Clinicopathologic Analysis of Breast Cancers with PIK3CA Mutations in Japanese Women

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Abstract

Purpose: Somatic mutations of PIK3CA, which encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase, have recently been shown to play an important role in the pathogenesis and progression of human breast cancers. In this study, the frequency of PIK3CA mutations and their relationship with clinicopathologic and biological variables were investigated in Japanese breast cancers.

Experimental Design: Mutational analysis of PIK3CA was done in 188 primary breast cancers of Japanese women. Relationship of these mutations with various clinicopathologic variables [histologic type, tumor size, histologic grade, lymph node status, estrogen receptor (ER)-α and progesterone receptor status, and prognosis], biological variables [phospho-AKT (pAKT) and HER2 expression determined by immunohistochemistry], and p53 mutation status was studied.

Results: Missense mutations of PIK3CA were found in 44 of 158 invasive ductal carcinomas, 4 of 10 invasive lobular carcinomas, 1 of 4 mucinous carcinomas, 2 of 2 squamous carcinomas, and 2 of 2 apocrine carcinomas, but no mutation was found in 12 noninvasive ductal carcinomas. PIK3CA-mutated tumors were found to be more likely to be ER-α positive (P < 0.05) and pAKT positive (P < 0.05). There was no significant association between PIK3CA mutations and p53 mutation status. PIK3CA mutations were significantly (P < 0.05) associated with a favorable prognosis, and multivariate analysis showed that PIK3CA mutation status was a significant (P < 0.05) prognostic factor independent of the other conventional prognostic factors.

Conclusions: The frequency of PIK3CA mutations in Japanese breast cancers is similar to that of Caucasian breast cancers. Association of PIK3CA mutations with positive pAKT and positive ER-α suggests that PIK3CA mutations might exert their effects through activation of the phosphatidylinositol 3-kinase/AKT/ER-α pathway. PIK3CA mutations seem to have a potential to be used as an indicator of favorable prognosis.

Phosphatidylinositol 3-kinase (PI3K) is an activator of AKT, which regulates many cellular processes implicated in tumorigenesis such as cell growth, cell survival, and cell migration (1–3). Actually, AKT has been shown to be frequently activated in various types of human tumors including breast cancers (4, 5), suggesting that the PI3K/AKT pathway plays an important role in the pathogenesis and progression of human breast cancers.

PI3K consists of heterodimers with catalytic subunits (p110α, p110β, or p110δ) and regulatory subunits (p85α, p85β, or p55γ; ref. 6). The catalytic subunits are composed of several modular domains: catalytic lipid kinase domain, helical domain, C2 domain, Ras-binding domain, and the NH2-terminal domain that interacts with the regulatory subunits. It is well established that PI3K is activated by autocrine or paracrine stimulation of receptor tyrosine kinases. Recently, in addition to this mechanism, somatic mutations of PIK3CA, which encodes the p110α catalytic subunit, have been shown to play an important role in the activation of PI3K in various human cancers (7–10). A high frequency of PIK3CA mutations has been reported in colorectal cancers, ovarian cancers, lung cancers, and breast cancers (7, 11–20). A great majority of somatic mutations in PIK3CA are missense mutations clustering in exons 9 and 20, which encode a part of the helical and kinase domains, respectively (7, 11, 13). In vitro studies have shown that the most frequently observed PIK3CA mutations in human breast cancers [i.e., E545K (exon 9) and H1047R (exon 20)] are associated with an increased kinase activity (8, 9), indicating that the PIK3CA mutations actually activate the PI3K pathway and thus are thought to be implicated in the pathogenesis and progression of breast cancers.

PIK3CA mutations, because they are found in 20% to 40% of breast cancers (11–14, 17), are considered to be one of the...
most commonly observed genetic changes besides p53 mutations and HER2 amplification. Although many reports have been available on the clinicopathologic characteristics of breast cancers with p53 mutations or HER2 amplification (21, 22), only a few reports have been available thus far on the clinicopathologic characteristics of breast cancers with PIK3CA mutations (11, 23). Elucidation of the characteristics of breast cancers with PIK3CA mutations seems to be important for the execution of personalized medicine in future. In addition, all the studies reported until now on PIK3CA mutations dealt with Caucasian breast cancers. It seems to be interesting to compare the frequency of PIK3CA mutations between Japanese and Caucasian breast cancers because breast cancer incidence in Japanese women is much lower (one fourth) than that of Caucasian women and, thus, contribution of PIK3CA mutations to pathogenesis of breast cancers might be different between two ethnicities. Therefore, in the present study, we have analyzed somatic mutations of PIK3CA in Japanese breast cancers as well as their relationship with the various clinicopathologic variables including patient prognosis. To further characterize breast cancers with PIK3CA mutations, correlation of PIK3CA mutations with phospho-AKT (pAKT) expression, HER2 overexpression, or p53 mutation status has also been studied.

Materials and Methods

Patients and surgical specimens. Tumor tissue samples were obtained from 188 primary breast cancer patients who underwent mastectomy or breast conserving surgery during the period from March 1998 to October 2002 at Osaka University Hospital. Tumor tissue samples were obtained from the surgical specimens and snap frozen in liquid nitrogen and kept at −80°C until use. Informed consent was obtained from each patient before surgery.

As adjuvant chemotherapy (Table 1), six cycles of CMF (cyclophosphamide 100 mg/d orally days 1-14 + methotrexate 40 mg/m² i.v. days 1 and 8 + 5-fluorouracil 600 mg/m² i.v. days 1 and 8 q4w) were given to 15 patients, four cycles of EC (epirubicin 60 mg/m² i.v. day 1 + cyclophosphamide 600 mg/m² i.v. day 1 q3w) were given to 12 patients, and four cycles of docetaxel 60 mg/m² i.v. day 1 q3w were given to 4 patients. One hundred patients were treated with adjuvant hormonal therapy [tamoxifen 20 mg/d (n = 77), tamoxifen 20 mg/d + goserelin 3.6 mg q4w (n = 23)]. Forty-seven patients were treated with combination of chemotherapy [CMF (n = 21), EC (n = 21), or other chemotherapies (n = 5)] and hormonal therapy [tamoxifen (n = 44), goserelin (n = 1), or tamoxifen + goserelin (n = 2)]. Ten patients received no adjuvant therapy. Duration of tamoxifen treatment was 5 years and that of goserelin treatment was 2 years in most cases. Indication for adjuvant treatment was decided essentially according to the St. Gallen recommendation (24).

Physical examination every 3 months for 2 years postoperatively and every 6 months thereafter, combined with blood test and chest X-ray examination every 6 months postoperatively, was done. The median follow-up period of these 188 patients was 64 months, ranging from 38 to 88 months. Forty-five patients developed recurrences (i.e., 16 developed bone metastases, 9 developed liver metastases, 5 developed brain metastases, 6 developed lung metastases, 4 developed soft tissue metastases, and 12 developed lymph node metastases). Ipsilateral breast recurrences after breast conserving surgery were not counted as recurrences.

Mutational analysis of PIK3CA. PCR amplification was done with the primers previously described for exons 1, 2, 4, 7, 9, 13, 18, and 20 of PIK3CA (7). Sequencing of the PCR products was done using an ABI 3300 automated capillary sequencer. We obtained the sequence data of PIK3CA gene from GenBank (accession no. NM_006218). Genomic DNA from corresponding normal tissue was subjected to sequence analysis to confirm that the nucleotide substitutions detected in tumor tissues are somatic in nature as for samples when nucleotide changes were detected in tumor tissues.

Immunohistochemistry of pAKT, HER2, and phospho-S6 expression. The expression of pAKT, HER2, and phospho-S6 (pS6) was studied by immunohistochemistry. In brief, for pAKT and pS6, endogenous peroxidases were quenched by incubating the sections for 10 min in 6% H₂O₂. After several washes in PBS-T, antigen retrieval was done by heating the samples in 10 mmol/L citrate buffer (pH 6.0) at 95°C for 40 min. After blocking serum (DAKO Diagnostics, Mississauga, Ontario, Canada) for 30 min, the samples were incubated with a polyclonal rabbit anti–pAKT (Ser473) antibody (1:100 dilution; Cell Signaling Technologies, Beverly, MA) or with a polyclonal anti–phospho-S6 ribosomal protein (1:50 dilution; Cell Signaling Technologies) at 4°C overnight. We then used the LSAB+ System (DAKO), which involved incubation with streptavidin treatment followed by secondary antibody for signal amplification (pAKT), or an avidin-biotin method (pS6). The positive reaction of pAKT was scored into four grades according to the intensity of the staining (0, none; 1+, weakly positive; 2+, moderately positive; and 3+, strongly positive) according to the method previously reported (25, 26). 0 and 1+ recorded as negative and 2+ and 3+ recorded as positive (Fig. 1). As for the cutoff level of pS6, we have scored both staining intensity and the percent of positive cells according to Allison scoring (27). The proportion score varies from 0 to 5 [0 (none or negative), 1 (<1/100), 2 (1/100-1/10), 3 (1/10-1/3), 4 (1/3-2/3), and 5 (>2/3)] and intensity score is the average intensity of all the positive cells (0, negative; 1, weak; 2, intermediate; and 3, strong). We classified positive when the total score that was obtained by summing proportion score and intensity score was ≥3. HER2 score was determined according to the DAKO system scale (DAKO Diagnostics, Vienna, Austria): HER2 negative (0 and 1+) and HER2 positive (2+ and 3+).

Frequency and location of PIK3CA mutations. For identify genomic abnormalities of p53, each exon-intron junction from exon 5 to exon 8 was screened using PCR-single-strand conformation polymorphism method or direct sequencing, following the method previously described (28). Nucleotide alterations detected by single-strand conformation polymorphism were determined by sequencing analysis.

Results

Frequency and location of PIK3CA mutations. Mutational analysis of PIK3CA was done in 188 primary breast cancers and, finally, 54 missense mutations were identified in total (Fig. 2). Because all the mutations were not detected in the corresponding normal tissues, these mutations were confirmed as somatic mutations. Of these 54 mutations, 17 and 29 mutations clustered in exon 9 and exon 20, respectively.
to histologic types is shown in Table 2. PIK3CA mutations were found in 44 of 158 (28%) invasive ductal carcinomas, 4 of 10 (40%) invasive lobular carcinomas, 1 of 4 (25%) mucinous carcinomas, 2 of 2 (100%) squamous carcinomas, and 2 of 2 (100%) apocrine carcinomas, but no mutation was found in 12 noninvasive ductal carcinomas.

The relationship of PIK3CA mutations with clinicopathologic variables is shown in Table 3. The frequency of PIK3CA mutations in ER-positive tumors (34%) was significantly ($P < 0.05$) higher than that in ER-negative tumors (19%), and the frequency of PIK3CA mutations in PR-positive tumors (33%) tended ($P = 0.09$) to be higher than that in PR-negative tumors (22%). PIK3CA mutation status was not significantly associated with menopausal status, tumor size, lymph node status, or histologic grade.

**Table 1.** Regimens used in postoperative adjuvant chemotherapy and/or hormonal therapy for breast cancer patients

<table>
<thead>
<tr>
<th>Mutation (+)</th>
<th>Mutation (-)</th>
<th>Total</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemotherapy</td>
<td>6</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>EC (4 cycles)$^*$</td>
<td>3</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>CMF (6 cycles)$^*$</td>
<td>3</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>TXT (4 cycles)$^*$</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Hormonal therapy</td>
<td>31</td>
<td>69</td>
<td>100</td>
</tr>
<tr>
<td>Tamoxifen$^1$</td>
<td>24</td>
<td>53</td>
<td>77</td>
</tr>
<tr>
<td>Chemotherapy + hormonal therapy</td>
<td>16</td>
<td>31</td>
<td>47</td>
</tr>
<tr>
<td>CMF (6 cycles) + tamoxifen</td>
<td>8</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td>EC (4 cycles) + tamoxifen</td>
<td>5</td>
<td>13</td>
<td>18</td>
</tr>
<tr>
<td>TXT + tamoxifen</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>EC (4 cycles) + goserelin</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EC (4 cycles) + tamoxifen + goserelin</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Others + tamoxifen</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>No therapy</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

$^*$Epirubicin 60 mg/m$^2$ i.v. day 1 + cyclophosphamide 600 mg/m$^2$ i.v. day 1 q3w.

$^1$Cyclophosphamide 100 mg/d orally days 1-14 + methotrexate 40 mg/m$^2$ i.v. days 1 and 8 + 5-fluorouracil 600 mg/m$^2$ i.v. days 1 and 8 q4w.

$^2$Docetaxel 60 mg/m$^2$ i.v. day 1 q3w.

$^3$Tamoxifen 20 mg/d.

$^4$Goserelin 3.6 mg q4w.

**Relationship of PIK3CA mutations with pAKT or HER2 expression and p53 mutation status.** Because it is suggested that PIK3CA mutations activate AKT function through its phosphorylation, we investigated the relationship between PIK3CA mutations and expression of pAKT. The frequency of PIK3CA mutations was significantly ($P < 0.05$) higher in pAKT-positive tumors (66%) than pAKT-negative tumors (40%; Table 4). We also studied the relationship between PIK3CA mutations and HER2 expression or p53 mutations (Table 4). HER2 expression and p53 mutations were not significantly associated with PIK3CA mutations.

**Fig. 1.** Representative results of immunohistochemical staining of pAKT, pS6, and PTEN in breast cancer tissues. A, pAKT ($\times$400); B, pS6 ($\times$400).
Relationship of pAKT expression with pS6 expression. To further confirm the downstream activation of PI3K/AKT pathway induced by the PIK3CA mutations, we have investigated pS6, a downstream target molecule of pAKT by immunostaining. As shown in Table 5, the frequency of pS6-positive tumors was significantly ($P < 0.05$) higher in pAKT-positive tumors (76%) than in pAKT-negative tumors (51%).

PIK3CA mutations and patient prognosis. The relationship of PIK3CA mutations with patient prognosis was analyzed in 176 invasive carcinomas. The relapse-free survival rates of patients with PIK3CA mutations were significantly ($P < 0.05$) better than those of patients without them in the total patients (Fig. 3A) as well as in the subset of patients with ER-$\alpha$-positive tumors (Fig. 3B). Univariate analysis showed that PIK3CA mutation status, tumor size, lymph node status, ER-$\alpha$ status, and PR status were significant ($P < 0.05$) prognostic factors, and multivariate analysis showed that PIK3CA mutation status, tumor size, lymph node status, and PR status were significant ($P < 0.05$) and mutually independent prognostic factors (Table 6).

Discussion

In the present study, we have identified PIK3CA mutations in 29% (54 of 188) of Japanese breast cancers including two novel missense mutations (N114T in exon 1 and Y698X in exon 13). Majority [83% (45 of 54)] of the mutations clustered in exon 9 and exon 20, helical and kinase domains, respectively. Not only the frequency but also the location of PIK3CA mutations is quite similar to that reported in Caucasian breast cancers (11). These results indicate that the contribution of PIK3CA mutations to pathogenesis and progression of breast cancers might be similar between two ethnicities.

No PIK3CA mutation was found in 12 noninvasive ductal carcinomas in the present study and Lee et al. reported PIK3CA mutations in only 2 of 15 (13%) noninvasive ductal carcinomas. These mutation frequencies in noninvasive ductal carcinomas seem to be slightly lower than those reported in invasive ductal carcinomas (20-40%). Because AKT stimulates tumor invasion by promoting the secretion of matrix metalloproteinases (29, 30) and the induction of epithelial-mesenchymal transition (31, 32), it is speculated that PIK3CA mutations led to activation of the PI3K/AKT pathway and to further proliferation, invasion, and metastasis.

Table 2. PIK3CA mutations and histologic types of breast cancers

<table>
<thead>
<tr>
<th>Histologic types</th>
<th>PIK3CA mutations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninvasive ductal carcinoma</td>
<td>0 of 12 (0)</td>
</tr>
<tr>
<td>Invasive carcinoma</td>
<td>54 of 176 (31)</td>
</tr>
<tr>
<td>Invasive ductal carcinoma</td>
<td>44 of 158 (28)</td>
</tr>
<tr>
<td>Invasive lobular carcinoma</td>
<td>4 of 10 (40)</td>
</tr>
<tr>
<td>Mucinous carcinoma</td>
<td>1 of 4 (25)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>2 of 2 (100)</td>
</tr>
<tr>
<td>Apocrine carcinoma</td>
<td>2 of 2 (100)</td>
</tr>
</tbody>
</table>

Table 3. PIK3CA mutations and clinicopathologic variables of breast cancers

<table>
<thead>
<tr>
<th>PIK3CA</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation status</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Premenopausal   | 29 (32) | 63 (68) | 92
| Postmenopausal  | 25 (26) | 71 (74) | 96 NS
| Tumor size (cm) |     |      |
| >2              | 19 (31) | 43 (69) | 62
| ≤2              | 35 (31) | 79 (69) | 114 NS
| Lymph node metastasis | | |
| Negative        | 33 (31) | 74 (69) | 107
| Positive        | 21 (31) | 46 (69) | 67 NS
| Unknown         | 2     | 2    |
| Histologic grade* | | |
| 1               | 9 (32) | 19 (68) | 28
| 2               | 37 (33) | 74 (67) | 111 NS
| 3               | 3 (14) | 19 (84) | 22
| Unknown         | 15    | 15   |
| ER              |     |      |
| Positive        | 42 (34) | 82 (66) | 124
| Negative        | 12 (19) | 52 (81) | 64 0.03
| PR              |     |      |
| Positive        | 38 (33) | 76 (67) | 114
| Negative        | 16 (22) | 57 (78) | 73 0.09
| Unknown         | 1     | 1    |

Abbreviation: NS, not significant.
*Noninvasive ductal carcinomas were excluded.
might play a certain role in the progression from noninvasive to invasive ductal carcinomas through the activation of AKT.

The frequency of PIK3CA mutations in breast cancers with histologic types other than ductal carcinomas has rarely been reported (13). Although the number of tumors with histologic types other than invasive ductal carcinomas is small in the present study, we have been able to show that PIK3CA mutations are found in 4 of 10 invasive lobular carcinomas, 1 of 4 mucinous carcinomas, 2 of 2 squamous carcinomas, and 2 of 2 apocrine carcinomas. These results indicate that PIK3CA mutations are implicated in the pathogenesis and progression of not only ductal carcinomas but also other types of breast cancers. Recently, Buttitta et al. (33) have reported that the frequency of PIK3CA mutations is higher in invasive lobular carcinomas than in invasive ductal carcinomas, being consistent with our present observation that the frequency of PIK3CA mutations was 40% in invasive lobular carcinomas and 28% in invasive ductal carcinomas. One characteristic phenotype of invasive lobular carcinomas is its high ER positivity (34, 35). This phenotype of invasive lobular carcinomas seems to be explained, at least in part, by the higher frequency of PIK3CA mutations, which are associated with ER-α-positive breast cancers.

It has been reported that the hotspot mutations in PIK3CA actually enhance the lipid kinase activity as compared with wild type, leading to the increased phosphorylation of AKT and the resultant transformation of normal breast epithelial cells to tumor cells by in vitro and in vivo studies (8, 9). To examine whether the PIK3CA mutations found in the present study actually activate AKT through phosphorylation in human breast cancers, immuno histochemical study using anti-pAKT specific antibody was done. As expected, the frequency of PIK3CA mutations was significantly (P < 0.05) higher in pAKT-positive tumors (66%) than in pAKT-negative tumors (40%), suggesting that activation of AKT through phosphorylation by PIK3CA mutations actually occurs in human breast cancers. Furthermore, to confirm whether the pAKT activates downstream targets, the relationship between pAKT and phosphorylation of S6, one of the target molecules phosphorylated by pAKT signaling, was investigated by immunostaining. The positive correlation between pAKT and pS6 might suggest a downstream activation of this signal transduction induced by PIK3CA mutations.

The PI3K/AKT pathway regulates the various important cell functions implicated in tumorigenesis including cell growth, cell survival, and cell migration. In the present study, we have found that PIK3CA mutations are significantly higher in ER-α-positive tumors than in ER-α-negative tumors. Saal et al. (11) also reported a significant association between PIK3CA mutations and ER-α tumors. A positive association between pAKT and ER-α was also shown by an immunohistochemical study in breast cancers (36). Recently, it has been shown that AKT phosphorylates Ser\(^{167}\) of ER-α and enhances the transcriptional activity of ER-α (37). Thus, it is speculated that, in tumor cells with PIK3CA mutations, the PI3K/AKT/ER-α pathway might be activated, resulting in the preferential growth of ER-α-positive tumors.

Because the effect of PIK3CA mutations on patient prognosis has rarely been studied, we have investigated the prognostic significance of PIK3CA mutations in the present study. PIK3CA mutations activate AKT through phosphorylation and the pAKT expression has been reported to be associated with poor prognosis (38). The reason for such an association is considered to be attributable to resistance of pAKT-positive tumors to adjuvant tamoxifen, being based on the findings that prognosis of pAKT-positive tumors is poorer than that of pAKT-negative tumors in the ER-α-positive group treated with adjuvant tamoxifen but not in that treated without adjuvant tamoxifen. Thus, we assumed that tumors with PIK3CA mutations would be associated with poor prognosis in the present study where almost all patients (93%) with ER-α-positive tumors had been treated with tamoxifen. Until now, only two reports have been available on the relationship between PIK3CA mutations and prognosis. Li et al. (23) reported a significant association of PIK3CA mutations with poor prognosis, but Saal et al. (11) failed to confirm such an association. In the present study, we have obtained an unexpected result that tumors with PIK3CA mutations are significantly associated with a favorable prognosis in the total patients as well as in the subset of patients with ER-α-positive tumors.

Our result that PIK3CA mutations are associated with a favorable prognosis seems to be inconsistent with the fact that pAKT-positive tumors are associated with poor prognosis (38). In PIK3CA-mutated tumors, the PI3K/AKT pathway is probably the principal pathway for carcinogenesis and progression. However, in pAKT-positive tumors, because AKT is activated not only by PIK3CA mutations but also by various growth factors, other pathways (e.g., extracellular signal-regulated kinase/mitogen-activated protein kinase pathway) are very likely to be activated in addition to the PI3K/AKT pathway. Therefore, it is not surprising that the biological behaviors of the PIK3CA-mutated tumors and pAKT-positive tumors are different. Very recently, it has been shown that breast cancer cells with PIK3CA mutations are more likely to respond to tamoxifen than those without them as opposed to the findings that pAKT is associated with a resistance to tamoxifen.

<p>| Table 4. Relationship between PIK3CA mutations and pAKT, HER2 expression, and p53 mutations |
|-----------------------------------------------|----------------|------------|------------|</p>
<table>
<thead>
<tr>
<th>PIK3CA</th>
<th>n</th>
<th>P</th>
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</tr>
</thead>
<tbody>
<tr>
<td>pAKT</td>
<td>Mutant (%)</td>
<td>Wild (%)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>19 (66)</td>
<td>10 (34)</td>
<td>29 0.03</td>
</tr>
<tr>
<td>Negative</td>
<td>19 (40)</td>
<td>28 (60)</td>
<td>47</td>
</tr>
<tr>
<td>HER2</td>
<td>Positive</td>
<td>9 (38)</td>
<td>15 (62)</td>
</tr>
<tr>
<td>Negative</td>
<td>39 (33)</td>
<td>80 (67)</td>
<td>119</td>
</tr>
<tr>
<td>p53</td>
<td>Mutant</td>
<td>9 (27)</td>
<td>24 (73)</td>
</tr>
<tr>
<td>Wild</td>
<td>45 (29)</td>
<td>110 (71)</td>
<td>155</td>
</tr>
<tr>
<td>Table 5. Relationship between pAKT expression and pS6 expression</td>
<td></td>
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<tr>
<td>-----------------------------------------------</td>
<td>----------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>pS6</td>
<td>Positive (%)</td>
<td>Negative (%)</td>
<td>P</td>
</tr>
<tr>
<td>pAKT</td>
<td>Positive</td>
<td>22 (76)</td>
<td>7 (24)</td>
</tr>
<tr>
<td>Negative</td>
<td>24 (51)</td>
<td>23 (49)</td>
<td>47</td>
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</tbody>
</table>
tamoxifen (39). We speculate that growth of tumor cells with PIK3CA mutations is highly dependent on estrogens due to the activation of the PI3K/AKT/ER-α pathway, and such cells are more likely to be growth inhibited by tamoxifen, and thus that PIK3CA-mutated tumors are associated with a favorable prognosis in patients treated with tamoxifen. Interestingly, recently, Yamashita et al. (37) have reported that phosphorylation of Ser167, which is induced by pAKT, is associated with a good response to hormonal therapy including tamoxifen. Ideally, the effect of PIK3CA mutations on prognosis would better be analyzed in ER-positive breast cancer patients treated separately with and without tamoxifen to clarify whether PIK3CA mutation status would serve as a prognostic factor or as a predictive factor of response to tamoxifen. However, because almost all ER-positive breast cancer patients had been treated with tamoxifen, such an analysis was unable to be done in the present study.

Because HER2 overexpression activates the PI3K/AKT pathway, tumors with HER2 amplification are speculated not to require a further activation of this pathway by PIK3CA mutations. However, Saal et al. (11) reported a significant positive association between HER2 overexpression and PIK3CA mutations, suggesting that more than one input activating the PI3K/AKT pathway might be necessary for carcinogenesis of breast cancer. In the present study, we have failed to show a significant association between HER2 overexpression and PIK3CA mutations. Because immunohistochemistry is not an accurate method for determination of HER2 amplification and only a limited number of breast cancers were analyzed in HER2 overexpression, our result needs to be interpreted with caution and should be confirmed by fluorescence in situ hybridization analysis of HER2 amplification using a larger number of tumors.

In conclusion, we have identified somatic missense mutations of PIK3CA in 54 of 188 (29%) Japanese breast cancers. Majority (83%) of the mutations clustered in exon 9 and exon 20, helical and kinase domains, respectively. PIK3CA mutations were significantly associated with ER-α-positive tumors, or pAKT-positive tumors. Patients with PIK3CA-mutated tumors showed a significantly more favorable prognosis than those with PIK3CA-nonmutated tumors. It is currently unknown whether PIK3CA mutation status serves as a prognostic factor or as a predictive factor of response to tamoxifen. Our preliminary results need to be confirmed by a future study including a larger number of patients with a longer follow-up period.

Table 6. Univariate and multivariate analyses of various prognostic factors

<table>
<thead>
<tr>
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<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR* (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>PIK3CA mutation</td>
<td>2.36 (1.10-5.06)</td>
<td>0.03</td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>6.25 (2.33-16.7)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Lymph node status</td>
<td>3.03 (1.69-5.56)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Histologic grade</td>
<td>1.27 (0.53-3.03)</td>
<td>0.59</td>
</tr>
<tr>
<td>ER status</td>
<td>2.01 (1.13-3.59)</td>
<td>0.02</td>
</tr>
<tr>
<td>PR status</td>
<td>2.94 (1.61-5.35)</td>
<td>0.0004</td>
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</table>

NOTE: Noninvasive ductal carcinomas were excluded. Abbreviation: CI, confidence interval.
*Hazard ratio of mutation negative against positive, large tumor (2.0 cm <) against small tumor (≤2.0 cm), lymph node positive against negative, histologic grade 2 + 3 against grade 1, ER-negative against ER-positive, and PR-negative against PR-positive.
References
