Activated Proteinkinase B in Breast Cancer

A. M. Shcherbakov, E. S. Gershtein, O. A. Anurova, and N. E. Kushlinskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 139, No. 5, pp. 570-572, May, 2005
Original article submitted March 16, 2005

The content of activated (phosphorylated) form of proteinkinase B in tumors and homologous tissues of 46 patients with breast cancer was measured by enzyme immunoassay. Activity of proteinkinase B was increased in tumors of 48% patients in comparison with homologous histologically unchanged tissue. Activity of proteinkinase B in hormone-dependent mammary tumors was significantly higher than in tumor tissue from patients with negative receptor status.

Key Words: *proteinkinase B; breast cancer; enzyme immunoassay*

Proteinkinase B (Akt) is a serine-threonine kinase playing an important role in the regulation of cell metabolism. Recent studies showed that Akt is the main down-stream effector of phosphatidylinositol-3 kinase (PI3K), an enzyme regulating proliferation, apoptosis, anoikis, protein synthesis, and cell survival [1,6]. The interaction of PI3K with specific lipid-binding Akt sequences, the so-called PH domains (PH: pleckstrin-homology) leads to conformation changes and activation of Akt molecule [6]. Impaired regulation of the PI3K/Akt signal route is closely interrelated with different mechanisms of malignant transformation and tumor resistance to some antineoplastic agents [1,8,9].

The interaction of PI3K regulatory subunit with cell tyrosine kinases, both nonreceptor (p60-src) and receptor ones (EGFR, VEGFR), is considered as a classical mechanism of activation of the PI3K/Akt signal route [11,13]. Experimental findings [3,13] indicate a possibility of PI3K and Akt interactions with the signal routes of estrogens, which suggests that increased Akt activity is one of the mechanisms underlying the development of hormone resistance of breast cancer [5,9].

N. N. Blokhin Russian Oncological Research Center, Russian Academy of Medical Sciences, Moscow. ***Address for correspondence:*** cesaro@front.ru. A. M. Shcherbakov

Despite numerous experimental data on the role of Akt in the maintenance of cell growth in breast tumor, its role in human tumors under clinical conditions remains unknown and published reports are contradictory. Three Akt isoforms are expressed in human tissues (Akt1/PKBα, Akt2/PKBβ, Akt3/PKBγ) [6]. The increase in Akt2 gene amplification was detected in 2.8% mammary tumors and increase of Akt2 kinase activity was observed in 30-40% cases [4,14]. Increased expression of Akt3 is attributed to the most aggressive hormone-independent phenotype of mammary tumors [10]. Changes in Akt1 expression and activity in tumors of different genesis are least studied. Recent results indicate possible activation of Akt1 in mammary (30-38% cases) and ovarian tumors (39% cases) [15].

We measured the content of activated (phosphorylated) Akt1 (phospho-Akt1) in tumors and adjacent intact tissues of patients with breast cancer and evaluated possible relationship between this parameter and the main clinical morphological factors and receptor status of the tumor.

MATERIALS AND METHODS

The study was carried out in 46 patients (35 to 75 years, median 55 years) with stages I-IV breast cancer treated at N. N. Blokhin Russian Oncological Research Center in 2002-2004. Specimens of tumor and

0007-4888/05/1395-0608

histologically intact mammary tissue (200-300 mg) were collected during surgery and immediately frozen in liquid nitrogen for further storage at -70°C.

Tissue samples for enzyme immunoassay were lyzed in 1:3 ratio in a buffer of the following composition: 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-phosphoglycerol, 1 mM sodium orthovanadate, and 1 μg/ml leupeptin. The lysates were centrifuged for 30 min at 22,000 rpm and 4°C (Optima™ TLX centrifuge, Beckman).

Activated (phosphorylated) Akt1 form (phospho-Akt1) was detected in lysates of mammary tissue specimens using PathScan Phospho-Akt1 (Ser473) Sandwich ELISA Kit (Cell Signaling Technology®) according to manufacturer's instruction. The measurements were carried out on a EL_X800 automated universal microplate analyzer (Bio-Tek Instruments, Inc.). The concentration of phospho-Akt1 in lysates was expressed in U/mg total protein (protein content was measured by the method of Lowry). Steroid hormone receptors in the tumors were detected by the standard radioligand method as described previously [2].

The values were compared using Student's *t* test, Pearson's correlation test (*r*), and Kendall's nonparametrical ranked correlation test (τ). The data were statistically processed using Statistica 6.0 and Origin 6.0 software.

RESULTS

Activated Akt1 (phospho-Akt1) form was detected in all specimens of breast cancer and corresponding histologically intact tissue. The level of phospho-Akt1 in mammary tumor tissue varied within a wide range (0.1-2.7 U/mg, median 0.4 U/mg); the levels in intact mammary tissues were 0.1-2.0 U/mg (median 0.4 U/mg). In 22 of 46 patients (48%) activity of Akt1 in the tumor was 2-470% higher than in homologous histologically intact tissue. The levels of phospho-Akt1 in mammary tumor and intact tissues were in significant positive correlation (r=0.5, p<0.05).

Clinical stage is a significant prognostic factor in breast cancer. As the study group consisted of just few patients, cases with disease stages I and IIa were united in one group (15 patients). Other groups were as follows: 10 patients with IIb stage and 9 with stages III and IV. Clinical diagnosis was confirmed by histological findings in all cases. The level of phospho-Akt1 in tumor tissue from patients with early stages of the disease varied from 0.1 to 2.7 U/mg (median 0.3 U/mg) and did not differ from that in tumors of patients with late stages (0.1-0.6 U/mg, median 0.4 U/mg). Activity of Akt1 in tumor tissues was elevated in comparison with homologous histologically intact mammary tissue in 40-60% patients in all groups. However, we found no clear-cut relationship between changes in this parameter and clinical stage of the disease. No significant relationship between phospho-Akt1 level and patient's age and menopausal status was detected either.

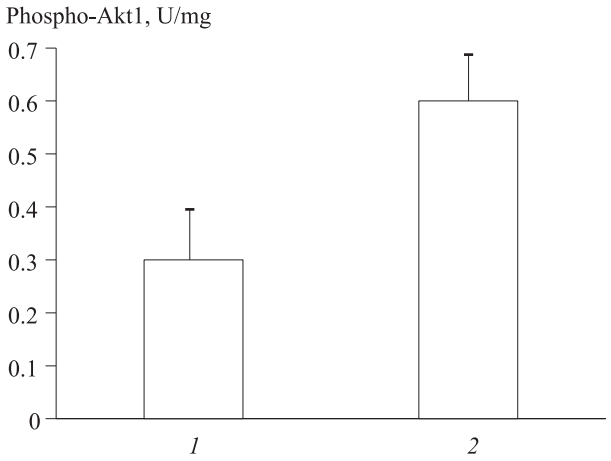

Fig. 1. Level of phospho-Akt1 in mammary tumor tissue in patients with different parameters of hormone dependence (level of estrogen receptors). *1*) patients with receptor-negative mammary tumors; *2*) patients with receptor-positive mammary tumors.

Further analysis included evaluation of possible differences in phospho-Akt1 levels in tumors of different histological structure. Increased Akt1 activity in tumor compared to histologically intact mammary tissue was observed in 11 of 22 patients (50%) with ductal invasive cancer, 4 of 6 (70%) patients with lobular invasive cancer, and in 3 of 6 (50%) patients with tumors of mixed histological structure. No significant differences in the levels of phospho-Akt1 in tissues from patients with different histological structure of the tumor were detected. The degree of tumor malignancy was as follows: 2 samples with first malignancy degree, 12 samples with second degree, and 20 with third degree. In group with second degree of tumor malignancy the level of phospho-Akt1 was higher than in histologically intact mammary tissue in 3 of 12 (25%) tumors, and in the group with third-degree malignancy the percentage of tumors with increased Akt1 activity increased to 55 (11 of 20 examined tumors). However, the differences between the groups were insignificant. Hence, no significant relationship between the degree of Akt1 activation and clinical morphological features of mammary tumors was detected.

The study of the relationship between phospho-Akt1 level and hormone dependence of the tumor (content of estrogen and progesterone receptors) was particularly interesting. The tumors were considered as receptor-positive, if the level of receptors surpassed 10 fmol/mg protein. The group of tumors positive for

estrogen receptors (ER) consisted of 23 specimens, the group of receptor-negative tumors consisted of 18 specimens. A significant correlation between the level of phospho-Akt1 and ER status (τ=0.23, p<0.05) was detected in mammary tumor tissue, activity (phosphorylation level) of Akt1 in ER-negative tumors being significantly lower than in receptor-positive tumors (p<0.05; Fig. 1). No relationship with the progesterone receptor status was revealed. These data are in line with the findings of recent research [13] and seem to indicate a possible involvement of estrogens into the regulation of PI3K/Akt signal route.

Hence, we demonstrated an increase of phospho-Akt1 (activated form of Akt1) level in 48% of studied mammary tumors, which is in line with the data on increased expression of PI3K (main inductor of Akt1) in mammary tumor tissue [7]. No significant relationship between Akt1 activation and the main clinical morphological characteristics of breast cancer were detected, but the level of phospho-Akt1 was significantly higher in ER-positive mammary tumors compared to receptor-negative tumors. We previously showed that PI3K expression was elevated in the majority (79%) of mammary tumors, but did not depend on their receptor status [7]. It can be hypothesized that the level of Akt1 activation is more closely related to hormone sensitivity of mammary cancer than the level of its inductor expression, and phospho-Akt1 is a very perspective marker of this disease. For instance, Akt is now regarded as a perspective target for purposeful antitumor therapy by many authors [5,8, 12], because modulation of this enzyme can be useful for overcoming drug, hormone, and radiation resistance of tumors.

The study was supported by the Russian Foundation for Basic Research (grant No. 03-03-32111).

REFERENCES

1. M. A. Krasil'nikov, E. V. Luzai, A. M. Shcherbakov, *et al.*, *Biokhimiya*, **69**, No. 3, 399-409 (2004).
2. N. E. Kushlinskii and E. S. Gershtein, *Mammologiya*, **3**, 15-25 (1996).
3. S. Ahmad, N. Singh, and R. I. Glazer, *Biochem. Pharmacol.*, **58**, No. 3, 425-430 (1999).
4. A. Bellacosa, D. de Feo, A. K. Godwin, *et al.*, *Int. J. Cancer*, **64**, No. 4, 280-285 (1995).
5. R. A. Campbell, P. Bhat-Nakshatri, N. M. Patel, *et al.*, *J. Biol. Chem.*, **276**, No. 13, 9817-9824 (2001).
6. T. F. Franke, C. P. Hornik, L. Segev, *et al.*, *Oncogene*, **22**, No. 56, 8983-8998 (2003).
7. E. S. Gershtein, V. A. Shatskaya, V. D. Ermilova, *et al.*, *Clin. Chim. Acta*, **287**, Nos. 1-2, 59-67 (1999).
8. W. Jin, L. Wu, K. Liang, *et al.*, *Br. J. Cancer*, **89**, No. 1, 185-191 (2003).
9. N. J. Jordan, J. M. Gee, D. Barrow, *et al.*, *Breast Cancer Res. Treat.*, **87**, No. 2, 167-180 (2004).
10. K. Nakatani, D. A. Thompson, A. Barthel, *et al.*, *J. Biol. Chem.*, **274**. No. 31, 21,528-21,532 (1999).
11. D. J. Price, T. Miralem, S. Jiang, *et al.*, *Cell Growth Differ.*, **12**, No. 3, 129-135 (2001).
12. K. Soderlund, G. Perez-Tenorio, and O. Stal, *Int. J. Oncol.*, **26**, No. 1, 25-32 (2005).
13. G. E. Stoica, T. F. Franke, A. Wellstein, *et al.*, *Mol. Endocrinol.*, **17**, No. 5, 818-830 (2003).
14. M. Sun, J. E. Paciga, R. I. Feldman, *et al.*, *Cancer Res.*, **61**, No. 16, 5985-5991 (2001).
15. M. Sun, G. Wang, J. E. Paciga, *et al.*, *Am. J. Pathol.*, **159**, No. 2, 431-437 (2001).