OncoTargets and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

The Study of PIK3CA Hotspot Mutations and Co-Occurring with EGFR, KRAS, and TP53 Mutations in Non-Small Cell Lung Cancer

YuXuan Zhang[1,2,*], Yuhong Shen[3,*], Jiayuan Wu[2], Jun Zhang[4], Chenxi Cao[5], Juanfen Mo[2], Yi Bao[2,5]

[1]The Fourth School of Clinical Medicine, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, 310000, People's Republic of China; [2]The Key Laboratory, the Second Affiliated Hospital of Jiaxing University, Jiaxing, Zhejiang, People's Republic of China; [3]Transfusion Center, the Second Affiliated Hospital of Jiaxing University, Jiaxing, Zhejiang, People's Republic of China; [4]The Department of Thoracic Surgery, the Second Affiliated Hospital of Jiaxing University, Jiaxing, Zhejiang, People's Republic of China; [5]The Department of Oncology, the Second Affiliated Hospital of Jiaxing University, Jiaxing, Zhejiang, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yi Bao, The Key Laboratory, The Department of Oncology, The Second Hospital of Jiaxing, 1518 Huanchen North Road, Zhejiang, People's Republic of China, 314000, Email ybao2011@zjxu.edu.cn; Juanfen Mo, The Key Laboratory, The Second Affiliated Hospital of Jiaxing University, 1518 Huancheng North Road, Jiaxing, Zhejiang, People's Republic of China, 314000, Email mojuanfen@163.com

Objective: PIK3CA-mutant non-small-cell lung cancer (NSCLC) is associated with other genetic mutations and may influence treatment strategies and clinical outcomes. We aimed to characterize PIK3CA mutations co-occurring with several major driver mutations using data from published cohorts and our medical center.

Materials and Methods: We analyzed NSCLC patients harboring PIK3CA mutations from The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering (MSK) databases and retrospectively identified NSCLC patients with PIK3CA-mutants at a single medical center from our electronic records. The Log rank test was used to determine the association between PIK3CA mutations and overall survival (OS) in NSCLC patients.

Results: Common hotspot mutations in PIK3CA were found in exon 9 (c.1633G > A, E545K, and c.1624G > A, E542K) and exon 20 (c.3140A > G, H1047R) in all cohorts. Co-occurring mutations of PIK3CA with EGFR, KRAS, and TP53 have been frequently observed in patients with NSCLC, with different percentages in these datasets generated by different background. PIK3CA mutations were observed to be significantly associated with poor OS in lung adenocarcinomas patients in the MSKCC cohort (hazard ratio [HR] = 0.519, 95% confidence interval [CI] = 0.301–0.896; P <0.05).

Conclusion: PIK3CA co-occurring mutations in other genes may represent distinct subsets of NSCLC. Further elucidation of the roles of PIK3CA hotspot mutations combined with other driver mutations, including EGFR and KRAS, is needed to guide effective treatment in patients with advanced NSCLC.

Keywords: co-occurring mutation, non-small cell lung cancer, PIK3CA, The Cancer Genome Atlas, Memorial Sloan Kettering Cancer Center

Introduction

Phosphoinositide-3-kinase (PI3Kα), which is composed of a catalytic subunit p110α and a regulatory subunit p85α, catalyzes the phosphorylation of phosphatidylinositol 4.5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3), which in turn activates multiple downstream effectors including AKT and mammalian target of rapamycin (mTOR).[1] The PI3K-AKT pathway is critical in cancer cells in the regulation of various cellular processes through its downstream targets, such as mTOR and glycogen synthase kinase 3 beta (GSK3β) involving effects on cell growth, proliferation, metabolism, and survival.[2]

PIK3CA is a gene encoding the p110α catalytic subunit of PI3Kα and related pathways are critical for malignant cell growth and other metabolic activities.[3] Gain-of-function mutations in p110α of PIK3CA are frequently observed in

Received: 11 March 2024
Accepted: 23 August 2024
Published: 11 September 2024

endometrial, colorectal, and breast cancer,[4,5] In an earlier study using a PCR-mass spectrometry assay, 9% of tumor sample from a total of 3252 cancer specimens contained PIK3CA mutations and with the highest rates in breast cancer (34%).[6] In non-small cell lung cancer (NSCLC), the highly prevalent and malignant disease globally, with an estimated 2.2 million new cases and 1.8 million deaths in 2020, comprise of lung squamous cell carcinoma (SqCC) and lung adenocarcinomas (ADC) mostly.[7] A higher prevalence of PIK3CA mutations has been reported in SqCC than ADC, with ~8.9% and 2.9%, respectively.[8]

PIK3CA mutations have been regarded as driver genes that are mutually exclusive of other driver mutation genes. Inhibitors blocking the activated forms of these enzymes have been widely investigated,[9] and recently published data showed the value of BYL719, a selective inhibitor of PI3Kα, in the treatment of HR (+), HER2 (–), and PIK3CA mutated advanced breast cancer.[10] However, this does not always appear to be the case, and data suggest that co-occurring PIK3CA mutations with other driver oncogenes such as EGFR seem to be present commonly in patients with NSCLC.[11] PIK3CA may act as a synergistic mutation rather than as a driver mutation, as certain mutations of PIK3CA are known to potentially act synergistically with other gene mutations to enhance the activation of the PI3K-Akt signaling pathway, which can lead to increase tumorigenesis and resistance to chemotherapy.[12,13] Additionally, although drugs targeting PIK3CA compounds have been developed for the treatment of tumors with PIK3CA-mutations,[14] no therapeutic drugs have been approved to treat patients harboring PIK3CA-mutation in NSCLC patients. Moreover, little is known about the impact of these combined co-mutations on the progression and survival of patients with NSCLC, as well as the value of combination therapy, in which two or more drugs are simultaneously applied to inhibit oncogenic kinase drivers.

The aim of this study was to better define molecular heterogeneity in PIK3CA mutants in NSCLC, especially the hotspot regions in exon 9 (c.1633G > A, E545K and c.1624G > A, E542K) and exon 20 (c.3140A > G, H1047R), which have been reported in a wide range of cancers, including lung cancer,[15] using published datasets comprising a large number of NSCLC patients, as well as the genetic features of NSCLC patients retrieved from our database. The functional consequence of the hotspot mutations on the activity of PIK3CA closely associates with PI3K-AKT signaling pathway involving cancer proliferation, survival, metastasis and chemo-drugs resistance. Moreover, co- occurring mutations in PIK3CA (E545K, E542K, and H1047R) and the mutational status of EGFR, KRAS, and TP53 were studied and compared in cohorts of different races and disease stages, and the prognostic significance of PIK3CA mutations in these published datasets was explored in NSCLC.

Materials and Methods

Clinical Cohorts

The Cancer Genome Atlas (TCGA) and Memorial Sloan Kettering Cancer Center (MSKCC) cohorts were retrieved from an online data repository comprising more than 2,000 patients retrieved from cBioPortal, a genomic site used to analyze subsets of cases with genetic mutations for clinical and genomic interests.[16]

The Cancer Genome Atlas (TCGA) NSCLC cohort, a database established in the year 2016, comprised the whole genome sequencing data from 660 lung adenocarcinomas (ADC) and 484 lung squamous cell carcinomas (SqCC) tumor/normal pairs which were included in this cohort. Among them, the majority of patients had previously untreated relatively early-stage NSCLC.

The Memorial Sloan Kettering Cancer Center (MSKCC) cohort, published in 2022, includes 1127 patients (969 lung ADC, 88 lung SqCC, and 70 unknown) with metastatic NSCLC and ctDNA-guided therapy.[17] In this cohort, data were obtained from MSKCC (n = 1002) and Sydney (n = 125). Tumor tissues from all patients were subjected to next-generation sequencing with MSK Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT).[18,19] Circulating ctDNA from patients with metastatic NSCLC was investigated by targeted plasma sequencing using the resolution ctDx lung platform.[20] In this cohort, genomic alterations in ctDNA not detected by time-matched tissue sequencing were reported in 25% of the patients.[17] To analyze the survival of PIK3CA mutations associated with overall survival (OS) in patients with ADC, a MSKCC cohort including 2298 metastatic ADC was analyzed.[21]

Another data including the genetic events, such as EGFR/KRAS/TP53/PIK3CA hotspot mutations, were retrieved from the electronic database of the Second Hospital of Jiaxing in 1093 lung adenocarcinomas and lung SqCC patients using probe-capture Next Generation Sequencing assay on the Illumina platform (Illumina NextSeq).

This section of study was approved by the Institutional Ethical Review Board of the Second Hospital of Jiaxing, and all patients provided specimens in this part of study with written informed consent (Ethical No: JXEY-2024-204-01).

Statistical Analyses

Statistical analyses were performed using GraphPad Prism (version 7.01, La Jolla, CA, USA). Statistical tests, including Wilcoxon and chi-square tests, were used to analyze the genomic data. Kaplan–Meier curve analysis of OS was performed using the Log rank test. All reported *P*-values were two-tailed, and statistical significance was set at $P < 0.05$.

Results

PIK3CA Mutations and Correlation of Prognosis in Pan-Lung Cancer from the TCGA Database

PIK3CA mutations were observed in 8.2% (94 of 1,144) of the TCGA dataset. Most mutations were missense mutations which are located in exon 9 (c.1633G > A E545K, E542K c.1624G > A) and exon 20 (c.3140A > G, H1047R), accounting for 26.7% (26/94), 20.2% (19/94), and 6.4% (6/94), respectively. Other frequently mutated somatic genes included EGFR, KRAS, and TP53 mutations, accounting for about 10.2%, 19.4%, and 67.7% of all the samples. We further analyzed the co-occurrence of EGFR, KRAS, and TP53 mutations, together with either of mutations among these three hotspots of PIK3CA, ranging from 3.9% (2/51) in EGFR, 5.9% (3/51) in KRAS, and 76.5% (39/51) in TP53. Table 1 presents the results. In this cohort, a trend toward a poor prognosis appeared to be associated with patients with PIK3CA mutations, although the difference was not statistically significant using Log rank test ($P = 0.0709$) (Figure 1). In a subsequent comparison of tumor mutation burden (TMB) between the PIK3CA mutation and wild-type groups, data showed that, in the PIK3CA altered group, the average values of TMB were significantly higher than those of the unaltered group using Wilcoxon test ($P < 0.05$) (Figure 2).

PIK3CA Mutations and Correlation of Prognosis in NSCLC from a MSKCC Cohort

A total of 1127 patients with stage IV NSCLC with distant metastases were included in the MSKCC cohort. From these 1127 patients, 5.8% (65/1127) patients harboring PIK3CA mutations, with E545K 23.1% (15/65), E542K 13.8% (9/65), and H1047R 6.2% (4/65), respectively. Other frequently somatic mutated genes included EGFR, KRAS, and TP53 mutations, accounting for about 27%, 22%, and 51% of all the samples. After further analyzing the co-occurring EGFR, KRAS, and TP53 mutations, together with these three hotspot mutations in PIK3CA, counting EGFR 28.6% (8/28), KRAS 39.3% (11/28), and TP53 60.7% (17/28), respectively. The data are presented in Table 2. The overall survival (OS) analysis suggested that PIK3CA mutations have a poor clinical outcome compared with wide types both in NSCLC and ADC MSKCC cohorts, but only observed $P < 0.05$ on the ADC MSKCC cohort (Figure 3A and B). In patients harboring PIK3CA (E545K, E542K, and H1047R), with or without EGFR mutations, the survival time and co-mutation with KRAS were no difference with KRAS wide types in the

Table 1 Co-Occurring Mutations in PIK3CA (E545K, E542K and H1047R) with EGFR, KRAS and TP53 respectively in NSCLC Samples Retrieved from TCGA Cohort

Types of combined mutations	PIK3CA E545K (N=26)	PIK3CA E542K (N=19)	PIK3CA H1047R (N=6)	Total (N=51)
EGFR	1	0	1	2 (3.9%)
KRAS	3	0	0	3 (5.9%)
TP53	19	14	6	39 (76.5%)

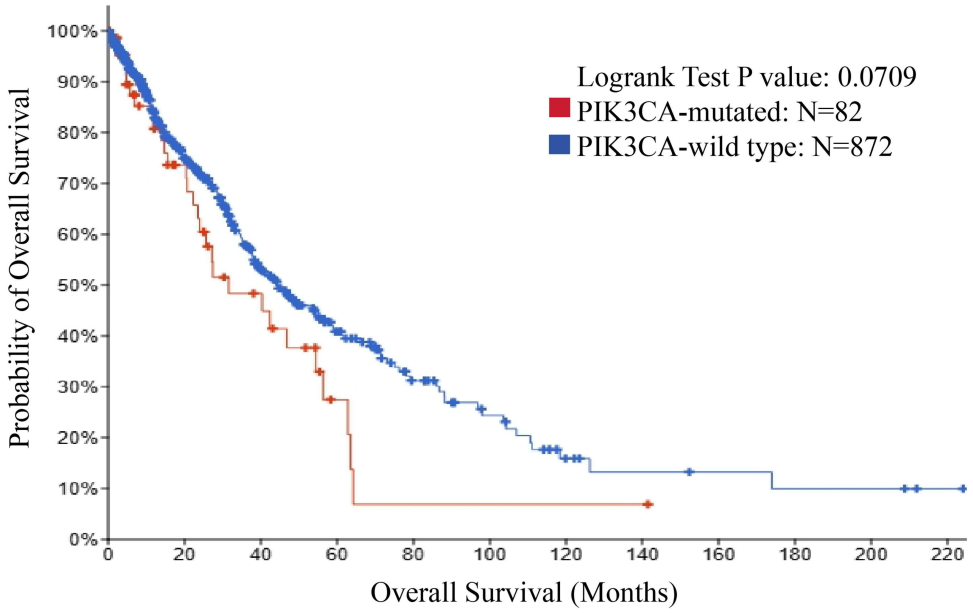

Figure 1 Comparing PIK3CA mutated group with the wide type group, no statistically significant difference of overall survival rates was observed between two groups, although a poor prognostic trend in PIK3CA mutated group, by analysing TCGA data, PIK3CA mutated group: N = 82; PIK3CA wide type group: N = 872.

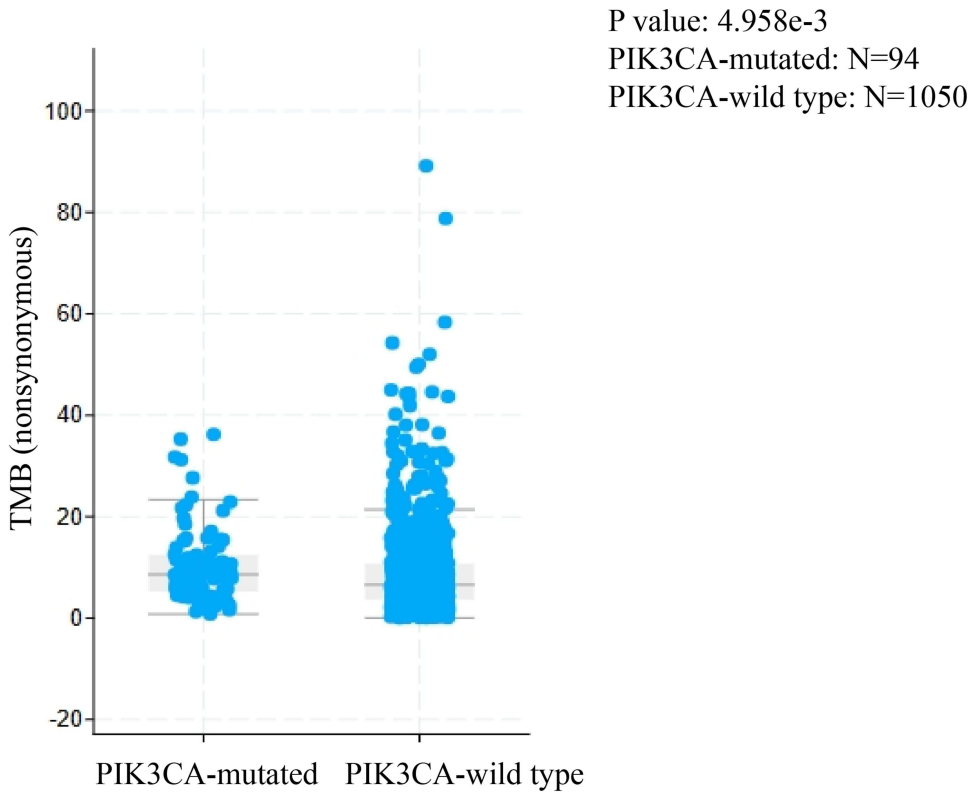

Figure 2 The tumor mutation burden (TMB) was significantly higher in the PIK3CA mutated group compared with the wide type group in the TCGA cohort using Wilcoxon Test, $P < 0.001$. PIK3CA mutated group: N = 94; PIK3CA wide type group: N = 1,050.

ADC MSKCC cohort. We further compared the tumor mutation burden (TMB) between the PIK3CA mutation and wild-type groups. In PIK3CA altered group, the average TMB values were significantly higher than those in the unaltered group using Wilcoxon test ($P < 0.05$) (Figure 4).

Table 2 Co-Occurring Mutations in PIK3CA (E545K, E542K and H1047R) with EGFR, KRAS and TP53 respectively in NSCLC Samples Retrieved from Metastatic MSK Cohort

Types of combined mutations	PIK3CA E545K (N=15)	PIK3CA E542K (N=9)	PIK3CA H1047R (N=4)	Total (N=28)
EGFR	2	4	2	8 (28.6%)
KRAS	8	1	2	11 (39.3%)
TP53	6	8	3	17 (60.7%)

Comparison of the Percentage Difference in the Co-Occurrence of EGFR, KRAS, and TP53 Together with PIK3CA Mutations Between These Two Online Cohorts

Next, we examined the percentage difference in the co-occurrence of EGFR, KRAS, and TP53, together with three hotspot mutations of PIK3CA in NSCLC from the TCGA and MSKCC cohorts. A higher percentage of co-occurrence of EGFR and KRAS together with PIK3CA mutations was observed in the MSKCC cohort than in the TCGA cohort, with statistical significance using Chi–square test ($P < 0.05$). No difference was observed in the co-occurrence of TP53 and PIK3CA mutations between the two cohorts ($P > 0.05$). Table 3 presents the results.

Co-Occurring PIK3CA Mutations in Our Medical Center in Comparison of the TCGA Cohort

In total, 1093 patients with stage I–IV NSCLC were included in this cohort. PIK3CA mutations were observed in 5.03% (55/1093), with E545K 34.5% (19/55), E542K 20.0% (11/55), and H1047R 12.7% (7/55), respectively. After further analyzing co-occurring EGFR, KRAS, and TP53 mutations, together with the three hotspot mutations of PIK3CA, counting EGFR as 56.8% (21/37), KRAS as 10.8% (4/37), and TP53 as 45.9% (17/37). The results are presented in Table 4.

We further compared the different percentages of co-occurring mutations in EGFR, KRAS, and TP53 with hotspot mutations (exon 9: c.1633G > A E545K, pE542K c.1624G > A; Exon 20: c.3140A > G, H1047R) in PIK3CA from TCGA and our cohorts. The similarity of these two datasets contained stage I–IV NSCLC, but different races. A statistically significantly higher percentage of co-concurring EGFR and PIK3CA mutations was observed in our cohort, with all patients originating from Asia compared with the TCGA cohort, the majority being Caucasian ($P < 0.001$). No difference was observed in the co-occurrence of KRAS and PIK3CA, as well as TP53 and PIK3CA mutations, between these two cohorts ($P > 0.05$). The results are presented in Table 5.

Discussion

In NSCLC, the co-occurring PIK3CA mutations (E545K, E542K, and H1047R) together with EGFR and KRAS mutations were higher in the metastatic MSKCC cohort than in the TCGA cohort. The TCGA cohort included most of the early-stage cases of NSCLC, in contrast to the MSKCC cohort. Interestingly, two cases of PIK3CA mutations that were only present in metastatic tumor tissues but not in primary sites were reported in the MSKCC cohort, suggesting that, although rare, PIK3CA-wild type tumor cells may switch to PIK3CA mutations in the progress of the disease; moreover, in a metastatic NSCLC dataset, patients with PIK3CA mutations were associated with a short survival time in metastatic lung adenocarcinomas. In contrast, analysing the TCGA data derived mostly from resectable NSCLC patients, PIK3CA mutations are likely to be associated with a poor prognosis, although statistically not significant. A higher percentage of co-occurring PIK3CA and EGFR mutations has been observed in Asian patients with NSCLC than in Caucasians. No difference was observed in co-occurring PIK3CA mutations (E545K, E542K, and H1047R) and KRAS and TP53 mutations in Asian NSCLC patients compared with Caucasians.

The oncogene KRAS mutations are the most common genetic driver in Caucasian patients with non-small cell lung cancer (NSCLC), appearing in approximately 35% of adenocarcinomas and 5% of squamous cell carcinomas.[20] Co-occurring genomic alterations, together with KRAS mutations, may be classified into subgroups in patients with NSCLC.

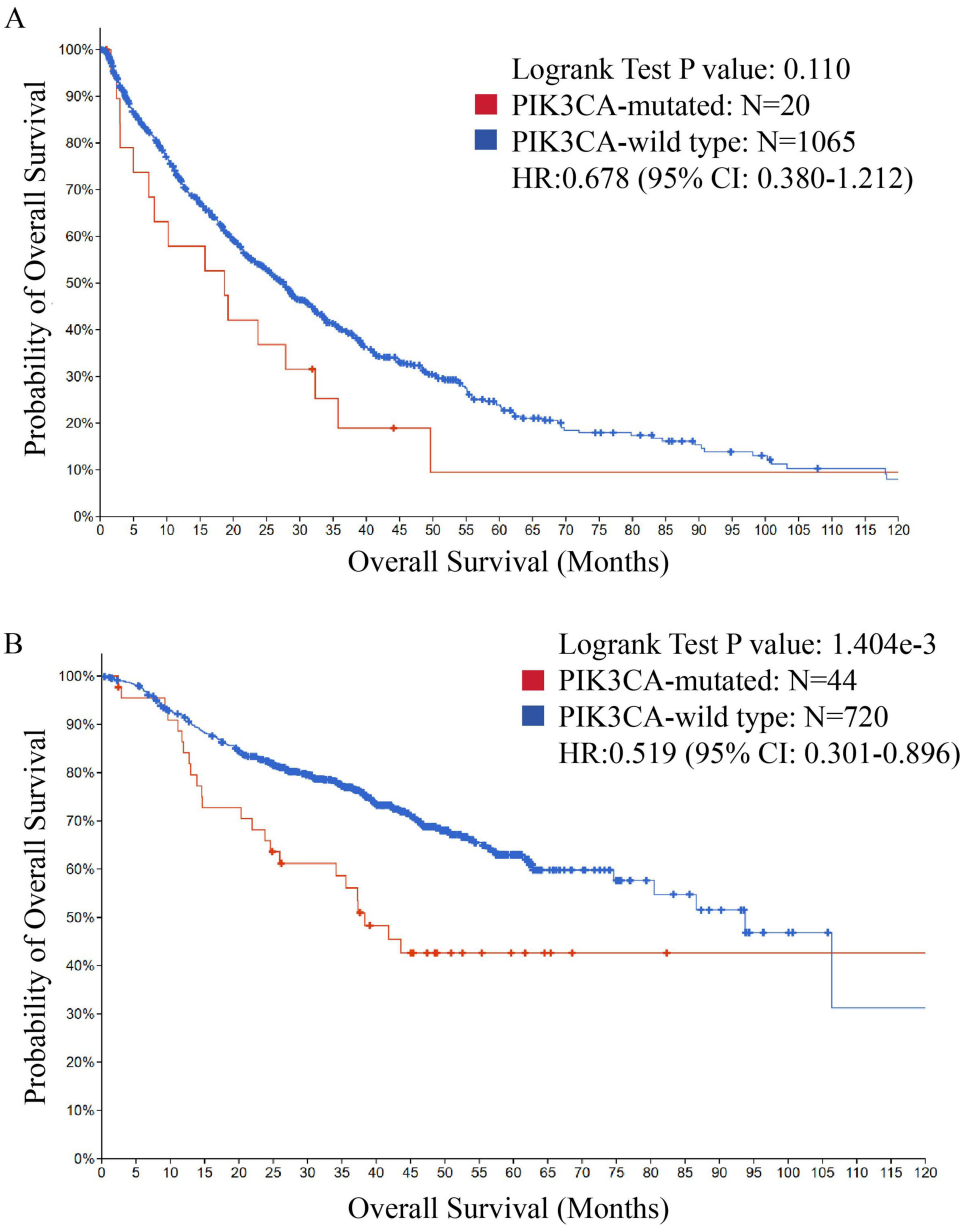

Figure 3 The overall survival (OS) analysis showed that PIK3CA mutations have a poor clinical outcome compared with wide types both in NSCLC (**A**) and ADC MSKCC cohorts (**B**), but only statistically significance in ADC MSKCC cohort using Log rank test. P < 0.05.(**A**) PIK3CA mutated group: N = 20; PIK3CA wide type group: N = 1065 (**B**) PIK3CA mutated group: N = 44; PIK3CA wide type group, N = 720.

PIK3CA encodes the p110α catalytic subunit of PI3K, a downstream enzyme of KRAS that drives tumorigenesis by upregulating the PI3K pathway. The oncogene KRAS mutations are the most common genetic driver in Caucasian patients with non-small cell lung cancer (NSCLC), appearing in approximately 35% of adenocarcinomas and 5% of squamous cell carcinomas;[22] and therefore, co-occurring KRAS and PIK3CA mutations may contribute tumor cell proliferation and survival and may be associated with lung cancer metastasis.[23,24] However, we were unable to find a significant association between survival time and PIK3CA mutations with or without co-occurring KRAS mutations in the MSKCC cohort, although PIK3CA or KRAS mutations alone indicated a short survival time. This may be because inhibitors targeting KRAS and PIK3CA were not commonly used in the previous majority datasets; for instance, sotorasib is currently approved for the treatment of patients with NSCLC harboring KRAS G12C mutation only.[25]

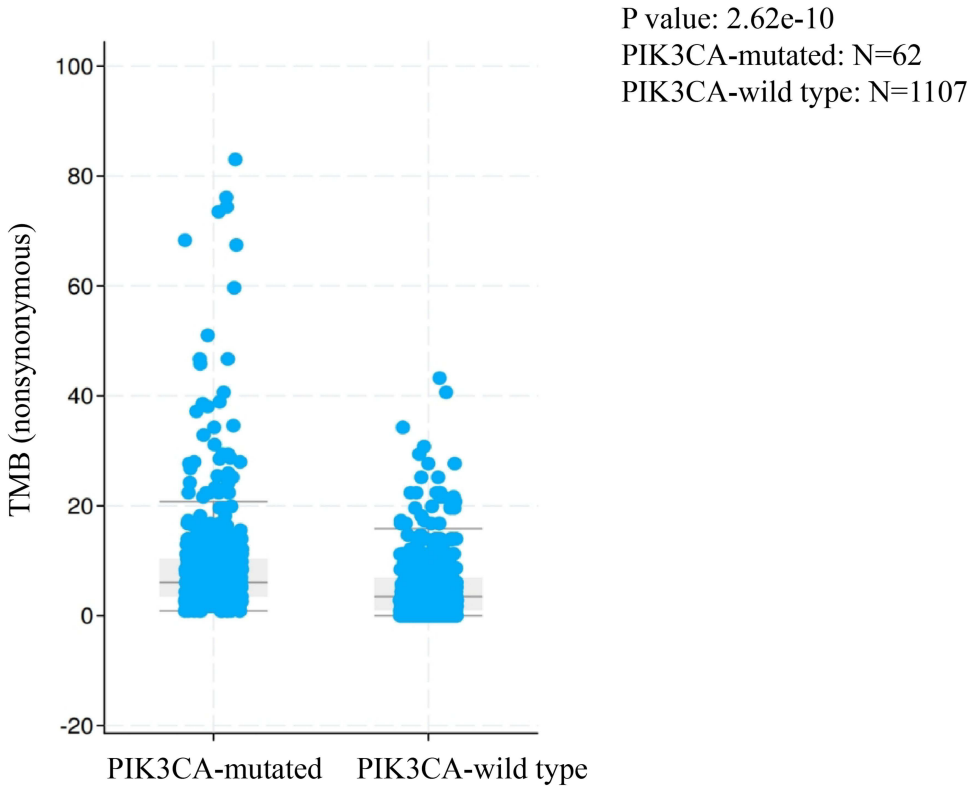

Figure 4 A statistically significant higher TMB value was observed in PIK3CA mutated group compared with wide type group in MSKCC cohort using Wilcoxon Test, $P < 0.001$. PIK3CA mutated group: N = 62; PIK3CA wide type group: N = 1107.

Although Pan-KRAS inhibitors that block a broad range of KRAS mutants, including G12A/C/D/F/V/S, G13C/D, etc have been investigated in patients with KRAS-driven cancers,[26] they are still in early clinical trials. Additionally, inhibitors, such as alpelisib that directly block PIK3CA mutations have been developed and approved by the FDA for the treatment of hormone receptor positive/human epidermal growth factor receptor 2 negative (HR+/HER2-) advanced breast cancer.[27] Moreover, given the poor clinical outcome of monotherapy by targeting PIK3CA mutations alone in

Table 3 Comparison of the Percentage Difference in the Co-Occurrence of EGFR, KRAS, and TP53 Together with PIK3CA Mutations (E545K, E542K and H1047R) Between These Two Online Cohorts

Types of combined mutations	TCGA Cohort (%)	MSK Cohort (%)	P values
EGFR/PIK3CA (E545K+E542K+H1047R)	3.9%	28.6%	0.0068*
KRAS/PIK3CA (E545K+E542K+H1047R)	5.9%	39.3%	0.0026*
TP53/PIK3CA (E545K+E542K+H1047R)	76.5%	60.7%	0.5368

Notes: *$P < 0.05$, significantly different from the values in TCGA Cohort using chi-square tests.

Table 4 Co-Occurring Mutations in PIK3CA (E545K, E542K and H1047R) with EGFR, KRAS and TP53 respectively in NSCLC Samples Retrieved from Our Cohort

Types of combined mutations	PIK3CA E545K (N=19)	PIK3CA E542K (N=11)	PIK3CA H1047R (N=7)	Total (N=37)
EGFR	11	7	3	21 (56.8%)
KRAS	1	0	3	4 (10.8%)
TP53	10	4	3	17 (45.9%)

Table 5 Comparison of the Percentage Difference in the Co-Occurrence of EGFR, KRAS, and TP53 Together with PIK3CA Mutations (E545K, E542K and H1047R) Between These Two Online Cohorts

Types of combined mutations	TCGA Cohort (%)	Our Cohort (%)	P values
EGFR/PIK3CA (E545K+E542K+H1047R)	3.9%	56.8%	< 0.001*
KRAS/PIK3CA (E545K+E542K+H1047R)	5.9%	10.8%	0.4376
TP53/PIK3CA (E545K+E542K+H1047R)	76.5%	45.9%	0.1578

Notes: *$P < 0.05$, significantly different from the values in TCGA Cohort using chi-square tests.

some breast cancer patients, fulvestrant, an estrogen receptor antagonist, is used to combine with alpelisib. In addition, the presence of co-occurring mutations in PIK3CA mutated NSCLC patients, a combination of chemical compounds targeting different mutations may be a strategy to improve treatment efficacy in the future. Finally, this study used fairly large samples retrieved from the MSKCC cohort; however, the size of each subset with co-occurring mutations was small, which may have led to reduced statistical power to detect differences in survival time. Additionally, co-mutation of PIK3CA with other genes in the PI3K-AKT signaling pathway, such as PTEN, add extra potential impact on cancer cell survival and resistance to chemotherapy due to a synergistic effect. Therefore, co-occurring PIK3CA and PTEN mutations may be valuable to investigate in depth.

Nevertheless, given the high frequency of anti-EGFR targeted therapy as a significant approach in NSCLC, the genomic landscape may need to be monitored in primary and metastatic tumor sites, as well as circulating ctDNA in the same patient, and adjustment to effective treatment may influence response and overall survival time, eventually changing existing guidelines. Further, successful studies targeting KRAS and PIK3CA mutant NSCLC may alter the results obtained from retrospective cohort studies.

In conclusion, our study provides genetic views of combined mutations of PIK3CA mutations with other driver genes in NSCLC patients. It is worthful for preclinical or clinical trials targeting EGFR/PIK3CA or KRAS/ PIK3CA activating mutations with drug combinations in NSCLC patients. NSCLC is a heterogeneous disease composed of distinct co-occurring molecular mutant subgroups and attention is needed with further assessments of therapeutic interventions.

Funding

This study was supported by the Science and Technology Bureau of Jiaxing (Grant Nos. 2020AD30081 and 2021AY30019).

Disclosure

The authors have no conflicts of interest to disclose for this work.

References

1. Vanhaesebroeck B, Stephens L, Hawkins P. Pi3k signalling: the path to discovery and understanding. *Nat Rev Mol Cell Biol*. 2012;13(3):195–203. doi:10.1038/nrm3290
2. Song M, Bode AM, Dong Z, et al. Akt as a therapeutic target for cancer. *Cancer Res*. 2019;79(6):1019–1031. doi:10.1158/0008-5472.CAN-18-2738
3. Fruman DA, Chiu H, Hopkins BD, et al. The pi3k pathway in human disease. *Cell*. 2017;170(4):605–635. doi:10.1016/j.cell.2017.07.029
4. Pavlidou A, Vlahos NF. Molecular alterations of pi3k/akt/mtor pathway: a therapeutic target in endometrial cancer. *Scientificworldjournal*. 2014;2014:709736. doi:10.1155/2014/709736
5. Di Cosimo S, Baselga J. Phosphoinositide 3-kinase mutations in breast cancer: a "good" activating mutation? *Clin Cancer Res*. 2009;15(16):5017–5019. doi:10.1158/1078-0432.CCR-09-1173
6. Stachler MD, Rinehart EM, Garcia E, et al. Pik3ca mutations are common in many tumor types and are often associated with other driver mutations. *Appl Immunohi Mol Morphol*. 2016;24(5):313–319. doi:10.1097/PAI.0000000000000195
7. Leiter A, Veluswamy RR, Wisnivesky JP. The global burden of lung cancer: current status and future trends. *Nat Rev Clin Oncol*. 2023;20(9):624–639. doi:10.1038/s41571-023-00798-3
8. Scheffler M, Bos M, Gardizi M, et al. Pik3ca mutations in non-small cell lung cancer (NSCLC): genetic heterogeneity, prognostic impact and incidence of prior malignancies. *Oncotarget*. 2015;6(2):1315–1326. doi:10.18632/oncotarget.2834
9. Tewari D, Patni P, Bishayee A, et al. Natural products targeting the pi3k-akt-mtor signaling pathway in cancer: a novel therapeutic strategy. *Semin Cancer Biol*. 2022;80:1–17. doi:10.1016/j.semcancer.2019.12.008

10. Chaft JE, Arcila ME, Paik PK, et al. Coexistence of pik3ca and other oncogene mutations in lung adenocarcinoma-rationale for comprehensive mutation profiling. *Mol Cancer Ther*. 2012;11(2):485–491. doi:10.1158/1535-7163.MCT-11-0692
11. Vanhaesebroeck B, Perry M, Brown JR, et al. Pi3k inhibitors are finally coming of age. *Nat Rev Drug Discov*. 2021;20(10):741–769. doi:10.1038/s41573-021-00209-1
12. Vasudevan KM, Barbie DA, Davies MA, et al. Akt-independent signaling downstream of oncogenic pik3ca mutations in human cancer. *Cancer Cell*. 2009;16(1):21–32. doi:10.1016/j.ccr.2009.04.012
13. Leontiadou H, Galdadas I, Athanasiou C, et al. Insights into the mechanism of the pik3ca e545k activating mutation using md simulations. *Sci Rep*. 2018;8(1):15544. doi:10.1038/s41598-018-27044-6
14. Yu Y, Xiao Z, Lei C, et al. Byl719 reverses gefitinib-resistance induced by pi3k/akt activation in non-small cell lung cancer cells. *Bmc Cancer*. 2023;23(1):732. doi:10.1186/s12885-023-11243-0
15. Beaver JA, Gustin JP, Yi KH, et al. Pik3ca and akt1 mutations have distinct effects on sensitivity to targeted pathway inhibitors in an isogenic luminal breast cancer model system. *Clin Cancer Res*. 2013;19(19):5413–5422. doi:10.1158/1078-0432.CCR-13-0884
16. Campbell JD, Alexandrov A, Kim J, et al. Distinct patterns of somatic genome alterations in lung adenocarcinomas and squamous cell carcinomas. *Nat Genet*. 2016;48(6):607–616. doi:10.1038/ng.3564
17. Jee J, Lebow ES, Yeh R, et al. Overall survival with circulating tumor DNA-guided therapy in advanced non-small-cell lung cancer. *Nat Med*. 2022;28(11):2353–2363. doi:10.1038/s41591-022-02047-z
18. Mandelker D, Zhang L, Kemel Y, et al. Mutation detection in patients with advanced cancer by universal sequencing of cancer-related genes in tumor and normal DNA vs guideline-based germline testing. *JAMA*. 2017;318(9):825–835. doi:10.1001/jama.2017.11137
19. Zehir A, Benayed R, Shah RH, et al. Erratum: mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med*. 2017;23(8):1004. doi:10.1038/nm0817-1004c
20. Reck M, Carbone DP, Garassino M, et al. Targeting kras in non-small-cell lung cancer: recent progress and new approaches. *Ann Oncol*. 2021;32(9):1101–1110. doi:10.1016/j.annonc.2021.06.001
21. Lengel HB, Mastrogiacomo B, Connolly JG, et al. Genomic mapping of metastatic organotropism in lung adenocarcinoma. *Cancer Cell*. 2023;41(5):970–985. doi:10.1016/j.ccell.2023.03.018
22. Wang L, Hu H, Pan Y, et al. Pik3ca mutations frequently coexist with egfr/kras mutations in non-small cell lung cancer and suggest poor prognosis in egfr/kras wildtype subgroup. *PLoS One*. 2014;9(2):e88291. doi:10.1371/journal.pone.0088291
23. Aredo JV, Padda SK, Kunder CA, et al. Impact of kras mutation subtype and concurrent pathogenic mutations on non-small cell lung cancer outcomes. *Lung Cancer*. 2019;133:144–150.
24. Skoulidis F, Li BT, Dy GK, et al. Sotorasib for lung cancers with kras p.g12c mutation. *N Engl J Med*. 2021;384(25):2371–2381. doi:10.1056/NEJMoa2103695
25. Kim D, Herdeis L, Rudolph D, et al. Pan-kras inhibitor disables oncogenic signalling and tumour growth. *Nature*. 2023;619(7968):160–166. doi:10.1038/s41586-023-06123-3
26. Andre F, Ciruelos E, Rubovszky G, et al. Alpelisib for pik3ca-mutated, hormone receptor-positive advanced breast cancer. *N Engl J Med*. 2019;380(20):1929–1940. doi:10.1056/NEJMoa1813904
27. Fernandes MS, Melo S, Velho S, et al. Specific inhibition of p110alpha subunit of pi3k: putative therapeutic strategy for kras mutant colorectal cancers. *Oncotarget*. 2016;7(42):68546–68558. doi:10.18632/oncotarget.11843