ONCOLOGY

Expression of Estrogen Receptors-α and -β in Primary Breast Neoplasms and Tumors Exposed to Neoadjuvant Hormonal Therapy

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We studied the content and expression of mRNA for estrogen receptors-α and -β in breast tumors before and after 3-month neoadjuvant hormone therapy with antiestrogen tamoxifen and/or aromatase inhibitors. Expression of estrogen receptors-α and -β was most often detected in ER^+PR^+ tumors and most significantly decreased in these neoplasms after exemestane therapy. Immunocytochemical and radioligand assays showed that tamoxifen and anastrozole have little effect on the number of estrogen receptors-α. The number of progesterone receptors in tumors decreased by the end of anastrozole therapy. Estrogen receptors-β were immunocytochemically revealed in 50% primary breast tumors. Anastrozole slightly decreased, while tamoxifen increased the incidence of these receptors. Interruption of signaling through estrogen receptors and suppression of estrogen biosynthesis had different effects on the receptor status of neoplasms and distribution of estrogen receptors-α and -β.

Key Words: *estrogen receptors-α and -β; breast cancer; hormonotherapy*

Estrogen signals are transduced through two receptor proteins, estrogen receptors-α (ER-α) and -β (ER-β). The role of these receptors in normal and pathological processes was revised. Much attention was given to this problem. However, the role of ER-β in hormonal carcinogenesis (*e.g.*, in breast tissue) and other processes remains unclear [7]. It was hypothesized that accumulation of ER-β in breast tumor tissue is associated with poor prognosis and low efficiency of hormonal therapy [10]. Further studies showed that ER-β can mediate the inhibition of proliferation and invasion of breast carcinoma cells [4] and have a favorable prognosis for the patients [6,7]. It is necessary to evaluate common and specific mechanisms of regulation of ER-α and ER-β by estrogens and antiestrogens. This problem was studied *in vitro* [9,11]. A relationship exists between the number of ER-α and ER-β and intensity of estrogen synthesis in primary breast carcinoma (aromatase activity) [1]. Here we studied the ER-α/ER-β ratio in breast cancer patients receiving antiestrogens and aromatase inhibitors.

MATERIALS AND METHODS

Breast tumor tissues were obtained by trephine biopsy before therapy and during surgery. The patients received neoadjuvant hormone therapy with aromatase inhibitors (exemestane/Aromasin, daily dose 25 mg; anastrozole/Arimidex, daily dose 1 mg) or antiestrogen (tamoxifen, daily dose 20 mg) for 3-4 months. The duration of menopause was not less than 2 years. Ali-

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TABLE 1. Expression of mRNA for ER-α and ER-β in Breast Tumors before and after Therapy with Steroid Aromatase Inhibitor Exemestane (%)

Tumor	Primary (*n*=24)			After exemestane therapy (*n*=12)		
	ER-α	ER-β	ER-α/ER-β	ER-α	ER-β	ER-α/ER-β
Total	59.1	31.8	1.9	50.0	25.0	2.0
ER^+PR^+ phenotype	72.7	54.5	1.3	55.5	22.2*	2.5
Other phenotypes	45.4	9.1	5.0	33.3	33.3	1.0

Note. *n*, number of examined tumors. *Significant differences from primary tumors.

quots of tumors were immediately frozen in liquid nitrogen to study expression of ER-α and ER-β. The number of ER and progesterone receptors (PR) was measured by the competitive radioligand method with tritium-labeled estradiol and progesterone (Amersham) [8]. Other samples were embedded into paraffin for morphological and immunocytochemical study.

Molecular and genetic studies of the expression of ER-α and ER-β involved the reverse transcription-polymerase chain reaction (RT-PCR). Samples of tRNA were isolated by the guanidine thiocyanate method from 22 primary neoplasms with various receptor phenotypes and 12 tumors of treated patients. The conditions for cDNA synthesis and amplification of reverse transcription products, structure of primers, and principles for evaluation of the results were described previously [1].

Immunocytochemical study of ER-β in 24 patients was performed before and after therapy with tamoxifen (*n*=8), anastrozole (*n*=9), or both preparations (*n*=7). Polyclonal N-19 antibodies (Santa Cruz Biotechnology) were used [5]. Sections (4 μ) were prepared from paraffin samples of tumors with the known content of ER-α and PR. Tissue sections were deparaffinized with xylene and alcohols. Endogenous peroxidase was suppressed by 3% H_2O_2 in methanol. Blockade of nonspecific binding and incubation with primary antibodies (dilution 1:100) were performed at 4°C overnight. Primary antibodies were not used in the reaction with control sections. The reaction with Vecstain secondary antibodies (biotinylated antibodies, Vector Laboratories, ABC kit), incubation with avidin-biotin-peroxidase complex, and detection of peroxidase with diamine benzidine were performed on the next morning. The results were assessed visually by 2 researchers and expressed as positive, moderately positive, and negative staining. The data for groups of positive and moderately positive staining were summarized. The results were classified as ER-β-positive and ER-β-negative, respectively.

RESULTS

Expression of mRNA for ER-α and ER-β genes was most typical of primary breast neoplasms (ER^+PR^+ phenotype), but was rarely detected in tumors with another receptor status (especially ER-β, Table 1). Probably, ER-β more different in tumors containing ER-α. The presence of ER-β can contribute to interruption of signaling through ER and lower induction of PR [1,7]. Neoadjuvant hormonal therapy with steroid aromatase inhibitor exemestane most significantly decreased expression of ER-α and ER-β in ER^+PR^+ tumors. It should be emphasized that expression of ER-β in other tumors did not decrease and even increased, which modified the ratio between the incidence of expressed of ER-α mRNA to ER-β mRNA (Table 1).

Competitive radioligand study showed that therapy with tamoxifen, nonsteroid aromatase inhibitor anastrozole, or both preparation for 3-4 months has little effect on the number of ER-α. The number of PR^+ tumors by the end of neoadjuvant hormonal therapy was much lower than in patients receiving anastrozole (Table 2), which is consistent with published

TABLE 2. Incidence of PR and ER-β after Neoadjuvant Therapy with Tamoxifen and Anastrozole

Preparation	PR^+		$ER\text{-}\beta^+$	
	before therapy	after therapy	before therapy	after therapy
Tamoxifen	77.7	50.0	37.5	77.7*
Anastrozole	66.6	22.2*	66.6	50.0
Tamoxifen and anastrozole	62.5	12.5*	42.8	50.0

Note. *Significant differences from pre-therapy parameters.

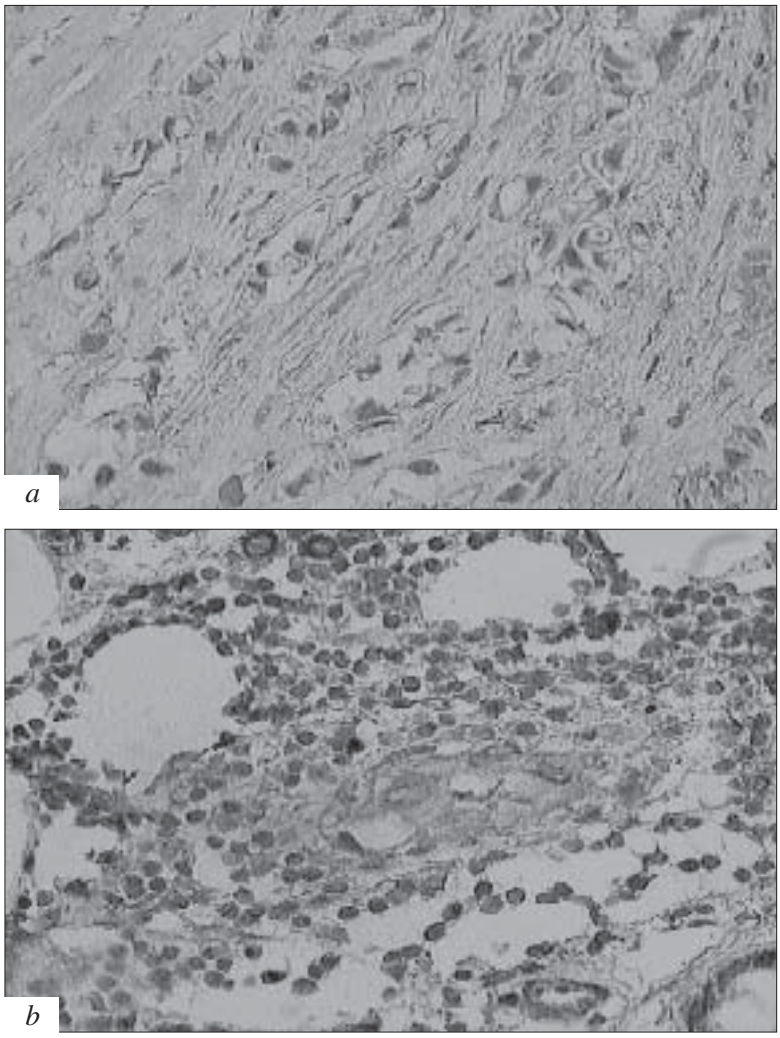

Fig. 1. Immunocytochemical detection of estrogen receptors-β (ER-β) in breast tumor sections (N-19 antibodies): negative reaction (*a*); ER-β in tumor tissue (*b*).

data [3]. Immunocytochemical study revealed ER-β in 50% primary breast neoplasms. Anastrozole slightly decreased, while tamoxifen increased the incidence of these receptors (Table 2).

The present study showed that interruption of signaling through ER by antiestrogen preparation tamoxifen and treatment with inhibitors of estrogen synthesis in tumor tissue have different effects on the receptor status (incidence of ER-β). Differences were revealed in the effect of steroid (exemestane) and nonsteroid aromatase inhibitors (anastrozole) on the distribution of ER-α and ER-β in the target tissue. Our results are consistent with published data that these preparations produce different endocrine effects [2-4]. The pharmacological approach confirmed the existence of functional differences between ER-α and ER-β [1,7]. These data can be used in clinical practice.

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REFERENCES

1. L. M. Bershtein, T. S. Zimarina, T. E. Poroshina, *et al*., *Byull. Eksp. Biol. Med*., **136**, No. 11, 550-552 (2003).
2. *Aromatase Inhibition and Breast Cancer*, Eds. W. R. Miller and R. J. Santen, New York, Basel (2001).
3. *Endocrine Therapy in Breast Cancer*, Eds. W. R. Miller and J. N. Ingle, New York, Basel (2002).
4. G. Lazennec, D. Bresson, A. Lucas, *et al*., *Endocrinology*, **142**, 4120-4130 (2001).
5. Y. Miyoshi, T. Taguchi, J. A. Gustafsson, and S. Noguchi, *Jpn. J. Cancer Res*., **92**, 1057-1061 (2001).
6. Y. Omoto, S. Inoue, S. Ogawa, *et al*., *Cancer Lett*., **163**, 207-212 (1998).
7. C. Palmieri, G. J. Cheng, S. Saji, *et al*., *Endocr. Relat. Cancer*, **9**, No. 1, 1-13 (2002).
8. M. Saez, P. Martin, and C. D. Chouver, *Cancer Res*., **38**, 3468-3473 (1978).
9. H. S. Seo, D. Larsimont, G. Querton, *et al*., *Int. J. Cancer*, **78**, No. 6, 760-765 (1998).
10. V. Speirs, A. T. Parkes, M. Kerin, and S. L. Atkin, *Cancer Res*., **59**, 525-528 (1999).
11. E. A. Vladusic, A. E. Hornby, F. K. Guerra-Vladusic, *et al*., *Oncol. Rep*., **7**, No. 1, 157-167 (2000).