The Lipid Kinase PI4KIIIβ Is Highly Expressed in Breast Tumors and Activates Akt in Cooperation with Rab11a

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Abstract
Emerging evidence now implicates phosphatidylinositol 4-kinases (PI4K), enzymes that generate PI(4)P from phosphatidylinositol (PtdIns), in cancer. In this study, we investigate the role of PI4KIIIβ, one of four mammalian PI4Ks, in breast cancer. Although PI4KIIIβ protein levels are low in normal breast tissue, we find that approximately 20% of primary human breast tumors overexpress it. Expression of PI4KIIIβ in breast carcinoma cells leads to increased Akt activation, dependent on increased PI(3,4,5)P3 production. However, a kinase-inactive version of PI4KIIIβ also led to increased Akt activation, and no changes in PI(4)P or PI(4,5)P2 lipid abundance were detected in the PI4KIIIβ-overexpressing cells. This implies that PI4KIIIβ regulates PI(3,4,5)P3 and Akt independent of PI(4)P production. We find that the PI4KIIIβ-binding protein, Rab11a, a small GTPase that regulates endosomal recycling, is involved in PI4KIIIβ-mediated activation of Akt, as RNAi depletion of Rab11a impairs Akt activation. Furthermore, ectopic PI4KIIIβ expression alters cellular Rab11a distribution and enhances recruitment of PI4KIIIβ and Rab11a to recycling endosomes. This work suggests that PI4KIIIβ affects PI3K/Akt signaling through Rab11a and endosomal trafficking, independent of its lipid kinase activity. Thus, PI4KIIIβ likely plays a role in breast oncogenesis and that cooperation between Rab11a and PI4KIIIβ represents a novel Akt activation pathway. Mol Cancer Res; 12(10); 1492–508. © 2014 AACR.

Introduction
Membrane phosphoinositide lipids, the phosphorylated derivatives of phosphatidylinositol (PtdIns), and the kinases/phosphatases that regulate their generation have critical roles in cancer. They do so by controlling apoptotic and cell survival signaling pathways and stimulating cell migration and cytokeratin remodeling (1–5). Although the kinase and phosphatase regulators of PI(3,4,5)P3 have been well studied in cancer, phosphatidylinositol 4-kinases (PI4Ks), proteins that generate PI(4)P from PtdIns, are now also emerging as important regulators of cancer (6). In particular, recent work points to a role for PI4KIIIβ, one of four mammalian PI4Ks, in breast cancer. First, Curtis and colleagues (7) showed that PI4KIIIβ, the 1q21 gene encoding PI4KIIIβ, was frequently amplified in breast tumors. On the basis of a large-scale transcriptional analysis of 2,000 tumors, the authors concluded that PIK4B is a novel breast cancer driver (7). Others have also shown that 1q21 is highly amplified in breast cancers (8–10). At a functional level, we have previously shown that PI4KIIIβ is likely to regulate breast epithelial morphogenesis because ectopic PI4KIIIβ expression disrupts in vitro three-dimensional acinar development in MCF10A cells (11). We have also identified PI4KIIIβ as a downstream effector of eukaryotic elongation factor 1 alpha 2 (eEF1A2), a transforming gene that is amplified and highly expressed in breast and ovarian cancer (12–15). Finally, PI4KIIIβ expression has also been shown to inhibit apoptosis in MDA-MB 231 cells (16). Taken together, these observations suggest a likely role for PI4KIIIβ in breast oncogenesis. However, the mechanism by which PI4KIIIβ might promote neoplasia is unclear.

In this report, we implicate PI4KIIIβ in the activation of Akt in breast cancer cells. The Akt serine/threonine kinase is a central regulator of multiple biologic processes, among them cell survival, proliferation, and growth (17, 18). Akt activity is increased in multiple human malignancies, with aberrant activation reported in as many as 70% of breast cancers (19–23). Akt activity is regulated, in large part, by phosphatidylinositol 3-kinase (PI3K)–generated PI(3,4,5)P3 and PI(3,4)P2 phosphoinositide lipids (18, 24). PI(3,4,5)P3 recruits Akt to the plasma membrane through its pleckstrin-homology (PH) domain, allowing phosphorylation at Thr308, within the activation loop of the protein, by 3-phosphoinositide-dependent protein kinase 1 (PDK1) and at Ser473, within the carboxyl-terminal tail, by the mammalian target of rapamycin complex 2 (mTORC2)
phosphorylates Akt (25–27). The tumor suppressors, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), and inositol polyphosphate 4-phosphatase (INPP4A) antagonize PI3K/Akt signaling by dephosphorylating PI(3,4,5)P3 at the D3 position, and PI(3,4)P2 at the D4 position, respectively (28–30).

In this report, we also show that approximately 20% of primary human breast tumors exhibit increased PI4KIIIβ protein expression relative to normal breast tissue. We find that ectopic PI4KIIIβ expression leads to Akt activation in a PI(3,4,5)P3-dependent manner. Surprisingly, PI4KIIIβ-mediated Akt activation is not dependent on PI4KIIIβ lipid kinase activity, its interaction with the small GTPase, Rab11a seems to be critical. Rab11a regulates slow endosomal recycling, and has previously been shown to interact with PI4KIIIβ (31, 32). Our work suggests that PI4KIIIβ has an important role in breast cancer and that cooperation between Rab11a and PI4KIIIβ represents a novel Akt activation pathway.

Materials and Methods

Cell culture

The BT549 and MCF10A cell lines were purchased from the American Type Culture Collection (ATCC). BT549 cells were grown in RPMI-1640 medium (Life Technologies) supplemented with 10% FBS (Life Technologies), 1 mmol/L sodium pyruvate (Life Technologies), 10 mmol/L HEPES buffer, and 0.023 IU/mL insulin (from bovine pancreas, # 1-5500; Sigma). MCF10A cells were grown in HEPES buffer, and 0.023 IU/mL insulin, 0.5 µg/mL hydrocortisone, and 100 ng/mL cholera toxin. BT549 cell lines stably expressing ectopic PI4KIIIβ were generated using a pLXSN retroviral system as previously described (33). Full-length human wild-type (WT; MGC-1921; ATCC) and kinase-dead (KD; D656A) PI4KIIIβ (gift from A. Hausser, University of Stuttgart, Stuttgart, Germany) were cloned into the pLXSN vector in the EcoRI/XhoI and HpaI/XhoI sites, respectively, for retroviral generation. Infected cells were selected in 0.4 mg/mL G418 (BioShop). Cell lines were cultured at 37°C in humidified atmosphere with 5% CO2.

Immunohistochemistry

Tumor tissue microarrays (TMA) were obtained from US Biomax. For TAM staining, the slide was sequentially deparaffinized (2 × xylene, 2 × 100% EtOH, 1 × 95% EtOH, 1 × 70% EtOH, and 1 × H2O), boiled in 0.1 mol/L sodium citrate (pH 6.0) for 10 minutes, and then allowed to cool in the citrate buffer for an additional 10 minutes. Slides were then rinsed twice in PBS/0.2% Tween 20 and blocked in 10% FCS/1% BSA/PBS/0.2% Tween 20 for 1 hour at room temperature in a humidified chamber. The slide was then incubated overnight at 4°C with PI4KIIIβ antibody (BD Biosciences) diluted at 1:200 in 1% BSA/PBS/0.2% Tween 20. The primary antibody was then removed by three rinses of 5 minutes each in PBS/0.2% Tween 20. Antibody staining was detected using biotinylated VECTASTAIN anti-mouse IgG antibody and either horseradish peroxidase or alkaline phosphatase according to the manufacturer’s instructions. Slides were counterstained using hematoxylin. Staining was visualized using a Zeiss Axio Imager.A1 microscope with images acquired using a Zeiss AxioCam MrRc5 camera and AxioVision 4.6 software. Scoring was done using a three-point visual system (low, moderate, and high) with representative images of each shown. Counting was performed in triplicate with discrepancies resolved by a fourth count.

siRNA and transfections

PI4KIIIβ-targeted siRNA sequence 1 is 5'-GGAGGU-GUUGGAGAAAGU-Ct-3’ (catalog number, AM51331; siRNA ID no., 283) and siRNA sequence 2 is 5'-GCACU-GUGCCCAACUAUGAtt-3’ (catalog number, AM51331; siRNA ID no., 184). The Rab11a-targeted siRNA sequence used was 5'-CAACAAUGGUGGUCUUAtt-3’ (catalog number, 442708; siRNA ID no., s16702). The Rab11b-targeted sequence is 5’-CGGACGCGAAGAAAGCCCAAAGCT-3’. siRNAs and the negative control siRNA were purchased from Life Technologies (Rab11a) or Integrated DNA Technologies (Rab11b). siRNA transfections were performed in 1% serum containing growth media with 10 nmol/L siRNA using Lipofectamine RNAiMAX (Invitrogen, Life Technologies) as per the manufacturer’s instructions. Lysates were collected either 48 or 72 hours after transfection for PI4KIIIβ and Rab11-targeted siRNA transfections, respectively. Cells were transfected with Akt-PH-GFP, FAPP1-PH-GFP (gifts from T. Balla, NIH, Bethesda, MD), Rab11a-GFP (gift from J. Presley, McGill University, Montréal, QC, Canada), GALT-CFP, and PI4KIIIβ-GFP (gift from A. Hausser, University of Stuttgart) using Lipofectamine LTX and Plus Reagents (Invitrogen, Life Technologies) according to the manufacturer’s instructions.

Adenoviral infection

Cells were plated (5 × 10^5 cells per 10-cm plate) 24 hours before infection. On the day of infection, cells were placed in 5 mL serum-free growth media with adenoviral particles at a multiplicity of infection (MOI) of 200. Cells were collected for Western blotting 24 hours after infection. The adenovirus expressing WT PTEN was kindly provided by M.J. Lee (Wayne State University, Detroit, MI).

Western blotting

Cells were plated (2 × 10^5 cells per 6-cm plate) 48 hours before lysis taking and serum depleted (placed in 1% serum containing growth media) 24 hours prior. Cells were lysed using radioimmunoprecipitation assay (RIPA) buffer (50 mmol/L Tris–Cl; pH 7.4, 1% Triton X-100, 1% sodium dodecyl sulfate (SDS), 1 mmol/L ethylenediaminetetraacetic acid (EDTA); pH 7.0, and 150 mmol/L NaCl) supplemented with protease and phosphatase inhibitor cocktail tablets (Roche Diagnostics). Protein concentrations were determined by Bradford protein assay (Bio-Rad). SDS sample buffer was added to 10 µg of protein lysate, resolved by SDS-PAGE,
transferred onto polyvinylidenedifluoride (PVDF) membrane (Millipore). PI4KIIIβ (BD Biosciences), pAkt, Akt, p-cRaf, cRaf (Cell Signaling Technology), Rab11a, Rab11b (Millipore), and β-actin (Sigma) antibodies were used according to the manufacturer’s instructions.

**Immunoprecipitation**

Cells were lysed in 50 mmol/L Tris, pH 7.5, 150 mmol/L NaCl, and 0.5% Triton X-100 supplemented with protease and phosphatase inhibitor cocktail tablets (Roche Diagnostics). Immunoprecipitation with PI4KIIIβ (Millipore) was performed with 5 μg of antibody per 1 mg/mL of lysate using Protein A agarose beads (Millipore).

**Immunofluorescence**

Protein immunostaining was performed on 1 × 10⁵ cells plated in 6-well plates (Corning) containing glass coverslips 24 hours later, cells were fixed with 3.7% formaldehyde for 15 minutes and either permeabilized with 0.1% Triton X-100 for 10 minutes and blocked with 3% FBS for 1 hour (PI4KIIIβ/Giantin) or permeabilized and blocked in 0.5% saponin, 3% FBS for 1 hour [transferrin receptor (TfR)]. Cells were then incubated with primary antibodies overnight in 3% FBS. The following primary antibodies were used as per the manufacturer’s instructions: PI4KIIIβ (BD Biosciences), Giantin (Abcam), and TfR (Abcam). This was followed by 1-hour incubation with Alexa Fluor 488– and Alexa Fluor 647–conjugated secondary antibodies in 3% FBS (1:200; Molecular Probes, Life Technologies) and a 20-minutes incubation with Hoechst 33258 dye (Sigma) for DNA labeling (0.5 μg/mL). All slides were mounted using fluorescent mounting media (Dako Cytomation). Fixed cell images were acquired by sequential excitation using an Olympus Fluoview FV1000 laser scanning confocal microscope with an Olympus UPLSAPO 100X/1.40 oil objective. Images were collected and Pearson correlation coefficient quantification was performed using Olympus software (FV1000, version 1.01a). Akt-PH-GFP reporter construct membrane recruitment and Rab11a-GFP distribution quantification were performed using ImageJ (NIH). Briefly, for the Akt-PH-GFP reporter, the integrated density of pixels for the GFP construct was measured at the cell periphery (defined as the outer 20% area of the cell) and divided by the total integrated density of pixels for the whole cell. Rab11a-GFP integrated density was measured at the Golgi, as defined by the area marked by GALT-CFP fluorescence, and converted to a proportion of the total integrated density of whole-cell Rab11a-GFP fluorescence. The proportion of extra-Golgi Rab11a-GFP was defined as the difference between whole-cell and Golgi-area–integrated density measurements, divided by whole-cell fluorescence. Background was subtracted from all integrated density measurements. PI(4)P (Echelon Biosciences) lipid antibody staining was performed on 3 × 10⁵ cells plated 24 hours before in 4-well plates (BD Biosciences) according to the Golgi and plasma membrane–specific immunostaining protocols as previously described (34).

**Live cell imaging**

For FAPP1-PH-GFP imaging in Pik93-treated cells, 1 × 10⁵ BT549 cells were plated on uncoated 35-mm glass bottom plates (MatTek P35G-0-20-C) 24 hours before transfection. Cells were transfected with FAPP1-PH-GFP using Lipofectamine LTX as per the manufacturer’s instructions (Life Technologies). Twenty-four hours after transfection, cells were treated with either DMSO or 250 nmol/L PIK93 in DMSO. Cell images were acquired by an ORCA-R2 Hamamatsu CCD camera in an Olympus VivaView FL microscope using the 20X objective and an X-cite eXacte mercury arc illumination with a GFP filter cube. Images were processed in VivaView FL software. For Rab11a/GALT imaging, Vector control, WT-PI4KIIIβ–expressing, and KD-PI4KIIIβ–expressing BT549 cells were plated on MatTek coverslips (MatTek Corporation) 24 hours before transfection. Cells were cotransfected with GFP-Rab11a and CFPS-Galt, using FuGENE HP extreme as per the manufacturer’s instructions (Roche Diagnostics). Time-lapse images were acquired 24 hours after transfection using an inverted confocal laser scanning microscope, LSM 510 (Zeiss), equipped with a stage heated to 37°C in a chamber containing 5% CO₂ (v/v)/95%/v/v air. GFP and CFPS were excited using 417 to 442 nm and 486 to 498 nm band-pass filters, respectively. Fluorescence emission spectra were collected using 455 to 475 nm (CFP) and 510 to 545 nm (GFP) band-pass filters. Images were acquired at a rate of 1/s. Captured images were converted to animation and exported to QuickTime movie using the ImageJ software (NIH software).

**Competitive ELISA and HPLC analysis**

We determined the relative amount of PI(3,4,5)P₃ present in the stable cell lines using a competitive PIP₃ Mass ELISA kit (Echelon Biosciences). Lipids were extracted and purified PIP₃-containing suspensions were treated according to the manufacturer’s instructions. The colorimetric signal was measured at 450 nm using the Tecan Spectra plate reader. For high-performance liquid chromatography (HPLC), cells were labeled in inositol-free media for 48 hours. Cells were extracted and separated as previously described (35). Lipid levels were normalized to total phosphatidylinositol levels. There were no differences in the total phosphatidylinositol between the groups.

**Results**

**High expression of PI4KIIIβ protein in primary human breast tumors**

To determine whether PI4KIIIβ expression might be altered in breast cancer, we analyzed PI4KIIIβ protein expression in 460 primary human breast tumors [371 invasive ductal carcinomas (IDC) and 89 medullary carcinomas]. For this purpose, we used a commercially available PI4KIIIβ antibody and commercially available human breast tumor tissue arrays. The antibody used for immunohistochemistry was verified for PI4KIIIβ-binding specificity, as siRNA knockdown of PI4KIIIβ in human breast cancer cells...
abolished antibody staining of the protein (Supplementary Fig. S1). Staining in each tissue section was categorized as low, moderate, or high. Representative photographs of the three categories are shown in Fig. 1. As shown in Table 1, PI4KIIIβ protein expression is detected at a low level in all normal breast tissue samples (73 of 73). The majority of primary human breast tumors assayed also express PI4KIIIβ protein at this low, but detectable, level (338 of 460). However, 27% of primary tumors assayed (81 of 371 IDC and 41 of 89 medullary carcinomas) expressed higher than normal levels of the protein, that is, either moderate or high. These results are statistically significant, yielding a P value of 0.0001 (Fisher exact test). There were no differences in the age of patients at diagnosis: 49 ± 11 in both the PI4KIIIβ normal and high groups. However, in IDC, there was a significantly increased probability of increased PI4KIIIβ protein expression in lower-grade stage I tumors (P = 0.008, Student t test). In the PI4KIIIβ-high group, 28% (21 of 75) of IDCs were stage I compared with only 10% (21 of 220) in the normal population. In the high group, 61% (46 of 75) were stage II compared with 78% (172 of 220). Eleven percent (8 of 75) of the PI4KIIIβ-high group were stage III, similar to the 12% (27 of 220) in the normal group. The increased expression of PI4KIIIβ protein in approximately one quarter of the primary tumors is consistent with the idea that high PI4KIIIβ expression has a functional role in breast oncogenesis.

**PI4KIIIβ activates Akt**

To investigate a potential role of PI4KIIIβ expression in breast cancer cell signaling, we generated BT549 human breast ductal carcinoma and MCF10A immortalized human breast epithelial cells that ectopically express PI4KIIIβ (Fig. 2A). In two independently generated BT549 and MCF10A cell lines with enhanced PI4KIIIβ expression, we detected an approximate 2-fold increase in Akt phosphorylation at Thr308 and at Ser473 as compared with the vector control (Fig. 2A and B). This increase in activating Akt phosphorylation was detected in the PI4KIIIβ-overexpressing BT549 cell lines in serum-free, low serum, and full serum media growth conditions (Supplementary Fig. S2). To determine whether this increase in Akt phosphorylation increases intracellular activity of the protein, we probed the cell lines for the level of phosphorylation of c-Raf, a known Akt kinase target that has been shown to shift human breast cancer cells from cell-cycle arrest to proliferation when

![Figure 1. PI4KIIIβ expression in primary breast tumors in a TMA. Representative images of PI4KIIIβ immunostaining in normal breast tissue and breast tumors classified as showing low, moderate, or high expression of PI4KIIIβ. Scale bars, 100 μm.](image)

### Table 1. Evaluation of PI4KIIIβ expression in breast cancer

<table>
<thead>
<tr>
<th>Breast tissue</th>
<th>PI4KIIIβ expression</th>
<th>Number of samples</th>
<th>Percentage of samples</th>
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</thead>
<tbody>
<tr>
<td>Normal (n = 73)</td>
<td>Low</td>
<td>73</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Moderate, high</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>All tumor (n = 460)</td>
<td>Low</td>
<td>338</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Moderate, high</td>
<td>122</td>
<td>27</td>
</tr>
<tr>
<td>IDC (n = 371)</td>
<td>Low</td>
<td>290</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>66</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>Medullary carcinoma (n = 89)</td>
<td>Low</td>
<td>48</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

NOTE: PI4KIIIβ protein expression was categorized as either low, moderate, or high for the microarray tissue samples stained. The n value indicates the number of each tissue type assayed.
Figure 2. PI4KIIIβ expression regulates Akt activity. A, Western blot analysis showing levels of phosphorylation of Akt at Ser473 and at Thr308 in two independent BT549 and MCF10A cell lines with increased PI4KIIIβ protein expression as compared with parental and vector controls. B, quantification of the relative increase in Akt phosphorylation over total Akt levels in the PI4KIIIβ-expressing cell lines as compared with the vector control. (Continued on the following page.)
phosphorylated at Ser259 (Fig. 2C; ref. 36). A 1.8-fold increase in c-Raf phosphorylation at Ser259 was detected in the PI4KIIIβ-overexpressing BT549 cell lines relative to the vector control, indicating a higher level of Akt kinase activity following PI4KIIIβ overexpression (Fig. 2D). Concordant with an increase in activation of the proliferative kinase Akt, as well as increased c-Raf phosphorylation, we found that BT549 cells overexpressing PI4KIIIβ proliferate at a faster rate than their parental or vector control counterparts. Parental and empty vector control cells had doubling times of 38.8 and 37.3 hours, respectively, while PI4KIIIβ-overexpressing cells had a doubling time of 17.2 hours (Fig. 2E). This indicates that elevated expression of PI4KIIIβ increases the in vitro proliferative capacity of breast cancer cells. We then determined whether a loss of PI4KIIIβ protein expression might lead to a decrease in Akt activation. To this end, BT549 parental cells were treated with two distinct siRNA sequences targeted to PI4KIIIβ (Fig. 2F). Concomitant with a decrease in PI4KIIIβ protein levels, we observed a consistent and significant decrease (~40%–50%) in Akt phosphorylation at Ser473 and Thr308 (Fig. 2G). These results indicate that PI4KIIIβ expression can regulate Akt activity.

PI4KIIIβ activates Akt via PI(3,4,5)P3 signaling

Because Akt activation is dependent, in large measure, on PI(3,4,5)P3 levels, we determined the effect of PI4KIIIβ expression on the overall cellular abundance of PI(3,4,5)P3 using a competitive ELISA. As shown in Fig. 3A, we observed a significant increase (~60%) in PI(3,4,5)P3 abundance in the PI4KIIIβ-overexpressing cell lines compared with the vector control. We then visualized the intracellular localization of PI(3,4,5)P3 using a fluorescent reporter composed of the PH domain of Akt fused to GFP (Akt-PH-GFP), which binds to PI(3,4,5)P3 and PI(4,5)P2 (37). Cell lines with increased PI4KIIIβ expression showed visibly increased plasma membrane recruitment of the reporter construct, indicating higher levels of PI(3,4,5)P3/PI(4,5)P2 at the cell membrane, where Akt activation is directed by these lipid species (Fig. 3B, top). When cells were transfected with GFP alone, no enhanced fluorescent recruitment could be observed at the cell membrane for the PI4KIIIβ-overexpressing cells as compared to the vector control (Fig. 3B, bottom). To quantify this reporter recruitment to the plasma membrane, we measured the percentage of the total fluorescent signal that was localized to the cell periphery (Fig. 3C). A significant increase ($P < 0.01, \text{Student } t$ test) in fluorescent intensity at the cell periphery was detected for the GFP-Akt-PH reporter in the vector control and PI4KIIIβ-overexpressing cells as compared with each cell line transfected with GFP alone (Fig. 3C). This indicates that the reporter is enriched in the cell periphery. Importantly, in PI4KIIIβ lines, 31% of total GFP-Akt-PH fluorescence was detected at the cell membrane compared with 19% of the same reporter in the vector control line. In GFP-expressing controls in both cell lines, approximately 14% of total GFP fluorescence was detected at the cell membrane in both cell lines. This significant increase (~10%; $P < 0.01, \text{Student } t$ test) indicates that PI4KIIIβ expression increases abundance of PI(3,4,5)P3/PI(3,4)P2 at the cell membrane. BT549 cells are PTEN-null, as they contain a truncation mutation in PTEN, which leads to the rapid degradation of the truncated protein (38, 39). Therefore, we next wanted to determine the effect of re-introducing WT PTEN on Akt activation, in the cell lines with enhanced PI4KIIIβ expression. Expression of WT PTEN by adenoviral infection led to a decrease in Akt phosphorylation in both the BT549 cells with enhanced PI4KIIIβ expression and the vector control cells (Fig. 3D). PTEN expression led to a decrease in Akt phosphorylation by approximately 50% in both the vector control and PI4KIIIβ-overexpressing cells (Fig. 3E). As PTEN is responsible for dephosphorylating PI(3,4,5)P3 into PI(4,5)P2, this result suggests that the increase in Akt activation in the PI4KIIIβ-expressing cells is dependent on cellular PI(3,4,5)P3 lipid levels.

PI4KIIIβ-mediated Akt activation is independent of its kinase function

PI(3,4,5)P3 is principally generated by the phosphorylation of PtdIns, at positions D4, D5, and D3 respectively, by PI4K, PI(4)P 5-kinase, and PI3K. We therefore hypothesized that enhanced PI4KIIIβ expression might increase the cellular abundance of PI(3,4,5)P3 by increasing intracellular concentrations of its precursors, PI(4)P and PI(4,5)P2. To test this idea, we measured the PI levels in the cell lines stably ectopically expressing PI4KIIIβ using HPLC separation of 3H-labeled inositol lipids. Surprisingly, no significant differences in the relative abundance of PI(4)P or PI(4,5)P2 lipids were detected in the PI4KIIIβ-overexpressing cell lines as compared with the vector or parental control cell lines (Fig. 4A and B). Thus, enhanced PI4KIIIβ expression seems to increase PI(3,4,5)P3 levels without having an impact on total cellular PI(4)P or PI(4,5)P2 abundance. To determine whether ectopic expression of

(Continued) Data, mean ± SE of the mean of three independent trials; statistical significance: * , $P < 0.05$ (Student $t$ test). C, Western blot analysis showing phosphorylation of c-Raf at Ser259 in two independently derived BT549 cell lines with increased PI4KIIIβ protein expression as compared with parental and vector controls. D, quantification of the relative increase in c-Raf phosphorylation at Ser259 over total c-Raf levels in the PI4KIIIβ-expressing cell lines as compared with the vector control. Data, mean ± SE of the mean of two independent trials for two independent cell lines ($n = 4$); statistical significance: * , $P < 0.05$ (Student $t$ test). E, PI4KIIIβ-overexpressing BT549 cells (closed symbols) proliferate at a faster rate than the parental and vector control cells (open symbols). Each point represents the average fold growth of cells at a given time point over the number of cells measured at the initial time point (0 hours) for three independent experiments for each cell line ± SD. F, Western blot analysis showing Akt phosphorylation at Ser473 and Thr308 in BT549 parental cells treated with two distinct siRNA sequences targeted to PI4KIIIβ as compared with cells treated with the negative control siRNA. G, quantification of relative decrease in Akt phosphorylation in cells treated with siRNA targeted to PI4KIIIβ as compared with cells treated with negative control siRNA. Data, mean ± SE of the mean of three independent experiments; statistical significance: * , $P < 0.05$ (Student $t$ test).
Figure 3. PI4K IIIβ expression activates Akt via PI(3,4,5)P3 signaling. A, PI(3,4,5)P3 abundance is shown as a concentration of pmol per 10^6 cells and is the mean ± SE of the mean of duplicate samples from three independent experiments in the case of the vector control and from two independent experiments for each of the BT549 PI4K IIIβ-overexpressing cell lines. Increased PI(3,4,5)P3 production in PI4K IIIβ-overexpressing cell lines as compared with the empty vector control is statistically significant (*, P < 0.05, Student t test). B, confocal images of BT549 vector control and PI4K IIIβ-overexpressing cells transfected with the PI(3,4,5)P3/PI(3,4)P2 reporter, Akt-PH-GFP (top) or GFP (bottom). Scale bars, 10 μm. C, quantification of the plasma membrane recruitment of the Akt-PH-GFP reporter in the vector and PI4K IIIβ-overexpressing cells presented as the mean ± SE of the mean for 20 cells from four independent experiments. GFP alone was used as a control. Statistical significance (**, P < 0.01, Student t test) is indicated. D, Western blot analysis showing p-Akt levels in vector control and PI4K IIIβ-expressing cells infected with adenovirus expressing WT PTEN as compared with untreated cells. E, quantification of the impact of PTEN expression on pAkt levels in vector control and PI4K IIIβ-expressing cells. Data, mean ± SE of the mean of three independent Western blot analyses; statistical significance: **, P < 0.01 (Student t test).
PI4KIIIβ might lead to an altered distribution of PI(4)P, cells were transfected with the PI(4)P-specific lipid reporter construct, GFP-FAPP1-PH (Fig. 4C). No changes in reporter construct localization were observed between the PI4KIIIβ-overexpressing cells as compared with vector controls, indicating that ectopic PI4KIIIβ expression does not lead to altered PI(4)P lipid distribution. These results were supported by lipid antibody immunostaining performed using organelle-specific staining protocols (34). Again there do not appear to be any gross changes in levels or distribution patterns of PI(4)P at the Golgi or plasma membrane in BT549 cells transfected with GFP-PI4KIIIβ as compared with untransfected cells in the same field (Supplementary Fig. S3). Together, these results suggest that ectopic PI4KIIIβ expression does not lead to a substantive change in PI(4)P lipid abundance or localization in BT549 cells.

In light of these findings, we then asked whether or not the kinase activity of PI4KIIIβ was required for Akt activation. To this end, we treated the BT549 cells stably expressing ectopic PI4KIIIβ with a drug that inhibits PI4KIIIβ, Pik93. This drug inhibits only the PI4KIIIβ isoform (and not PI4KIIIα) at a molar concentration of 250 nmol/L, though at this molar range Pik93 also inhibits PI3K (40–42). Treatment of cells with Pik93 had no effect on PI4KIIIβ-mediated Akt activation, as the same increase in Akt phosphorylation is observed in PI4KIIIβ-expressing cells as compared with vector control cells, when treated with DMSO (vehicle control) or Pik93 (Fig. 4D). To ensure that Pik93 did in fact inhibit PI4KIIIβ in our cell system, we followed the distribution of the PI(4)P lipid reporter, FAPP1-PH-GFP, in BT549 parental cells treated with either DMSO or Pik93 (Supplementary Fig. S4). Pik93 inhibition of PI4KIIIβ has previously been shown to lead to a loss of FAPP1-PH-GFP Golgi localization (43). We found that treatment of cells with 250 nmol/L of Pik93 led to loss of Golgi reporter fluorescence by 30 minutes (Supplementary Fig. S4). In contrast, FAPP1-PH-GFP remained primarily Golgi localized in DMSO-treated cells. This reflects the loss of PI(4)P lipid at the Golgi due to Pik93 inhibition of PI4KIIIβ. As treatment with Pik93 had no effect on PI4KIIIβ-mediated...
Akt activation, this indicates that PI4KII β catalytic activity is not required for its role in Akt activation.

To further investigate the catalytic role of PI4KII β in Akt activation, we next generated BT549 cells ectopically expressing a KD PI4KII β. KD-PI4KII β contains a substitution of an aspartic acid residue for alanine at position 656, rendering it catalytically inactive (44). Previous work has demonstrated that the PI4K activity of KD-PI4KII β is <0.2% that of WT-PI4KII β (32, 44). Two independently derived cell lines expressing KD-PI4KII β showed significant increases (~2-fold) in Akt phosphorylation at Thr308 and at Ser473 as compared with the vector control and at levels comparable with those observed in BT549 cells ectopically expressing WT-PI4KII β (Fig. 5A and B). Similar results were found in MCF10A cells (Supplementary Fig. S5). In addition, BT549 cells ectopically expressing KD-PI4KII β proliferate at a faster rate than vector control cells (Fig. 5C). Both KD-PI4KII β– and WT-PI4KII β–expressing cells had a doubling time of approximately 27 hours, as compared with vector control cells, which had a doubling time of 47 hours. This further indicates that PI4KII β activates Akt independently of its catalytic activity.

Because it has been previously reported that kinase activation is not required for the Golgi localization of the soluble PI4KII β protein (32), we wanted to verify that ectopic expression of KD-PI4KII β did not alter the subcellular localization of PI4KII β protein in our cell system. We found that in all cell lines, the PI4KII β protein colocalized with the cis-medial Golgi marker Giantin and could also be observed in the cytoplasm (Fig. 5D). As expected, the Golgi and cytoplasmic pools of PI4KII β appear more abundant in cells ectopically expressing either WT- or KD-PI4KII β as compared with vector controls. No differences in PI4KII β cellular distribution were detected between the cells ectopically expressing either WT- or KD-PI4KII β.

We next investigated whether KD-PI4KII β–expressing cells showed increased PI(3,4,5)P3 plasma membrane abundance, similar to WT-PI4KII β–expressing cells. To this end, cells were transfected with the PI(3,4,5)P3/PI(3,4)P2 reporter construct, Akt-PH-GFP. A significant increase in cell membrane association of the GFP-Akt-PH reporter was detected in the KD-PI4KII β–expressing cells, as compared with vector controls (Fig. 5E and F). This increase in PI(3,4,5)P3 reporter plasma membrane recruitment is similar in amplitude to that observed in WT-PI4KII β–overexpressing cells (Fig. 3C). Thus, ectopic expression of a kinase-inactive PI4KII β also has the capacity to increase the plasma membrane abundance of PI(3,4,5)P3 and increase Akt activation in breast cancer cells, to similar levels observed in cells overexpressing the WT-PI4KII β protein.

A role for Rab11a in PI4KII β–mediated Akt activation

As we determined that the kinase function of PI4KII β is not necessary for Akt activation, we speculated that an accessory PI4KII β-binding protein was involved in the activation process. The endosomal trafficking protein Rab11a was an attractive candidate as it had already been shown to bind to PI4KII β independently of its kinase function (32, 45). We first verified that PI4KII β and Rab11a interact in our cells lines by coimmunoprecipitation. Rab11a coimmunoprecipitated with PI4KII β in the vector control, WT-PI4KII β–overexpressing and KD-PI4KII β–expressing cell lines, with a greater amount of Rab11a found with PI4KII β in the cell lines ectopically expressing WT- or KD-PI4KII β, suggesting that a proportion of exogenous PI4KII β interacts with Rab11a in these cell lines (Fig. 6A). This interaction between PI4KII β and Rab11a was visualized by immunofluorescence in WT-overexpressing and KD-PI4KII β–expressing BT549 cells as well as vector controls, each of which were transfected with Rab11α-GFP (Fig. 6B). In these cell lines, PI4KII β and Rab11a were both found in a pericentriolar region (yellow arrows). Importantly, WT-PI4KII β and KD-PI4KII β cells showed substantial colocalization between Rab11a and PI4KII β in peripheral areas of the cell (high-magnification panels, white arrows). Intensity tracings of the high-magnification images showed visible correlation between Rab11a and PI4KII β signals (Supplementary Fig. S6A). To quantitate colocalization between the two proteins, we counted the number of peripheral Rab11a vesicles that had visible PI4KII β. In WT-PI4KII β–overexpressing cells, 69% ± 11% of Rab11a-positive vesicles contained PI4KII β (Supplementary Fig. S6B). Similarly, in KD-PI4KII β–expressing cells 73% ± 9% of Rab11a-positive vesicles contained PI4KII β. This coassociation was significantly higher than the 15% ± 9% coassociation in the vector controls (Supplementary Fig. S6B). In whole cells, the Pearson correlation coefficient (r) for PI4KII β and Rab11a-GFP shows a significant increase in the colocalization of these two proteins in WT-PI4KII β–overexpressing and KD-PI4KII β–expressing cells compared with vector control cells (Supplementary Fig. S6C). For these experiments, we chose cells with...
Figure 6. PI4KIIIβ interacts with Rab11 in vector control, WT-PI4KIIIβ–overexpressing, and KD-PI4KIIIβ–expressing cells. A, coimmunoprecipitation (Co-IP) was performed on whole-cell lysates of vector control, WT-PI4KIIIβ–expressing, and KD-PI4KIIIβ–expressing BT549 cells. Each lane contains 10 μg of total protein. Coimmunoprecipitation was performed using a PI4KIIIβ-specific antibody. Agarose beads alone were used as a negative control. Interacting proteins were identified by Western blot analysis using PI4KIIIβ and Rab11a antibodies. B, top, confocal images of Rab11a (green) and PI4KIIIβ (magenta) in the vector control, WT-PI4KIIIβ–expressing, and KD-PI4KIIIβ–expressing BT549 cell lines. Scale bars, 5 μm. Bottom, higher magnification of yellow inset in the merge panel marking Rab11a vesicles (white arrow). Scale bars, 1 μm. C, top, confocal images of PI4KIIIβ (green) and the recycling endosome marker, TfR (magenta), in the vector control, WT-PI4KIIIβ–overexpressing, and KD-PI4KIIIβ–expressing BT549 cell lines. Scale bars, 5 μm. Bottom, higher magnification of yellow inset in the merge panel with TfR vesicles marked (white arrow). Scale bars, 1 μm.
equivalent levels of moderate Rab11a-fluorescence at an unchanged laser intensity and detector sensitivity. This was done to preclude the possibility that differences in Rab11a expression between the lines might be affecting colocalization. Thus, ectopically expressed PI4KIIIβ colocalizes with Rab11a.

To determine whether PI4KIIIβ and Rab11a were colocalizing in recycling endosomes, the stably expressing BT549 cell lines were immunostained for the recycling endosome marker TIR. PI4KIIIβ was found to colocalize with TIR to a greater degree in WT-PI4KIIIβ–overexpressing and KD-PI4KIIIβ–expressing cells compared with vector control cells (Fig. 6C, white arrows). Intensity tracings of the high-magnification images show visible correlation between TIR and PI4KIIIβ signals (Supplementary Fig. S7A). In WT-PI4KIIIβ–overexpressing cells, 57% ± 13% of TIR-positive vesicles contained with PI4KIIIβ (Supplementary Fig. S7B). In KD-PI4KIIIβ–expressing cells, 48% ± 11% of TIR-positive vesicles contained with PI4KIIIβ. This was significantly higher than the 8% ± 4% containing in the vector controls. Furthermore, a significant increase in the Pearson correlation coefficient (r) for PI4KIIIβ and TIR was also detected in the WT-PI4KIIIβ–overexpressing and KD-PI4KIIIβ–expressing cells, as
**Figure 8.** Rab11a is implicated in PI4KIIIβ-mediated Akt activation. A, left, siRNA depletion of Rab11a decreases AKT activation in WT-PI4KIIIβ-overexpressing and KD-PI4KIIIβ-expressing BT549 cells as compared with cells treated with the negative control siRNA. Right, relative decrease in Akt phosphorylation in cells treated with siRNA targeted to Rab11a as compared with cells treated with negative control siRNA. Data, mean ± SE of the mean of three independent trials; statistical significance: *, P < 0.05 (Student t test). (Continued on the following page.)
compared with the vector control cells (Supplementary Fig. S7C). Taken together, these data suggest that PI4KIIIβ is recruited to Rab11a containing recycling endosomes in cells with increased PI4KIIIβ expression, either WT or KD.

We then examined the localization of Rab11a in the expressing cell lines. Cells were transfected with Rab11a-GFP and Galt-CFP, to mark the Golgi. Rab11a is reported localized to the trans Golgi network (TGN), early and recycling endosomes (31). Figure 7A presents still images taken during live cell imaging, and show that Rab11a distribution is altered in cells ectopically expressing either WT- or KD-PI4KIIIβ. In vector control cells, Rab11a is detected primarily in the Golgi, in WT-PI4KIIIβ–expressing and KD-PI4KIIIβ–expressing cells, Rab11a shows a broader punctate distribution in addition to Golgi localization. Rab11a Golgi versus extra-Golgi distribution was quantified and showed a significant shift from Golgi to extra-Golgi localization in WT-PI4KIIIβ–overexpressing and KD-PI4KIIIβ–expressing cells, as compared with vector control cells (P < 0.05, Student t test; Fig. 7B). This indicates that with either WT or a KD mutant, enhanced expression of PI4KIIIβ relocates Rab11a from the Golgi to a broader punctate cellular distribution, suggesting that enhanced PI4KIIIβ expression shifts Rab11a localization from the Golgi onto endosomes.

To determine whether the PI4KIIIβ–Rab11a interaction might be involved in PI4KIIIβ–mediated Akt activation, the vector control, WT–expressing, and KD–expressing cell lines were treated with siRNA against Rab11a. Depletion of Rab11a protein led to a consistent and significant decrease (~50%) in Akt phosphorylation in both cell lines (Fig. 8A and B). In these experiments, Rab11a knockdown was approximately 60%. Similar results were found in the MCF10A cells, where approximately 70% knockdown of Rab11a leads to a significant, approximately 60%, decrease in Akt activity (Fig. 8B). This effect is specific for Rab11a because knockdown of the related Rab11b protein (~60% knockdown) had no discernible effect on Akt phosphorylation (Fig. 8C). Furthermore, Rab11a knockdown (~60%) had no effect on the overall abundance of P(3,4,5)P3, lipid in BT549 cells (Fig. 8D). Taken together, this work is consistent with the idea that Rab11a is important in mediating the activation of Akt signaling by PI4KIIIβ.

To further investigate the importance of Rab11a in breast cancer, we next determined whether there is evidence of Rab11a overexpression during breast tumorigenesis. To this end, we measured Rab11a protein expression in human breast tumor samples. As shown in Fig. 8E, Rab11a is expressed at a moderate level in 100% (18 of 18) of normal breast epithelia and 97% (156 of 161) of infiltrating ductal carcinomas. The absence of breast tumors highly expressing Rab11a indicates that any involvement of Rab11a in oncogenesis is likely through expression-independent mechanisms.

**Discussion**

A role for PI4Ks in cancer is now emerging (6). For example, PI4KIIIα has been reported highly expressed in malignant melanoma, fibrosarcoma, bladder, thyroid, and breast cancer and its expression has been shown to promote tumor angiogenesis (46). In addition, high levels of PI4KIIIα expression are also associated with invasive and metastatic pancreatic cancer development (47). The recent finding that PI4KIIIβ is genetically amplified in a subset of primary human breast tumors points to a specific role for PI4KIIIβ in breast cancer (7). We have also previously implicated PI4KIIIβ in breast oncogenesis as the protein is bound and activated by the breast cancer oncogene eEF1A2, and its high expression disrupts in vitro three-dimensional mammary epithelial morphogenesis (11–15). Supporting a role for PI4KIIIβ in breast cancer development, we show here that PI4KIIIβ protein levels are increased in approximately 20% of primary human breast tumors assayed, consistent with the idea that high PI4KIIIβ expression plays a role in breast cancer neoplastic development. Given the high, but not absolute, concordance between protein expression of a gene and its amplification (48, 49), it is likely that the breast tumors highly expressing PI4KIIIβ also have PI4KB gene amplifications. However, because we have not measured gene copy number in our samples, it is possible that enhanced PI4KIIIβ expression is being driven through other mechanisms other than gene amplification.

At the functional level, we found that enhanced PI4KIIIβ expression increases Akt activation in BT549 cells and the loss of PI4KIIIβ by siRNA silencing decreases Akt activity. This suggests that endogenous PI4KIIIβ is involved in regulating Akt activity and that increased PI4KIIIβ expression, as would occur in tumor cells, further augments Akt signaling. We also show that this PI4KIIIβ–dependent increase in Akt activity itself is dependent on increased P(3,4,5)P3 production. Although PI3K-dependent modes of Akt activation have been reported (50), our data support the hypothesis that PI4KIIIβ expression affects Akt activation through the canonical PI3K pathway. We also show that PI4KIIIβ–mediated Akt activation is lost upon expression of PTEN, the phosphatase that antagonizes PI3K signaling.
This suggests that PI4KIIIβ expression may affect Akt activation only in a PTEN-null background. A loss of PTEN expression has been reported in 30% to 50% of breast cancers, with PTEN loss correlating with lymph node metastasis and disease-related death (51, 52). It is possible that a combination of enhanced PI4KIIIβ expression and PTEN deficiency may contribute to the aggressive nature of PTEN-null breast tumors.

We found that the enhanced PI(3,4,5)P3 production and Akt activation due to increased PI4KIIIβ expression is unlikely to be driven by changes in abundance or intracellular localization of PI(4)P and PI(4,5)P2. No changes in PI(4)P and PI(4,5)P2 lipid abundance were detected in cells overexpressing PI4KIIIβ, nor were we able to detect substantial changes in cellular lipid distribution using immunofluorescence. This was a surprise to us, though it remains formally possible that a higher rate of PI4P generation occurs, but only in a local and transitory manner. Small and/or compartmentalized changes in PI(4)P lipid composition may not have been detectable by HPLC analysis or dramatic enough to be observable by reporter construct imaging or lipid immunostaining. However, the fact that the PI4KIIIβ inhibitor, Plk93, had no effect on PI4KIIIβ-mediated Akt activation and the ability of a catalytically inactive PI4KIIIβ protein to activate Akt rule out a necessary role for PI(4)P in PI4KIIIβ-dependent Akt activation.

Our data suggest that PI4KIIIβ activates Akt in conjunction with Rab11a. We demonstrate that in BT549 cells, Rab11a interacts with PI4KIIIβ in the vector control, WT-PI4KIIIβ-overexpressing, and KD-PI4KIIIβ-expressing cell lines. Moreover, downregulation of Rab11a (using RNAi) inhibits Akt activation. Although it is possible that PI4KIIIβ and Rab11a function in parallel pathways of Akt activity, we favor the hypothesis that PI4KIIIβ activates Akt in concert with Rab11a at the endosomal membranes. Consistent with this idea, PI4KIIIβ was found to colocalize more strongly with the recycling endosomal marker, TR, in both the WT-overexpressing and KD-PI4KIIIβ–expressing cell lines. These results suggest that in the overexpressing cell lines, PI4KIIIβ is recruited to recycling endosomes, as they traffic through the TGN. Coupled to our observation that the distribution of Rab11a is altered (showing a broader punctate cellular distribution) in the WT-PI4KIIIβ–overexpressing and KD-PI4KIIIβ–expressing cell lines, these results suggest that in the overexpressing cell lines, PI4KIIIβ is recruited to recycling endosomes, as they traffic through the TGN. Coupled to our observation that the distribution of Rab11a is altered (showing a broader punctate cellular distribution) in the WT-PI4KIIIβ–overexpressing and KD-PI4KIIIβ–expressing cell lines, these results suggest that enhanced expression of PI4KIIIβ leads to greater endosomal recruitment of Rab11a in BT549 cells.

In Drosophila, the PI4KIIIβ homolog, four-wheel drive (Fwd), has been shown to regulate Rab11a recruitment and trafficking events. More specifically, Fwd is required for Rab11a localization to secretory granules at the midzone of dividing spermatocyte cells, allowing successful completion of cytokinesis (45). Of interest to our study, a noncatalytic role for Fwd in Rab11a regulation was determined as expression of a KD mutant of Fwd, which also binds to Rab11, partially rescues male Drosophila spermatocyte cytokinesis defects in fwd-null flies. Therefore, there is a conserved role for Rab11a as a downstream effector of PI4KIIIβ in the regulation of cell trafficking. We propose that PI4KIIIβ-dependent recruitment of Rab11a could enhance PI3K and Akt signaling on endosomal vesicles exiting the TGN. Endosomal membranes are now recognized as sites of signal transduction. A number of receptor tyrosine kinases (RTK) have been demonstrated to signal from endosomes (53). For example, in adipocytes, insulin-stimulated PI3K activation via phosphorylation of insulin receptor substrate (IRS-1) occurs preferentially in internal membranes rather than at the plasma membrane (54). Endosome-specific signaling of EGFR has also been shown to activate PI3K/Akt signaling (55, 56). In addition, the endosomal proteins EEA1 and App1 (adaptor protein, phosphotyrosine interaction, PH domain, and leucine zipper containing 1) serve as scaffolding proteins that promote endosomal Akt recruitment, phosphorylation, and activation (57, 58). Garcia-Regalado and colleagues (59) showed that the Lipophosphatidic acid-stimulated interaction between Rab11a and the heterotrimeric G protein subunit Gβγ on endosomes contributes to the recruitment and activation of PI3K and Akt on these endosomal compartments. In addition, Sato and colleagues (60) demonstrated that PI(3,4,5)P3 was rapidly produced at the plasma membrane in response to platelet-derived growth factor (PDGR), followed by accumulation in endomembranes of Chinese hamster ovary (CHO) cells. They further showed that PI(3,4,5)P3 was produced in situ in endomembranes, a process stimulated by RTK endocytosis. Together, this research demonstrates an important role for endosome-localized PI3K/Akt activation. We believe that the interaction between PI4KIIIβ and Rab11a at the TGN and on endosomal membranes is likely regulating Akt activation on endosomes themselves. Because we have not observed any alterations in PI(3,4,5)P3 abundance following Rab11a knockdown, we speculate that Rab11a may serve as an endosomal scaffolding nexus for proteins that activate and phosphorylate Akt directly and that increased expression of PI4KIIIβ increases the number of these scaffolding complexes. Rab GT-Pases have been previously linked to breast cancer oncogenesis in their role as central regulators of cell trafficking (61–63).

In conclusion, we find that PI4KIIIβ expression is increased in a subset of breast tumors, and that enhanced PI4KIIIβ expression in BT549 breast cancer cells activates Akt signaling. Enhanced PI4KIIIβ expression leads to PI3K/Akt activation independent of its kinase function, but likely via its interaction with the endosomal trafficking protein Rab11a. This study reveals a novel role for PI4KIIIβ expression in breast cancer pathogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

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The Lipid Kinase PI4KIIIβ Is Highly Expressed in Breast Tumors and Activates Akt in Cooperation with Rab11a

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