PIK3CA Mutation Is Associated with a Favorable Prognosis among Patients with Curatively Resected Esophageal Squamous Cell Carcinoma

Hironobu Shigaki1, Yoshifumi Baba1,2, Masayuki Watanabe1, Asuka Murata1, Takatsugu Ishimoto1, Masaaki Iwatsuki1, Shiro Iwagami1, Katsuhiko Nosho2, and Hideo Baba1

Abstract

Purpose: PIK3CA encodes the catalytic subunit of PI3K, p110α. Mutant PIK3CA stimulates the AKT pathway and promotes cancer cell proliferation. PIK3CA mutations have been associated with poor prognosis in patients with colorectal or lung cancer. In contrast, the relationship between PIK3CA mutations and favorable prognoses has been shown in breast cancer. However, the influence of PIK3CA mutations on the prognosis of patients with esophageal squamous cell carcinoma (ESCC) remains unclear.

Experimental Design: Using a nonbiased database of 219 curatively resected ESCCs and eight esophageal cancer cell lines, we evaluated PIK3CA mutational status by pyrosequencing. The expression of p53 and phosphorylated AKT (i.e., AKT activation) was evaluated by immunohistochemistry.

Results: PIK3CA mutations in exon 9 and/or 20 were detected in 46 cases (21%). No ESCC cell line harbored PIK3CA mutations. PIK3CA mutations were significantly associated with phosphorylated AKT expression, but not with p53 expression, sex, age at surgery, tobacco use, alcohol use, or histologic grade. Compared with wild-type PIK3CA cases, patients with PIK3CA mutations in exons 9 and/or 20 experienced significantly better disease-free survival [log-rank $P = 0.0089$; univariate HR: $0.37$, 95% confidence interval (CI): $0.15–0.75$, $P = 0.0042$; multivariate HR: $0.34$, 95% CI: $0.10–0.86$, $P = 0.021$] and overall survival [log-rank $P = 0.012$; univariate HR: $0.38$, 95% CI: $0.16–0.78$, $P = 0.0060$; multivariate HR: $0.35$, 95% CI: $0.10–0.90$, $P = 0.028$].

Conclusion: PIK3CA mutations in ESCC are associated with longer survival, suggesting its role as a prognostic biomarker. Future studies are needed to confirm this association and to elucidate the exact mechanisms by which PIK3CA mutations affect tumor behavior. Clin Cancer Res; 19(9); 1–9. ©2013 AACR.

Introduction

Esophageal squamous cell carcinoma (ESCC), the major histologic type of esophageal cancer in East Asian countries, is one of the most aggressive malignant tumors (1). Despite the development of multimodality therapies, the prognosis of patients remains poor, even for those who undergo complete resection of their carcinomas (2–4). The limited improvement in treatment outcome provided by conventional therapies has prompted us to seek innovative strategies for treating ESCCs, especially those that are molecularly targeted. The identification of new prognostic or predictive molecular markers for ESCC may improve the risk-adapted therapeutic strategies and help stratify patients in future clinical trials for treatment with drugs targeting these molecular changes (5, 6).

Activation of the phosphoinositide 3-kinase (PI3K)/AKT signaling pathway plays an important role in the development of a variety of human carcinomas, and several efforts are underway to define therapeutic inhibitors of this signaling pathway (7, 8). PI3K interacts with phosphatidylinositol-3-phosphate at the membrane and catalyzes the phosphorylation of AKT, which activates the downstream signaling pathway (8). Mutations in the PIK3CA gene, which encodes the p110α catalytic subunit of PI3K (7, 8), have been identified in many human cancers (9). The majority of activating PIK3CA mutations map to 3 sites (codons 542 and 545 in exon 9 and codon 1,047 in exon 20; ref. 10). In studies of ESCC, PIK3CA mutation has been detected in 2.2% to 11.8% of analyzed cases (11–14), and its prognostic role has been still unknown. Previous studies...
Translational Relevance

PIK3CA mutations and subsequent activation of the AKT pathway play a crucial role in human neoplasms. PIK3CA mutations have been associated with poor prognosis in patients with colorectal or lung cancer. In contrast, the relationship between PIK3CA mutations and favorable prognoses has been shown in breast cancer. However, no large-scale study has examined the prognostic impact of PIK3CA mutations in esophageal squamous cell carcinoma (ESCC). In this study, we quantified the PIK3CA mutations in exons 9 and 20 using a nonbiased database of 219 curatively resected ESCCs and pyrosequencing technology. This is, by far, the largest study on the prognostic role of PIK3CA mutations in ESCC to date, and it shows that PIK3CA mutations in ESCC are associated with favorable prognoses. Our data suggest that PIK3CA mutational status can have a potential role as a prognostic biomarker.

in large numbers of cases (more than 200) have shown that PIK3CA mutations are associated with unfavorable prognosis in patients with colorectal (15–19) or lung cancer (20). On the other hand, a study using 590 patients with breast cancer showed the relationship between PIK3CA mutation and a favorable prognosis, although it was not statistically significant in multivariate analysis (ref. 21; Table 1). Given accumulating evidence on vital roles of the PIK3/AKT pathway in cancer cells, we hypothesized that PIK3CA-wild-type ESCCs and PIK3CA mutation ESCCs might behave differently.

To test this hypothesis, we quantified the PIK3CA mutations in 219 samples of curatively resected ESCCs using pyrosequencing, and examined the prognostic significance of PIK3CA mutations in ESCC. In addition, we also evaluated the relationship between PIK3CA mutations, phosphorylated AKT (p-AKT) expression, which was the most successful biomarker for evaluating the status of AKT activation, and p53 expression, which was one of the important molecular alterations in ESCC.

Materials and Methods

Study subjects

A total of 235 patients with ESCC who underwent curative resection at Kumamoto University Hospital between April 2005 and December 2011 were enrolled in this study. Fifteen patients were excluded because of the unavailability of adequate tissue samples. We initially quantified PIK3CA mutations in 220 cancer specimens, and obtained valid results in 219 (99.5%) of the cases. Thus, a total of 219 ESCCs were ultimately included in this study. Patients were observed at 1- to 3-month intervals until death or 31 March 2012, whichever came first. Tumor staging was carried out by the American Joint Committee on Cancer Staging Manual (7th edition; ref. 22). Eighty-two patients received preoperative treatment [50 patients; chemotherapy (cisplatin, 5-fluorouracil either with or without docetaxel), 5 patients; radiation therapy, 27 patients; chemoradiotherapy]. Disease-free survival was defined as the length of time after surgical treatment of the cancer during which the patient survived with no sign of cancer recurrence. Overall survival was defined as the time between the date of the operation and the date of death. Cancer-specific survival was defined as the time between the date of operation and the date of death, which was confirmed to be attributable to ESCC. In our cohort, the 5-year overall survival rates of patients treated by esophagectomy were 83.9% for stage I, 59.7% for stage II, and 36.7% for stage III. These rates were comparable with those from the “Comprehensive Registry of Esophageal Cancer in Japan” (79.5% for stage I, 59.7% for stage II, and 36.7% for stage III).

Table 1. Studies on prognostic significance of PIK3CA mutations in several types of cancers

<table>
<thead>
<tr>
<th>Author</th>
<th>Type of cancer</th>
<th>Prognostic effect</th>
<th>Sample size</th>
<th>HR (95% CI)</th>
<th>Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liao et al. (2012)</td>
<td>Colon cancer</td>
<td>Unfavorable</td>
<td>1,170</td>
<td>3.51 (1.28–9.62; CS)</td>
<td>Pyrosequencing</td>
<td>(15)</td>
</tr>
<tr>
<td>Farinha et al. (2011)</td>
<td>Colon cancer</td>
<td>Unfavorable</td>
<td>685</td>
<td>N/A</td>
<td>Single nucleotide primer extension, real-time PCR</td>
<td>(16)</td>
</tr>
<tr>
<td>De Roock et al. (2010)</td>
<td>Colon cancer</td>
<td>Unfavorable</td>
<td>743</td>
<td>2.27 (1.10–4.66; PFS)</td>
<td>MassARRAY system</td>
<td>(17)</td>
</tr>
<tr>
<td>He et al. (2009)</td>
<td>Rectum cancer</td>
<td>Unfavorable</td>
<td>240</td>
<td>3.4 (1.2–9.2; LR)</td>
<td>Direct sequencing</td>
<td>(18)</td>
</tr>
<tr>
<td>Kalinsky et al. (2009)</td>
<td>Breast cancer</td>
<td>Favorable</td>
<td>590</td>
<td>0.7 (0.4–1.2; CS)</td>
<td>MassARRAY system</td>
<td>(21)</td>
</tr>
<tr>
<td>Ogino et al. (2009)</td>
<td>Colon cancer</td>
<td>Unfavorable</td>
<td>450</td>
<td>2.23 (1.21–4.11; CS)</td>
<td>Pyrosequencing</td>
<td>(19)</td>
</tr>
<tr>
<td>Kawano et al. (2006)</td>
<td>Lung cancer</td>
<td>Unfavorable</td>
<td>235</td>
<td>N/A</td>
<td>Direct sequencing</td>
<td>(20)</td>
</tr>
<tr>
<td>Current study</td>
<td>ESCC</td>
<td>Favorable</td>
<td>219</td>
<td>Pyrosequencing</td>
<td></td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: CS, cancer-specific survival; DFS, disease-free survival; LR, local recurrence; N/A, not available; OS, overall survival; PFS, progression-free survival; RFS, relapse-free survival.
I, 58.9% for stage II, and 39.8% for stage III), certainly supporting the absence of bias in our database. Written informed consent was obtained from each subject, and the study procedures were approved by the Institutional Review Board. The term "prognostic marker" is used throughout this article according to the reporting recommendations for tumor marker prognostic studies (REMARK) guidelines (23).

Cell lines

The TE-1, TE-4, TE-8, TE-9, TE-11, TE-14, TE-15 (obtained from the Institute of Development, Aging, and Cancer, Tohoku University, Tohuku, Japan), and KYSE30 (obtained from Japanese Collection Research Bioresources) human esophageal cancer cell lines were cultured in a medium supplemented with 10% FBS in 5% CO2 atmosphere at 37°C.

Genomic DNA extraction

Genomic DNA was extracted from 220 paraffin-embedded tissue specimens of surgically resected esophageal cancers. Tumors areas were marked on hematoxylin and eosin–stained slides by one pathologist (Y. Baba). Genomic DNA was extracted from tumor lesions enriched with neoplastic cells, without adjacent normal tissue, using an FFPE Kit (Qiagen). DNA was also extracted from ESCC cell lines using a QI Amp DNA Mini Kit (Qiagen).

Pyrosequencing for PIK3CA mutations

The exon 9 PCR primers were as follows PIK3CA 9-F, 5'-biotin-AACAGCTCAAGAAGCAATTTCTACACG-3; PIK3CA 9-R, 5'-ACCTGTGACTCATAGAACCTTTGTTCCA-3. Each PCR mix contained the forward and reverse primers (10 µmol/L each), 0.2 µmol/L each of dNTP Mix with dUTP, 0.2 U of AmpEraser (Uracil-N glycosylase; Applied Biosystems), 2 mmol/L MnCl2, 1 x PCR buffer, 0.9 U of Amplitaq Gold 360 (Applied Biosystems), and 3.5 µl of template Whole Genome Amplification (WGA) product in a total volume of 35 µL. The exon 20 PCR primers were: PIK3CA 20-F, 5'-biotin-CATCGTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGACTGAC

PIK3CA Mutations in Esophageal Cancer

www.aacrjournals.org Clin Cancer Res; 19(9) May 1, 2013

Published OnlineFirst March 26, 2013; DOI: 10.1158/1078-0432.CCR-12-3559

OF3

Downloaded from clincancerres.aacrjournals.org on November 1, 2017. © 2013 American Association for Cancer Research.
HR according to PIK3CA mutation status, sex (male vs. female), age at surgery (continuous variable), tobacco use (yes vs. no), alcohol use (yes vs. no), year of diagnosis (2005–2008 vs. 2009–2011), preoperative treatment (yes vs. no), and histologic grade (G1 vs. G2–4). A backward stepwise elimination with a threshold of $P = 0.2$ was used to select variables in the final model. For cases with missing information in any of the categorical variables [tobacco use (1.4%) and alcohol use (2.3%)], we included those cases in a majority category of that variable in the initial model. After the selection was complete, we assigned separate missing indicator variables to those cases with missing information in the final model. An interaction was assessed by including the cross product of the PIK3CA variable and another variable of interest (without data-missing cases) in a multivariate Cox model, and thereafter the Wald test was conducted.

Results

PIK3CA mutational status in ESCC

Among 219 patients who had undergone curative resection of stage I to III ESCC, we examined PIK3CA exon 9 and 20 mutations by pyrosequencing technology (Fig. 1). PIK3CA mutations were detected in 46 (21%) of 219 cases; 11 cases in exon 9 only, 21 cases in exon 20 only, and 14 cases in both exon 9 and 20. The most common mutation was the c.3140A > G (p.H1047R) mutation, which was present in 35 tumors, followed by c.1633G > A (p.E545K) in 16 tumors (Supplementary Table S1). All ESCC cell lines (TE-1, TE-4, TE-8, TE-9, TE-11, TE-14, TE-15, and KYSE30) showed wild-type statuses in both exon 9 and 20.

PIK3CA mutations and patients characteristics

PIK3CA mutations were significantly associated with p-AKT expression ($P = 0.0043$). In contrast, there was no
significant relationship between PIK3CA mutations and p53 expression. PIK3CA mutations were not significantly associated with any clinical, epidemiologic, or pathologic characteristics (Table 2).

**PIK3CA mutations and patient survival**

We assessed the influence of PIK3CA mutations on clinical outcome in patients with curatively resected ESCC. During the follow-up of the 219 patients, there were a total of 71 esophageal cancer recurrences, 70 deaths, and 52 deaths confirmed to be attributable to esophageal cancer. The median follow-up time for censored patients was 2.2 years.

In the Kaplan–Meier analysis, patients with PIK3CA mutations experienced significantly longer disease-free survival (log rank \( P = 0.0089 \)), cancer-specific survival (log rank \( P = 0.021 \)), and overall survival (log rank \( P = 0.012 \)) than those with wild-type PIK3CA (Fig. 2). In the univariate Cox regression analysis, compared with patients with wild-type PIK3CA tumors, those patients with PIK3CA-mutated

### Table 2. PIK3CA mutational status in esophageal cancers, clinical features, and tumor features

<table>
<thead>
<tr>
<th>Clinical, epidemiologic, or pathologic feature</th>
<th>Total, N</th>
<th>Mutant</th>
<th>Wild-type</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>219</td>
<td>46</td>
<td>173</td>
<td>0.10</td>
</tr>
<tr>
<td>Mean age ± SD</td>
<td>66.9 ± 8.8</td>
<td>64.7 ± 9.2</td>
<td>67.5 ± 8.7</td>
<td>0.10</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Male</td>
<td>193 (88%)</td>
<td>39 (85%)</td>
<td>154 (89%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>26 (12%)</td>
<td>7 (15%)</td>
<td>19 (11%)</td>
<td></td>
</tr>
<tr>
<td>Tobacco use</td>
<td></td>
<td></td>
<td></td>
<td>0.72</td>
</tr>
<tr>
<td>Yes</td>
<td>160 (74%)</td>
<td>35 (76%)</td>
<td>35 (74%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>56 (26%)</td>
<td>11 (24%)</td>
<td>11 (26%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td></td>
<td>0.65</td>
</tr>
<tr>
<td>Yes</td>
<td>177 (83%)</td>
<td>37 (80%)</td>
<td>140 (84%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>35 (17%)</td>
<td>9 (20%)</td>
<td>26 (16%)</td>
<td></td>
</tr>
<tr>
<td>Year of operation</td>
<td></td>
<td></td>
<td></td>
<td>0.83</td>
</tr>
<tr>
<td>2005–2008</td>
<td>116 (53%)</td>
<td>25 (54%)</td>
<td>91 (53%)</td>
<td></td>
</tr>
<tr>
<td>2009–2011</td>
<td>103 (47%)</td>
<td>21 (46%)</td>
<td>82 (47%)</td>
<td></td>
</tr>
<tr>
<td>Preoperative treatment</td>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Present</td>
<td>82 (37%)</td>
<td>15 (33%)</td>
<td>67 (39%)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>137 (63%)</td>
<td>31 (67%)</td>
<td>106 (61%)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
<td>0.22</td>
</tr>
<tr>
<td>IA</td>
<td>29 (13%)</td>
<td>9 (20%)</td>
<td>20 (12%)</td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>46 (21%)</td>
<td>11 (24%)</td>
<td>35 (16%)</td>
<td></td>
</tr>
<tr>
<td>IIA</td>
<td>18 (8.2%)</td>
<td>6 (13%)</td>
<td>12 (6.9%)</td>
<td></td>
</tr>
<tr>
<td>IIB</td>
<td>45 (21%)</td>
<td>7 (15%)</td>
<td>38 (22%)</td>
<td></td>
</tr>
<tr>
<td>IIIA</td>
<td>33 (15%)</td>
<td>7 (15%)</td>
<td>26 (15%)</td>
<td></td>
</tr>
<tr>
<td>IIIB</td>
<td>19 (8.7%)</td>
<td>1 (2.2%)</td>
<td>18 (10%)</td>
<td></td>
</tr>
<tr>
<td>IIIC</td>
<td>29 (13%)</td>
<td>5 (11%)</td>
<td>24 (14%)</td>
<td></td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>G1</td>
<td>80 (37%)</td>
<td>16 (35%)</td>
<td>64 (37%)</td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>109 (50%)</td>
<td>26 (57%)</td>
<td>83 (48%)</td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>25 (11%)</td>
<td>3 (6.5%)</td>
<td>22 (13%)</td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>5 (2.3%)</td>
<td>1 (2.2%)</td>
<td>4 (2.3%)</td>
<td></td>
</tr>
<tr>
<td>Molecular feature</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP53 expression</td>
<td></td>
<td></td>
<td></td>
<td>0.064</td>
</tr>
<tr>
<td>Positive</td>
<td>117 (53%)</td>
<td>19 (41%)</td>
<td>98 (57%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>102 (47%)</td>
<td>27 (59%)</td>
<td>75 (43%)</td>
<td></td>
</tr>
<tr>
<td>p-AKT expression</td>
<td></td>
<td></td>
<td></td>
<td>0.0043</td>
</tr>
<tr>
<td>Positive</td>
<td>79 (36%)</td>
<td>25 (54%)</td>
<td>54 (31%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>140 (64%)</td>
<td>21 (46%)</td>
<td>119 (69%)</td>
<td></td>
</tr>
</tbody>
</table>

NOTE: “%” indicates the proportion of cases with a specific clinical, pathologic, epidemiologic, or molecular feature among each group (wild-type or mutant).
tumors experienced significant improvement in the disease-
recurrence rate [HR: 0.37; 95% confidence interval (CI): 0.15–0.75; \( P = 0.0042 \)], in cancer-specific mortality [HR: 0.35; 95% CI: 0.12–0.81; \( P = 0.011 \)], and in overall mortality (HR: 0.38; 95% CI: 0.16–0.78; \( P = 0.0060 \); Table 3). In the multivariate Cox model adjusted for the clinical, epidemiologic, and pathologic features, PIK3CA mutations were found to be associated with an improvement in the disease-recurrence rate (multivariate HR: 0.34; 95% CI: 0.10–0.86; \( P = 0.021 \)), cancer-specific survival (multivariate HR: 0.33; 95% CI: 0.075–1.02; \( P = 0.054 \)), and overall survival (multivariate HR: 0.35; 95% CI: 0.10–0.90; \( P = 0.028 \); Table 3). Other features (sex, age at surgery, tobacco use, alcohol use, year of diagnosis, and histologic grade) were not significantly associated with patient survival in the multivariate analyses.

We also assessed PIK3CA exon 9 mutations and exon 20 mutations separately. However, there was no significant difference in mortality between exon 9 and exon 20 mutations (log-rank \( P = 0.59 \)). In addition, patients with concomitant PIK3CA mutations in exons 9 and 20 experienced a similar prognosis with those with PIK3CA mutation in either exon 9 or 20 alone (log rank \( P = 0.98 \); Supplementary Fig. S2).

Survival analyses of the interaction between PIK3CA mutations and other variables
We also examined whether the influence of PIK3CA mutations on cancer-specific survival was modified by any of the clinical, pathologic, and epidemiologic variables evaluated. The effect of PIK3CA mutations was not significantly modified by tobacco use, alcohol use, year of diagnosis, tumor grade, or tumor stage (all \( P > 0.29 \); Fig. 3).

Survival analyses for patients with or without preoperative therapy
The relationship between preoperative chemotherapy and/or radiotherapy and PIK3CA mutation is not known. Therefore, we conducted the additional survival analyses for 2 subgroups; one is a cohort without preoperative therapy \((n = 137)\) and another is a cohort with preoperative treatment \((n = 82)\). Patients with PIK3CA mutations experienced better disease-free survival compared with wild-type PIK3CA cases both in cohort without preoperative treatment (log rank \( P = 0.80 \); univariate HR: 0.41, 95% CI: 0.12–1.03; \( P = 0.058 \)) and cohort with preoperative treatment (log rank \( P = 0.64 \); univariate HR: 0.34, 95% CI: 0.083–0.96; \( P = 0.041 \); Supplementary Fig. S3). Notably, we did not observe a modifying effect of the preoperative treatment on the relationship between PIK3CA mutation and the recurrence rate (\( P \) for interaction = 0.76, Fig. 3).

Discussion
PIK3CA mutations and subsequent activation of the AKT pathway are considered to play a crucial role in human neoplasms (24–26). The PI3K/AKT pathway has emerged as a central node in cancer cell signaling pathways downstream of growth factors, cytokines, and other cellular stimuli (7, 8). We conducted this study to examine the prognostic impact of PIK3CA mutations among 219 patients with curatively resected ESCCs. We found that PIK3CA mutations in ESCCs are associated with a good prognosis, suggesting that PIK3CA mutational status may be a biomarker that can be used to identify patients who will experience a favorable clinical outcome.

Examining biomarkers or prognostic factors is important in cancer research (27–31). Previous studies examining the relationship between PIK3CA mutations and prognosis in human cancers have yielded variable results (Table 1; all \( n \geq 200 \)). In addition, the relationship
between PIK3CA mutations and a better prognosis has been shown in several small-sized studies (all \( n < 200 \)) of breast cancer and ovarian cancer (32–35). This discrepancy might be due to differences in the tumor histologic type. A study of ESCC showed that PIK3CA mutational status is not associated with patient survival; however, this study was limited by its small sample size (\( n = 87 \); ref. 11). The importance of large-scale studies cannot be emphasized enough, because small studies with null results have much higher likelihood of being unpublished than small studies with “significant” results, leading to publication bias. In contrast to the previous study, our study examined PIK3CA mutational status in a much larger nonbiased cohort of ESCCs. Nonetheless, our finding of the correlation between PIK3CA mutations and favorable prognosis in esophageal cancer needs to be confirmed by independent studies in the future.

We also found the significant relationship between PIK3CA mutation and p-AKT expression (i.e., AKT activation) in ESCCs. This finding is in agreement with previous results on colorectal cancer, bladder cancer, and pancreas neoplasms (36–38). There was no perfect correlation between PIK3CA mutation and p-AKT, in part because there were other mechanisms of AKT activation (i.e., PTEN loss and/or methylation, PIK3CA amplification, and PIK3RI mutation). Given the well-known roles of the AKT pathway in tumor proliferation, invasion, and survival (8, 39–41), one could expect that PIK3CA mutations would imply poor clinical outcome. It is very common to assume that the presence of oncogene activation or tumor suppressor inactivation implies aggressive tumor behavior. However, this presumption does not always hold true. Esophageal cancers develop through the accumulation of multiple genetic and/or epigenetic alterations, and each tumor has its own unique combination of these molecular aberrations. Tumors harboring mutant PIK3CA may activate the AKT pathway, whereas tumors harboring wild-type PIK3CA may not. To acquire malignant characteristics, wild-type PIK3CA tumors require alternative molecular aberrations to the AKT pathway. These alternative aberrations may lead to more aggressive phenotypes that the AKT pathway actually does.

Another possible explanation for the relationship between PIK3CA mutations and good prognosis may be due to “tumor suppressive” roles of the AKT pathway (42). In an experimental study using primary esophageal epithelial cells, inducible activation of AKT results in growth arrest and a senescent phenotype in normal epithelial cells (43). That AKT activation induces growth arrest is reminiscent of the effects of oncogenic Ras in primary human cells. Indeed, as with oncogenic Ras, activation of AKT in primary cells may represent an antitumorigenic effect (44, 45). In addition, one study on colorectal cancer reported that p-AKT expression is associated with low-stage cancer and good prognosis (36). Future studies are necessary to elucidate the biologic mechanisms by which PIK3CA mutation activation affects esophageal cancer behavior.

Interestingly, we detected PIK3CA mutations in 21% of ESCCs. This frequency is slightly higher compared with those of previous studies on ESCCs. This difference might be due to a difference in the patient cohorts or the methods used to assess PIK3CA mutation. In the current study, we

### Table 3. PIK3CA status in esophageal cancer and patient mortality

<table>
<thead>
<tr>
<th>PIK3CA Status</th>
<th>Total, ( N )</th>
<th>Disease-free survival</th>
<th>Cancer-specific survival</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>173</td>
<td>1 (referent)</td>
<td>1 (referent)</td>
<td>1 (referent)</td>
</tr>
<tr>
<td>Mutant</td>
<td>46</td>
<td>0.37 (0.15–0.75)</td>
<td>0.35 (0.12–0.81)</td>
<td>0.38 (0.16–0.78)</td>
</tr>
<tr>
<td>( P )</td>
<td></td>
<td>0.0042</td>
<td>0.011</td>
<td>0.0060</td>
</tr>
</tbody>
</table>

Figure 3. Stratified analysis of PIK3CA mutation and cancer-specific mortality. Log (adjusted HR) and 95% CI for PIK3CA–mutated tumors (vs. PIK3CA wild-type tumors) in various strata are shown.
used pyrosequencing, which has been shown to be more sensitive than regular Sanger sequencing in KRAS mutation analysis (46). Pyrosequencing is a nonelectrophoretic nucleotide extension sequencing technology that can be used for mutation detection in tumors. Pyrosequencing assay for PIK3CA mutation detection is certainly useful, because most activating PIK3CA mutations cluster in the hotspots of exons 9 and 20, affecting the functionally important helical and kinase domains.

In summary, our large cohort study suggests that PIK3CA mutations are associated with a favorable clinical outcome in stage I to III esophageal cancer, supporting its role as a prognostic biomarker. Future studies are needed to confirm this association, and to elucidate the exact mechanisms by which PIK3CA mutation affects tumor behavior.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References

Authors’ Contributions
Conception and design: H. Shigaki, Y. Baba, H. Baba
Development of methodology: H. Shigaki, K. Nosho
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Shigaki, Y. Baba, A. Murata, S. Iwagami, H. Baba
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Shigaki, Y. Baba, M. Watanabe, H. Baba
Writing, review, and/or revision of the manuscript: H. Shigaki, Y. Baba, M. Watanabe, H. Baba
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Shigaki
Study supervision: H. Shigaki, M. Watanabe, T. Ishimoto, M. Iwatsuki, H. Baba

Grant Support
This work was supported in part by the Japan Society for the Promotion of Science Grant-in-Aid for Scientific Research, grant number 23591941.

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 November 17, 2012; revised February 10, 2013; accepted March 3, 2013, published OnlineFirst March 26, 2013.


PIK3CA Mutation Is Associated with a Favorable Prognosis among Patients with Curatively Resected Esophageal Squamous Cell Carcinoma

Hironobu Shigaki, Yoshifumi Baba, Masayuki Watanabe, et al.

Clin Cancer Res Published OnlineFirst March 26, 2013.

Updated version Access the most recent version of this article at: doi:10.1158/1078-0432.CCR-12-3559

Supplementary Material Access the most recent supplemental material at: http://clincancerres.aacrjournals.org/content/suppl/2013/04/05/1078-0432.CCR-12-3559.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, contact the AACR Publications Department at permissions@aacr.org.