Phosphatidylinositol 3-kinase/AKT Signalling Pathway Components in Human Breast Cancer: Clinicopathological Correlations

ELENA S. GERSHTEIN[1], ALEXANDER M. SCHERBAKOV[1], VALENTINA A. SHATSKAYA[2], NICOLAI E. KUSHLINSKY[1] and MIKHAIL A. KRASIL'NIKOV[2]

[1]Laboratory of Clinical Biochemistry, Institute of Clinical Oncology and [2]Laboratory of Molecular Endocrinology, Institute of Carcinogenesis, Russian N. N. Blokhin Cancer Research Centre RAMS, 115478 Moscow, Russia

Abstract. *Background: The phosphatidylinositol 3-kinase (PI3K)/Akt signalling pathway plays a major role in the regulation of breast cancer growth and survival, but the clinical value of its components in human tumours is unclear. Patients and Methods: PI3K was analysed using Western blotting with monoclonal antibodies to the p85 subunit in tumour and adjacent mammary gland samples from 33 breast cancer patients. Activated Akt1 (pAkt1) expression was quantified in 46 sample pairs by a direct sandwich ELISA assay. Results: Tumour PI3K expression was increased in 79% of the investigated sample pairs and was not associated with the main clinico-pathological features. Only 49% of breast cancers had increased pAkt1, but the frequency of its elevation was positively associated with tumour size and histological grade, and controversially related to estrogen and progesterone receptor status. Conclusion: Increased PI3K, but not pAkt1, expression appears to be a widespread feature of human breast cancer indicating the different roles of the two components of one signalling system.*

The phosphatidylinositol 3-kinase/Akt signalling pathway controls many of the intracellular processes that are deregulated in human cancer (1-3). It has particularly become a focus of interest because of its ability to inhibit apoptosis and mediate cell survival by acting as a transducer of signals from growth factor receptors, especially ErbB2 (1, 4, 5). Akt kinase was also shown to be a downstream effector of estrogen-dependent proliferation and survival in hormone-responsive breast carcinoma cells (6-9).

Correspondence to: Dr. Elena S. Gershtein, Laboratory of Clinical Biochemistry, Institute of Clinical Oncology, Russian N. N. Blokhin Cancer Research Centre RAMS, Kashirskoye shosse 24, Moscow 115478, Russia. Tel: +7 495 324 1159, Fax: +7 495 324 6352, e-mail: esgershtein@gmail.com

Key Words: Phosphatidylinositol 3-kinase, phospho-Akt1, Western blotting, ELISA, breast cancer, mammary gland.

Though experimental data on the role of the PI3K/Akt pathway in the regulation of breast cancer growth, survival, hormone, drug and radiosensitivity were so convincing that these signalling proteins are now regarded as prospective molecular targets for the improvement of breast cancer therapies (10-13), the literature describing Akt and, particularly, PI3K expression and/or activation in clinical samples are insufficient and rather equivocal (14-26). The role of the PI3K/Akt system in the growth and progression of human breast tumours remains unclear.

Earlier we demonstrated that PI3K expression was increased in the majority of breast cancer samples with various grades of malignancy and hormone sensitivity (14); in the present study we compare these data with the results of quantitative ELISA assessment of activated Akt1 expression in a comparative series of breast cancer and adjacent mammary gland samples collected at surgery.

Patients and Methods

Tumour and adjacent unchanged mammary gland samples from breast cancer patients were collected by a pathologist during routine in-surgical examination of the dissected gland, then frozen in liquid nitrogen and stored at –70°C until processed.

Thirty-three patients aged from 34 to 80 years were included in the group for PI3K analysis and 46 patients aged 35-75 years comprised the group for pAkt1 measurement. The patient distribution according to TNM classification is shown in Table I. Tumour histology was assessed according to WHO guidelines.

Tumour estrogen receptor (ER) and progesterone receptor (PgR) content was detected using a standard radioligand dextran-coated charcoal (DCC) assay as described elsewhere (14). The cut-off value for both ER and PgR was 10 fmol/mg protein.

The study meets the requirements of the Ethical Committee of the Russian N.N. Blokhin Cancer Research Centre.

Western blot analysis of PI3K and total Akt. In immunoblotting experiments, monoclonal antibodies to the p85 subunit of PI3K (Sigma Chemical Co, St. Louis, MO, USA) and to total Akt (Anti-Akt antibody, 05-591, Upstate, USA) were used. Tissue samples were lysed in buffer pH 7.5 containing 50 mM Tris-HCl, 1% NP-40,

Table I. *TNM distribution of the patients from the two study groups.*

TNM	PI3K		pAkt1	
	n	%	n	%
T1N0M0	–	–	2	4
T2N0M0	9	27	17	37
T1N1M0	2	6	–	–
T2N1M0	10	30	14	30
T2N2M0	2	6	4	9
T3N0M0	5	15	–	–
T3N1M0	2	6	3	7
T4N0-2M0	3	10	5	11
T2N1M1	–	–	1	2
Total	33	100	46	100

150 mM NaCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 0.1 mM Na-orthovanadate and 1% aprotinin. Electrophoresis of samples containing 60-100 μg of protein was performed in 10% polyacrylamide gel and the proteins were transferred onto nitrocellulose membranes (Hybond-C Extra; Amersham BS, Inc., Chalfont, UK) blocked with 5% non-fat milk (Nestle, Vevey, Switzerland) for 1 h. Incubation with the first antibody was performed overnight at 4˚C (dilution 1:2000 in Tris-buffered saline (TBS): 150 mM NaCl, 10 mM Tris-HCl, pH 7.4). After washing in TBS containing 0.1% Tween 20 (Sigma Chemical Co) membranes were exposed to horseradish peroxidase-coupled antibodies (PIERCE, ImmunoPure Antibody, Rockford, IL, USA) for 1.5 h at 20˚C (1:6000 in TBS). Finally, the membranes were rewashed and developed with the enhanced chemiluminescence (ECL) system (Amersham BS). Quantification of the data was carried out with the help of the Image Quant for Windows (version 3.3) software and normalised by total protein content. The intensity of the bands was expressed in relative units (rU; the intensity of the slightest band was taken as 1.0). The level of PI3K expression was then qualitatively graded into four categories: no expression (–), slight expression (+) 1.0 – 1.5 rU, moderate expression (++) 1.5-2.0 rU, pronounced expression (+++) 2.0 – 2.5 rU, and high expression (++++) >2.5 rU.

ELISA measurement of pAkt1. Tissue samples were pulverized in liquid nitrogen and lysed in a 3-fold volume of 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM sodium ortho-vanadate, 1 μg/ml leupeptin, and then centrifuged at 20,000 xg, at 4˚C for 30 min (OptimaTM TLX; Beckman, Fullerton, CA, USA). Supernatants were collected and stored in aliquots at –70˚C until assayed. A direct PathScan™ PhosphoAkt1 (Ser473) Sandwich ELISA Kit (Cell Signalling Technology®, Boston, MA, USA) was used for the assessment of phosphorylated Akt1 levels. The procedure was conducted according to the manufacturer's instructions and the results were read using a ELX800 microplate reader (Bio-Tek Instruments, Winooski, Vermont, USA). The pAkt1 level was expressed in relative optical units (u) per 1 mg protein quantified by the Lowry assay.

Statistics. Quantitative parameters were compared using Student's *t*-test and nonparametric Mann-Whitney, median and sign tests.

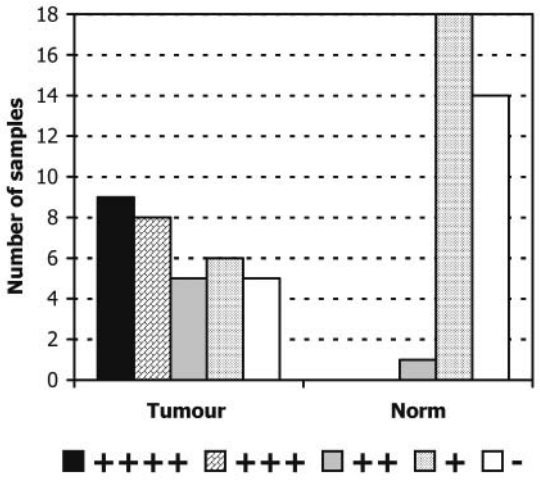

Figure 1. *Distribution of samples with various PI3K expression levels in breast cancer (Tumour) and histologically unchanged adjacent mammary gland tissues (Norm) of 33 patients. After immunoblotting with monoclonal antibodies to the p85 regulatory subunit (Sigma Chemical Co, St. Louis, MO, USA) the level of PI3K expression was attributed to one of four categories: ++++ (high expression), +++ (pronounced expression), ++ (moderate expression), + (slight expression) and - (no expression).*

Associations between groups were assessed using the Pearson (r), Spearman's rank correlation (R) and gamma correlation (γ) tests. *P* value of <0.05 was considered statistically significant.

Results

Western-blot analysis of PI3K expression in cancerous and non-cancerous samples. Lysates of breast cancer samples and corresponding histologically unchanged mammary gland tissue were normalised by protein content and separated by SDS-PAGE in parallel runs. PI3K was detected in 28 out of 33 (85%) breast cancer samples studied. In 22 (66%) of the tumour samples expression of PI3K was characterised as from moderate to high, and in 51% of the tumours pronounced or high PI3K expression was observed (Figure 1). Only 19 (58%) histologically unchanged mammary gland samples of the same patients were PI3K-positive; in most of them (18) the intensity of the signal was low (Figure 1).

Most of the investigated sample pairs were characterised by an increased level of PI3K in the tumour as compared to histologically unchanged adjacent tissue. The extent of PI3K activation (increase of the intensity of PI3K signal in the tumour sample over the level observed in histologically unchanged mammary gland of the same patient) was attributed to one of the following categories: strong, moderate, or weak. Strong activation was observed in 10 (30.3%) of the tumours, moderate in 9 (27.3%), weak in 7 (21.2%), and in 7 tumours (21.2%) no change in the PI3K expression as compared to non-cancerous gland tissue was observed.

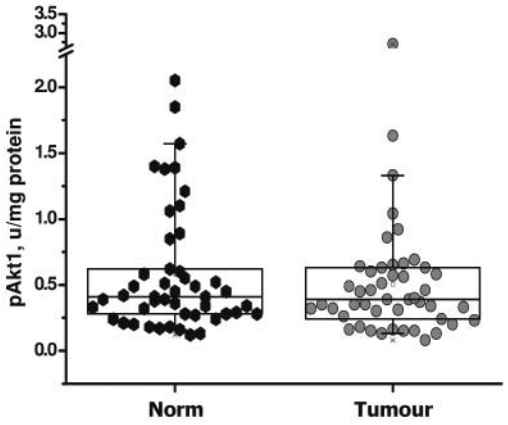

Figure 2. *Phosphorylated Akt1 levels in breast cancer (Tumour) and histologically unchanged adjacent mammary gland tissues (Norm). Results of direct PathScan™ PhosphoAkt1 (Ser473) Sandwich ELISA Kit (Cell Signalling technology®, USA) measurements in tissue extracts. Boxes represent upper and lower quartile range with median indicated, whiskers show the 95% confidence interval.*

No statistically significant associations were revealed between the level of PI3K expression in tumour tissue and/or the extent of its activation and clinical stage, tumour size histologic type or grade, lymph node involvement, tumour ER and/or PgR status, patient's age, or menopausal status.

ELISA measurement of pAkt1 concentrations in breast tumours and adjacent tissues. Measurable levels of pAkt1 were detected using the specific ELISA kit in all breast cancer and adjacent mammary gland samples (Figure 2). pAkt1 was elevated in only 49% of the tumours and the difference between cancerous and non-cancerous tissues was not statistically significant. Moreover, tumour pAkt1 positively correlated with the corresponding normal tissue values (r= 0.5; $p<0.0001$).

The associations of the ELISA-detected pAkt1 levels and the frequency of pAkt1 elevation in the tumour as compared to adjacent tissue with the main breast cancer clinicopathological features are given in Table II. The activated Akt1 level was significantly higher in stage IIb as compared to stage I-IIa breast cancer samples ($p<0.05$) and was more frequently elevated in large (>5 cm) than in small tumours (71 and 42%, respectively; $\gamma=0.54$, $p<0.05$). It was also more frequently elevated in grade III than in grade II tumours (57 *vs.* 15%; $\gamma=0.50$; $p<0.01$).

The phosphorylated Akt1 level was also significantly higher in PgR-negative than in PgR-positive tumours ($p<0.05$), and increased pAkt1 as compared to adjacent tissue was found in 59% of PgR-negative and only in 30% of PgR-positive breast cancers ($\gamma=-0.53$, $p<0.01$). On the other hand, the mean pAkt1 level was significantly higher in ER-positive than in ER-negative breast cancer samples (0.64±0.12 and 0.35±0.04 u/mg protein, respectively; $p<0.05$; Student's *t*-test).

Table II. *pAkt1 content and frequency of elevation in breast cancer depending on clinicopathological factors.*

Patient/tumour characteristic	n	pAkt1	
		u/mg protein median (25-75%) range	% T>N
Menopausal status			
Premenopausal	17	0.48 (0.31-0.63)	64
Perimenopausal	3	0.40 (0.33-0.49)	40
Postmenopausal	26	0.42 (0.24-0.81)	33
Clinical stage			
I-IIa	19	0.35 (0.26-0.49)	50
IIb	14	0.56 (0.33-1.04)[1]	36
III-IV	13	0.39 (0.31-0.46)	44
Tumour size			
T1-2 ≤5 cm	38	0.40 (0.31-0.59)	42
T3-4 >5 cm	8	0.46 (0.15-0.63)	71[3]
Lymph node status			
N0	21	0.36 (0.28-0.53)	53
N+	25	0.46 (0.31-0.63)	43
Histological type			
Ductal invasive carcinoma	24	0.39 (0.30-0.63)	48
Lobular invasive carcinoma	6	0.50 (0.18-0.86)	67
Mixed	6	0.34 (0.16-0.58)	50
Histological type			
I	2	0.48 (0.38-0.58)	100
II	13	0.74 (0.34-1.39)	15
III	21	0.40 (0.24-0.49)	57[4]
ER status			
Positive	24	0.49 (0.27-0.76)	46
Negative	18	0.35 (0.18-0.46)	44
PgR status:			
Positive	23	0.34 (0.18-0.63)	30
Negative	17	0.46 (0.35-0.60)[2]	59[5]

[1]difference between stages I-IIa and IIb, $p<0.05$ (Mann-Whitney test); [2]$p<0.05$, Mann-Whitney test; [3]$p<0.05$, $\gamma=0.54$, gamma correlation test; [4]$p<0.01$, $\gamma=0.50$, gamma correlation test; [5]$p<0.01$, $\gamma=-0.53$, gamma correlation test.

Western-blot analysis of total Akt expression in cancerous and non-cancerous samples. Six pairs of samples were available for comparative Western blot analysis of the total Akt expression (Figure 3 a). Marked elevation of total Akt in the tumour as compared to adjacent tissue was revealed in patients 3 and 5, and moderate elevation in patient 6. Correspondingly (Figure 3 b), patients 3 and 5 were characterised by pronounced pAkt1 increase in the tumour tissue (2- and 3.4-fold, respectively); in patient 6 pAkt1 levels in the tumour and adjacent tissue were similar; in patients 1, 2 and 4, with no total Akt elevation on Western blots, pAkt1 levels in the tumour were even lower than in adjacent tissue.

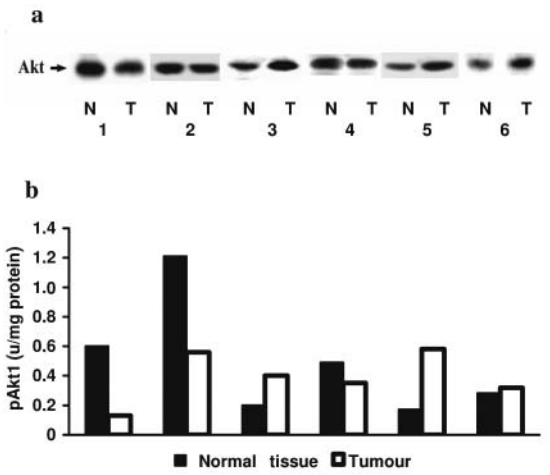

Figure 3. *(a) Western blot analysis of the total Akt (staining with Upstate, anti-Akt antibody, 05-591, USA) in breast cancer patients: T - tumour tissue, N - histologically unchanged adjacent mammary gland tissue of the same patient; the numbers indicate individual patients. (b) pAkt1 levels in the same tissues as measured with the PathScan™ PhosphoAkt1 (Ser473) Sandwich ELISA Kit.*

Discussion

Studies on cell cultures have demonstrated that the activation of the PI3K/Akt signal transduction pathway might be a necessary prerequisite for oncogenic transformation of the mammary glands (27). Many authors have published data indicating a critical role of Akt activation (imposed by its phosphorylation) in conferring a broad-spectrum chemoresistance (10) including resistance to tamoxifen (11) and herceptin (5), and radioresistance (12) on breast cancer cells. Thus, the PI3K/Akt signalling pathway is now accepted and actively explored as a novel molecular target for therapies that might help to overcome resistance to known therapeutic regiments and improve the outcome of patients with breast cancer. Much less is known about the expression and activation state of these signalling proteins in human breast cancer tissue and their role in disease prognosis and treatment sensitivity.

Except for our original publication in 1999 (14), we found no literature describing the expression of PI3K in clinical breast cancer, while there was rather an abundance of publications devoted to the clinical role of Akt (16-23). The majority of the authors used immunostaining for one or more of three known Akt isoforms and/or for phosphorylated Akt (15-23), and five research groups demonstrated the independent unfavourable prognostic value of elevated pAkt for breast cancer outcome either in a random group (15) or in various subsets of patients, namely those receiving endocrine or radiotherapy (16-18), node-negative patients (19, 20) or patients with HER2-overexpressing tumours (21).

These rather promising, but insufficient clinical data prompted us to further explore the role of Akt activation in clinical breast cancer and to compare the data with that previously obtained for PI3K. To our knowledge, this is the first study where a direct Sandwich ELISA Kit specific for Akt1 phosphorylated at Ser473 was used to assess the activation state of this kinase in the tumours and adjacent tissues of breast cancer patients, and the second publication where quantitative data on the pAkt level in breast cancer tissue are presented. In the sole quantitative investigation published in 2005 (21), a chemiluminiscence-linked immunosorbent assay (CLISA) for pAkt was used that differs from the method applied by us not only in its principle of detection, but also in the specificity of the pAkt isoforms measured: while in the CLISA system described in this paper all three Akt isoforms were detected, the design of the PathScan kit allowed us to specifically measure pAkt1.

For the interpretation of the results of both the semi-quantitative Western-blot PI3K detection and quantitative pAkt1 ELISA, we assessed two parameters: the tumour expression level and the frequency of elevation in the tumour as compared to adjacent mammary gland tissue. While PI3K was detected in 85% of breast cancer and only in about one half of adjacent mammary gland samples, measurable pAkt1 was found in all tissues studied. In contrast, PI3K expression increased in almost 80% of breast cancer samples, but only 49% of the tumours included in our study were characterized by an increased pAkt1 level as compared to adjacent normal tissue.

As described in detail in another publication (14), no statistically significant associations were revealed between the level of PI3K expression in tumour tissue and/or the extent of its activation and breast cancer main clinicopathological features including steroid receptor status and epidermal growth factor receptor expression. Meanwhile, the frequency of pAkt1 elevation was positively associated with the tumour malignancy grade: increased pAkt1 was almost 4-fold more often observed in grade III. It was also higher in more advanced (stage IIb) than in early stage breast cancer. Comparison with steroid receptor content determined using a radioligand technique revealed contradictory relationships between pAkt1 expression and two types of steroid receptors: the pAkt1 level was weakly, but significantly positively associated with ER-status, the mean Akt1 phosphorylation level in ER-positive tumours being higher than in ER-negative ones. On the other hand, no difference in the frequency of pAkt1 elevation between ER-positive and ER-negative tumour samples was found. In contrast to ER, in PgR-positive tumours the median pAkt1 level was 1.35-fold lower than in PgR-negative ones, and increased tumour pAkt1 was found twice as frequently in PgR-negative as in PgR-positive tumours. To explain these apparent contradictions, we hypothesize that the increased

pAkt1 in PgR-negative tumours might indicate impaired ER functional activity imposed by Akt kinase activation. This assumption indirectly corresponds with data demonstrating that ER-positive patients with a negative status for Akt were more sensitive to tamoxifen than ER-positive and Akt-positive patients (17). It is also substantiated by the experimental data indicating that constitutively active PI3K or Akt induced ER activity (6-8), but reduced PgR levels and activity in cultured breast cancer cells (28).

The discrepancy revealed between the clinicopathological expression patterns of PI3K and pAkt1 – two components of one signalling pathway – were rather surprising. We supposed that it might either reflect the different regulation of these two components of one signalling system in breast cancer, or be a result of the fact that in the former case (PI3K) we measured the basal expression of the enzyme's regulatory subunit, while in the latter (Akt1) the activated phosphoprotein level. However, Western blot experiments performed on a limited available number of sample pairs revealed that the increase of pAkt1 coincided with the increase of total Akt expression, indicating that pAkt1 elevation might predominantly reflect the changes in the total protein levels. This observation corresponds with the data of other authors (16, 17) who demonstrated using immunohistochemistry that pAkt staining was significantly positively associated with both Akt1 and Akt2 expression.

Conclusion

We conclude that despite the cooperative role of PI3K and Akt in the regulation of cell growth and survival their involvement in the clinical course of breast cancer might be quite different. Further follow-up and investigations are needed to clarify whether the activity of either of these signalling proteins is related to breast cancer prognosis in general or to the sensitivity of patients to various types of endocrine therapy and to assess the possibility of using corresponding inhibitors in breast cancer therapy.

Acknowledgements

This work was supported in part by the Russian Foundation for Basic Research, grants 03-03-32111 and 04-04-48458. We thank Dr. O. Anurova, Department of Pathology, Russian N. N. Blokhin Cancer Research Centre RAMS, for help with tissue collection and histological revision.

References

1 Fry MJ: Phosphoinositide 3-kinase signalling in breast cancer: how big a role might it play? Breast Cancer Res *3*: 304-312, 2001.

2 El-Deiry WS: Akt takes centre stage in cell-cycle deregulation. Nat Cell Biol *3*: E71-E73, 2001.

3 Kumar R and Hung MC: Signaling intricacies take center stage in cancer cells. Cancer Res *65*: 2511-2515, 2005.

4 Franke TF, Hornik CP, Segev L, Shostak GA and Sugimoto C: PI3K/Akt and apoptosis: size matters. Oncogene *22*: 8983-8998, 2003.

5 Le XF, Lammayot A, Gold D, Lu Y, Mao W, Chang T, Patel A, Mills GB and Bast RC Jr: Genes affecting the cell cycle, growth, maintenance, and drug sensitivity are preferentially regulated by anti-HER2 antibody through phosphatidylinositol 3-kinase-AKT signalling. J Biol Chem *280*: 2092-2104, 2005.

6 Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Ali S and Nakshatri H: Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. J Biol Chem *276*: 9817-9824, 2001.

7 Stoica GE, Franke TF, Wellstein A, Czubayko F, List HJ, Reiter R, Morgan E, Martin MB and Stoica A: Estradiol rapidly activates Akt via the ErbB2 signaling pathway. Mol Endocrinol *17*: 818-830, 2003.

8 Jordan NJ, Gee JM, Barrow D, Wakeling AE and Nicholson RI: Increased constitutive activity of PKB/Akt in tamoxifen resistant breast cancer MCF-7 cells. Breast Cancer Res Treat *87*: 167-180, 2004.

9 Sun M, Paciga JE, Feldman RI, Yuan Z, Coppola D, Lu YY, Shelley SA, Nicosia SV and Cheng JQ: Phosphatidylinositol-3-OH kinase (PI3K)/AKT2, activated in breast cancer, regulates and is induced by estrogen receptor alpha (ERalpha) *via* interaction between ERalpha and PI3K. Cancer Res *61*: 5985-5991, 2001.

10 Knuefermann C, Lu Y, Liu B, Jin W, Liang K, Wu L, Schmidt M, Mills GB, Mendelsohn J and Fan Z: HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. Oncogene *22*: 3205-3212, 2003.

11 Johnston SR, Head J, Pancholi S, Detre S, Martin LA, Smith IE and Dowsett M: Integration of signal transduction inhibitors with endocrine therapy: an approach to overcoming hormone resistance in breast cancer. Clin Cancer Res *9*: 524S-532S, 2003.

12 Liang K, Jin W, Knuefermann C, Schmidt M, Mills GB, Ang KK, Milas L and Fan Z: Targeting the phosphatidylinositol 3-kinase/Akt pathway for enhancing breast cancer cells to radiotherapy. Mol Cancer Ther *2*: 353-360, 2003.

13 Crowder RJ and Ellis MJ: Treating breast cancer through novel inhibitors of the phosphatidylinositol 3'-kinase pathway. Breast Cancer Res *7*: 212-214, 2005.

14 Gershtein ES, Shatskaya VA, Ermilova VD, Kushlinsky NE and Krasil'nikov MA: Phosphatidylinositol 3-kinase expression in human breast cancer. Clin Chim Acta *287*: 59-67, 1999.

15 Vestey SB, Sen C, Calder CJ, Perks CM, Pignatelli M and Winters ZE: Activated Akt expression in breast cancer: correlation with p53, Hdm2 and patient outcome. Eur J Cancer *41*: 1017-1025, 2005.

16 Perez-Tenorio G, Stal O and members of the Southeast Sweden Breast Cancer Group: Activation of AKT/PKB in breast cancer predicts a worse outcome among endocrine treated patients. Br J Cancer *86*: 540-545, 2002.

17 Stal O, Perez-Tenorio G, Akerberg L, Olsson B, Nordenskjold B, Skoog L and Rutqvist LE: Akt kinases in breast cancer and the results of adjuvant therapy. Breast Cancer Res *5*: R37-R44, 2003.

18 Kirkegaard T, Witton CJ, McGlynn LM, Tovey SM, Dunne B, Lyon A and Bartlett JM: AKT activation predicts outcome in breast cancer patients treated with tamoxifen. J Pathol *207*: 139-146, 2005.

19 Schmitz KJ, Otterbach F, Callies R, Levkau B, Holscher M, Hoffmann O, Grabellus F, Kimmig R, Schmid KW and Baba HA: Prognostic relevance of activated Akt kinase in node-negative breast cancer: a clinicopathological study of 99 cases. Mod Pathol *17*: 15-21, 2004.

20 Schmitz KJ, Grabellus F, Callies R, Otterbach F, Wohlschlaeger J, Levkau B, Kimmig R, Schmid KW and Baba HA: High expression of focal adheson kinase (p125FAK) in node-negative breast cancer is related to overexpression of HER-2/neu and activated Akt kinase but does not predict outcome. Breast Cancer Res *7*: R194-R203, 2005.

21 Cicenas J, Urban P, Vuaroqueaux V *et al*: Increased level of phosporylated Akt measured by chemiluminiscence-linked immunisorbent assay is a predictor of poor prognosis in primary breast cancer overexpressing ErbB-2. Breast Cancer Res *7*: R394-R401, 2005.

22 Panigrahi AR, Pinder SE, Chan SY, Paish EC, Robertson JF and Ellis IO: The role of PTEN and its signalling pathways, including AKT, in breast cancer: an assessment of relationships with other prognostic factors and with outcome. J Pathol *204*: 93-100, 2004.

23 Zhou X, Tan M, Stone Hawthorne V *et al*: Activation of the Akt/mammalian target of rapamycin/4E-BP1 pathway by ErbB2 overexpression predicts tumor progression in breast cancers. Clin Cancer Res *10*: 6779-6788, 2004.

24 Bellacosa A, de Feo D, Godwin AK *et al*: Molecular alterations of the AKT2 oncogene in ovarian and breast carcinomas. Int J Cancer *64*: 280-285, 1995.

25 Zinda MJ, Johnson MA, Paul JD *et al*: AKT-1, -2, and -3 are expressed in both normal and tumor tissues of the lung, breast, prostate, and colon. Clin Cancer Res *7*: 2475-2479, 2001.

26 Nakatani K, Thompson DA, Barthel A *et al*: Up-regulation of Akt3 in estrogen receptor-deficient breast cancers and androgen-independent prostate cancer lines. J Biol Chem *274*: 21528-21532, 1999.

27 Amundadottirl L and Leder P: Signal transduction pathways activated and required for mammary carcinogenesis in response to specific oncogenes. Oncogene *16*: 737-746, 1998.

28 Cui X, Zhang P, Deng W *et al*: Insulin-like growth factor-I inhibits progesterone receptor expression in breast cancer cells *via* the phosphatidylinositol 3-kinase/Akt/ mammalian target of rapamycin pathway: progesterone receptor as a potential indicator of growth factor activity in breast cancer. Mol Endocrinol *17*: 575-588, 2003.

Received December 13, 2006
Revised March 15, 2007
Accepted March 26, 2007