ONCOLOGY

Expression of Phosphatidylinositol-3 Kinase in Lung Cancer

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The expression of phosphatidylinositol-3 kinase in tumors and homologous tissues from 29 patients with lung cancer, 5 patients with lung metastases of various tumors, and some non-tumorous pulmonary diseases was studied by Western blot analysis. The expression of phosphatidylinositol-3 kinase was increased in these tumors in comparison with histologically intact lung tissue in 5 patients with non-small-cell cancer. In 20 patients expression of phosphatidylinositol-3 kinase was the same as in homologous tissue and in 4 patients it was decreased. No relationship between phosphatidylinositol-3 kinase expression and clinical and morphological characteristics of lung cancer was revealed.

Key Words: *phosphatidylinositol-3 kinase; lung cancer; Western blot analysis*

Phosphatidylinositol-3 kinase (PI-3K), the enzyme phosphorylating the inositol ring in the D-3 position, is now considered to be one of the most important regulatory proteins, as it is situated at the crossing of various signal pathways and controls cell functions [1, 11], in particular proliferation [9,11], apoptosis [7], migration [10], and cytoskeleton reorganization [6]. PI-3K consists of two subunits: catalytic (p110) and regulatory (p85) [4]. Phosphatidylinositol mono-, bi-, and triphosphates (main PI-3K products) are precursors of second messengers inositol phosphates and diacylglycerol [8]. Activation of PI-3K is mediated by various mechanisms, including phosphorylation by activated growth factor receptors after their interaction with the corresponding ligands [11]. PI-3K plays a special role in tumor transformation. It possesses intrinsic oncogenic activity [3] and forms complexes with some viral and cellular oncoproteins, whose transforming potential can be realized only in the presence of PI-3K in the cell [2].

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Despite numerous persuasive experimental data on the role of PI-3K in carcinogenesis, its importance for the growth and progress of human tumors remains poorly understood. We previously showed [5] that in the majority of breast cancer patients PI-3K expression in tumors increased in comparison with homologous histologically intact tissue and that these changes did not depend on the main clinical and morphological characteristics of the tumor and its sensitivity to hormones [5].

The aim of the present study was to find out whether the enhanced expression of PI-3K was characteristic of tumors of different origin and aggressiveness. To this end we carried out Western blot analysis of tumors and homologous intact tissues in patients with lung cancer (LC).

MATERIALS AND METHODS

Tumor specimens and histologically intact lung tissue (100-300 mg) were collected during operations from 34 patients treated in Thoracal Oncology Department of N. N. Blokhin Cancer Research Center: 29 patients with LC, 3 patients with metastases of other malignant

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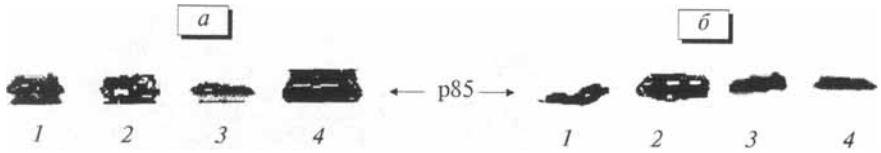

Fig. 1. Expression of phosphatidylinositol-3 kinase in tumor (*2*, *4*) and homologous normal tissue (*1*, *3*). *a*) patients with lung cancer; *b*) patient with breast cancer (*1*, *2*) and patient with lung cancer (*3*, *4*).

tumors to the lungs, 1 patient with tuberculosis, and 1 patient with Boeck sarcoid. The specimens were immediately frozen in liquid nitrogen and stored at -70°C.

Twenty-four men and 5 women aged 44-70 years (mean age 59.5 years) were included in the study. Twelve patients presented with stage I, 14 with stage III, and 3 with stage IV of the disease. Squamous-cell cancer was diagnosed in 19 and adenocarcinoma in 7, other patients had various forms of LC including operable small-cell lung cancer. Central LC was found in 15 and peripheral LC in 10 patients.

For immunoblotting, tissue specimens were treated with a buffer containing: 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM dithiotreitol, 0.1 mM sodium orthovanadate, 1 mM phenylmethylsulfonyl fluoride, 1% aprotinine, and 1% NP-40 detergent (1:2 tissue-buffer ratio) and centrifuged in an Optima TLX centrifuge (Beckman) for 15 min at 15,000 rpm and 4°C. Aliquots containing 100 μg protein were separated by electrophoresis in 8% PAAG, and proteins were transferred to nitrocellulose filters. The filters were hybridized with first antibodies to PI-3K regulatory subunit p85 (Sigma) and the resultant complexes were developed using ECL reagent (Amersham). The blots were scanned using an Image Quant software and after scanning classified into 4 categories: high, medium, low, or no expression.

RESULTS

Figure 1, *a* illustrates a typical experiment for 2 tumors and normal tissues. One patient had increased expression of PI-3K in the tumor in comparison with intact lung tissue (tracks *3* and *4*), while in the other PI-3K levels in both samples were virtually the same (tracks *1* and *2*).

In all patients PI-3K was found in both the tumor and normal lung tissue irrespective of the diagnosis, but high level of its expression was observed rarely: in only one adenocarcinoma (Fig. 1, *a*, track *4*) and lung tuberculosis. Expression of PI-3K was moderate in 11 samples of intact lung tissue, in 11 samples of LC, in 1 metastasis, and in 1 Boeck sarcoidosis. Activation of PI-3K was detected in 5 patients with LC and was pronounced in only 1 patient (Fig. 1, *a*, tracks *3* and *4*). In 20 patients with LC the expression of PI-3K was the same in the tumor and homologous tissue, and in 4 patients it was lower than in normal lung tissue. Interestingly, PI-3K expression was enhanced in tuberculosis and Boeck sarcoidosis, but not in lung metastases.

Our findings on PI-3K expression in non-small-cell LC principally differed from those in breast cancer: PI-3K was activated in 79% breast cancer specimens and in half of these its expression was high or very high, while in 42% intact breast tissue samples it was absent [5]. Figure 1, *b* presents typical Western blots for breast cancer (pronounced activation of PI-3K, tracks *1* and *2*) and LC samples (no changes, tracks *3* and *4*).

All LC patients presented with stage III with high expression of PI-3K in the tumor; there were 3 adenocarcinomas and 2 squamous-cell cancers. Two patients had highly differentiated and 2 moderate differentiated tumors, in 1 patients (with the highest activation of PI-3K) adenocarcinoma was characterized by a mixed differentiation type. No appreciable activation of PI-3K was detected in one examined sample of small-cell LC, though experiments with several cultures showed that high basal activity of PI-3K in these cells promotes their active proliferation [9].

On the whole, no significant relationships between the expression and activation of PI-3K and clinical and morphological features of LC were found.

Hence, activation of PI-3K during tumor transformation is organ- or tissue-specific. In spite of the fact that PI-3K plays an important role in the regulation of cell growth and survival and possesses a high oncogenic potential (which previously allowed us to regard increased expression of PI-3K as a fundamental characteristics of breast cancer), only 18% non-small-cell lung cancer specimens expressed PI-3K and this expression was negligible and did not correlated with clinical manifestations of the disease.

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REFERENCES

1. M. A. Krasil'nikov, *Biokhimiya*, **65**, No. 1, 68-75 (2000).
2. L. Amundadottirl and P. Leder, *Oncogene*, **16**, 737-746 (1998).
3. H. Chang, M. Aoki, D. Fruman, *et al.*, *Science*, **276**, 1848-1850 (1997).

4. J. Escobedo, S. Navankasattusas, W. Kavanaugh, *et al., Cell,* **65,** 75-82 (1991).
5. E. S. Gershtein, V. A. Shatskaya, N. E. Kushlinsky, *et al., Clin. Chim. Acta,* **287,** 59-67 (1999).
6. R. Kapeller, R. Chakrabarti, L. C. Cantley, *et al., Mol. Cell. Biol.,* **13,** 6052-6063 (1993).
7. S. Kennedy, A. Wagner, S. Conzen, *et al., Genes Dev.,* **11,** 701-713 (1997).
8. S. J. Leevers, B. Vanhaesebroeck, and M. D. Waterfield, *Curr. Opin. Cell. Biol.,* **11,** 219-225 (1999).
9. S. M. Moore, R. C. Rintoul, T. R. Walker, *et al., Cancer Res.,* **22,** 5239-5247 (1998).
10. B. Vanhaesebroeck, G. E. Jones, W. E. Allen, *et al., Nature Cell Biol.,* **1,** 69-71 (1999).
11. B. Vanhaesebroeck and M. D. Waterfield, *Exp. Cell. Res.,* **253,** 239-254 (1999).