysed using RIPA buffer and protein concentration was detected using the BCA™ protein assay kit (Beyotime, China). Cell lysates (20 μg) were separated on a 8%–20 % SDS-PAGE gel and transferred to a 0.45 μm PVDF membrane (Millipore), and 5 % nonfat milk was used to block the PVDF membrane for 1 h *PIK3CA* (Beyotime; 1:1000) and β-actin (Wanleibio, China, 1:2000) primary antibodies were incubated overnight at 4 °C and incubated with horseradish peroxidase-labeled goat anti-rabbit IgG (Beyotime, 1:2000) for 60 min at 15°C–25 °C. An ECL-Star kit (Beyotime) was used to detect proteins.

2.8. MTT assay

si-negative control and si-*PIK3CA* cells were plated in a 96-well microplate at 2000 cells per well for 48 h and 20 μl of 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di- phenytetrazoliumromide (MTT) reagent (5 mg/ml, Wanleibio) was added to the wells and the cells were incubated at 37 °C for 4 h. The supernatant was discarded and 150 μl of dimethyl sulphoxide was added to each well. The absorbance was measured at 570 nm.

2.9. Statistical methods

Clinical data were analyzed using GraphPad Prism version 8.0.1 (GraphPad Software Inc.) and SPSS 22.0, and statistical software (IBM SPSS Statistics, USA). Chi-square test, or Fisher's exact test were used for comparison of qualitative data groups; kappa test was used for consistency analysis; Pearson correlation coefficient was used for the correlation analysis; receiver operating characteristic (ROC) curve was used to assess the sensitivity and specificity. Student's t-test (two-tailed) was used to analyze si-NC and si-*PIK3CA* data. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Distribution of PIK3CA mutations

Of the 810 samples, 46 had *PIK3CA* mutations (5.68 %): 5.19 % (21/405) in ctDNA and 6.17 % (25/405) in paired tissues. *PIK3CA*

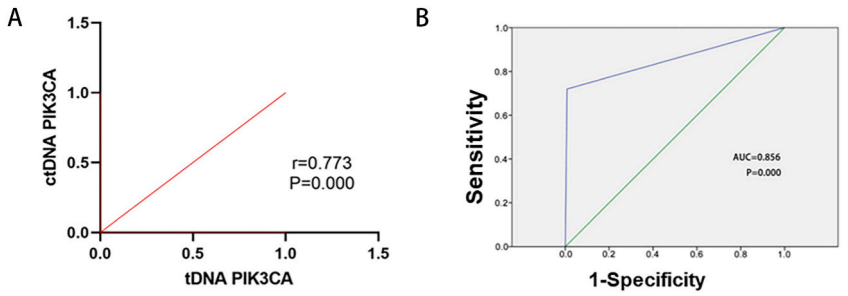

Fig. 2. Comparative analyses of *PIK3CA* mutations in ctDNA and paired tissues. A. Consistency of *PIK3CA* mutations between ctDNA and tissue; B. ROC curves of *PIK3CA* mutations between ctDNA and tissue; ctDNA, circulating tumor DNA.

was the most common mutation subtype of *PI3K* family in ctDNA and paired tissues (36 % and 27 %, respectively), and the three main mutation types of *PIK3CA* were p.Glu542Lys, p.Glu545Lys, and p.His1047Arg(Fig. 1A–B). Thirty-six of 688 samples had *PIK3CA* mutations: 5.23 % in lung adenocarcinoma, 4.65 % (16/344) in ctDNA, and 5.81 % (20/344) in paired tissues. The mutation rate of *PI3K*CA was 5.36 % in lung squamous cell carcinoma, 7.14 % (2/28) in ctDNA, and 3.57 % (1/28) in paired tissues; however, *PIK3CA* mutations were not detected in the SCLC ctDNA samples (Fig. 1C). Fourteen lung adenocarcinoma tissue datasets (N = 7194) and four lung squamous cell carcinoma datasets (N = 1256) in the cBioPortal database were analyzed, and *PIK3CA* was found at a higher frequency in squamous cell carcinoma (12.93 %) than in adenocarcinoma (5.76 %). The mutation rate of *PIK3CA* in adenocarcinoma was consistent with that in the database but was lower in squamous cell carcinoma than in the database (Fig. 1D).

3.2. Consistency analysis of PIK3CA mutations in tissues and ctDNA

PIK3CA mutation status in ctDNA was positively correlated with that in matched tissues ($r = 0.773$, $P = 0.000$). Using tissue *PIK3CA* mutation results as the gold standard, the mutation consistency rate was 97.53 % (kappa: 0.770, $P = 0.000$) (Fig. 2A), true negative rate (TNR) was 99.2 %, sensitivity/true positive rate (TPR) was 72.0 %, and negative predictive value (NPV) and positive predictive value (PPV) were 0.982 and 0.857, respectively (AUC = 0.856, $P = 0.000$) (Fig. 2B). The consistency rate was 98.26 % (kappa = 0.824, $P = 0.000$) for lung adenocarcinoma (TPR, 75 %; TNR, 99.69 %; NPV, 0.9848; PPV, 0.9375). The consistency rate was 96.43 % (kappa: 0.65, $P = 0.000$) for lung squamous cell carcinoma (TPR, 100 %; TNR, 96.30 %; NPV, 1; PPV, 0.5).

3.3. TP53 and EGFR were the most common concomitant genomic alterations

TP53 was the most frequent concomitant gene with *PIK3CA* mutations (72 %) and *EGFR* (41 %) was the most common concomitant driver gene mutation (Fig. 3A), which was consistent with the concomitant *PIK3CA* mutations in ctDNA *TP53* (71 % and 38 %) and paired tissue *EGFR* (72 % and 44 %) (Fig. 3B–C).

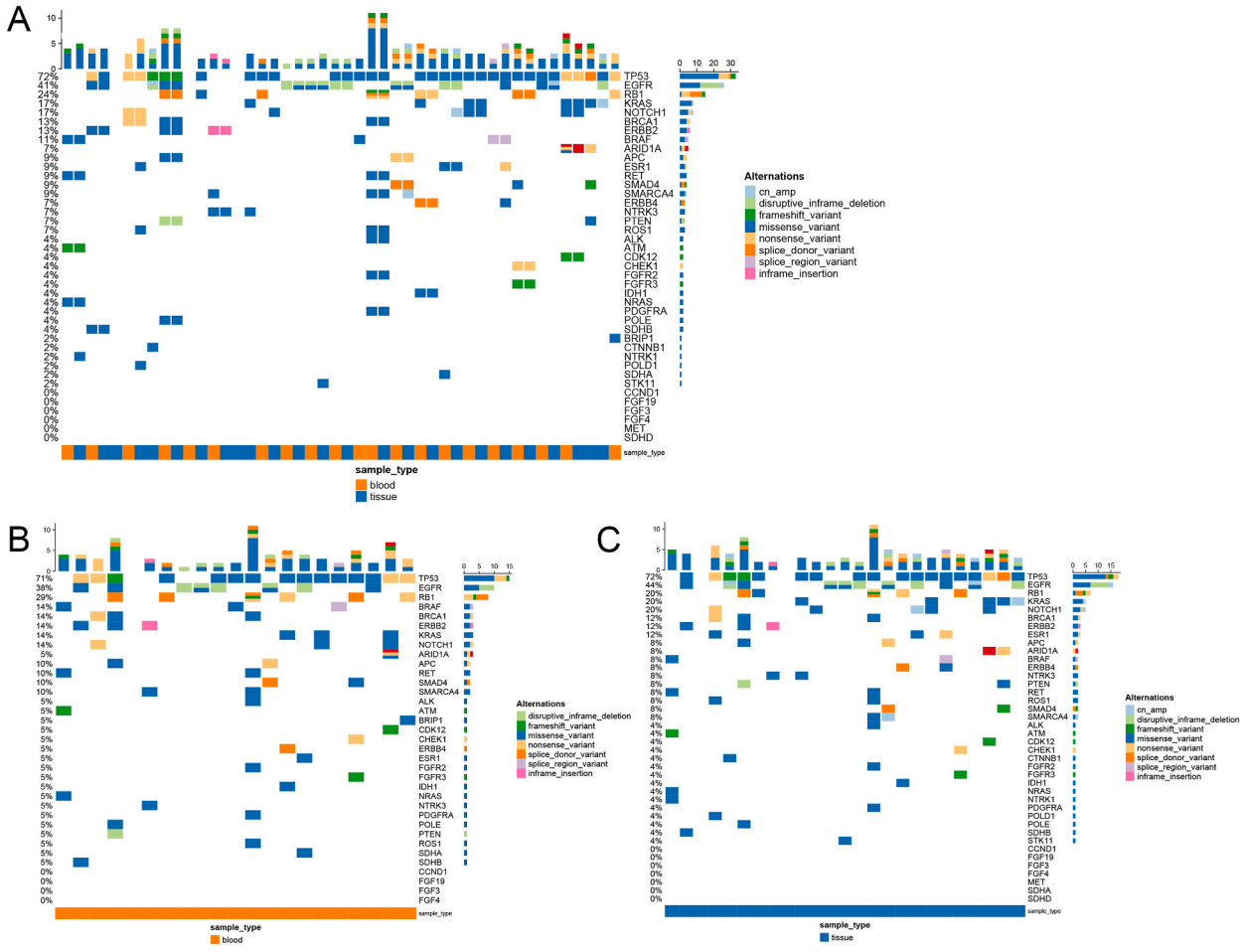

Fig. 3. Concomitant genomic alteration heatmap of patients with *PIK3CA* mutations for A. all samples, B. ctDNA, and C. tissue.

3.4. Patients with PIK3CA and PI3K pathway mutations had high TMB

PIK3CA mutations in ctDNA (47.6 %) and matched tissue patients (44.0 %) had high TMB ($P = 0.000$, $P = 0.001$). *PI3K* pathway *(PIK3/AKT/mTOR/RAS/PTEN)* changes were also significantly associated with high TMB ($P = 0.000$) (Table 1, Fig. 4A–D) and bTMB showed a correlation with tTMB in lung cancer patients with *PIK3CA* mutations ($r = 0.5986$, $P = 0.0041$) (Fig. 4E) but was not correlated with altered *PI3K* pathway ($r = -0.03183$, $P = 0.7620$) (Fig. 4F).

3.5. Correlation between PIK3CA mutations and clinicopathological factors

PIK3CA mutations were uncorrelated with sex, age, smoking, PS score, and pathological classification (Table 2), but *PI3K* pathway alteration was associated in male patients older than 60 and smoking ($P < 0.05$), while contrary to tissue, ctDNA *PI3K* pathway alteration was associated with histologic subtype ($P = 0.036$) (Supplementary Table 2). *PI3K* pathway was also correlated with male sex ($P = 0.022$), old age ($P = 0.007$), and smoking (P = 0.001) for concordant patients (Table 3). Seven patients with t*PIK3CA*^{mut}/ctDNA *PIK3CA*^{wt} harbored adenocarcinoma subtype stage IV (71.4 %), with low PS score (≤1) (85.7 %) and TMB (71.4 %) (Table 4). We analyzed lung cancer biomarkers, including carcinoembryonic antigen (CEA), neuron-specific enolase (NSE), cytokeratin 19 (CK19), and found that *PIK3CA* mutations were not associated with CEA, NSE, and CK19 expression (Supplementary Table 3).

3.6. Downregulation of PIK3CA inhibited A549 and H3255 cell proliferation

siRNA was used to knockdown *PIK3CA* expression in NSCLC cells. Compared with small interference negative control (si-NC), si-*PIK3CA*#1 notably downregulated *PIK3CA* expression in A549, H358, H2228, H3255, and H1975 cells (Fig. 5A), and the **uncropped** images were showed in Supplementary Figs. 1A–1D. *PIK3CA* knockdown decreased cell viability of KRAS p.Gly12Ser mutant cells (A549) and *EGFR* p.Leu858Arg mutant cells (H3255) compared with si-NC (P = 0.011 and P = 0.016, respectively) (Fig. 5B).

3.7. PIK3CA mutation maybe resistance mechanisms to EGFR-TKIs in EGFR-mutant NSCLC

PIK3CA mutation was not associated with overall survival (OS) and Recurrence free survival (RFS) in Lung adenocarcinoma (LUDC)and lung squamous cell carcinoma (LUSC) in the Kaplan-Meier Plotter database (Supplementary Figs. 2A–2D). Furthermore, we explore WebGestalt web, found *PIK3CA* gene was enrich in *EGFR* tyrosine kinase inhibitor resistance pathway (Supplementary Fig. 3), in our *PIK3CA* mutation data, the eight patients with *PIK3CA* mutation received *EGFR*-TKI treatment, three patients showed Stable disease (SD), four patients were progressive disease (PD), only one patient showed partial response (PR), the disease control rate (DCR)was 50 %, and overall response rate (ORR) was only 12.5 % (Supplementary Table 4).

4. Discussion

PIK3CA mutations have reached level 1 evidence for predicting the benefit of alpelisib, which is approved by the FDA, in breast cancer but not in lung cancer [20]. *PIK3CA* mutations have been found in tumors, especially in NSCLC [21–24], but whether they are consistent in NSCLC patient tissues and blood remains unclear. NGS is considered to be a reliable and promising method for detecting PIK3CA mutations [25,26]. Based on this new analysis, as expected, we found that blood *PIK3CA* mutations were highly consistent with tissue *PIK3CA* mutations and that *PIK3CA* mutations co-existed with other genomic changes.

According to previous studies, 6.33 % and 4.76 % *PIK3CA* mutations are observed in lung cancer tissues and ctDNA. and, which is similar to the frequencies of 6.17 % and 5.19 % in our study. Consistent with Huang et al. [27], we found that the most common *PIK3CA* mutation subtypes were p.Glu542Lys, p.Glu545Lys, and p.His1047Arg in the tissues or ctDNA. The mutation rate of *PIK3CA* in adenocarcinoma was similar to that reported previously, but our study indicated a lower frequency in squamous cell carcinoma than that reported previously [28], and we found that the small sample size of squamous cell carcinoma resulted in a result bias.

One major finding of our study was that ctDNA and tissue samples first reported 97.53 % concordance for *PIK3CA* in lung cancer, which was superior to the concordance of data (85.2 %) from ctDNA and tissue in lung cancer by NGS [29]. However, the tissue concordance rate was 36.6 % for 520 pan-cancer-related gene panels [30].

Table 1
The relationships between TMB with *PIK3CA* mutation and *PI3K* pathway alteration in lung cancer.

	bTMB (Mutations/mb)		P	tTMB (Mutations/mb)		
	≥10	<10		≥10	<10	
PIK3CA						
Mut	10(47.6 %)	11(52.4 %)	0.000	11(44.0 %)	14(56.0 %)	0.001
Wt	54(14.1 %)	330(85.9 %)		68(17.9 %)	312(82.1 %)	
***PI3K* pathway**[a]						
altered	35(37.6 %)	58(62.4 %)	0.000	44(35.8 %)	79(64.2 %)	0.000
unaltered	29(9.3 %)	283(90.7 %)		35(12.4 %)	247(87.6 %)	

[a] , *PIK3/AKT/mTOR/RAS/PTEN*; TMB, high tumor mutational burden; Mut, mutation; Wt, wide-type.

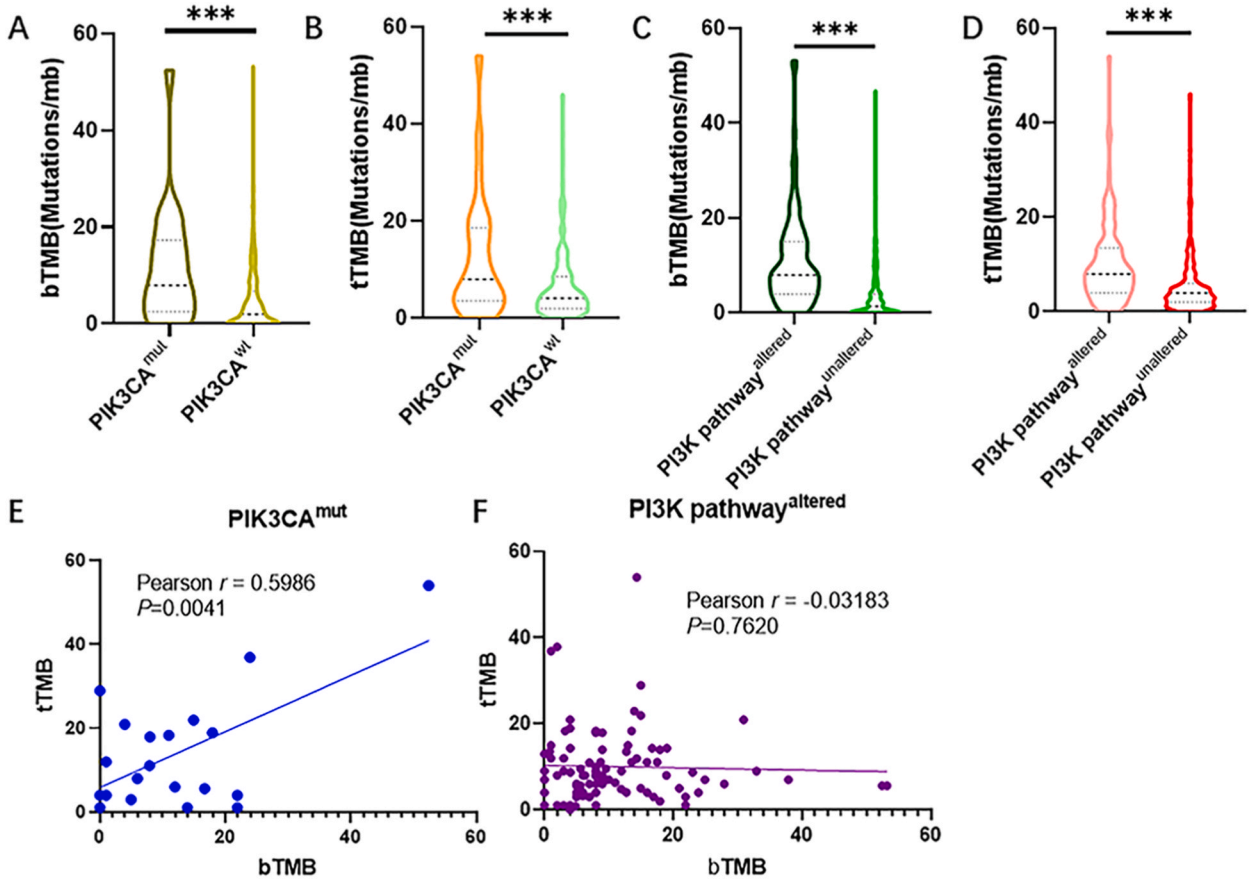

Fig. 4. bTMB and tTMB in lung cancer patients with *PIK3CA* mutations or *PI3K* pathway alterations. A. bTMB in lung cancer patients with *PIK3CA* mutations. B. tTMB in lung cancer patients with *PIK3CA* mutations. C. bTMB in lung cancer patients with *PI3K* pathway alterations. D. tTMB in lung cancer patients with *PI3K* pathway alterations. E. Correlation between bTMB and tTMB in lung cancer patients with *PIK3CA* mutations. F. Correlation between bTMB and tTMB in lung cancer patients with *PI3K* pathway alterations. ***, $P < 0.001$.

In recent years, studies have focused on the clinical role of *PIK3CA* mutation status in lung cancer; therefore, we evaluated the differences in clinical characteristics between NSCLC patients with *PIK3CA* mutations and *PI3K*-pathway alterations. Matthias Scheffler et al. indicated that age and *PIK3CA* mutations were not correlated [6], which was similar to our *PIK3CA* mutation data in tissues; however, we observed a correlation between *PIK3CA* mutations and old age in ctDNA.

To date, the effect of *PIK3CA* mutations on lung cancer treatment remains controversial. A study reported that *PIK3CA* mutations can predict a higher TMB distribution [31]; however, a subsequent study found that *PIK3CA* mutations indicate poor benefit from immunotherapy [32,33]. Previous studies have found that tumors with high TMB may trigger stronger antitumor immune responses [34,35]. A single-arm phase II multicenter clinical trial showed that high bTMB and tTMB levels could effectively benefit from immunotherapy in paired NSCLC samples [36]. Our results showed that *PIK3CA* mutations were associated with high TMB levels in NSCLC plasma or tissue, and this finding may predict the benefit of immunotherapy as a screening biomarker. Patients from different regions may have different clinical characteristics but the underlying mechanisms remain unclear. Therefore, additional studies with larger sample sizes are required to confirm these results.

Another interesting observation is that *PI3K* pathway gene alterations, but not *PIK3CA* mutations, were significantly associated with smoking history and pathological type in our samples or concordant samples. Interestingly, activation of the PI3-kinase pathway may strengthen the lymph node metastasis of tumor cells, and *PIK3CA* mutations are associated with deficient-PTEN [37]. However, the detailed underlying mechanisms remain unclear. Toschi et al. indicated that patients with a history of smoking were more likely to have *PIK3CA* mutations than non-smokers, and we observed this in tissue samples; meanwhile, *PIK3CA* overexpression was observed in male patients or patients with low differentiation [38]. However, Iijima et al. reported a negligible association between *PIK3CA* and sex, which is consistent with our conclusion that *PIK3CA* mutations in tissues and blood are not associated with sex [39]. In addition, we found that blood *PIK3CA* mutations were more likely to be found in older adults, with no differences in tissues, which is partially consistent with the results reported by Li et al. who showed that total *PIK3CA* mutations were associated with age [7]. Furthermore, we found that patients with t*PIK3CA*^{mut}/ctDNA *PIK3CA*^{wt} harbored an adenocarcinoma subtype, low PS score, and low TMB. In our data, the patients received *EGFR*-TKI treatment with *PIK3CA* mutation showed lower DCR and ORR than the patients without *PIK3CA*

Table 2
The clinical characteristics with *PIK3CA*^{mut} lung cancer patients.

	CtDNA-*PIK3CA*			t*PIK3CA*		
	Mut	Wt	*P*	Mut	Wt	*P*
Sex						
male	13(6.2 %)	197(93.8 %)	0.344	13(6.2 %)	197(93.8 %)	0.988
female	8(4.1 %)	187(95.9 %)		12(6.2 %)	183(93.8 %)	
Age						
<60 year	5(2.8 %)	175(97.2 %)	0.051	9(5.0 %)	171(95.0 %)	0.380
≥60 year	16(7.1 %)	209(92.9 %)		16(7.1 %)	209(92.9 %)	
Stage						
I-III	4(6.3 %)	59(93.7 %)	0.885	5(7.9 %)	58(92.1 %)	0.527
IV	17(5.0 %)	325(95.0 %)		20(5.8 %)	322(94.2 %)	
Smoke History						
Never	11(4.2 %)	252(95.8 %)	0.382	13(4.9 %)	250(95.1 %)	0.200
Former	7(8.0 %)	81(92.0 %)		9(10.2 %)	79(89.8 %)	
Current	3(5.6 %)	51(94.4 %)		3(5.6 %)	51(94.4 %)	
PS score						
0–1	18(5.2 %)	327(94.8 %)	1.000	22(6.4 %)	323(93.6 %)	0.906
2–3	3(5.0 %)	57(95.0 %)		3(5.0 %)	57(95.0 %)	
Histologic subtype						
Adenocarcinoma	16(4.7 %)	328(95.3 %)	0.231	20(5.8 %)	324(94.2 %)	0.302
Squamous Cell carcinoma	2(7.1 %)	26(92.9 %)		1(3.6 %)	27(96.4 %)	
Small Cell Lung Cancer	0(0)	9(100 %)		1(11.1 %)	8(88.9 %)	
Sarcoma	1(14.3 %)	6(85.7 %)		1(14.3 %)	6(85.7 %)	
Other	2(11.8 %)	15(88.2 %)		2(11.8 %)	15(88.2 %)	

Mut, mutation; Wt, wild type.

Table 3
The clinical characteristics with lung cancer patients for concordant *PIK3CA*^{mut}/*PI3K*pathway variation.

	PIK3CA		*P* value	*PI3K* pathway[a]		
	Mut	Mt		Mut	Mt	*P* value
Sex						
male	10(4.9 %)	194(95.1 %)		56(27.5 %)	148(72.5 %)	
female	8(4.2 %)	183(95.8 %)	0.734	34(17.8 %)	157(82.2 %)	0.022
Age						
<60 year	5(2.8 %)	171(97.2 %)		29(16.5 %)	147(83.5 %)	
≥60 year	13(5.9 %)	206(94.1 %)	0.143	61(27.9 %)	158(72.1 %)	0.007
Stage						
I-III	3(5.0 %)	57(95.0 %)		13(21.7 %)	47(78.3 %)	
IV	15(4.5 %)	320(95.5 %)	1.000	77(23.0 %)	258(77.0 %)	0.823
Smoke History						
Never	10(3.9 %)	249(96.1 %)		44(17.0 %)	215(83.0 %)	
Former	6(7.1 %)	78(92.9 %)		30(35.7 %)	54(64.3 %)	
Current	2(3.8 %)	50(96.2 %)	0.440	16(30.8 %)	36(69.2 %)	0.001
PS score						
0–1	16(4.7 %)	321(95.3 %)		77(22.8 %)	260(77.2 %)	
2–3	2(3.4 %)	56(96.6 %)	0.922	13(22.4 %)	45(77.6 %)	0.942
Histologic subtype						
Adenocarcinoma	15(4.4 %)	323(95.6 %)		69(20.4 %)	269(79.6 %)	
Squamous Cell carcinoma	1(3.7 %)	26(96.3 %)		9(33.3 %)	18(66.7 %)	
Small lung cancer cell	0(0 %)	8(100 %)		3(37.5 %)	5(62.5 %)	
Sarcoma	1(14.3 %)	6(85.7 %)		3(42.9 %)	4(57.1 %)	
Other	1(6.7 %)	14(93.3 %)	0.496	6(40.0 %)	9(60.0 %)	0.059

[a] , *PIK3/AKT/mTOR/RAS/PTEN*; Mut, mutation; Wt, wide-type.

mutation [40].

5. Conclusion

We reported one of the largest datasets of lung cancer patients with *PIK3CA* mutations in blood samples and matched tissues in a cohort of Chinese population and analyzed concomitant genomic alterations. Moreover, we found that *PIK3CA* mutations were correlated with TMB. The findings provide valuable data for the possibility of replacing tissues with liquid specimens. However, this retrospective study had a few limitations. The number of patients with lung squamous cell carcinoma was small. A prospective study with a larger sample size is required to explore the prognostic value of *PIK3CA*.

Table 4
Characteristics of patients with t*PI3KCA*^{mut}/ctDNA*PI3KCA*$^{w.t}$.

	t*PI3KCA*^{mut}/ctDNA*PI3KCA*^{wt} (N = 7)
Sex	
male	3(42.9 %)
female	4(57.1 %)
Age	
<60 year	3(42.9 %)
≥60 year	4(57.1 %)
Stage	
I-III	2(19.6 %)
IV	5(71.4 %)
Smoke History	
Never	3(42.9 %)
Former/Current	4(57.1 %)
PS score	
0–1	6(85.7 %)
2–3	1(14.3 %)
Histology type	
Adenocarcinoma	5(71.4 %)
Squamous Cell carcinoma	0(0 %)
Other	2(28.6 %)
TMB	
≥10	2(28.6 %)
<10	5(71.4 %)

PS, performance status, TMB, high tumor mutational burden; Mut, mutation; wt, wide-type.

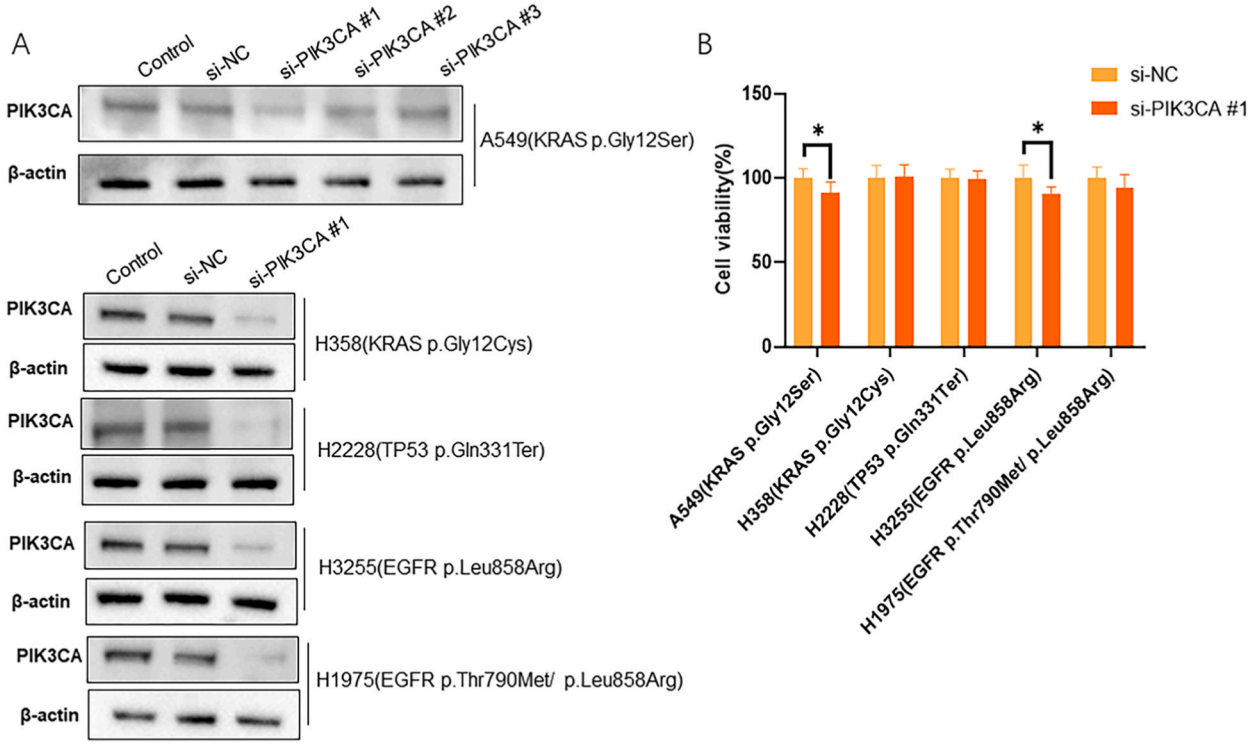

Fig. 5. *PIK3CA* knockdown inhibited human NSCLC viability. A. Western blot analysis of *PIK3CA* expression in A549, H358, H2228, H3255, and H1975 cells, which were transfected with si-NC and si-*PIK3CA*. β-actin was used as the loading control. B. MTT assay detected cell viability after *PIK3CA* knockdown. *$P < 0.05$.

Ethical statement

This study was reviewed and approved by the Medical Ethics Committee of Jilin Cancer Hospital with the approval number: 202309-10-01, dated Sep 14, 2023. Individual consent was waived for this study because of its design as a retrospective analysis.

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Data availability statement

Data will be made available on request to the corresponding author. The datasets used during the current study are openly available from the Kaplan-Meier Plotter (http://kmplot.com/analysis/), WebGestalt (https://www.webgestalt.org/) and cBioPortal (https://www.cbioportal.org/).

CRediT authorship contribution statement

Yan Liu: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Hui Li:** Writing – original draft, Supervision, Formal analysis. **Xiang Li:** Methodology, Investigation. **Tingting Zhang:** Resources, Methodology. **Yang Zhang:** Resources, Methodology. **Jing Zhu:** Resources, Methodology. **Heran Cui:** Investigation, Formal analysis, Data curation. **Rixin Li:** Formal analysis, Data curation. **Ying Cheng:** Writing – review & editing, Validation, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e34013.

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