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Expression of Phospho-Akt and PTEN Proteins in Human Breast Cancer in Relation to Tumor Progression and Patient Survival

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Phosphatidylinositol 3-kinase (PI3-kinase) controls mitogenesis, cellular growth and transformation in a variety of cancers. The serine-threonine kinase Akt is a downstream target of PI3-kinase, and phosphorylated Akt (Phospho-Akt) inhibits apoptosis. Phosphatase and tensin homolog detected on chromosome ten (PTEN) is a tumor suppressor that antagonizes PI3-kinase activity, negatively regulates its downstream-target, Akt, inhibits phosphorylation of Akt, and medicates cell-cycle arrest and apoptosis. To clarify whether the PI3-kinase/Akt pathway and PTEN relate to breast cancer, we examined the expression of pathway-related proteins such as Phospho-Akt and PTEN in clinical specimens. Immunohistochemical analysis was performed on tissue specimens surgically obtained from 221 patients with breast cancer. The association of Phospho-Akt and PTEN expression with clinicopathological variables and the prognosis of patients were analyzed. Of 221 breast carcinomas, positive Phospho-Akt expression was observed in 91 (41.1%) and positive PTEN expression in 119 (53.8%). Phospho-Akt expression and loss of PTEN expression significantly correlated with tumor staging, tumor size and lymph node metastasis. Patients with Phospho-Akt-positive tumors had significantly inferior disease-free survival or over-all survival to those with Phospho-Akt-negative tumors, while those with PTEN positive tumors were better than those with PTEN negative tumors. Moreover, patients with Phospho-Akt-positive and PTEN-negative tumors had a significantly inferior disease-free survival and over-all survival compared to those with Phospho-Akt-negative and PTEN-positive tumors. Multivariate analysis revealed that expression of Phospho-Akt and tumor size were the independent factors ($P = 0.024$). We demonstrated that the expression of Phospho-Akt significantly correlated with tumor progression and patients survival with breast cancer. Phospho-Akt/PTEN expression status is possibly a definitive prognostic factor in clinical breast cancer.

Key words: breast cancer; immunohistochemistry; phosphatase and tensin homolog detected on chromosome ten; phosphorylated-Akt; prognosis

Aberrant functions of phosphatidylinositol 3-kinase (PI3-kinase) contribute to mitogenesis, cellular growth and transformation in a variety of cancers including breast cancer (Carpenter et al., 1990; Varticovski et al., 1994). PI3-kinase is activated by insulin, various growth factors

Abbreviations: ER, estrogen receptor; PBS, phosphate-buffered saline; PgR, progesterone receptor; PI3-kinase, phosphatidylinositol 3-kinase; Phospho-Akt, phosphorylated-Akt; PTEN, phosphatase and tensin homolog detected on chromosome ten

and cytokines (Burgering and Coffer, 1995), and catalyzes the phosphorylation of inositol phospholipids at the 3 position to generate phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate. Serine-threonine kinase Akt is a downstream target of PI3-kinase. Akt is phosphorylated at Thr-308 and Ser-473 by PI3-kinase, thus Akt is converted to phosphorylated Akt (Phospho-Akt) (Franke et al., 1997), which phosphorylates BAD, caspase-9, Forkhead transcription factors and IκB kinase α which correlate with apoptosis (Datta et al., 1997; Ozes et al., 1999). Akt has also been shown to inhibit the Raf-MEK-ERK pathway through phosphorylation of Raf-1 in myotubes, and to overcome constitutively activated MAPK-induced cell-cycle arrest in MCF7 cells (Rommel et al., 1999). Akt is thus an important regulator of cell proliferation and survival. Amplification of genes encoding Akt isoforms has been found in several types of human cancers. Phospho-Akt plays an important role in the development and/or progression of a subset of human cancers.

In addition, mutations in the phosphatase and tensin homolog deleted on chromosome ten (PTEN), one of the most frequently mutated tumor suppressor genes on 10q23.3, results in elevated Akt activity. Germline mutations of PTEN/MMAC1/TEP1 has been reported to correlate with Cowden syndrome, Bannayan-Riley-Ruvalcade, proteus and proteus-like syndromes (Marsh et al., 1999; Zhou et al., 2001). PTEN antagonizes PI3-kinase activity, negatively regulates its downstream-target Akt and mediates cell-cycle arrest and apoptosis. Specifically, cell line analyses have shown that PTEN appears to suppress breast cancer growth by down-regulation of PI3-kinase, with resultant G1 arrest and cell death (Weng et al., 1999). Studies of embryonic stem cells have shown that cells featuring mutations of the PTEN gene exhibited an increased growth rate and an advanced entry into S-phase (Sun et al., 1999). The accelerated G1/S transition was accompanied by down-regulation of p27, a major inhibitor of G1 cyclin-dependent kinases. Overall, results have demonstrated that PTEN regulates cell cycle progression and survival.

Breast cancer is the most common cancer in women, accounting for about 30% of all female malignancies, and is a major cause of morbidity and mortality in the female population (Landis et al., 1998). In this report, to clarify whether the PI3-kinasse/Akt pathway and PTEN are related to breast cancer, we examined the expression of pathway-related proteins such as Phospho-Akt and PTEN in clinical specimens of breast cancer.

Materials and Methods

Specimen collection

Specimens, embedded in paraffin, which had been collected from patients with breast cancer who underwent surgery at Tottori University Hospital, Yonago, and Tottori Red Cross Hospital, Tottori, between 1987 and 2002, were examined. There were 221 post-operated patients, aged from 25 to 87 years (mean, 55.5), with a mean follow-up of 67.8 months (range, 7–180). Fifty-three (23.9%) patients had a relapse and 35 (15.8%) died of the cancer, while 176 (79.6%) were alive at the time of the completion of the study (August 2003). Cancer was residual in 8 patients and the disease free survival of 13 patients was unknown. Eighty-one (41.2%) patients were at stage I, 59 (26.7%) stage IIA, 34 (15.4%) stage IIB, 27 (12.2%) stage IIIA, 1 (0.5%) stage IIIB and 9 (4.1%) stage IV. The clinicopathological findings were determined according to tumor-node-metastasis (Stmad, 1999). The patients were given adjuvant chemotherapy according to St. Gallen recommendations from 1998.

Histopathological examination

Resected specimens were fixed in 10% buffered formalin for 48 h, and then were cut into 5-mm slices. Slices were embedded in paraffin, and the tissue paraffin blocks were cut into 4-μm-thick sections for hematoxylin-eosin and immunostaining.

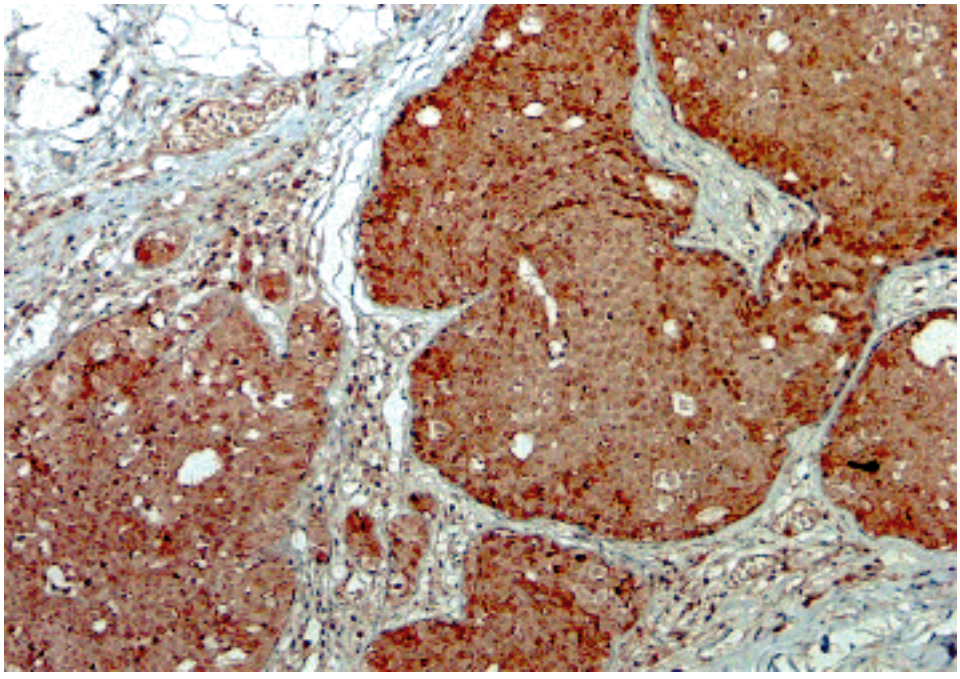

Fig. 1. Immunohistochemical staining patterns for Phospho-Akt expression in breast cancer. Phospho-Akt antigen exists in both the cytoplasm and nucleus (× 100).

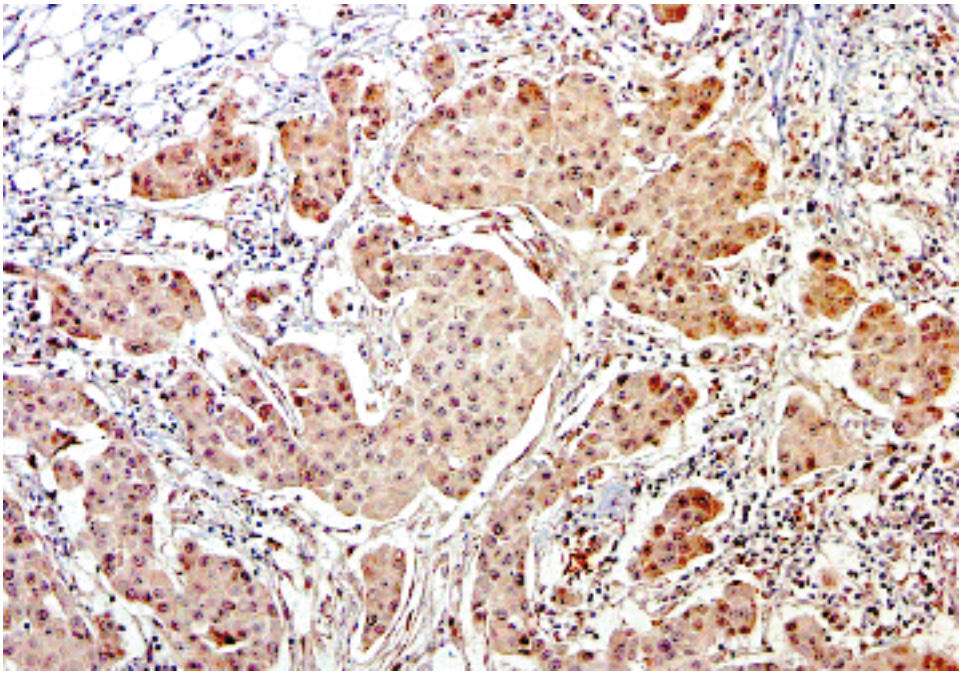

Fig. 2. PTEN expression in breast cancer. PTEN expression is found in the cytoplasm (× 100).

Immunohistochemistry for Phospho-Akt and PTEN

The 4 μm-thick sections were dewaxed using xylene and transferred to alcohol. Deparaffinized tissue sections underwent antigen retrieval with the target retrieval solution at pH 6.1 (Dako, Grostrup, Denmark) in an autoclave at 120˚C for 15 min. The slides for immunohistochemistry for PTEN were placed in citric acid buffer (10 mM)

and heated in an autoclave at 120˚C for 15 min. Endogenous peroxidase activity was blocked by incubating sections with 0.3% hydrogen peroxide in methanol for 10 min at room temperature. Slides were then washed 3 times in phosphate-buffered saline (PBS) and incubated in 10% normal goat serum for 1 h to reduce nonspecific antibody binding. Then slides were incubated overnight at 4˚C with the following primary antibodies: polyclonal antibodies raised against Phospho-Akt (Ser 473) (diluted 1:50; Cell Signaling Technology, Danvers, MA) and PTEN (diluted 1:100; Upstate Biotechnology, Lake Placid, NY). After washing the slides with PBS, biotinylated antibodies were applied as second antibody: anti-rabbit immunoglobulin (IgG + IgA + IgM) conjugated with biotin for 1 h , followed by incubation with a sterptvidin-peroxidase complex (Histofine ABC kit; Nichirei, Tokyo, Japan) for 1 h at room temperature. The reaction products were visualized with diaminobenzidine as the chromogen and the slides were counterstained with hematoxylin. All immunostained sections were evaluated in a blinded manner with no knowledge of the clinical and pathological factors. Intensity is designated as negative when less than 5% of tumor cells are stained and positive when over 5% of tumor cells are stained.

Statistical analysis

The χ^2 test was used to determine the significance of the association between different variables. The comparison of Phospho-Akt and PTEN expression with disease-free survival and over-all survival were performed by univariate and multivariate analysis using the Kaplan-Meier test and Cox proportion hazard model. The level of significance was set at $P < 0.05$.

Results

Phospho-Akt and PTEN expression

The immunoreactivity of Phospho-Akt was predominantly observed in the cytoplasm and the nucleus (Fig. 1). The expression of Phospho-Akt protein was seen in 91 (41.1%) of the breast tumors. Phospho-Akt expression significantly correlated with staging, tumor size and lymph node metastasis (Table 1). PTEN expression was found in the cytoplasm of breast cancer cells; shown in Fig. 2. Of the 221 breast tumors, 119 (53.8%) showed PTEN protein expression, which significantly correlated inversely with tumor stage, tu-

Table 1. Immunohistochemical staining for Phospho-Akt and PTEN in relation to the clinical stage in the current study

Variable	Phospho-Akt			PTEN		
	Negative	Positive	*P* value	Negative	Positive	*P* value
Age	60.7 ± 10.6	48.6 ± 10.4	< 0.05	56.0 ± 14.0	54.7 ± 10.3	0.96
Size						
2 cm ≥	80	31	< 0.05	32	79	< 0.05
2 cm <	50	60		70	40	
Lymph node metastasis						
Absent	102	32	< 0.05	47	87	< 0.05
Present	28	59		55	32	
Histological stage						
I	71	20	< 0.05	24	67	< 0.05
IIA	35	24		27	32	
IIB	12	22		25	9	
IIIA	10	7		21	6	
IIIB	0	1		0	1	
IV	2	7		5	4	

mor size and lymph node metastasis. There was no significant association between Phospho-Akt expression and loss of PTEN expression, although a tendency was observed (Table 2, $P = 0.08$).

Table 2. Association between Phospho-Akt and PTEN expression

	Positive	Negative	*P* value
Phospho-Akt	91	120	0.08
PTEN	119	102	

Disease-free survival and over-all survival rates

Patients with Phospho-Akt-positive tumor showed a significantly inferior disease-free survival rate compared to those with a negative tumor (Fig. 3A). Figure 3B shows the over-all survival curves for patients with breast cancer stratified by Phospho-Akt expression. Patients with Phospho-Akt-positive tumors showed significantly shorter over-all survivals than those with Phospho-Akt-negative tumors. Furthermore, patients with PTEN-positive expression had significantly better disease-free survival rate than those with PTEN-negative expression (Fig. 4A). There was a significant difference in over-all survival between patients with PTEN-positive and -negative expres-

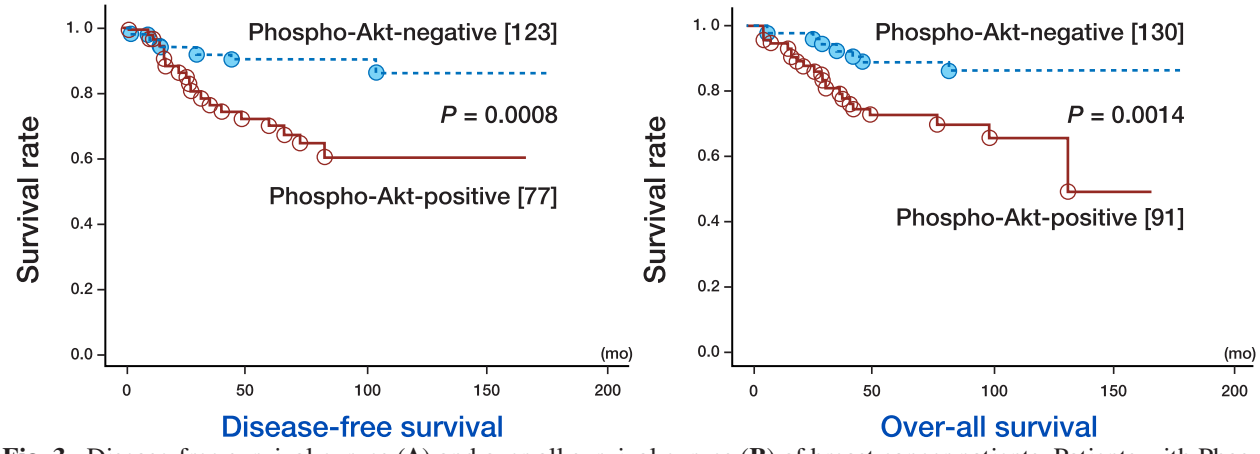

Fig. 3. Disease-free survival curves (**A**) and over-all survival curves (**B**) of breast cancer patients. Patients with Phospho-Akt positive tumors are significantly inferior to those with Phospho-Akt negative tumors in both disease-free survival and over-all survival. mo, month. [ ], number of subjects.

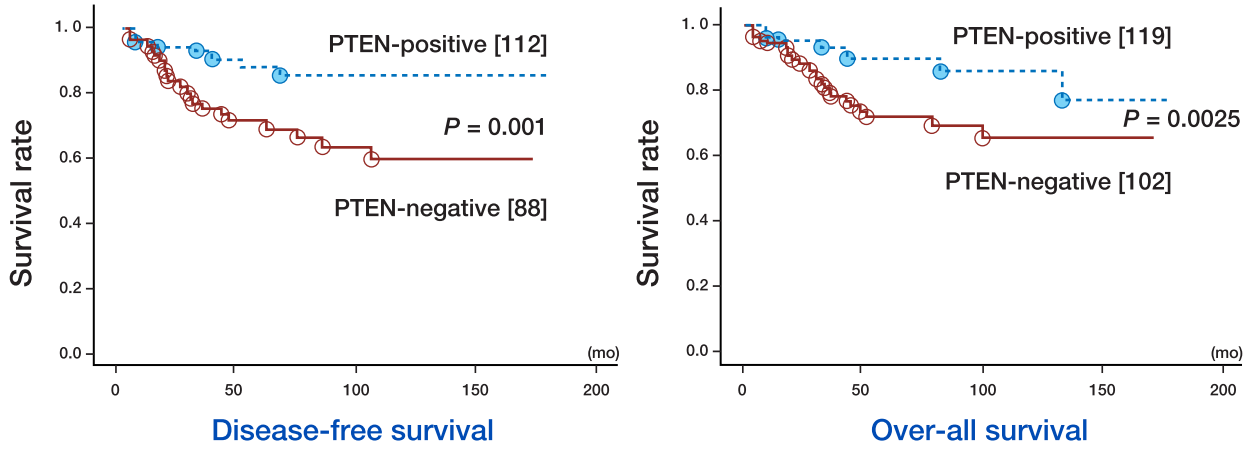

Fig. 4. Disease-free survival curves (**A**) and survival curves (**B**) of breast cancer patients. Patients with PTEN positive tumors show higher results than those with PTEN negative tumors in both disease-free survival and over all survival. mo, month. [ ], number of subjects.

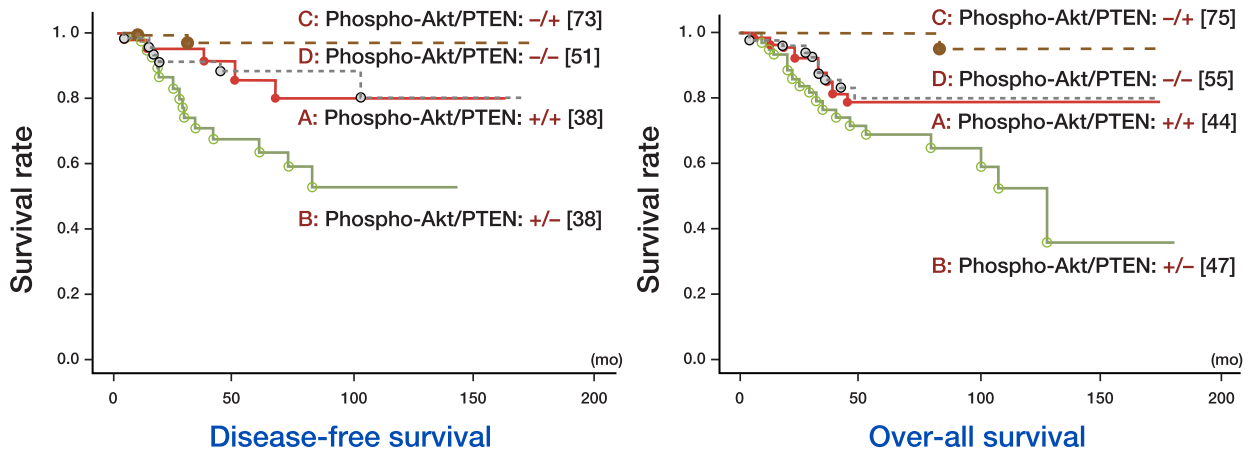

Fig. 5. Disease-free survival curves (**A**) and over-all survival curves (**B**) of breast cancer patients. The disease-free survival curve and over-all survival curve of patients who had tumors in Group B were significantly lower than those of patients who had tumors in Group C. mo, month. [ ], number of subjects; +, positive; –, negative.

sions (Fig. 4B). For further analysis of survival in relation to Phospho-Akt and PTEN expression, patients were classified into the following groups: Group A, positive expression in both Phospho-Akt and PTEN (Fig. 5A: $n = 38$, Fig. 5B: $n = 44$); Group B, positive in Phospho-Akt and negative in PTEN (Fig. 5A: $n = 38$, Fig. 5B: $n = 47$); Group C, negative in Phospho-Akt and positive in PTEN (Fig. 5A: $n = 73$, Fig. 5B: $n = 75$); and Group D, negative in both Phospho-Akt and PTEN (Fig. 5A: $n = 51$, Fig. 5B: $n = 55$). Disease-free survival and over-all survival of patients in each group are shown in Fig. 5. For both disease-free survival and over-all survival, patients in Group B had worse results than those in the other groups. Both disease-free survival and over-all survival of patients in Group C were significantly better than those in the other groups. Additionally, there was no significant association between Groups A and D in disease-free survival ($P = 0.9176$) and over-all survival time ($P = 0.8144$).

Multivariate analysis

To determine which of the many covariates in the factors listed in Table 3 were important prognostic factors of disease-free survival, a multivariate analysis with a Cox regression analysis was done. The analysis revealed that expression of Phospho-Akt and tumor size were the independent factors.

Discussion

Considerable evidence indicates that PI3-kinase regulated signaling pathways play important roles in a variety of cellular processes (Khwaja et al., 1998) and that the protein kinase, Akt, and PTEN are important mediators of these effects (Cross et al., 1995; Gingras et al., 1998; Hajduch et al., 1998; Van Weeren et al., 1998). The role of PI3-kinase in the suppression of apoptosis and the ability of Akt to directly phosphorylate key mediators of the apoptotic response provide strong

Table 3. Multivariate analysis by the Cox proportion hazard model for disease-free survival in 200 patients with breast cancer

	Hazard ratio	95% CI	*P* value
Age	0.978	0.951–1.007	0.140
Size (2 cm ≥/2 cm <)	0.243	0.086–0.683	0.007
Lymph-node metastasis	0.861	0.360–2.062	0.737
Phospho-AKT	0.368	0.154–0.877	0.024
PTEN	0.588	0.255–1.356	0.212

CI, confidence interval.

circumstantial evidence for the importance of the PI3-kinase-Akt pathway in the aberrant behavior of cancer cells. The tumor suppressor PTEN acts as a lipid phosphatase, regulates the PI3-kinase/Akt-signaling pathway, and modulates cell cycle progression and cell survival. Recent studies have shown that the most critical tumor-cell survival pathways are those mediated by PI3-kinase (Chan et al., 1999; Ling-Ping et al., 1999).

In the current study, Phospho-Akt was observed in cytoplasm and nucleus of tumor cells. Staining patterns were consistent with reports that activated Akt could translocate to the nucleus in ectopically Akt-overexpressing cells (Andjelkovic et al., 1997; Meier et al., 1997). As reported previously, the PI3-kinase pathway is an essential survival signaling pathway for various human cancers, including carcinomas of the colon, prostate, ovary, pancreas and lung (Maulik et al., 2002; Mei Sun et al., 2001; Roy et al., 2002).

It is known that patients with both estrogen receptor (ER)- and progesterone receptor (PgR)-negative tumors experienced significantly shorter disease-free survival compared to those who's tumors were positive in either or both. The relationship between a dysregulated PI3-kinase pathway and reduced ER/PgR expression in human breast cancers has been reported previously (Depowski et al., 2001; Shi et al., 2003). Kappes et al. (2001) reported that there was a significant correlation between PI3-kinase pathway abnormalities and the hormone receptors. However, there was no apparent correlation between these factors in our study (data not shown), though the precise mechanism behind this observation is not entirely clear. It has been noted by others that increased Akt mRNA correlates with ER-negative breast tumors (Nakatani et al., 1999) and increased ER α activity, which results in tamoxifen resistance (Campbell et al., 2001).

It is reported that a loss of expression of the PTEN gene protein product is associated with a poor outcome in breast cancer (Depowski et al., 2001). In the current study, PTEN expression inversely correlated with the progression of breast cancer. Patients with PTEN positive breast cancer survived significantly longer than those with a PTEN negative tumor. Furthermore, Phospho-Akt expression and loss of PTEN expression were frequently observed in patients with advanced breast cancer. Patients with Phospho-Akt-negative and PTEN-positive tumors showed the best prognoses, whereas those with Phospho-Akt positive and PTEN negative tumors showed the worst. Therefore, monitoring the status of Phosph-Akt and PTEN protein expression might predict survival in patients with breast cancer.

The Akt phosphorylation status in each carcinoma case can monitor aspects of malignancies such as cell proliferation rate, resistance to chemotherapy and irradiation, invasion and metastasis, and patient prognosis (Brognard et al., 2001; Clark et al., 2002; Itoh et al., 2002; Semba et al., 2002). At present, no specific inhibitor for Akt is available, though the PI3-kinase inhibitor, LY294002, is widely used in laboratories. It can be speculated that LY294002 inhibits some other downstream effectors of PI3-kinase in addition to Akt; the MAPK cascade and small G protein, Rac/Cdc42, are reported to be downstream of Akt (Cross et al., 1995). Izuishi et al. (2000) reported that LY294002 administration with tumor embolization may be a new therapeutic strategy for patients with pancreatic cancer. These findings suggest the possibility of treating human malignancy using the PI3-kinase inhibitor. Our results indicate that activation of the PI3-kinase pathway is a common occurrence in human breast cancers, especially in more advanced stages. Thus, regulating the PI3-kinase pathway could be of benefit and may increase the effectiveness of therapies for patients with breast cancer.

In conclusion, Phospho-Akt/PTEN expression status could be a definitive prognostic factor in clinical breast cancer. Further studies concerning the regulation of the PI3-kinase pathway in breast cancer are urgently required.

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References

1 Andjelkovic M, Alessi DR, Meier R. Role of translocation in the activation and function of protein kinase B. J Biol Chem 1997;272:31515–31524.

2 Brognard J, Clark AS, Ni Y, Dennis PA. Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation. Cancer Res 2001;61:3986–3997.

3 Burgering BM, Coffer PJ. Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. Nature 1995;376:599–602.

4 Campbell RA, Bhat-Nakshatri P, Patel NM, Constantinidou D, Nakshatri H. Phophatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. J Biol Chem 2001;276:9817–24.

5 Carpenter CL, Duckworth BC, Auger KR, Cohen B, Schaffausen BS, Cantley LC. Purificatin and characterization of phosphoinositide 3-kinase from rat liver. J Biol Chem 1990;265:19704–19711.

6 Chan TO, Rittenhouse SE , Tsichlis PN. AKT/PKB and other D3 phosphoinositide-regulated kinase: kinase activation by phosphoinostitide-dependent phosphoryylation. Annu Rev Biochem 1999;68:965–1014.

7 Clark AS, West K, Streicher S, Dennis PA. Constitutive and inducible Akt activity promotes resistance to chemotherapy, trastuzumab, or tamoxifen in breast cancer cells. Mol Cancer Ther 2002;1:707–717.

8 Cross DA, Alessi DR, Cohen P, Andjelkovich M, Hemming BA. Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature (Lond.) 1995;378:785–789.

9 Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y, et al. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 1997;91:231–241.

10 Depowski PL, Rosenthal SI, Ross JS. Loss of expression of the PTEN gene protein product is associated with poor outcome in breast cancer. Mod Pathol 2001;14:672–676.

11 Franke TF, Kaplan DR, Cantley LC, Toxer A. Direct regulation of the Akt proto-oncogene product by phosphatidylinositol 3,4-bisphosphate. Science 1997;275:665–668.

12 Gingras AC, Kennedy SG, O·Leary MA, Sonenberg N, Hay N. 4E-BP1, a repressor of mRNA translation, is phosphorylated and inactivated by the Akt (PKB) signaling pathway. Genes Dev 1998;12:502–513.

13 Hajduch E, Alessi DR, Hemming BA, Hundal HS. Constitutive activation of protein kinase Bα by membrane targeting promotes glucose and system A amino acid transport, protein synthesis, and inactivation of glycogen synthase kinase 3 in L6 muscle cells. Diabetes 1998;47:1006-1013.

14 Itoh N, Semba S, Ito M, Takeda H, Kawata S, Yamakawa M. Phosphorylation of Akt/PKB is required for suppression and cancer cell apoptosis and tumor progression in human colorectal carcinoma. Cancer 2002;94:3127–3134.

15 Izuishi K, Kato K, Ogura T, Kinoshita T, Esumi H. Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical target for cancer therapy. Cancer Res 2000;60:6201–6207.

16 Kappes H, Goemann C, Bamberger AM, Loning T, Milde-Langosch K. PTEN expression in breast and endometrial cancer: correlation with steroid hormone receptor status. Pathobiology 2001;69:136–142.

17 Khwaja A, Lehmann K, Marte BM, Downward J. Phosphoinositide-3 kinase induces scattering and tubulogenesis in epithelial cells through a novel pathway. J Biol Chem 1998;273:18793–18801.

18 Landis SH, Murray T, Bolden S, Wingon S. Cancer statistics. CA Cancer J Clin 1998;48:6–29.

19 Liang-Ping W, Wendy M, Charis E. PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. C ancer Res 1999;59:5808–5814.

20 Marsh DJ, Kum JB, Lunetta KL, Bennett MJ, Gorlin RJ, Ahmed SF, et al. PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. Hum Mol Genet 1999;8:1461–1472.

21. Maulik G, Madhiwala P, Brooks S, Ma PC, Kijima T, Tibaldi EV, et al. Activated c-Met signals through PI3K with dramatic effects on cytoskeletal functions in small cell lung cancer. J Cell Mol Med 2002;6: 539–53.

22 Mei Sun, Gen Wang, June E Paciga, Richard I Feldman, Zeng-Qiang Yuan, Xiao-Ling Ma, et al. AKT1/PKBα kinase is frequently elevated in human cancers and its constitutive activation is required for oncogenic transformation in NIH3T3 cells. Am J Pathology 2001;159:431–437.

23 Meier R, Alessi DR, Cron P. Mitogenic activation, phosphorylation, and nucler translocation of protein kinase Bβ. J Biol Chem 1997;272:30491–30497.

24 Nakatani K, Thompson DA, Barthel A. Up-regulation of Akt3 in estrogen receptor-deficient breast

cancers and androgen-independent prostate cancer lines. J Biol Pathol 1999;274:21528–21532.
25 Ozes ON, Mayo LD, Gustin JA, Pfeffer SR, Pfeffer LM, Donner DB. NFκB activation by tumor necrosis factor requires the Akt serine-theronine kinase. Nature 1999;401:82–85.
26 Rommel C, Clarke BA, Zimmermann S, Nunez L, Rossman R, Reid K, et al. Differentiation stage-specific inhibition of the Raf-MEK-ERK pathway by Akt. Science 1999;286:1738–1741.
27 Roy HK, Olusola BF, Clemens DL, Karolski WJ, Ratashak A, Lynch HT, et al. AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis. Carcinogenesis 2002;23:201–205.
28 Semba S, Itoh N, Ito M, Harada M, Yamakawa M. The in vitro and in vivo effects of 2-(4-Morpholinyl)-8-phenylchromone (LY294002), a specific inhibitor of phsphatidylinositol 3-kinase, in human colon cancer cells. Clin Cancer Res 2002;8:1957–1963.
29 Shi W, Zhang X, Pintilie M, Ma N, Miller N, Banerjee D, et al. Dysregulated PTEN-PKB and negative receptor status in human breast cancer. Int J Cancer 2003;104:195–203.
30 Stmad P. The 5th edition of the TNM classification malignant tumor of the breast. Cesk Gynecol 1999; 64:54–57.
31 Sun H, Lesche R, Li DM, Liliental J, Zhang H, Gao J, et al. PTEN modulates cell cycle survival by regulating phosphatidylinositol 3,4,5-triphosphate and Akt/protein kinase B signaling pathway. Proc Natl Acad Sci USA 1999;96:6199–6204.
32 Van Weeren PC, de Bruyn KM, de Vries-Smits AM, van Lint J, Burgering BM. Essential role for protein kinase B (PKB) in insulin-induced glycogen synthase kinase 3 inactivation. Characterization of dominant-negative mutant of PKB. J Biol Chem 1998;273:13150–13156.
33 Varticovski L, Harrison-Findik D, Keeler ML, Susa M. Role of PI3-kinase in mitogenesis. Biochim Biophys Acta 1994;1226:1–11.
34 Weng LP, Smith WM, Dahia PL, Ziebold U, Gil E, Lees JA, et al. PTEN suppresses breast cancer cell growth by phosphatase activity-dependent G1 arrest followed by cell death. Cancer Res 1999;59:5808–5814.
35 Zhou XP, Hampel H, Thiele H, Gorlin RJ, Hennekam RC, Parisi M, et al. Association of germline mutation in the PTEN tumor suppressor gene and Proteus and Proteus–like syndrome. Lancet 2001;358:210–211.

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