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

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**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): 2451–9. ©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 PI3K/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).

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>Fariña et al. (2011)</td>
<td>Colon cancer</td>
<td>Unfavorable</td>
<td>685</td>
<td>2.68 (1.24–5.77; OS)</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>3.30 (1.46–7.45; OS)</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></td>
<td>Pyrosequencing</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.
l, 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, Tohoku, 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 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 QiAmp DNA Mini Kit (Qiagen).

Pyrosequencing for PIK3CA mutations
The exon 9 PCR primers were as follows PIK3CA 9-F, 5'-biotin-AACAGGTCAAGAAATCTTCTACG-3'; PIK3CA 9-R, 5’-ACCTGTGACCTCATAGAAAATCTTT-3'. Each PCR mix contained the forward and reverse primers (10 μmol/L each), 0.2 mmol/L each of dNTP Mix with dUTP, 0.2 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-C, 5'-biotin-TACTGCTTCTTGCTCTCGC-3’; and PIK3CA 20-R, 5'-GAATCTCTTTCTTGTCTTCA-3’. Each PCR mix contained the same components described for the exon 9 PCR reactions except 2.5 μL of template WGA product was used in a total volume of 25 μL.

PCR conditions consisted of initial denaturing at 96°C (10 minutes) for AmpErase UNG; initial denaturing at 94°C (10 minutes) for AmpliTaq Gold 360; 50 cycles of 95°C (30 seconds) for annealing (30 seconds; 60°C for PIK3CA exon 9, 57°C for PIK3CA exon 20) (30 seconds); and final extension at 72°C (7 minutes). The PCR products were electrophoresed on agarose gels to confirm successful amplification of the 81 (exon 9) and 74 bp (exon 20) products.

PIK3CA pyrosequencing was carried out using the PyroMark Q24 System (Qiagen) according to the manufacturer’s instructions. All forward sequencing results were confirmed by reverse sequencing. In the PIK3CA exon 9 pyrosequencing assay, we routinely confirmed the presence of a mutation by 3 different sequencing primers and by the creation of frameshifted reading of a mutant sequence relative to a wild-type sequence in a program. Representative pyrograms of wild-type and mutant exon 9 and 20 are shown in Fig. 1. The primer PIK3CA 9-RS1 (5’-CCATAGAAAATCTTCTAC-3’; nucleotide dispensation order, ATCGACTACGTGACGTGACTGACG; nucleotide dispensation order, ATACACATGCATGACGTGACTGACG) could detect the c.1634A>G mutation. The primer PIK3CA 9-RS2 (5’-TCTCCCTGCTCCTAGTTT-3’; nucleotide dispensation order, ATAGCGACTACGTGACGTGACTGACG) could detect the most common mutation (c.1624G>A). For PIK3CA exon 20, we used the primer PIK3CA 20-RS (5’-GGTTCCAGGCCACCA-3’; nucleotide dispensation order, CGTACGATCTGTGCTCATATGCATGCTAGCGATG) to detect various exon 20 mutations (c.3140A>G, c.3139C>T). Nucleotide dispensation orders were designed so that if any of the common mutations were present, it caused a shift in the reading frame and resulted in additional new peak or peaks (indicated by arrowheads in Fig. 1) following the mutated nucleotide.

Immunohistochemistry
For p53 staining, we used the anti-p53 antibody [Mouse monoclonal anti-p53 (PAb1801 Ab-2), Santa Cruz Biotechnology]. The second antibody used was a ready-for-use antimouse EnVision Peroxidase system (Dako Japan Inc.). For p-AKT staining, we used the pan-AKT antibody [Rabbit monoclonal anti-Phospho-Akt (Ser473; 736E11), Cell Signaling Technology] and a subsequent reaction was carried out with the Vectastain ABC Elite avidin/biotin/peroxidase kit (Vector Laboratories Inc.). In each case, we recorded nuclear p53 expression and cytoplasmic p-AKT expression as no expression, weak expression, moderate expression, or strong expression compared with normal esophageal epithelial cells. The positivity of p53 and p-AKT was defined as the presence of weak to strong expression. Among 219 esophageal cancers, we observed p53 expression in 117 tumors (53%) and p-AKT expression in 79 tumors (36%); Supplementary Fig. S1).

Statistical methods
For the statistical analyses, we used the JMP (Version 9, SAS Institute) and the SAS software programs (Version 9.1, SAS Institute). All P values were 2-sided. For the survival analysis, the Kaplan–Meier method was used to assess the survival time distribution, and the log-rank test was used. To assess the independent effect of the PIK3CA mutations on mortality, the tumor stage (IA+IB, IIA+IIB, IIA+IIB+III) was used as a stratifying (matching) variable in Cox models using the “strata” option in the SAS “proc phreg” command to avoid residual confounding and overfitting. We constructed a multivariate, stage-stratified Cox proportional hazard model to compute a
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, 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

Figure 1. PIK3CA exon 9 and exon 20 pyrograms (antisense strand). A, wild-type exon 9 sequenced with the 9-RS1 primer. B, The c.1634A > G mutation (arrow) causes a shift in the reading frame and results in a new peak at A (arrowhead), which serves as a quality assurance. C, wild-type exon 9 sequenced with the 9-RS2 primer. D, the c.1624G > A mutation (arrow) causes a shift in the reading frame and results in a new peak at A (arrowhead). E, wild-type exon 9 sequenced with the 9-RS3 primer. F, the c.1633G > A mutation (arrow) causes a shift in the reading frame and results in new peaks (arrowheads). G, wild-type exon 20. H, the c.3139C > T mutation (arrow) causes a shift in the reading frame and results in a new peak at T (arrowhead). I, the c.3140A > G mutation (arrow) causes a shift in the reading frame and results in a new peak at G (arrowhead). Mut, mutant; WT, wild-type.
significant relationship between PIK3CA mutations and p53 expression. PIK3CA mutations were not significantly associated with any clinical, epidemiologic, or pathologic characteristics (Table 2).

**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></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></td>
</tr>
<tr>
<td>Male (88%)</td>
<td>193</td>
<td>39 (85%)</td>
<td>154 (89%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Female (12%)</td>
<td>26</td>
<td>7 (15%)</td>
<td>19 (11%)</td>
<td></td>
</tr>
<tr>
<td>Tobacco use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (74%)</td>
<td>160</td>
<td>35 (76%)</td>
<td>35 (74%)</td>
<td>0.72</td>
</tr>
<tr>
<td>No (26%)</td>
<td>56</td>
<td>11 (24%)</td>
<td>11 (26%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (83%)</td>
<td>177</td>
<td>37 (80%)</td>
<td>140 (84%)</td>
<td>0.65</td>
</tr>
<tr>
<td>No (17%)</td>
<td>35</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></td>
</tr>
<tr>
<td>2005–2008 (53%)</td>
<td>116</td>
<td>25 (54%)</td>
<td>91 (53%)</td>
<td>0.83</td>
</tr>
<tr>
<td>2009–2011 (47%)</td>
<td>103</td>
<td>21 (46%)</td>
<td>82 (47%)</td>
<td></td>
</tr>
<tr>
<td>Preoperative treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present (37%)</td>
<td>82</td>
<td>15 (33%)</td>
<td>67 (39%)</td>
<td>0.44</td>
</tr>
<tr>
<td>Absent (63%)</td>
<td>137</td>
<td>31 (67%)</td>
<td>106 (61%)</td>
<td></td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IA (13%)</td>
<td>29</td>
<td>9 (20%)</td>
<td>20 (12%)</td>
<td>0.22</td>
</tr>
<tr>
<td>IB (21%)</td>
<td>46</td>
<td>11 (24%)</td>
<td>35 (16%)</td>
<td></td>
</tr>
<tr>
<td>IIA (8.2%)</td>
<td>18</td>
<td>6 (13%)</td>
<td>12 (6.9%)</td>
<td></td>
</tr>
<tr>
<td>IIB (21%)</td>
<td>45</td>
<td>7 (15%)</td>
<td>38 (22%)</td>
<td></td>
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<tr>
<td>IIIA (15%)</td>
<td>33</td>
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<tr>
<td>IIIB (8.7%)</td>
<td>19</td>
<td>1 (2.2%)</td>
<td>18 (10%)</td>
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<tr>
<td>IIIC (13%)</td>
<td>29</td>
<td>5 (11%)</td>
<td>24 (14%)</td>
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<td>Histologic grade</td>
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<td></td>
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<tr>
<td>G1 (37%)</td>
<td>80</td>
<td>16 (35%)</td>
<td>64 (37%)</td>
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<tr>
<td>G2 (50%)</td>
<td>109</td>
<td>26 (57%)</td>
<td>83 (48%)</td>
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<tr>
<td>G3 (11%)</td>
<td>25</td>
<td>3 (6.5%)</td>
<td>22 (13%)</td>
<td></td>
</tr>
<tr>
<td>G4 (2.3%)</td>
<td>5</td>
<td>1 (2.2%)</td>
<td>4 (2.3%)</td>
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</tr>
<tr>
<td>Molecular feature</td>
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<tr>
<td>TP53 expression</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Positive (53%)</td>
<td>117</td>
<td>19 (41%)</td>
<td>98 (57%)</td>
<td>0.064</td>
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<tr>
<td>Negative (47%)</td>
<td>102</td>
<td>27 (59%)</td>
<td>75 (43%)</td>
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<tr>
<td>p-AKT expression</td>
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</tr>
<tr>
<td>Positive (36%)</td>
<td>79</td>
<td>25 (54%)</td>
<td>54 (31%)</td>
<td>0.0043</td>
</tr>
<tr>
<td>Negative (64%)</td>
<td>140</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).

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
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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 \((n \equiv 137)\) and another is a cohort with preoperative treatment \((n \equiv 82)\). Patients with PIK3CA mutations experienced better disease-free survival compared with wild-type PIK3CA cases both in cohort without preoperative treatment (log rank \( P = 0.080 \); univariate HR: 0.41, 95% CI: 0.12–1.03; \( P = 0.058 \)) and cohort with preoperative treatment (log rank \( P = 0.064 \); 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 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 \((n \equiv 137)\) and another is a cohort with preoperative treatment \((n \equiv 82)\). Patients with PIK3CA mutations experienced better disease-free survival compared with wild-type PIK3CA cases both in cohort without preoperative treatment (log rank \( P = 0.080 \); univariate HR: 0.41, 95% CI: 0.12–1.03; \( P = 0.058 \)) and cohort with preoperative treatment (log rank \( P = 0.064 \); 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
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 \( \text{PIK3CA} \) 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 \( \text{PIK3CA} \) mutational status in a much larger nonbiased cohort of ESCCs. Nonetheless, our finding of the correlation between \( \text{PIK3CA} \) mutations and favorable prognosis in esophageal cancer needs to be confirmed by independent studies in the future.

We also found the significant relationship between \( \text{PIK3CA} \) 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 \( \text{PIK3CA} \) mutation and p-AKT, in part because there were other mechanisms of AKT activation (i.e., \( \text{PTEN} \) loss and/or methylation, \( \text{PIK3CA} \) amplification, and \( \text{PIK3RI} \) mutation). Given the well-known roles of the AKT pathway in tumor proliferation, invasion, and survival (8, 39–41), one could expect that \( \text{PIK3CA} \) 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 \( \text{PIK3CA} \) may activate the AKT pathway, whereas tumors harboring wild-type \( \text{PIK3CA} \) may not. To acquire malignant characteristics, wild-type \( \text{PIK3CA} \) 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 \( \text{PIK3CA} \) 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 \( \text{Ras} \) in primary human cells. Indeed, as with oncogenic \( \text{Ras} \), 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 \( \text{PIK3CA} \) mutation activation affects esophageal cancer behavior.

Interestingly, we detected \( \text{PIK3CA} \) 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 \( \text{PIK3CA} \) mutation. In the current study, we...
used pyrosequencing, which has been shown to be more sensitive than regular Sanger sequencing in KRAS mutation analysis (46). Pyrosequencing is a non-electrophoretic 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

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