

Correlating Phosphatidylinositol 3-Kinase Inhibitor Efficacy with Signaling Pathway Status: *In silico* and Biological Evaluations

Shingo Dan¹, Mutsumi Okamura¹, Mariko Seki¹, Kanami Yamazaki¹, Hironobu Sugita¹, Michiyo Okui^{2,3}, Yumiko Mukai¹, Hiroyuki Nishimura⁴, Reimi Asaka², Kimie Nomura², Yuichi Ishikawa², and Takao Yamori¹

Abstract

The phosphatidylinositol 3-kinase (PI3K) pathway is frequently activated in human cancers, and several agents targeting this pathway including PI3K/Akt/mammalian target of rapamycin inhibitors have recently entered clinical trials. One question is whether the efficacy of a PI3K pathway inhibitor can be predicted based on the activation status of pathway members. In this study, we examined the mutation, expression, and phosphorylation status of PI3K and Ras pathway members in a panel of 39 pharmacologically well-characterized human cancer cell lines (JFCR39). Additionally, we evaluated the *in vitro* efficacy of 25 PI3K pathway inhibitors in addition to conventional anticancer drugs, combining these data to construct an integrated database of pathway activation status and drug efficacies (JFCR39-DB). *In silico* analysis of JFCR39-DB enabled us to evaluate correlations between the status of pathway members and the efficacy of PI3K inhibitors. For example, phospho-Akt and KRAS/BRAF mutations prominently correlated with the efficacy and the inefficacy of PI3K inhibitors, respectively, whereas *PIK3CA* mutation and PTEN loss did not. These correlations were confirmed in human tumor xenografts *in vivo*, consistent with their ability to serve as predictive biomarkers. Our findings show that JFCR39-DB is a useful tool to identify predictive biomarkers and to study the molecular pharmacology of the PI3K pathway in cancer. *Cancer Res*; 70(12): 4982–94. ©2010 AACR.

Introduction

Phosphatidylinositol 3-kinases (PI3K) are lipid kinases that phosphorylate phosphoinositide at position D3 of the inositol ring (1, 2). The catalytic subunit of class I PI3K is composed of four isoforms (p110 α , p110 β , p110 δ , and p110 γ , encoded by *PIK3CA*, *PIK3CB*, *PIK3CD*, and *PIK3CG*). Among these isoforms, *PIK3CA* is often activated in cancer by gain-of-function hotspot mutations (3, 4) and gene amplification (5, 6). On the other hand, phosphatase and tensin homologue deleted on chromosome 10 (PTEN) is a lipid phosphatase that dephosphorylates PIP3 at position D3 of the inositol ring to generate PIP2. *PTEN* has been shown to be a tumor suppressor gene and is often inactivated by deletion or mutation

in cancer (7–9). Activation of PI3K and PTEN loss trigger sequential phosphorylation of the PI3K downstream signal cascade, including Akt and mammalian target of rapamycin (mTOR), and mediates a survival signal, as well as tumor proliferation (9). Therefore, the PI3K pathway is thought to be a promising therapeutic target.

LY294002 and wortmannin are first-generation PI3K inhibitors, but neither has progressed to clinical trials because of cytotoxicity to liver and skin (10, 11). We previously reported a selective PI3K inhibitor, ZSTK474, which has potent antitumor activity and low toxicity *in vivo* (12). Subsequently, several PI3K inhibitors have been developed, and some including NVP-BEZ235 and GDC-0941 have already entered clinical trials (13–15). In addition to PI3K inhibitors, antitumor compounds targeting Akt and mTOR have been developed (15–18).

To develop a molecular-targeted anticancer drug, it is highly desirable to develop predictive biomarkers for stratifying patients susceptible to the drug. In fact, mutations of *EGFR* and *KRAS* are used to predict the efficacy of gefitinib in lung cancer and that of cetuximab in colorectal cancer, respectively (19–22). Moreover, Engelman and colleagues recently showed that tumor growth triggered by *KRAS* mutant exhibited resistance to NVP-BEZ235 (23). On the other hand, dysregulation of *PIK3CA* and PTEN has been reported to be involved in resistance to EGFR-targeted therapies (24–26). As mentioned above, *PIK3CA* mutation and PTEN loss trigger

Authors' Affiliations: ¹Division of Molecular Pharmacology, Cancer Chemotherapy Center, and ²Department of Pathology, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan and ³Department of Biomedical Engineering, Toin Human Science and Technology Center, and ⁴Department of Biomedical Engineering, Toin University of Yokohama, Yokohama, Japan

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

Corresponding Author: Takao Yamori, Division of Molecular Pharmacology, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, 3-8-31 Ariake, Koto-ku, Tokyo 135-8550, Japan. Phone: 81-3-3520-0111 ext. 5432; Fax: 81-3570-0484; E-mail: yamori@jfccr.or.jp.

doi: 10.1158/0008-5472.CAN-09-4172

©2010 American Association for Cancer Research.

activation of the kinase cascade of the PI3K downstream pathway. However, it is not well understood whether the efficacy of PI3K pathway inhibitors could be predicted by these upstream abnormalities and downstream activities of the PI3K pathway.

We previously established a panel of 39 human cancer cell lines (JFCR39) derived from various organs (27–29). JFCR39, as well as NCI60 developed by National Cancer Institute, has been used as an *in vitro* tool to measure “fingerprints” of cytotoxic compounds. Fingerprints are defined as the patterns of differential drug efficacy across a panel of cell lines and have been found to reflect mechanisms of drug action (27–30). ZSTK474 is a compound that we identified as a new PI3K inhibitor based on the similarity with the fingerprint of LY294002 (12). In other words, each of the JFCR39 cell lines showed similar responses to ZSTK474 and LY294002, and the responses varied from cell line to cell line. This suggested that the status of biological pathways determining a cancer cell's response to PI3K inhibitors (which may include the PI3K pathway) is heterogeneous across these cell lines.

In the present study, taking advantage of JFCR39, we examined the mutation and/or expression of upstream regulators of the PI3K and Ras pathways, including four PI3K isoforms (*PIK3CA*, *PIK3CB*, *PIK3CD*, and *PIK3CG*), *PTEN*, the ERBB family receptor tyrosine kinases (RTKs), *KRAS* and *BRAF*, and the phosphorylation of downstream pathway members including Akt, TSC2, GSK-3, mTOR, S6K1, mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) kinase (MEK)-1/2, and ERK1/2. Next, we examined the fingerprints of 25 compounds targeting the PI3K pathway as well as other conventional anticancer drugs. We then combined the PI3K and Ras pathway database and the drug database to develop an integrated database of pathway status and drug efficacies (JFCR39-DB). Using this database, we first evaluated the functional relationships among these pathway inhibitors and those among drugs by comparing their fingerprints. Second, we evaluated the correlation between PI3K pathway members and PI3K pathway inhibitors to identify candidate biomarkers for predicting their efficacy.

Materials and Methods

Cell lines and cell culture

A panel of 39 human cancer cell lines, termed JFCR39, were previously described (27, 28, 31). Cells were grown in RPMI 1640 (Wako Pure Chemical Industries Ltd.) supplemented with 1 µg/mL kanamycin and 5% (v/v) fetal bovine serum (Moregate Exports) and incubated at 37°C in a humidified atmosphere supplemented with 5% CO₂. Authentication of cell lines was done by short tandem repeat analysis using PowerPlex16 Systems (Promega; data not shown).

Amplification of genomic DNA fragments for sequencing

Extraction of genomic DNA was done using DNeasy blood and tissue kit (Qiagen) according to the manufac-

turer's instructions. Amplification of genomic DNA fragments was done using Pfu Ultra High-Fidelity DNA polymerase (Agilent Technologies), FastStart High Fidelity PCR System (Roche Applied Science), or AccuPrime Taq DNA Polymerase High Fidelity (Invitrogen). Polymerases and primer sequences used in each reaction are shown in Supplementary Table S1.

Nucleotide sequence analysis

Sequencing reactions were done using BigDye Terminator v3.1 and dGTP BigDye Terminator v3.0 (Applied Biosystems) according to the manufacturer's instructions. Primers were shown in Supplementary Table S1. Nucleotide sequences were analyzed using a 3130 Genetic Analyzer (Applied Biosystems) and sequence files were edited using 4 Peaks software (Mekentosj B.V.).

Detection of lipid kinase activities of PI3K p110α mutants

Determination of lipid kinase activities of PI3K p110α was described as previously (32). In brief, HEK293T cells were transfected with pFLAG-PIK3CA (with or without mutation) and pc-PIK3R1 using Lipofectamine 2000 (Invitrogen). After 48 hours of incubation, cells were harvested and the lysates were immunoprecipitated by using FLAG-Tagged Protein Immunoprecipitation Kit (Sigma-Aldrich). To detect kinase activity, we used the PI3K-HTRF assay kit (Millipore) and EnVision 2103 Multilabel Reader (Perkin-Elmer).

Preparation of total cell extract

Cells were resuspended in lysis buffer [10 mmol/L Tris-HCl (pH 7.4)], 50 mmol/L NaCl, 0.5% w/v NP40, 0.1% w/v SDS, 50 mmol/L sodium fluoride, 30 mmol/L sodium pyrophosphate, 50 mmol/L sodium orthovanadate, 5 mmol/L EDTA, 0.1 trypsin inhibitor unit/mL aprotinin, and 1 mmol/L phenylmethylsulfonyl fluoride] and lysed by sonication in an ice bath. Concentrations of proteins in the extracts were determined using a protein assay kit (Pierce).

Immunoblot analysis

Equal amounts of protein were subjected to SDS-PAGE and the separated proteins were transferred onto an Immobilon FL polyvinylidene difluoride membrane (Millipore). The membrane was incubated with a primary antibody. The antibodies for PI3K p110α, Akt, phospho-Akt (T308, S473), phospho-GSK-3 (S9), phospho-TSC2 (T1462), phospho-mTOR (S2448), phospho-S6K1 (T389), phospho-MEK1 (S217/S221), and phospho-ERK1/2 (T202/Y204) were purchased from Cell Signaling Technologies. The antibodies for PI3K p110β, p110γ, and p110δ were purchased from Millipore. The antibody for PTEN was purchased from BD Biosciences Pharmingen. Bound antibody was quantitatively detected using an appropriate antimouse or rabbit immunoglobulin secondary antibody labeled with Alexa Fluor 680 (Invitrogen) and the Odyssey Infrared Imaging System (LI-COR). Data shown are median values of three independent experiments.

Table 1. Missense mutations of PI3K isoforms, *PTEN*, *KRAS*, and *BRAF* in JFCR39

| ID | Origin | Cell line | <i>PIK3CA</i> | <i>PIK3CB</i> | <i>PIK3CD</i> | <i>PIK3CG</i> | <i>PTEN</i> | <i>KRAS</i> | <i>BRAF</i> |
|-----------------------|----------------|------------|--------------------|----------------|-------------------|-------------------------|---|-------------|-------------|
| 01 | Lung ca. | NCI-H23 | | | | P538L | | G12C | |
| 02 | | NCI-H226 | | | | S442Y (SNP) | | | |
| 03 | | NCI-H522 | | | | | | | |
| 04 | | NCI-H460 | E545K (genomic) | | | | | Q61H | |
| 05 | | A549 | | | | | | <u>G12S</u> | |
| 06 | | DMS273 | | | | M259I | <u>K128N</u> | | |
| 07 | | DMS114 | | | | | | | |
| 11 | Colorectal ca. | HCC2998 | I391M (SNP) | R149Q R562Q | | T857A R273H L466M | Y46C R130Q F341V G129* K267fs(del.-1)*9 | A146T | |
| 12 | | KM-12 | | | | | | | |
| 13 | | HT-29 | P449T | | | S442Y(SNP) | | | V600E |
| 15 | | HCT-15 | E545K D549N | R628Q | S174L | | | G13D | |
| 16 | | HCT-116 | H1047R | | <u>Ex.16 del.</u> | | G13D | | |
| 21 | Gastric ca. | St-4 | | | | S442Y(SNP) | <u>E291*</u> | G12A | |
| 22 | | MKN1 | E545K | | | | | | |
| 23 | | MKN7 | | | T456A (SNP) | <u>A621S</u> | | | |
| 24 | | MKN28 | | | T456A (SNP) | <u>A621S</u> | | | |
| 25 | | MKN45 | | | | | | | |
| 26 | | MKN74 | | | T456A (SNP) | <u>A621S</u> | | | |
| 31 | Breast ca. | HBC-4 | | E1051K | | S442Y(SNP) | | | |
| 32 | | BSY-1 | H1047R | | | | <u>Ex.1-9 del.</u> | | |
| 34 | | HBC-5 | | | | | | | |
| 35 | | MCF-7 | E545K | | | <u>S442Y(SNP)</u> | | | |
| 36 | | MDA-MB-231 | | | | | | G13D | G464V |
| 41 | Ovarian ca. | OVCAR-3 | | | | | | | |
| 42 | | OVCAR-4 | | | | | | | |
| 43 | | OVCAR-5 | | | | | | | |
| 44 | | OVCAR-8 | | | | <u>N522S</u> | | <u>G12V</u> | |
| 45 | | SK-OV-3 | H1047R | | | | | | |
| 51 | Brain ca. | U251 | | | | | E242fs(ins.+2)*15 | | |
| 52 | | SF-268 | | | | | | | |
| 53 | | SF-295 | | | S312C | | <u>R233*</u> | | |
| 54 | | SF-539 | | | | | <u>Ex.1-9 del.</u> | | |
| 55 | | SNB-75 | | | | | | | |
| 56 | | SNB-78 | L719F | | | | <u>T26fs(SD del.)</u> | | |
| 61 | Renal ca. | RXF-631L | | | | S442Y(SNP) | | | |
| 62 | | ACHN | | | | | | | |
| 71 | Melanoma | LOX-IMVI | | | | | | | V600E |
| 91 | Prostate ca. | DU-145 | | A686T | | S442Y(SNP) | | | |
| 92 | | PC-3 | | | S312C | S442Y(SNP) | <u>Ex.3-9 del.</u> | | |
| Total # (without SNP) | | | 10 (9) | 4 | 7 (4) | 15 (7) | 10 | 9 | 3 |

NOTE: Underline indicates a homozygous mutation.

Drugs

ZSTK474 was kindly provided by Zenyaku Kogyo Co. Ltd. LY294002, PI103, PI3K α inhibitor IV, PI3K γ inhibitor (AS605240), and Akt inhibitors II, III, IV, V (triciribine), VIII,

IX, X, and XI were purchased from Calbiochem. TGX221 and perifosine were purchased from Cayman. GDC-0941 and IC87114 were obtained from Symansis. RAD001 and CCI779 were purchased from LC Laboratories. Wortmannin,

PX-866, NVP-BEZ235, and rapamycin were obtained from Kyowa Medex, Sigma, Selleck, and Wako Pure Chemical, respectively.

Determination of drug efficacy

Drug efficacy was assessed as changes in total cellular protein after 48 hours of drug treatment using a sulforhodamine B assay. Assays were done in duplicate and the GI_{50} was calculated as described previously (27, 33).

Animal experiments

Animal care and treatment were done in accordance with the guidelines of the animal use and care committee of the Japanese Foundation for Cancer Research and conformed to the NIH Guide for the Care and Use of Laboratory Animals. Female nude mice with BALB/c genetic backgrounds were purchased from Charles River Japan. Mice were maintained under specific pathogen-free conditions and provided with

sterile food and water *ad libitum*. Human tumor xenografts were generated by s.c. inoculating nude mice with 3 mm × 3 mm × 3 mm tumor fragments of human cancer cells. When the tumors became 100 to 300 mm³ in size, ZSTK474 was p.o. administered at 100, 200, and 400 mg/kg of body weight following the indicated schedule. The length (*L*) and width (*W*) of the subcutaneous tumor mass were measured by calipers in live mice, and the tumor volume (TV) was calculated as $TV = (L \times W^2)/2$. Percent treated/control [T/C (%)] was calculated as $(TV_{\text{with drug}}/TV_{\text{control}}) \times 100$. To assess toxicity, we measured the body weight of the tumor-bearing mice. Mice were finally sacrificed and tumors were excised and frozen in liquid N₂.

Results

Characterization of the mutation status of PIK3Cs, PTEN, KRAS, and BRAF in JFCR39 cell lines

We first examined the mutation status of PI3K isoforms in the JFCR39 cell lines (Table 1; Supplementary Table S2). Analysis of genomic sequences of *PIK3CA* on exon 9 and exon 20 revealed the hotspot mutations (E545K in four cell lines and H1047R in three cell lines). Analysis of the full coding sequence of cDNA revealed four additional missense mutations: I391M, P449T, D549N, and L719F. Evaluation of relative kinase activity of these mutants revealed that mutant P449T exhibited gain of function (>2-fold) compared with wild-type PI3K α (Fig. 1A). The E545K mutation found in NCI-H460 genomic DNA was not detected in the cDNA, suggesting that the allele with the E545K mutation was hardly transcribed in NCI-H460 cells. Therefore, we concluded that seven cell lines [HCT-116, SK-OV3, BSY-1 (H1047R), MKN-1, MCF7 (E545K), HCT-15 (E545K/D549N), and HT-29 (P449T)] expressed a gain-of-function mutant of *PIK3CA*. With regard to two hotspot mutants (E545K and H1047R), we examined the effect of three representative PI3K inhibitors on their enzymatic activity and found no striking difference in their efficacies compared with wild-type p110 α (Fig. 1B).

In addition to *PIK3CA*, we examined the genomic sequences of all coding exons of *PIK3CB*, *PIK3CD*, and *PIK3CG* genes and found five missense mutations in *PIK3CB*, three in *PIK3CD*, and eight in *PIK3CG* (Table 1; Supplementary Table S2). Most of these mutations were not registered in the dbSNP database established by The National Center for Biotechnology Information. Therefore, this study is the first report showing that cancer cells harbor missense mutations in *PIK3CB*, *PIK3CG*, and *PIK3CD* as well as in *PIK3CA*.

Next, we proceeded to examine the genomic sequences of the *PTEN* gene and found that the *PTEN* gene was deleted in three cell lines (BSY-1, SF539, and PC-3), and seven had missense or frameshift mutations.

We examined the genomic sequences of exon 1 (including G12 and G13) and exon 2 (Q61) of *KRAS* and exon 15 (V600) and exon 11 (G464, G466, and G468) of *BRAF* (Table 1). Eight of 39 cell lines had a hotspot mutation in the G12, G13, or Q61 residue. On the other hand, the *BRAF* mutation was observed in three cell lines including LOX-IMV1.

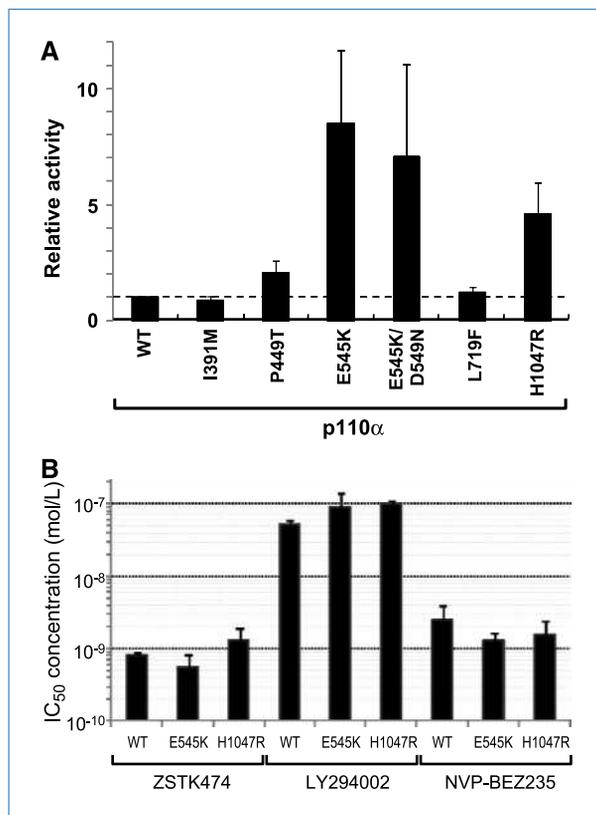
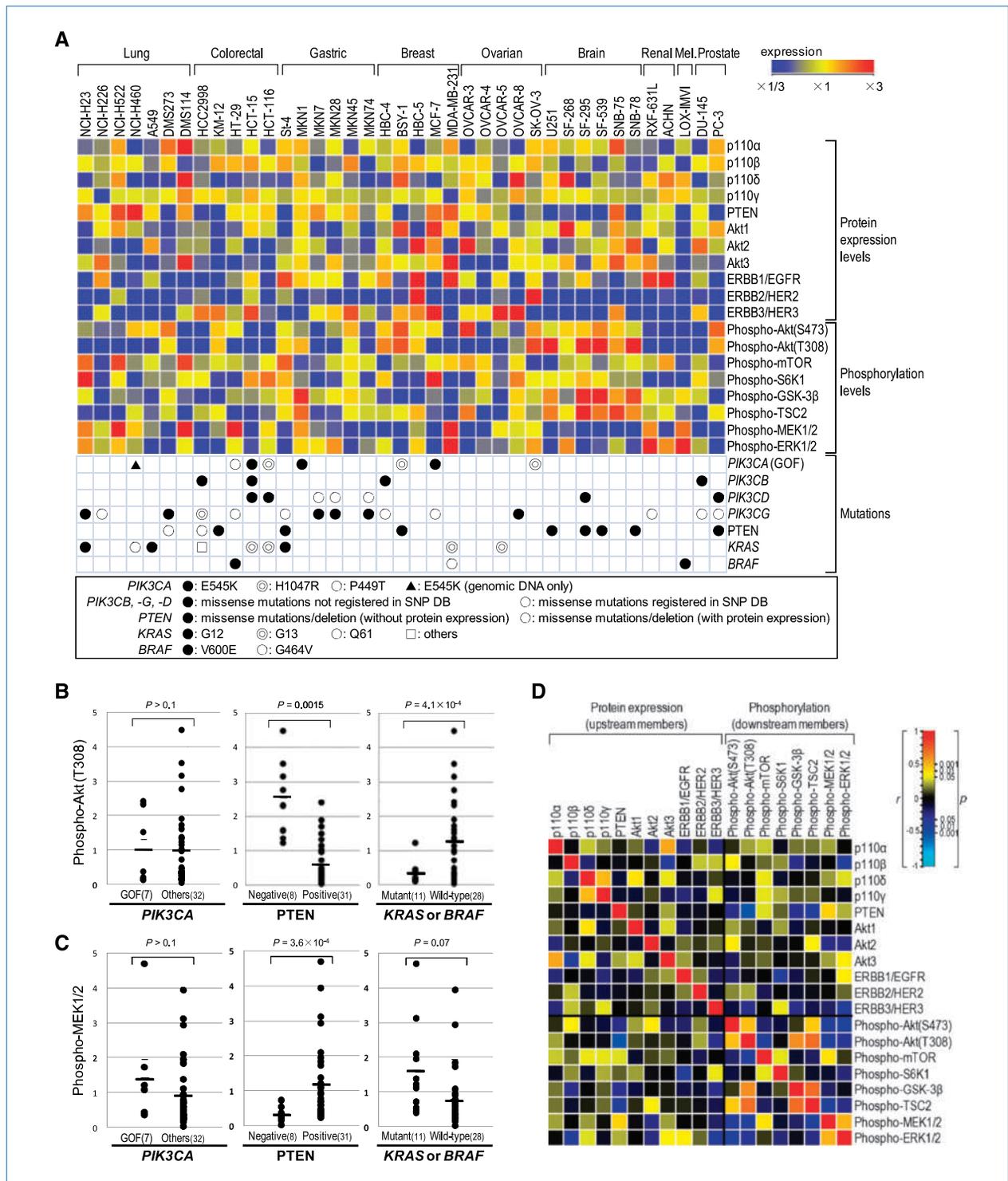


Figure 1. Relative kinase activities of p110 α mutants and the inhibitory effect of PI3K inhibitors against hotspot mutants of p110 α . A, recombinant FLAG-tagged PI3K p110 α /p85 α protein complex produced in 293T cells was immunoprecipitated, and the immunoprecipitates were used for the quantitative PI3K-HTRF assay (32). B, effect of three PI3K inhibitors (ZSTK474, LY294002, and NVP-BEZ235) on the enzymatic activity of two hotspot mutants (E545K and H1047R) p110 α compared with the wild-type. No striking difference was observed in the IC₅₀ concentrations of these inhibitors between wild-type and mutant p110 α .



Expression of PI3K isoforms, PTEN, Akt isoforms, and ERBB-family RTKs

We next examined the protein expression of upstream members of the PI3K pathway that would affect downstream activity, including PI3K isoforms (p110 α / β / γ / δ), PTEN, Akt isoforms (Akt1/2/3), and ERBB-family RTKs (EGFR/ERBB2/ERBB3; Fig. 2A; Supplementary Data). As expected, p110 α and p110 β were widely expressed, whereas p110 γ and p110 δ , which were thought to be expressed preferentially in leukocytes, were unexpectedly expressed in most JFCR39 cell lines derived from solid tumors. PTEN expression was undetectable in all of three cell lines with the deletion and in five of seven cell lines with a mutation, whereas all of 29 cell lines lacking a mutation or deletion expressed a certain amount of PTEN protein (Supplementary Fig. S1). Some of the cell lines exhibited overexpression of Akt isoforms; SF268 and MCF-7 highly expressed Akt1, whereas OVCAR3 and HBC5 highly expressed Akt2. EGFR was expressed in a wide variety of cell lines, but in some cell lines including NCI-H522 and MCF-7, EGFR expression was absent or present at an extremely low level. ERBB3 was highly expressed in a wide variety of cell lines derived from ovarian, gastric, breast, and colorectal cancer cell lines, but not in most brain cancer cell lines. On the other hand, ERBB2 was highly expressed in two cell lines (SK-OV-3 and HBC5).

Activation status of downstream members of the PI3K pathway

We examined the phosphorylation levels of PI3K downstream effectors including Akt (phosphorylated on T308 and S473), mTOR (S2448), S6K1 (T389), GSK-3 β (S9), and TSC2 (T1462; Fig. 2A). We also examined Ras downstream effectors, phosphorylated MEK1/2 (S217/S221) and ERK1/2 (T202/Y204). Interestingly, the expression pattern of phosphorylated Akt (T308) was highly correlated with phosphorylated TSC2 ($r = 0.70$) and GSK-3 β ($r = 0.60$), but not with phosphorylated mTOR and S6K1 (Fig. 2A; Supplementary Fig. S2). On the other hand, expression levels of phosphorylated Akt (T308) had a significant negative correlation with phosphorylated ERK1/2 ($r = 0.39$).

Correlation between upstream abnormalities and phosphorylation of downstream effectors in the PI3K pathway

Correlation analysis between upstream abnormalities and downstream activities revealed some interesting results (Fig. 2B and C; Supplementary Table S3). For example, phosphorylation levels of Akt (T308) ($P = 0.0015$) and TSC2 ($P = 0.011$) were significantly higher and those of MEK1/2 ($P = 3.6 \times 10^{-4}$) and ERK1/2 ($P = 2.7 \times 10^{-5}$) were significantly lower in eight PTEN-negative cell lines than those in 31 PTEN-expressing cell lines. This suggested that PTEN loss conferred activation of PI3K downstream factors and inactivation of the MAPK pathway. In contrast, phosphorylation levels of Akt (T308) ($P = 4.1 \times 10^{-4}$) and TSC2 ($P = 0.0021$) were significantly lower in cell lines having a mutation in either the *KRAS* or the *BRAF* gene, suggesting inac-

tivation of the PI3K/Akt pathway in these cell lines. However, mutation of *PIK3CA* did not have significant associations with downstream activation, including phosphorylation levels of Akt and MEK.

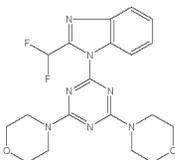
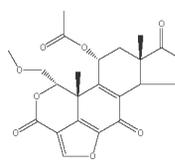
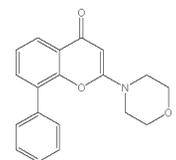
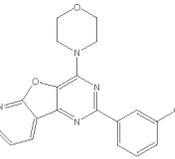
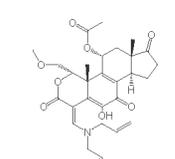
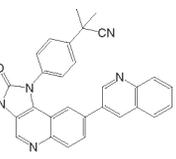
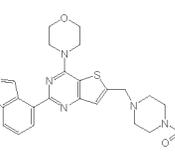
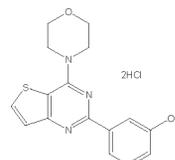
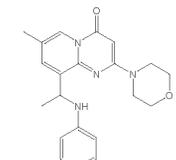
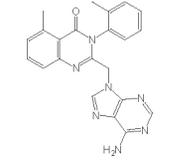
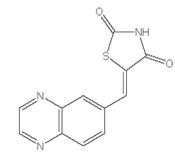
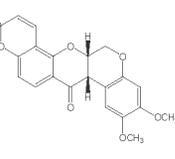
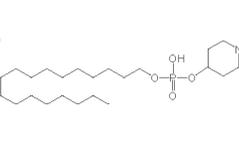
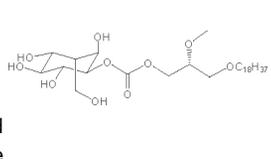
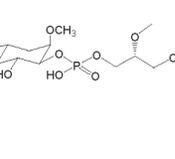
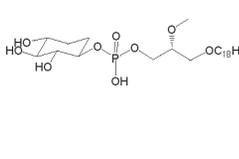
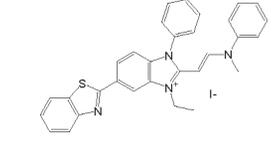
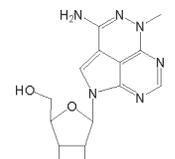
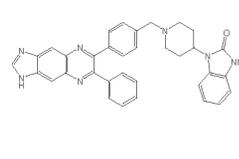
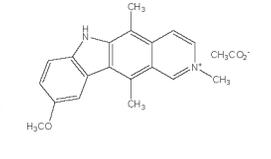
Determination of efficacy patterns of 25 PI3K pathway inhibitors across JFCR39 cell lines, or fingerprints, and evaluation of their modes of action from their fingerprints

We next examined the efficacy of 25 PI3K pathway inhibitors (Table 2) in each of the JFCR39 cell lines. Then, we compared the fingerprints of PI3K inhibitors with those of other conventional anticancer drugs by cluster analysis (Fig. 3A; Supplementary Fig. S3A). Interestingly, 10 of 11 PI3K inhibitors were tightly clustered and the cluster also included Akt inhibitor VIII (AKTi-1/2) and rapamycins, suggesting similarity in the mechanisms of action across these compounds. Moreover, their fingerprints were clearly different from those of the remainder of the 10 Akt inhibitors, all of three MEK inhibitors, and other conventional anticancer drugs. Furthermore, comparison of the fingerprints of 15 PI3K pathway inhibitors in the cluster revealed that some pairs, including ZSTK474/GDC-0941 ($r = 0.86$), wortmannin/PX-866 ($r = 0.81$), and PI103/PI3K α inhibitor IV ($r = 0.80$), exhibited extremely high correlations, suggesting a close similarity in the molecular mechanisms of action between each pair of compounds (Supplementary Fig. S3B–D).

Construction of an integrated database and correlations between the status of pathway members and the efficacy of PI3K inhibitors

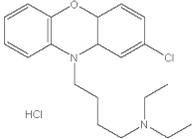
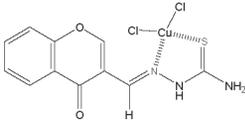
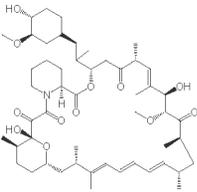
We have thus far studied the drug efficacy data and the signal pathway data with regard to JFCR39 cell lines. We then combined these data to develop an integrated database (JFCR39-DB). Using JFCR39-DB, we studied the relationship between the activation status of the PI3K pathway and the efficacy of PI3K pathway inhibitors. First, we examined the correlation between the mutation status of upstream members and drug efficacy. The Student *t* test revealed no significant differences in the efficacies of all of 25 PI3K pathway inhibitors examined, in seven cell lines expressing a gain-of-function mutant of PI3K α , and in the remainder of the 32 cell lines (Fig. 3B; Supplementary Table S4). As mentioned before, we examined the effect of three representative PI3K inhibitors on the enzymatic activity of two hotspot mutants of PI3K α and found no striking difference in their efficacies compared with wild-type p110 α (Fig. 1B). These results suggest that cancer cells expressing mutant PI3K α are susceptible to PI3K inhibitors to a similar extent as those expressing wild-type PI3K α . Moreover, PTEN status did not correlate with the efficacy of PI3K pathway inhibitors. In addition, the mutation status of other PI3K isoforms did not exhibit striking correlations either. On the other hand, cell lines having a mutation in either *KRAS* or *BRAF* exhibited resistance to several PI3K pathway inhibitors including ZSTK474 (Fig. 3B; Supplementary Table S4). The present results suggest that *KRAS/BRAF*

Table 2. The 25 PI3K pathway inhibitors used in this study and their profiles and structures (15–18)

| PI3K inhibitors | | | | | |
|---|---|---|--|--|---|
| ZSTK474 (4.8×10^{-7} mol/L) Selective PI3K inhibitor Preclinical |  | Wortmannin (9.7×10^{-6} mol/L) PI3K/mTOR/MLCK inhibitor Preclinical |  | LY294002 (8.1×10^{-6} mol/L) PI3K/mTOR/CK2 inhibitor Preclinical |  |
| PI103 (1.8×10^{-7} mol/L) PI3K/mTOR inhibitor Preclinical |  | PX866 (1.4×10^{-6} mol/L) PI3K $\alpha/\delta/\gamma$ inhibitor; derivative of wortmannin Phase I |  | NVP-BE2235 (8.6×10^{-9} mol/L) PI3K/mTOR inhibitor Phase I/II |  |
| GDC-0941 (5.0×10^{-7} mol/L) Selective PI3K inhibitor Phase I |  | PI3K α inhibitor IV (1.0×10^{-6} mol/L) PI3K α/β inhibitor; derivative of PI103 Preclinical |  | TGX221 (1.0×10^{-5} mol/L) Selective PI3K β inhibitor Preclinical |  |
| IC87114 (CAL-101); 8.3×10^{-5} mol/L) Selective PI3K δ inhibitor Phase I |  | AS605240 (PI3K γ inhibitor; 9.5×10^{-6} mol/L) Selective PI3K γ inhibitor Preclinical |  | | |
| Akt inhibitors | | | | | |
| Deguelin (7.6×10^{-6} mol/L) Selective Akt inhibitor; derivative of rotenone Preclinical |  | Perifosine (9.1×10^{-6} mol/L) Lipid-based PI analogue Phase II |  | Akt inhibitor (1.3×10^{-5} mol/L) Lipid-based PI analogue Preclinical |  |
| Akt inhibitor II (8.6×10^{-6} mol/L) Lipid-based PI analogue Preclinical |  | Akt inhibitor III (1.9×10^{-5} mol/L) Lipid-based PI analogue Preclinical |  | Akt inhibitor IV (2.8×10^{-7} mol/L) Preclinical |  |
| Akt inhibitor V (tricitrine/VQD-002; 2.2×10^{-5} mol/L) Tricyclic nucleotide Phase I/II (as tricitrine phosphate) |  | Akt inhibitor VIII (AKTi-1/2; 8.6×10^{-6} mol/L) Allosteric Akt1/2 inhibitor Preclinical |  | Akt inhibitor IX (8.0×10^{-7} mol/L) Preclinical |  |

(Continued on the following page)

Table 2. The 25 PI3K pathway inhibitors used in this study and their profiles and structures (15–18) (Cont'd)

| Akt inhibitors | | | |
|--|---|--|--|
| Akt inhibitor X (3.6×10^{-6} mol/L) Preclinical |  | Akt inhibitor XI (1.1×10^{-5} mol/L) Preclinical |  |
| mTOR inhibitors | | | |
| Rapamycin (1.7×10^{-7} mol/L) Allosteric inhibitor of mTORC1 In clinical use |  | Everolimus (RAD001; 3.6×10^{-8} mol/L) Rapalogue In clinical use | Temsirolimus (CCI779; 2.0×10^{-7} mol/L) Rapalogue In clinical use |

NOTE: Numbers in parentheses are mean GI₅₀ values across the JFCR39 cell lines.

mutation would be a biomarker for resistance to these PI3K pathway inhibitors.

Correlation of the efficacy of PI3K pathway inhibitors with protein expression levels of PI3K isoforms, Akt isoforms, and ERBB-family RTKs

We next correlated the expression levels of the above proteins with the efficacy of PI3K pathway inhibitors (Fig. 3A). As a result, we found that expression of p110 α had a slight positive correlation with four PI3K inhibitors including IC87114 and LY294002. As for p110 β , we found that its high expression correlated with the efficacy of several Akt inhibitors including perifosine. As for Akt isoforms, we found that high expression of Akt2 was associated with the efficacy of five PI3K pathway inhibitors including TGX-221 and LY294002. Correlation analysis between the expression levels of ERBB-family RTKs and the efficacy of PI3K inhibitors revealed no significant correlations with PI3K pathway inhibitors. However, high expression of EGFR correlated with resistance to other classes of anticancer drugs such as navelbine ($r = -0.49$) and mitoxantrone ($r = -0.43$), suggesting that EGFR would confer resistance to these drugs in cancer cells.

Correlation of the efficacy of PI3K pathway inhibitors with phosphorylation levels of the PI3K/Akt and Ras/MAPK pathways

Lastly, we correlated the phosphorylation levels of downstream members of the PI3K pathway with drug efficacy (Fig. 3A). Of note, expression of Akt phosphorylated at S473 had significant correlations with 11 of 25 inhibitors including ZSTK474 ($r = 0.44$; Fig. 3C) and wortmannin ($r = 0.52$). In addition, phosphorylated TSC2 correlated with 7 of 25 inhibitors, including Akt inhibitor IX ($r = 0.41$) and CCI779 ($r = 0.40$). This result suggests that expression levels of Akt phosphorylated at S473 and phosphorylated TSC2 would be predictive markers for these PI3K pathway inhibitors.

Phosphorylated Akt levels and KRAS/BRAF mutation correlated with the *in vivo* efficacy of ZSTK474

We have thus far studied correlations between the status of pathway members and the efficacy of PI3K inhibitors *in vitro*. The most prominent associations were that phosphorylated Akt correlated with the efficacy whereas *KRAS/BRAF* mutation correlated with the inefficacy of PI3K inhibitors including ZSTK474. To test these correlations *in vivo*, we inoculated 24 transplantable cell lines of JFCR39 and examined the antitumor effect of ZSTK474 (Supplementary Fig. S4). We first confirmed that the *in vitro* efficacy pattern across the 24 cell lines significantly correlated with the *in vivo* efficacy pattern (200 mg/kg; $P = 0.02$), suggesting that the *in vivo* efficacy of ZSTK474 reflected its *in vitro* efficacy (Supplementary Fig. S5). Based on these data, we examined the involvement of phosphorylated Akt and *KRAS/BRAF* mutation in the efficacy of ZSTK474 *in vivo* (Fig. 4). The Student *t* test revealed that cell lines having a hotspot mutation in either *KRAS* (G12, G13, and Q61) or *BRAF* (V600) exhibited inefficacy of ZSTK474 ($P = 0.04$; Fig. 4B). Cell lines with PTEN loss such as PC-3 and BSY-1 exhibited susceptibility to ZSTK474, but the difference was not statistically significant across the 24 cell lines. On the other hand, expression levels of phosphorylated Akt in the xenografted tumors significantly correlated with susceptibility to ZSTK474 (Fig. 4C). These results suggest that phosphorylated Akt and *KRAS/BRAF* hotspot mutation could be used as a biomarker for predicting the efficacy of PI3K inhibitors in the clinic.

Discussion

In this study, taking advantage of the JFCR39 cell line panel, we examined the drug efficacy of PI3K pathway inhibitors as well as the status of PI3K and Ras pathway members, and combined them to develop an integrated database. This

database, designated as JFCR39-DB, enabled us to evaluate correlations between the status of pathway members and drug efficacy, as well as correlations among pathway members and among inhibitors *in silico*. First, comparison of the status of pathway members revealed that PTEN loss significantly correlated with upregulated phosphorylation levels of PI3K downstream members and with downregulated phosphorylation levels of Ras downstream members, whereas *KRAS/BRAF* mutation exhibited an opposite tendency. Second, comparison of drug efficacies revealed that most PI3K inhibitors and rapamycins were tightly clustered and were clearly different from other classes of anticancer compounds, suggesting a similarity in the mechanisms of action across these compounds in the cluster. Third, correlation analysis between the status of pathway members and drug efficacy revealed that phosphorylated Akt and *KRAS/BRAF* hotspot mutation correlated with the efficacy and the inefficacy of PI3K inhibitors, respectively. These correlations were confirmed in xenografted human tumors *in vivo*, suggesting that they could serve as predictive biomarkers for PI3K inhibitors.

Correlation analysis among the pathway members revealed that PTEN loss significantly correlated with the phosphorylation levels of Akt, GSK-3 β , and TSC2, but *PIK3CA* mutation did not exhibit such correlations, suggesting that PTEN loss was directly connected with activation of these downstream pathway members. Similar results were recently shown by Vasudevan and colleagues using NCI60 cell lines (34) and by Stemke-Hale and colleagues using clinical specimens and cell lines of human breast cancer (35), indicating the significance of PTEN loss in the activation of the PI3K pathway. Furthermore, we showed that PTEN loss was not accompanied by activation of mTOR and S6K1, suggesting the existence of upstream molecule(s) other than Akt that may regulate the activation of mTOR and S6K1. Indeed, mTOR is regulated by energy stress via AMPK and by hypoxia via HIF1 α , as well as by growth factor stimulation via PI3K/Akt (13). Interestingly, PTEN loss was strongly associated with inactivation of MAPK pathway components such as MEK1/2 and ERK1/2. Previously, Zimmermann and Moelling reported that phosphorylation of Raf by Akt inhibited activation of the Ras/Raf/MAPK pathway (36), which supports our observation. On the other hand, mutation in either *KRAS* or *BRAF* was strongly correlated with inactivation of PI3K/Akt, rather than activation of the MAPK pathway. This result strongly suggests that gain-of-function mutations of *KRAS* and *BRAF* confer inactivation on Akt and its downstream pathway.

In this study, we performed a mutation analysis of all coding exons of *PIK3CB*, *PIK3CD*, and *PIK3CG* isoforms, which are thought to be rarely mutated in cancer (3). Unexpectedly, we found missense mutations in each isoform that were not registered in the SNP database. Although we could not determine whether they were cancer-specific somatic mutations or derived from germline mutations that have not yet been registered in the SNP database, this is the first report showing that cancer cells harbor missense mutations in these PI3K isoforms. The kinase activities of these mutants compared with their wild-type analogues are under investigation. Cancer cells having these mutations did not exhibit hyperphosphorylation of Akt and its downstream factors, suggesting that they did not activate the canonical PI3K pathway via Akt.

We previously showed that the fingerprints of antitumor compounds across the JFCR39 cell panel can allow evaluation of similarities in the cellular mechanism of action by which drugs exert their antitumor activity (28, 29). Comparison of drug fingerprints revealed that most PI3K inhibitors and rapamycins were tightly clustered and their fingerprints were clearly different from those of other classes of anticancer compounds, suggesting that they act by similar mechanisms, probably by blocking the PI3K/mTOR pathway. Moreover, a fine comparison of the fingerprints of these PI3K pathway inhibitors revealed that ZSTK474 and GDC-0941, both of which are specific class I PI3K inhibitors that do not inhibit other PI3K-related protein kinases including mTOR (15, 37–39), show similar fingerprints ($r = 0.86$). On the other hand, NVP-BEZ235, which is a more potent inhibitor of mTOR than of PI3K (40), exhibited high correlations with the mTOR inhibitor rapamycin ($r = 0.78$), and the correlation was much higher than those with ZSTK474 ($r = 0.67$) and GDC-0941 ($r = 0.46$). Thus, these results suggest that comparison of fingerprints may accurately distinguish the cellular target(s) that determine(s) the susceptibility of cancer cells to these drugs.

Using the integrated PI3K database, we correlated the status of these pathway members with the efficacy of PI3K inhibitors. We first found that either gain-of-function mutation of *PIK3CA* or PTEN loss did not exhibit significant correlation with the efficacy of PI3K inhibitors. Brachmann and colleagues recently reported that NVP-BEZ235 selectively induced apoptosis in *PIK3CA*-mutant breast cancer cells (41). However, in the present study, *PIK3CA*-mutant cell lines did not exhibit hypersensitivity but were susceptible to PI3K inhibitors to a similar extent as those with wild-type *PIK3CA*. Indeed, similar results were obtained in *in vivo* experiments using 24

Figure 3. Fingerprints of 67 compounds including 25 PI3K pathway inhibitors and other conventional anticancer agents and their correlations with the activation status of PI3K pathway members. A, top, the GI_{50} in each cell line was determined and log transformed. The compounds were clustered on the basis of their correlations with other compounds (average-linkage clustered with Pearson correlation metric). Cluster analysis was done by using GeneSpring GX (Agilent Technologies). A yellow point represents the average $\log GI_{50}$ for each compound across JFCR39. A red point and a blue point represent sensitivity and resistance by 10-fold, respectively. Bottom, a heat map of correlation between the fingerprints of 67 anticancer compounds and those of PI3K pathway members. A red point (high positive Pearson correlation coefficient, $P < 0.001$) indicates that the compound tends to be more effective against cell lines that express more of the protein; a blue point (high negative correlation, $P < 0.001$) indicates the opposite tendency. B, difference in the efficacy of ZSTK474 between cell lines with and without *PIK3CA* gain-of-function mutation, loss of PTEN expression, and *KRAS/BRAF* mutation. C, scatter plots of JFCR39 cell lines showing a significant correlation between phospho-Akt (S473) and the efficacy of ZSTK474.

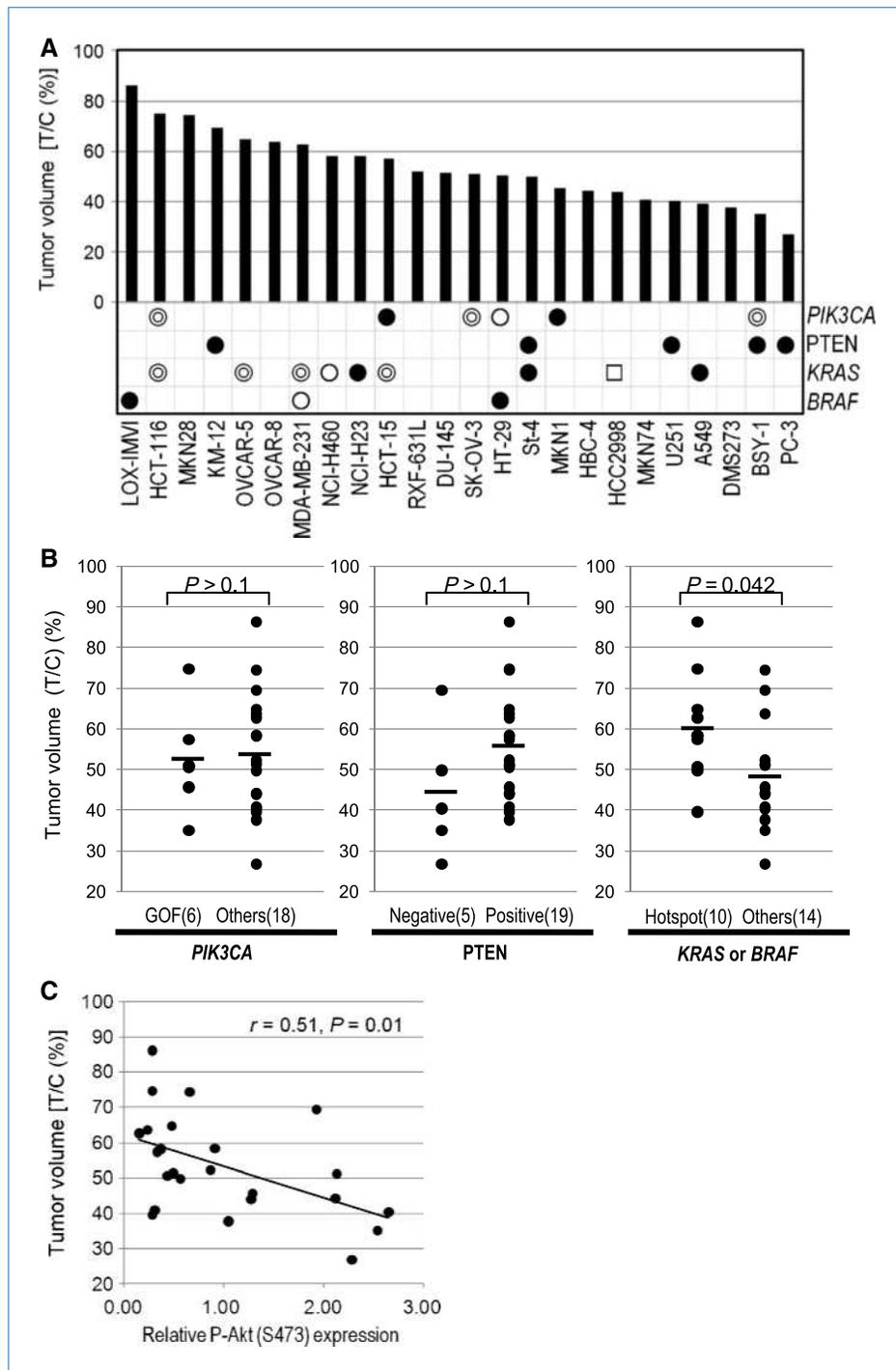


Figure 4. *In vivo* efficacy of ZSTK474 toward human tumors xenografted in nude mice and the activation status of PI3K pathway members. A, 24 transplantable cell lines of JFCR39 were inoculated in nude mice and the *in vivo* efficacy of ZSTK474 (200 mg/kg) was examined (T/C%). The mutation status of *PIK3CA* (closed circle: E545K, double circle: H1047R, open circle: P449T), *PTEN* (closed circle: mutation or deletion without protein expression), *KRAS* (closed circle: G12, double circle: G13, open circle: Q61, open square: A146), and *BRAF* (closed circle: V600E, open circle: G464V) were indicated. B, difference in the *in vivo* efficacy of ZSTK474 between cell lines with and without *PIK3CA* gain-of-function mutation, loss of *PTEN* expression, and *KRAS/BRAF* hotspot mutation. C, scatter plots of 24 cancer cell lines showing significant correlations between phospho-Akt (S473) expressed in tumor sample and the *in vivo* efficacy of ZSTK474.

transplantable human cancer cell lines xenografted in nude mice (Fig. 4B). We further showed that PI3K inhibitors including ZSTK474 and NVP-BEZ235 inhibit the enzymatic activity of gain-of-function mutant of p110 α to an extent comparable to the wild-type (Fig. 1B). These results suggest that such PI3K inhibitors can be used for these cancers. On the other hand, cell lines with mutation in either *KRAS* or *BRAF* exhibited resistance to five PI3K inhibitors including ZSTK474. *In vivo*

studies, we showed that *KRAS/BRAF* mutation exhibited a significant correlation with efficacy of ZSTK474 using 24 xenografted tumors. Similar results were recently reported by Ihle and colleagues by using PX-866 and 13 human tumor xenografts (42) and by Engelman and colleagues using NVP-BEZ235 and a mouse tumor model ectopically expressing a *KRAS* mutant (23), suggesting the significance of *KRAS/BRAF* mutation in the inefficacy of these PI3K inhibitors.

Of the PI3K pathway downstream members examined in this study, it should be noted that Akt phosphorylated at S473 had significant correlations with 6 of 11 PI3K inhibitors including ZSTK474. Moreover, the correlation between phosphorylated Akt levels and the efficacy of ZSTK474 was confirmed in *in vivo* experiments using 24 human cancer xenografts. This result indicated that the expression level of Akt phosphorylated at S473 could be a candidate biomarker for predicting the efficacy of PI3K inhibitors. The reason why Akt phosphorylated at S473, but not T308, exhibited this correlation is unknown. Phosphorylation of the S473 residue of Akt is known to be catalyzed by the TORC2 complex (whose components include mTOR and rictor) and is required, in addition to T308, for full activation of Akt (43). It is intriguing that the fully activated form of Akt correlated with the efficacy of PI3K inhibitors.

In summary, we constructed an integrated database of PI3K and Ras pathway members and the efficacy of PI3K pathway inhibitors in JFCR39 (JFCR39-DB). *In silico* correlation analysis using JFCR39-DB enabled us to extract two candidates, phospho-Akt and *KRAS/BRAF* mutation, as predictive biomarkers for efficacy of PI3K inhibitors from various pathway members examined. Moreover, we confirmed these correlations by *in vivo* studies using 24 xenografted tumors. The utility of these candidate biomarkers should be validated through clinical studies in future. In addition, JFCR39-DB enabled us to study functional relationships

among pathway members and those among drugs. Therefore, JFCR39-DB described here is a useful tool to identify predictive biomarkers, as well as to study the molecular pharmacology of the PI3K pathway in cancer.

Disclosure of Potential Conflicts of Interest

T. Yamori: commercial research grant, Zenyaku Kogyo Co., Ltd. The other authors disclosed no potential conflicts of interest.

Acknowledgments

We thank Yumiko Nishimura and Yoshimi Ohashi for their technical assistance and Zenyaku Kogyo Co. Ltd. for providing us with ZSTK474.

Grant Support

National Institute of Biomedical Innovation, Japan, grant 5-13 (T. Yamori); Grants-in-Aid of the Priority Area "Cancer" from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, nos. 18015049 and 20015048 (T. Yamori); Grants-in-Aid for Scientific Research (B), no. 17390032, and (A), no. 22240092, from the Japan Society for the Promotion of Science (T. Yamori); the Kobayashi Institute for Innovative Cancer Chemotherapy (T. Yamori); and Grants-in-Aid for Young Scientists (B) from the Japan Society for the Promotion of Science, nos. 20790087 and 22700929 (S. Dan).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 11/16/2009; revised 03/25/2010; accepted 04/22/2010; published OnlineFirst 06/08/2010.

References

- Cantley LC. The phosphoinositide 3-kinase pathway. *Science* 2002; 296:1655–7.
- Engelman JA, Luo J, Cantley LC. The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 2006;7:606–19.
- Samuels Y, Velculescu VE. Oncogenic mutations of PIK3CA in human cancers. *Cell Cycle* 2004;3:1221–4.
- Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science* 2004;304:554.
- Ma YY, Wei SJ, Lin YC, et al. PIK3CA as an oncogene in cervical cancer. *Oncogene* 2000;19:2739–44.
- Shayesteh L, Lu Y, Kuo WL, et al. PIK3CA is implicated as an oncogene in ovarian cancer. *Nat Genet* 1999;21:99–102.
- Li J, Yen C, Liaw D, et al. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997;275:1943–7.
- Steck PA, Pershouse MA, Jasser SA, et al. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Genet* 1997;15:356–62.
- Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat Rev Cancer* 2002;2:489–501.
- Hu L, Zaloudek C, Mills GB, Gray J, Jaffe RB. *In vivo* and *in vitro* ovarian carcinoma growth inhibition by a phosphatidylinositol 3-kinase inhibitor (LY294002). *Clin Cancer Res* 2000;6:880–6.
- Ihle NT, Williams R, Chow S, et al. Molecular pharmacology and antitumor activity of PX-866, a novel inhibitor of phosphoinositide-3-kinase signaling. *Mol Cancer Ther* 2004;3:763–72.
- Yaguchi S, Fukui Y, Koshimizu I, et al. Antitumor activity of ZSTK474, a new phosphatidylinositol 3-kinase inhibitor. *J Natl Cancer Inst* 2006;98:545–56.
- Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. *Nat Rev Cancer* 2009;9:550–62.
- Hoeflich KP, O'Brien C, Boyd Z, et al. *In vivo* antitumor activity of MEK and phosphatidylinositol 3-kinase inhibitors in basal-like breast cancer models. *Clin Cancer Res* 2009;15:4649–64.
- Kong D, Yamori T. Advances in development of phosphatidylinositol 3-kinase inhibitors. *Curr Med Chem* 2009;16:2839–54.
- Ihle NT, Powis G. Take your PIK: phosphatidylinositol 3-kinase inhibitors race through the clinic and toward cancer therapy. *Mol Cancer Ther* 2009;8:1–9.
- Knight ZA, Gonzalez B, Feldman ME, et al. A pharmacological map of the PI3-K family defines a role for p110 α in insulin signaling. *Cell* 2006;125:733–47.
- Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. *Nat Rev Drug Discov* 2009;8:627–44.
- Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304:1497–500.
- Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350: 2129–39.
- Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;66:3992–5.
- Siena S, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009;101:1308–24.
- Engelman JA, Chen L, Tan X, et al. Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. *Nat Med* 2008;14:1351–6.
- Jhawer M, Goel S, Wilson AJ, et al. PIK3CA mutation/PTEN expression status predicts response of colon cancer cells to the epidermal

- growth factor receptor inhibitor cetuximab. *Cancer Res* 2008;68:1953–61.
25. Sartore-Bianchi A, Martini M, Molinari F, et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. *Cancer Res* 2009;69:1851–7.
 26. Markman B, Atzori F, Perez-Garcia J, Taberero J, Baselga J. Status of PI3K inhibition and biomarker development in cancer therapeutics. *Ann Oncol* 2010;21:683–91.
 27. Yamori T, Matsunaga A, Sato S, et al. Potent antitumor activity of MS-247, a novel DNA minor groove binder, evaluated by an *in vitro* and *in vivo* human cancer cell line panel. *Cancer Res* 1999;59:4042–9.
 28. Yamori T. Panel of human cancer cell lines provides valuable database for drug discovery and bioinformatics. *Cancer Chemother Pharmacol* 2003;52 Suppl 1:S74–9.
 29. Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. *Nat Rev Cancer* 2006;6:813–23.
 30. Paull KD, Shoemaker RH, Hodes L, et al. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. *J Natl Cancer Inst* 1989;81:1088–92.
 31. Dan S, Tsunoda T, Kitahara O, et al. An integrated database of chemosensitivity to 55 anticancer drugs and gene expression profiles of 39 human cancer cell lines. *Cancer Res* 2002;62:1139–47.
 32. Sugita H, Dan S, Kong D, Tomida A, Yamori T. A new evaluation method for quantifying PI3K activity by HTRF assay. *Biochem Biophys Res Commun* 2008;377:941–5.
 33. Monks A, Scudiero D, Skehan P, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer Inst* 1991;83:757–66.
 34. Vasudevan KM, Barbie DA, Davies MA, et al. AKT-independent signaling downstream of oncogenic PIK3CA mutations in human cancer. *Cancer Cell* 2009;16:21–32.
 35. Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al. An integrative genomic and proteomic analysis of PIK3CA, PTEN, AKT mutations in breast cancer. *Cancer Res* 2008;68:6084–91.
 36. Zimmermann S, Moelling K. Phosphorylation and regulation of Raf by Akt (protein kinase B). *Science* 1999;286:1741–4.
 37. Kong D, Dan S, Yamazaki K, Yamori T. Inhibition profiles of phosphatidylinositol 3-kinase inhibitors against PI3K superfamily and human cancer cell line panel JFCR39. *Eur J Cancer* 2010;46:1111–21.
 38. Raynaud FI, Eccles SA, Patel S, et al. Biological properties of potent inhibitors of class I phosphatidylinositide 3-kinases: from PI-103 through PI-540, PI-620 to the oral agent GDC-0941. *Mol Cancer Ther* 2009;8:1725–38.
 39. Folkes AJ, Ahmadi K, Alderton WK, et al. The identification of 2-(1*H*-indazol-4-yl)-6-(4-methanesulfonyl-piperazin-1-ylmethyl)-4-morpholin-4-yl-t hieno[3,2-*d*]pyrimidine (GDC-0941) as a potent, selective, orally bioavailable inhibitor of class I PI3 kinase for the treatment of cancer. *J Med Chem* 2008;51:5522–32.
 40. Serra V, Markman B, Scaltriti M, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res* 2008;68:8022–30.
 41. Brachmann SM, Hofmann I, Schnell C, et al. Specific apoptosis induction by the dual PI3K/mTOR inhibitor NVP-BEZ235 in HER2 amplified and PIK3CA mutant breast cancer cells. *Proc Natl Acad Sci U S A* 2009;106:22299–304.
 42. Ihle NT, Lemos R, Jr., Wipf P, et al. Mutations in the phosphatidylinositol-3-kinase pathway predict for antitumor activity of the inhibitor PX-866 whereas oncogenic Ras is a dominant predictor for resistance. *Cancer Res* 2009;69:143–50.
 43. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 2005;307:1098–101.

Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

Correlating Phosphatidylinositol 3-Kinase Inhibitor Efficacy with Signaling Pathway Status: *In silico* and Biological Evaluations

Shingo Dan, Mutsumi Okamura, Mariko Seki, et al.

Cancer Res Published OnlineFirst June 8, 2010.

Updated version Access the most recent version of this article at:
doi:[10.1158/0008-5472.CAN-09-4172](https://doi.org/10.1158/0008-5472.CAN-09-4172)

Supplementary Material Access the most recent supplemental material at:
<http://cancerres.aacrjournals.org/content/suppl/2010/06/08/0008-5472.CAN-09-4172.DC1>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link <http://cancerres.aacrjournals.org/content/early/2010/06/04/0008-5472.CAN-09-4172>. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.