Histol Histopathol (2022) 37: 1177-1184

http://www.hh.um.es

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE **Open Access**

High expression of the phosphoinositide 3-kinase p110γ isoform can predict poor prognosis of non-small cell lung cancer

Ina Jung[1], Hyoun Wook Lee[1], Mee Sook Roh[2], Jae Seok Lee[1], Kisu Kim[1], Kyungeun Kim[3], Tae Gyu Kim[4] and Hyun-Yeol Nam[5]

[1]Department of Pathology, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, [2]Department of Pathology, Dong-A University College of Medicine, Busan, [3]Department of Pathology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, [4]Department of Radiation Oncology, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine and [5]Department of Nuclear Medicine, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, Changwon, South Korea

Summary. The protein p110γ is an isoform of the catalytic subunit of class I phosphoinositide 3-kinases (PI3Ks). PI3Ks are involved in the regulation of cell survival, growth, proliferation, and migration and have been implicated in the oncogenesis of various cancers. In this study, p110γ expression in non-small cell lung cancer (NSCLC) and its association with clinicopathological factors and patient survival were evaluated. A total of 230 NSCLC tumors were immunohistochemically stained for p110γ. Of these, 174 (75.7%) and 56 (24.3%) were placed in the low and high expression groups, respectively. The positive rate of p110γ was significantly higher in adenocarcinoma than in squamous cell carcinoma ($p<0.001$). Advanced stage NSCLCs showed higher p110γ expression than those at an early stage ($p=0.002$). Irrespective of the histological tumor type, the patients with high p110γ expression had significantly worse overall survival than those with low p110γ expression ($p=0.004$). p110γ expression was an independent poor prognostic factor in the multivariate analysis. Our results suggest that p110γ may be involved in the development and progression of NSCLC, and that p110γ has promising potential as a prognostic factor or novel therapeutic target for NSCLC.

Key words: Immunohistochemistry, Non-small cell lung cancer, PI3K, p110γ, Prognosis, Therapeutic target

Corresponding Author: Prof. Hyoun Wook Lee, Department of Pathology, Samsung Changwon Hospital, Sungkyunkwan University School of Medicine, 158, Paryong-ro, Masanhoewon-gu, Changwon 51353, South Korea. e-mail: sudowo@naver.com
DOI: 10.14670/HH-18-480

Introduction

Phosphoinositide 3-kinases (PI3Ks) catalyze the phosphorylation of lipids known as phosphoinositides in the 3′-hydroxyl position of their inositol rings in response to extracellular stimuli and generate phosphatidylinositol-3,4,5-triphosphate (PIP_3). PIP_3 plays a pivotal role in the activation of several signaling molecules, including Akt and its downstream effector mammalian target of rapamycin (mTOR). The activated PI3K/Akt/mTOR pathway is involved in diverse signaling cascades for cell growth, proliferation, survival, motility, and morphology (Kasto et al., 2001; Vanhaesebroeck et al., 2001; Engelman et al., 2006; Falasca and Maffucci, 2014). PI3Ks are grouped into three classes based on their structure and substrate specificity. Among them, class I PI3Ks are heterodimeric enzymes comprising a catalytic subunit and a regulatory subunit. The catalytic subunits of class I PI3Ks encompass four different isoforms: p110α, p110β, p110γ, and p110δ. These four isoforms have non-redundant functions and different expression patterns in different cell types. While p110α and p110β are ubiquitously expressed, p110γ and p110δ are expressed predominantly in cells of hematopoietic lineage (Zhao

Abbreviations. AC, Adenocarcinoma; CLL, Chronic lymphocytic leukemia; FDA, Food and Drug Administration; FL, Follicular lymphoma; HCC, Hepatocellular carcinoma; mTOR, Mammalian target of rapamycin; NSCLC, Non-small cell lung cancer; OS, Overall survival; PDAC, Pancreatic ductal adenocarcinoma; PI3K, Phosphoinositide 3-kinases; PIP3, Phosphatidylinositol-3,4,5-triphosphate; PTEN, Phosphatase and tensin homolog; RCC, Renal cell carcinoma; SCC, Squamous cell carcinoma; SLL, Small lymphocytic lymphoma; TMA, Tissue microarray

and Vogt, 2008; Falasca and Maffucci, 2014).

Aberrant upregulation of the PI3K catalytic subunits is associated with carcinogenesis in various human cancers. Gain-of-function mutations or amplification of *PIK3CA*, a gene encoding p110α, are the most common and well-known genetic abnormalities implicated in cancer development and progression (Samuels and Ericson, 2006; Marone et al., 2008; Zhao and Vogt, 2008; Shi et al., 2012). Somatic mutations of the genes encoding the other PI3K isoforms have also been identified; however, their prevalence and functional relevance in human cancers are limited and unclear. Instead, accumulating data suggest that overexpression of the wild-type non-α catalytic subunits, rather than gain-of-function mutations, can sufficiently contribute to oncogenic transformation (Kang et al., 2006; Falasca and Maffucci, 2014).

p110γ is primarily expressed in leukocytes, including lymphocytes and natural killer cells, and regulates their differentiation, migration, transformation, proliferation, and survival, and is involved in immune, inflammatory, and allergic responses (Martin et al., 2008; Thomas et al., 2008; Saudemont et al., 2009; Beer-Hammer et al., 2010). In addition, p110γ plays a key role in the proliferation, angiogenesis, metabolism, metastasis, and tumor-associated inflammation in certain cancers, particularly hematologic malignancies (Cui et al., 2014; Falasca and Maffucci, 2014). Duvelisib, a dual oral inhibitor of p110γ and p110δ, was recently approved by the Food and Drug Administration (FDA) for relapsed or refractory chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), and follicular lymphoma (FL) (Blair, 2018). Recent studies have indicated that p110γ has specific functions in the regulation of cancer cell proliferation and progression in pancreatic ductal adenocarcinoma (PDAC), hepatocellular carcinoma (HCC), and renal cell carcinoma (RCC). In preclinical cell line studies on these cancers, small molecular inhibition of p110γ significantly suppressed cancer cell proliferation and induced cell death (Edling et al., 2010; Guerreiro et al., 2011; Dituri et al., 2012; Emmanouilidi et al., 2019; Torres et al., 2019; Wang et al., 2019). However, p110γ expression and its druggable potential in lung cancer require further investigation, as only limited data are available.

In this study, we investigated p110γ expression in non-small cell lung cancer (NSCLC) and the correlation between its expression and clinicopathological factors, including patient survival. This study aimed to assess the potential of p110γ expression as a predictive biomarker for PI3K inhibitor treatment and a prognostic biomarker of NSCLC.

Materials and methods

Patients and tissue samples

This study included 230 patients with adenocarcinoma (AC) or squamous cell carcinoma (SCC) who underwent complete resection without neoadjuvant chemotherapy at the Dong-A University Medical Center and Samsung Changwon Hospital from January 2007 to December 2012. Demographic and clinicopathological data were collected from medical records and pathological reports. The clinical stage was determined based on the 7th edition of the American Joint Committee on Cancer TNM staging system (Tsim et al., 2010). Follow-up data were included until March 2020, or until death or loss of follow-up with the patient. The study was approved by the Institutional Review Board of our medical institution, which waived the requirement for informed consent.

Of the 230 patients with NSCLC, 157 were men and 73 were women. The mean patient age at the time of diagnosis was 64.4±9.0 years. Histologically, there were 141 AC (61.3%) and 89 SCC (38.7%) tumors. One-hundred-thirteen tumors (49.1%) were well differentiated, seventy (30.4%) were moderately

Table 1. Clinicopathological characteristics of the 230 patients with non-small cell lung cancer.

Characteristics	No.	(%)
Total	230	(100.0)
Age (mean ± SD)	64.43±8.95	
Sex		
Male	157	(68.3)
Female	73	(31.7)
Tumor size (cm, mean ± SD)	3.72±1.90	
Histology		
Adenocarcinoma	141	(61.3)
Squamous cell carcinoma	89	(38.7)
Histological grade		
Well differentiated	113	(49.1)
Moderately differentiated	70	(30.4)
Poorly differentiated	47	(20.4)
Pleural invasion		
Present	80	(34.8)
Absent	150	(65.2)
Lymphovascular invasion		
Present	72	(31.3)
Absent	158	(68.7)
Marginal status		
Involved	21	(9.1)
Uninvolved	209	(90.9)
Lymph node metastasis		
Present	95	(41.3)
Absent	135	(58.7)
Distant metastasis		
Present	13	(5.7)
Absent	219	(94.3)
TNM stage		
I	97	(42.2)
II	59	(25.7)
III	61	(26.5)
IV	13	(5.7)

SD, standard deviation.

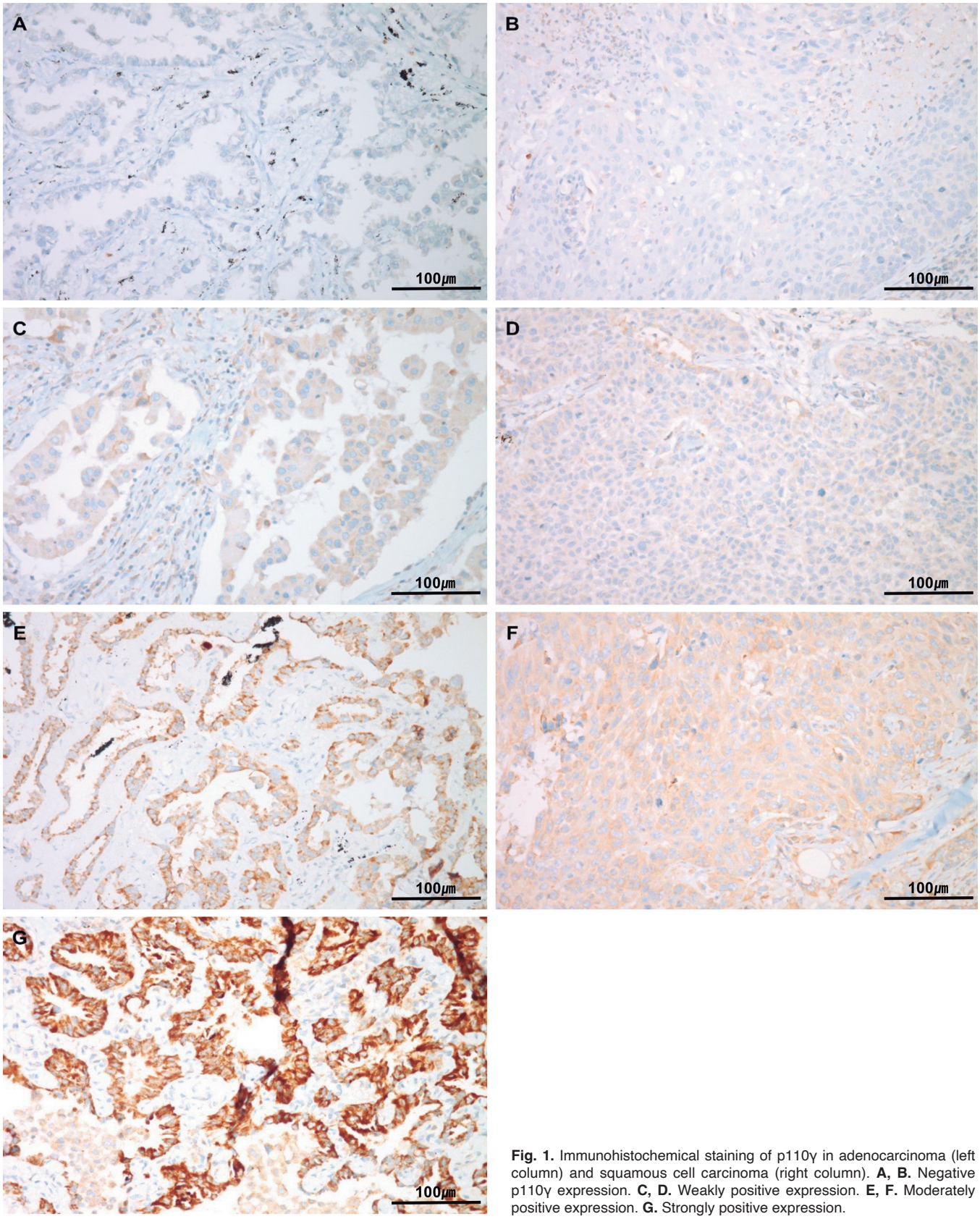

Fig. 1. Immunohistochemical staining of p110γ in adenocarcinoma (left column) and squamous cell carcinoma (right column). **A, B.** Negative p110γ expression. **C, D.** Weakly positive expression. **E, F.** Moderately positive expression. **G.** Strongly positive expression.

differentiated, and forty-seven (20.4%) were poorly differentiated. The mean tumor size was 3.7±1.9 cm. Ninety-seven tumors (42.2%) were stage I, fifty-nine (25.7%) were stage II, sixty-one (26.5%) were stage III, and thirteen (5.7%) were stage IV. Pleural invasion, lymphovascular invasion, and nodal metastasis were detected in 80 (34.8%), 72 (31.3%), and 95 cases (41.3%), respectively. Thirteen patients (5.7%) had distant metastasis at the time of diagnosis. Twenty-one tumors (9.1%) involved surgical resection margins. The clinicopathological characteristics are summarized in Table 1.

Tissue microarray and immunohistochemistry

Representative areas of the tumors were marked on hematoxylin and eosin-stained slides and used for tissue microarray (TMA) construction. Tissue cores 2 mm in diameter were removed from donor paraffin blocks and placed in blank recipient paraffin blocks. Two cores per tumor were arrayed. The TMA blocks were sectioned at 4 μm for immunohistochemical staining using a BenchMark XT automated staining platform (Roche-Ventana, Tucson, AZ, USA). All sections were deparaffinized and pretreated with Cell Conditioning 1 solution (Roche-Ventana) for 30 min at 100°C. Sections were washed with reaction buffer followed by incubation with a primary antibody against p110γ (clone D-12, 1:100, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 32 min at 37°C. An ultraView Universal DAB kit (Roche-Ventana) was used to detect the primary antibody according to the manufacturer's instructions, followed by counterstaining with hematoxylin (Roche-Ventana).

Immunostained slides were evaluated by two independent pathologists (Jung I and Lee HW) who were blinded to the clinicopathological information. Discrepant cases were discussed with the use of a multi-head microscope until an agreement was reached. Cases were considered positive when ≥10% of the tumor cells expressed p110γ. The staining intensity of the positive cases was scored as 1 (weak), 2 (moderate), or 3 (strong). For statistical analyses, the negative and weakly positive cases constituted the low expression group, while the moderately and strongly positive cases constituted the high expression group.

Statistical analysis

Statistical analyses were performed using SPSS Ver. 18 (SPSS Inc., Chicago, IL, USA). To evaluate possible relationships between p110γ expression and various clinicopathological parameters, Fisher's exact test for categorical variables and the Mann–Whitney test for ordinal variables were used. The impact of various parameters on overall survival (OS) was analyzed using the Kaplan–Meier method, and differences were compared using the log-rank test. Multivariate analysis for OS was performed using the Cox proportional hazards model. Statistical significance was set at $p<0.05$.

Results

Correlation between p110γ expression and clinicopathological factors

Of the tumors, 172 (74.8%) showed cytoplasmic granular expression of p110γ with variable staining intensity. p110γ expression was weak in 116 tumors, moderate in 51, and strong in 5 (Fig. 1). Based on the previously described immunostaining interpretation, 174 (75.7%) and 56 (24.3%) tumors were classified into the low and high expression groups, respectively. The p110γ expression rate was significantly higher in AC than in

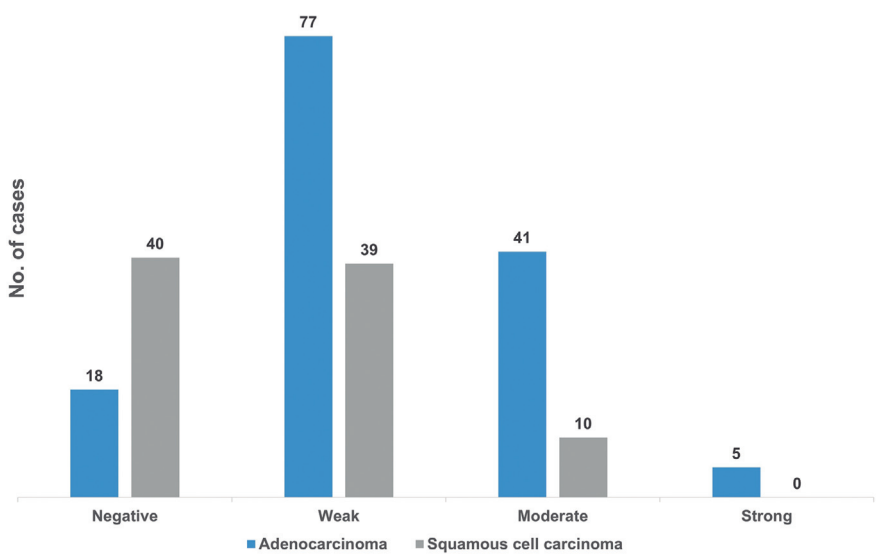

Fig. 2. Difference in p110γ expression between adenocarcinoma and squamous cell carcinoma.

SCC (p<0.001). Out of the 141 ACs, 123 (87.2%) showed weak to strong positive expression for p110γ and 46 (32.6%) were classified into the high expression group, while 49 (55.1%) of the 89 SCCs showed weak or moderately positive expression, none of the SCCs showed strong positive p110γ expression. Only 10 SCCs (11.2%) showed moderate p110γ expression and were classified into the high expression group (Fig. 2). Advanced stage NSCLCs showed higher p110γ expression than those at an early stage (p=0.002). When it was stratified based on histological type, this positive correlation between stage and p110γ expression remained statistically significant in both AC (p=0.021) and SCC (p=0.012). Other clinicopathological factors were not significantly associated with p110γ expression levels (Table 2).

Survival analysis

The median follow-up period was 38 months (range, 1–159 months). The deaths of 90 (39.1%) of the 230 patients in this study were confirmed, and 76 (33.0%) were lost during the follow-up period. In a univariate survival analysis for all patients, male sex (p=0.003), larger tumor size (p<0.001), lymphovascular invasion (p=0.010), nodal metastasis (p=0.010), distant metastasis (p=0.001), positive resection margin (p=0.001), and higher TNM stage (p<0.001) were significantly correlated with worse OS. High p110γ expression was also significantly associated with a shorter OS (Fig. 3A; p=0.004). When stratified by histological type, the prognostic impact of p110γ expression on OS remained significant in both AC (Fig. 3B; p=0.007) and SCC (Fig. 3C; p=0.013).

Multivariate Cox regression analysis, including p110γ and clinicopathological factors significantly correlated with OS in univariate analysis, showed that high p110γ expression, male sex, larger tumor size, involved resection margin, distant metastasis, and advanced TNM stage were independent prognostic factors for OS (Table 3).

Discussion

The PI3K/Akt/mTOR signaling cascade is one of the most important and well-established intracellular pathways that regulates cell growth, proliferation, survival, metabolism, angiogenesis, and motility (Kasto et al., 2001; Vanhaesebroeck et al., 2001; Engelman et al., 2006; Falasca and Maffucci, 2014). Abnormal activation of this pathway can contribute to the development and progression of tumors; therefore, its inhibition may be an attractive therapeutic strategy for cancer treatment. Several pan-PI3K inhibitors and isoform-selective PI3K inhibitors have been investigated in clinical trials (Yang et al., 2019; Mishra et al., 2021). Compared to pan-PI3K inhibitors, isoform-selective PI3K inhibitors demonstrated improved specificity and reduced toxicity. To date, four isoform-selective PI3K

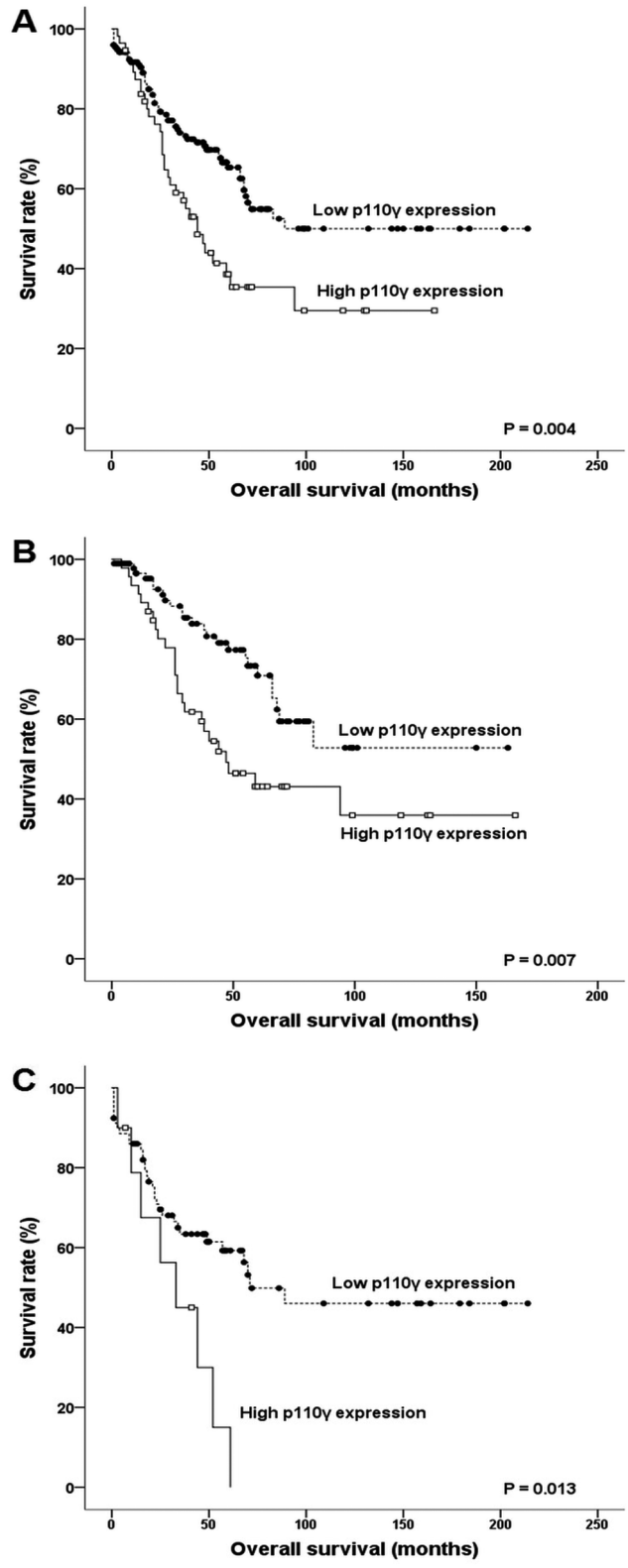

Fig. 3. Overall survival curves according to p110γ expression levels in all patients **(A)**, in patients with adenocarcinoma **(B)**, and in patients with squamous cell carcinoma **(C)**.

inhibitors, namely alpelisib, idelalisib, umbralisib, and duvelisib, have been approved by the FDA (Mishra et al., 2021). Alpelisib, an oral selective p110α inhibitor, was approved by the FDA for the treatment of patients with *PIK3CA*-mutated, hormonal receptor-positive, HER2-negative advanced breast cancer who had previously received endocrine therapy. Idelalisib and umbralisib, oral specific inhibitors targeting p110δ, have been approved by the FDA for the treatment of patients with relapsed or refractory CLL/SLL, FL, or marginal zone lymphoma. Duvelisib, a dual oral inhibitor of p110γ and p110δ, was also approved for relapsed or refractory CLL/SLL and FL (Blair, 2018; Mishra et al., 2021).

Isoform p110γ is primarily localized in immune cells; therefore, studies on oncogenic functions and druggable potential of p110γ have been limited to hematologic malignancies, such as lymphoma, leukemia, or plasma cell myeloma (Göckeritz et al., 2015; Pillinger et al., 2016; Piddock et al., 2017; Ksionda et al., 2018). However, emerging evidence suggests that p110γ could be involved in the carcinogenesis of various solid cancers and may be a promising therapeutic target. In several preclinical cell line studies of PDAC, specific p110γ inhibitors reduced tumor cell growth and proliferation, and sensitized tumor cells to chemotherapeutic agents, such as gemcitabine (Edling et al., 2010; Emmanouilidi et al., 2019; Torres et al., 2019). Similar results have also been reported in HCC, RCC, and medulloblastoma (Guerreiro et al., 2011; Dituri et al., 2012; Wang et al., 2019).

With regard to NSCLC, the majority of studies on aberrant activation of the PI3K/Akt/mTOR pathway have focused on amplification or mutation of *PIK3CA* and genetic or epigenetic inactivation of phosphatase and tensin homolog (*PTEN*), a negative regulator of Akt (Solomon and Pearson, 2009; Martinez-Martí and Felip, 2012; Scrima et al., 2012; Wang et al., 2014). Only a limited number of studies have examined PI3K protein expression, particularly isoform p110α, and its correlation with clinicopathological factors and associated molecular alterations in NSCLC (Scrima et al., 2012; Wang et al., 2014; Usul Afsar et al., 2015). Trigka et al. (2013) assessed p110γ expression using a Histo-score, a different interpretation method from ours, in 102 NSCLCs including 39 ACs, 48 SCCs, and 15 tumors of other types. Sixty-two (60.7%) were positive for p110γ and 20 of them showed nuclear expression in contrast to our study wherein nuclear expression was not observed. This discrepancy might have resulted from the use of the primary antibody for p110γ from a different manufacturing company and different clone. The median

Table 2. Correlation between p110γ expression level and clinicopathological factors in the 230 patients with non-small cell lung cancer.

Clinicopathological factors	p110γ expression, No. (%)		p-value
	Low	High	
Total	174 (75.7)	56 (24.3)	
Age (years)			
<65	78 (73.6)	28 (26.4)	0.539
≥65	96 (77.4)	28 (22.6)	
Sex			
Male	118 (75.2)	39 (24.8)	0.870
Female	56 (76.7)	17 (23.3)	
Tumor size (cm)			
≤3	78 (70.3)	33 (29.7)	0.090
3	96 (80.7)	23 (19.3)	
Histology			
Adenocarcinoma	95 (67.4)	46 (32.6)	0.001
Squamous cell carcinoma	79 (88.8)	10 (11.2)	
Histological grade			
Well differentiated	86 (76.1)	27 (23.9)	0.979
Moderately differentiated	53 (75.7)	17 (24.3)	
Poorly differentiated	35 (74.5)	12 (25.5)	
Pleural invasion			
Present	58 (72.5)	22 (27.5)	0.424
Absent	116 (77.3)	34 (22.7)	
Lymphovascular invasion			
Present	50 (69.4)	22 (30.6)	0.185
Absent	124 (78.5)	34 (21.5)	
Marginal status			
Involved	16 (76.2)	5 (23.8)	1.000
Uninvolved	158 (75.6)	51 (24.4)	
Lymph node metastasis			
Present	69 (72.6)	26 (27.4)	0.436
Absent	105 (77.8)	30 (22.2)	
Distant metastasis			
Present	7 (63.6)	4 (36.4)	0.469
Absent	167 (76.3)	52 (23.7)	
TNM stage			
I and II	128 (82.1)	28 (17.9)	0.002
III and IV	46 (62.2)	28 (37.8)	

Table 3. Multivariate analysis for overall survival in the 230 patients with non-small cell lung cancer.

Factor	Parameter	Hazard ratio (95 % CI)	p-value
p110γ expression	High Low	1.962 (1.233-3.120)	0.004
Sex	Male Female	2.081 (1.235-3.505)	0.006
Tumor size (cm)	3 ≤3	2.251 (1.408-3.598)	0.001
Lymphovascular invasion	Present Absent	1.398 (0.849–2.300)	0.188
Marginal status	Involved Uninvolved	2.678 (1.430-5.018)	0.002
Lymph node metastasis	Present Absent	1.101 (0.624-1.942)	0.740
Distant metastasis	Present Absent	2.480 (1.013-6.074)	0.047
TNM stage	III and IV I and II	1.927 (1.057-3.512)	0.032

CI, confidence interval.

values and ranges of p110γ histoscores in AC and SCC were 0 (0–200) and 0 (0–90), respectively. The detailed distribution or difference of histoscores in AC and SCC was not provided in the article. There was no significant association found between p110γ expression and clinicopathological factors including histological type, stage, and cancer-specific survival, however, there was a positive correlation between p110γ and Akt and its downstream effectors.

In this study, NSCLCs showed cytoplasmic expression of p110γ with variable staining intensity. Regardless of the staining intensity, p110γ was more frequently expressed in AC than in SCC (87.2% vs 55.1%). Tumors from the high (moderate to strong) expression group were also higher in AC than in SCC (32.6% vs 11.2%). Notably, SCCs did not show strong p110γ expression and only 10 SCCs were moderately positive for p110γ. This difference between both tumor types suggests that p110γ expression may be more closely related with the carcinogenesis of AC than SCC. NSCLCs at an advanced stage (III or IV) showed higher p110γ expression than those at an early stage (I or II). Irrespective of the histological tumor type, the patients with high p110γ expression had significantly worse OS than those with low p110γ expression. In the multivariate statistical analysis to adjust for the confounding effects of well-established prognostic factors, such as TNM stage, lymphovascular invasion, or positive resection margin, on patient outcomes, p110γ expression was an independent poor prognostic factor. These results indicate that p110γ may be implicated in the development and progression of NSCLC, and that it has promising potential as a prognostic factor or novel therapeutic target for NSCLC. Based on our results, isoform-selective inhibition of p110γ requires application in NSCLC treatment in the near future.

To our knowledge, this is the first study to show that p110γ expression significantly correlates with various clinicopathological factors and patient survival in NSCLC. Our results suggest that p110γ plays a critical role in tumorigenesis and is a poor prognostic biomarker for NSCLC. However, larger prospective studies with a standardized interpretation method for p110γ immunostaining are required to verify these results. Isoform-selective inhibition of p110γ could be a novel and attractive therapeutic strategy for NSCLC treatment as it has fewer side effects than that of pan-PI3K inhibition. Therefore, further studies on the use of p110γ-specific inhibitors and the possibility of p110γ immunostaining as a prognostic or predictive biomarker in NSCLC are warranted.

Acknowledgements. This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science, and Technology (NRF-2017R1C1B5018270).

Conflict of interest statement. The authors declare no conflicts of interest.

References

Beer-Hammer S., Zebedin E., von Holleben M., Alferink J., Reis B., Dresing P., Degrandi D., Scheu S., Hirsch E., Sexl V., Pfeffer K., Nürnberg B. and Piekorz R.P. (2010). The catalytic PI3K isoforms p110gamma and p110delta contribute to B cell development and maintenance, transformation, and proliferation. J. Leukoc. Biol. 87, 1083-1095.

Blair H.A. (2018). Duvelisib: first global approval. Drugs 78, 1847-1853.

Cui W., Cai Y., Wang W., Liu Z., Wei P., Bi R., Chen W., Sun M. and Zhou X. (2014). Frequent copy number variations of PI3K/AKT pathway and aberrant protein expressions of PI3K subunits are associated with inferior survival in diffuse large B cell lymphoma. J. Transl. Med. 12, 10.

Dituri F., Mazzocca A., Lupo L., Edling C.E., Azzariti A., Antonaci S., Falasca M. and Giannelli G. (2012). PI3K class IB controls the cell cycle checkpoint promoting cell proliferation in hepatocellular carcinoma. Int. J. Cancer 130, 2505-2513.

Edling C.E., Selvaggi F., Buus R., Maffucci T., Di Sebastiano P., Friess H., Innocenti P., Kocher H.M. and Falasca M. (2010). Key role of phosphoinositide 3-kinase class IB in pancreatic cancer. Clin. Cancer Res. 16, 4928-4237.

Emmanouilidi A., Fyffe C.A., Ferro R., Edling C.E., Capone E., Sestito S., Rapposelli S., Lattanzio R., Iacobelli S., Sala G., Maffucci T. and Falasca M. (2019). Preclinical validation of 3-phosphoinositide-dependent protein kinase 1 inhibition in pancreatic cancer. J. Exp. Clin. Cancer Res. 38, 191.

Engelman J.A., Luo J. and Cantley L.C. (2006). The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. Nat. Rev. Genet. 7, 606-619.

Falasca M. and Maffucci T. (2014). Targeting p110gamma in gastro-intestinal cancers: attack on multiple fronts. Front. Physiol. 5, 391.

Göckeritz E., Kerwien S., Baumann M., Wigger M., Vondey V., Neumann L., Landwehr T., Wendtner C.M., Klein C., Liu N., Hallek M., Frenzel L.P. and Krause G. (2015). Efficacy of phosphatidylinositol-3 kinase inhibitors with diverse isoform selectivity profiles for inhibiting the survival of chronic lymphocytic leukemia cells. Int. J. Cancer 137, 2234-2242.

Guerreiro A.S., Fattet S., Kulesza D.W., Atamer A., Elsing A.N., Shalaby T., Jackson S.P., Schoenwaelder S.M., Grotzer M.A., Delattre O. and Arcaro A. (2011). A sensitized RNA interference screen identifies a novel role for the PI3K p110γ isoform in medulloblastoma cell proliferation and chemoresistance. Mol. Cancer Res. 9, 925-935.

Kang S., Denley A., Vanhaesebroeck B. and Vogt P.K. (2006). Oncogenic trans- formation induced by the p110beta, -gamma, and -delta isoforms of class I phosphoinositide 3-kinase. Proc. Natl. Acad. Sci. USA 103, 1289-1294.

Katso R., Okkenhaug K., Ahmadi K., White S., Timms J. and Waterfield M.D. (2001). Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. Annu. Rev. Cell Dev. Biol. 17, 615-675.

Ksionda O., Mues M., Wandler A.M., Donker L., Tenhagen M., Jun J., Ducker G.S., Matlawska-Wasowska K., Shannon K., Shokat K.M. and Roose J.P. (2018). Comprehensive analysis of T cell leukemia signals reveals heterogeneity in the PI3 kinase-Akt pathway and limitations of PI3 kinase inhibitors as monotherapy. PLoS One 13, e0193849.

Marone R., Cmiljanovic V., Giese B. and Wymann M.P. (2008).

Targeting phosphoinositide 3-kinase: moving towards therapy. Biochim. Biophys. Acta 1784, 159-185.

Martin A.L., Schwartz M.D., Jameson S.C. and Shimizu Y. (2008). Selective regulation of CD8 effector T cell migration by the p110 gamma isoform of phosphatidylinositol 3-kinase. J. Immunol. 180, 2081-2088.

Martinez-Martí A. and Felip E. (2012). PI3K Pathway in NSCLC. Front. Oncol. 1, 55.

Mishra R., Patel H., Alanazi S., Kilroy M.K. and Garrett J.T. (2021). PI3K Inhibitors in cancer: clinical implications and adverse effects. Int. J. Mol. Sci. 22, 3464.

Piddock R.E., Loughran N., Marlein C.R., Robinson S.D., Edwards D.R., Yu S., Pillinger G.E., Zhou Z., Zaitseva L., Auger M.J., Rushworth S.A. and Bowles K.M. (2017). PI3Kδ and PI3Kγ isoforms have distinct functions in regulating pro-tumoural signalling in the multiple myeloma microenvironment. Blood Cancer J. 7, e539.

Pillinger G., Loughran N.V., Piddock R.E., Shafat M.S., Zaitseva L., Abdul-Aziz A., Lawes M.J., Bowles K.M. and Rushworth S.A. (2016). Targeting PI3Kδ and PI3Kγ signalling disrupts human AML survival and bone marrow stromal cell mediated protection. Oncotarget 7, 39784-39795.

Samuels Y. and Ericson K. (2006). Oncogenic PI3K and its role in cancer. Curr. Opin. Oncol. 18, 77-82.

Saudemont A., Garçon F., Yadi H., Roche-Molina M., Kim N., Segonds-Pichon A., Martín-Fontecha A., Okkenhaug K. and Colucci F. (2009). p110γ and p110δ isoforms of phosphoinositide 3-kinase differentially regulate natural killer cell migration in health and disease. Proc. Natl. Acad. Sci. USA 106, 5795-5800.

Scrima M., De Marco C., Fabiani F., Franco R., Pirozzi G., Rocco G., Ravo M., Weisz A., Zoppoli P., Ceccarelli M., Botti G., Malanga D. and Viglietto G. (2012). Signaling networks associated with AKT activation in non-small cell lung cancer (NSCLC): new insights on the role of phosphatydil-inositol-3 kinase. PLoS One 7, e30427.

Shi J., Yao D., Liu W., Wang N., Lv H., Zhang G., Ji M., Xu L., He N., Shi B. and Hou P. (2012). Highly frequent PIK3CA amplification is associated with poor prognosis in gastric cancer. BMC Cancer 12, 50.

Solomon B. and Pearson R.B. (2009). Class IA phosphatidylinositol 3-kinase signaling in non-small cell lung cancer. J. Thorac. Oncol. 4, 787-791.

Thomas M.S., Mitchell J.S., DeNucci C.C., Martin A.L. and Shimizu Y. (2008). The p110gamma isoform of phosphatidylinositol 3-kinase regulates migration of effector CD4 T lymphocytes into peripheral inflammatory sites. J. Leukoc. Biol. 84, 814-823.

Torres C., Mancinelli G., Cordoba-Chacon J., Viswakarma N., Castellanos K., Grimaldo S., Kumar S., Principe D., Dorman M.J., McKinney R., Hirsch E., Dawson D., Munshi H.G., Rana A. and Grippo P.J. (2019). p110γ deficiency protects against pancreatic carcinogenesis yet predisposes to diet-induced hepatotoxicity. Proc. Natl. Acad. Sci. USA 116, 14724-14733.

Trigka E.A., Levidou G., Saetta A.A., Chatziandreou I., Tomos P., Thalassinos N., Anastasiou N., Spartalis E., Kavantzas N., Patsouris E. and Korkolopoulou P. (2013). A detailed immunohistochemical analysis of the PI3K/AKT/mTOR pathway in lung cancer: correlation with PIK3CA, AKT1, K-RAS or PTEN mutational status and clinicopathological features. Oncol. Rep. 30, 623-636.

Tsim S., O'Dowd C.A., Milroy R. and Davidson S. (2010). Staging of non-small cell lung cancer (NSCLC): a review. Respir. Med. 104, 1767-1774.

Usul Afsar C., Sahin B., Gunaldi M., Kılıc Bagir E., Gumurdulu D., Burgut R., Erkisi M., Kara I.O., Paydas S., Karaca F. and Ercolak V. (2015). Expression of fibroblast growth factor receptor 1, fibroblast growth factor 2, phosphatidyl inositol 3 phosphate kinase and their clinical and prognostic significance in early and advanced stage of squamous cell carcinoma of the lung. Int. J. Clin. Exp. Pathol. 8, 9760-9771.

Vanhaesebroeck B., Leevers S.J., Ahmadi K., Timms J., Katso R., Driscoll P.C., Woscholski R., Parker P.J. and Waterfield M.D. (2001). Synthesis and function of 3-phosphorylated inositol lipids. Annu. Rev. Biochem. 70, 535-602.

Wang L., Hu H., Pan Y., Wang R., Li Y., Shen L., Yu Y., Li H., Cai D., Sun Y. and Chen H. (2014). 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 9, e88291.

Wang X., Huang S., Xin X., Ren Y., Weng G. and Wang P. (2019). The antitumor activity of umbelliferone in human renal cell carcinoma via regulation of the p110γ catalytic subunit of PI3Kγ. Acta Pharm. 69, 111-119.

Yang J., Nie J., Ma X., Wei Y., Peng Y. and Wei X. (2019). Targeting PI3K in cancer: mechanisms and advances in clinical trials. Mol. Cancer 18, 26.

Zhao L. and Vogt P.K. (2008). Class I PI3K in oncogenic cellular transformation. Oncogene 27, 5486-5496.

Accepted June 8, 2022