Expression of Estrogen Receptors-β and Aromatase Activity in Primary Mammary Gland Tumors

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Expression of estrogen receptors α and β was studied by the PCR method in 22 primary receptor-positive or receptor-negative breast carcinomas obtained during surgical intervention from patients aged 41-77 years and activity of aromatase in the same specimens was evaluated by the formation of tritiated water from labeled androgen precursor. Expression of estrogen receptors α and β was more often detected in receptor-positive tumors characterized by lower aromatase activity. The authors conclude that the intensity of local production of estrogens can be one of the regulators of their expression, limiting this expression in case of more active production of estrogen in the tumor. On the other hand, there were no differences in the expression of estrogen β-receptor gene or in detection of this receptor by immunocytochemical method in primary tumors lacking one of these receptors, and hence, this form of estrogen receptors is less involved in induction of progesterone receptors than α-receptors.

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

Estrogen α-receptors (ER-α) were considered to be the only type of estrogen receptors for a long time. This receptor protein belongs to the so-called nuclear receptors. After binding the appropriate ligand and dimerization it is translocated into the nucleus and induces expression of estrogen-dependent genes. Recently discovered estrogen β-receptor (ER-β) presumably acts not only via the genomic pathway but, similarly to ER-α, through membrane receptors. On the other hand, ER-β differs from ER-α by its functional characteristics, including the involvement in transfer of the estrogen-induced mitogen signal [8], which explains the necessity of a comparative study of ER-α and ER-β in receptor-negative and receptor-positive tumors, first of all in breast cancer. This latter fact is explained by the role of ER in hormonal carcinogenesis in the mammary gland and clinical prediction of the disease course and outcome and selection of effective individual hormone therapy [2-4]. It is also noteworthy that the data on the relationship between ER-α content in tumor tissue and production of estrogens in this tissue (local estrogen production) are scanty [1,5,9], while for ER-β such data are virtually absent.

MATERIALS AND METHODS

Breast tumor specimens were collected during surgical intervention in 22 patients (aged 41-77 years) who received no treatment before surgery. Aliquots of resected tumors were immediately frozen in liquid nitrogen for subsequent measurements of ER and progesterone receptors (PR), aromatase (estrogen synthetase) activity, and expression of ER-α and ER-β. From 12 other tumors (in addition to measurements of ER and PR) 4-μ sections were made, deparaffinated, and immunocytochemical staining for ER-β with N-19 polyclonal antibodies (Santa Cruz Biotechnology) was carried out by the method described by Y. Miyoshi *et al.* [10]. The results were evaluated in points.

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ER and PR were measured by the radiocompetitive method [11] using labeled estradiol and PR (Amersham). Aromatase activity was evaluated by the formation of heavy water from androstendione labeled with tritium in 1β-position by the modified method of N. Tilson-Mallett *et al.* [13].

Total RNA for molecular genetic studies was isolated from breast tumor tissue by the guanidine thiocyanate method. cDNA was synthesized under the following conditions: RNA was added in the reaction mixture (20 μl) to a final concentration of 0.1 μg/μl. The concentrations of other components were as follows: 50 mM Tris HCl (pH 8.3), 40 mM KCl, 8 mM $MgCl_2$, 1 M DTT, 500 μM dNTP, and 2.5 μM random primers (Sigma). The samples containing RNA and random primers were incubated at 72°C for 10 min, after which they were transferred onto ice, and RNase inhibitor was added to a final concentration of 1 U/μl and reverse transcriptase (of the Enhanced avian RT type, Sigma) was added to a final concentration of 1 U/μl. The mixture was incubated for 1 h at 43°C. The reaction efficiency was controlled by amplification of glyceraldehyde-3-phosphate dehydrogenase gene.

The reverse transcription product (1 μl) was amplified by PCR in 20 μl of reaction mixture of the following composition: 20 μM Tris HCl (pH 8.55), 16 μM $(NH_4)_2SO_4$, 2.5 μM $MgCl_2$, 200 μM dNTP, 0.3 μM oligonucleotides, and 0.125 U Taq DNA polymerase (Gelikon). Expression of ER-α and ER-β gene was studied as recommended by E. F. Foley *et al.* [6]. The following primers were used: 5"GCCTTGTTG GATGCTGAG 3' and 5' GAGGCACACAAACTCC TC 3' (size of amplified product 393 b. p.) for ER-α, and 5' CCTGCTGTGATGAATTACAG 3' and 5' TT CTCTGTCTCCGCACAAG 3' (product size 540 b. p.) for ER-β. The reaction conditions for ER-α: 1 min denaturing at 95°C, 1 min annealing at 58°C, 1 min synthesis at 72°C (37 cycles); for ER-β: 1 min denaturing at 95°C, 1 min annealing at 61°C, 1.5 min synthesis at 72°C (45 cycles). The resultant PCR products were analyzed in 2% agarose gel stained with ethidium bromide and photographed (Fig. 1). The results were evaluated visually in points as positive, weakly positive, and negative.

RESULTS

Based on the results of detection of steroid hormone receptors (the sample was considered "positive" if it contained 10 fmol/mg receptor protein), the tumors were subdivided into 2 groups: 1) receptor-positive tumors ER^+PR^+ (n=11) and 2) tumors (n=11) laking one (ER^+PR^- and ER^-PR^+) or both receptors (ER^-PR^-). Further analysis showed markedly higher incidence of ER-α (72.7%) and ER-β (54.5%) expression in group 1 tumors in comparison with group 2 tumors (45.5 and 9.1%, respectively). By contrast, aromatase activity in group 1 tumors was virtually 2-fold lower than in group 2 (Table 1).

Analysis of 12 other tumors with ER^+PR^- and ER^-PR^+ phenotypes (6 tumors of each phenotype) showed positive or weakly positive immunocytochemical staining for ER-β with equal frequency (3 of 6 cases). PCR analysis of ER-β expression in similar tumors also showed no appreciable differences: ER-β expression was not detected in 5 ER^+PR^- tumors and was detected in only 1 of 5 ER^-PR^+ carcinomas. On the other hand, in tumors containing PR but lacking ER aromatase activity tended to increase (13.3 fmol/mg protein/h *vs.* 8.7 fmol/mg protein/h).

Our findings and published reports [7,12] indicate that ER-β expression is most often detected in the tumors with ER-α expression. Previous data on the relationship between ER expression and aromatase activity in mammary carcinomas concerned only ER-α [1,5,9]. Experiments on breast cancer cell strains showed that addition of estradiol into the incubation medium inhibitied expression of ER-α, but stimulated expression of ER-β [14].

Our data indicate that higher activity of aromatase in breast tumors is associated with more rare expression of both ER-α and ER-β. This suggests that the regulatory effect of local estrogen production under *in vivo* conditions can be directed to both ER types. Despite similar incidence of immunocytochemical detection of ER-β in ER^+PR^- and ER^-PR^+ tumors, the latter phenotype was associated with higher aromatase activity. This probably indicates that intratumor estrogens retain the capacity to PR induction even in the

TABLE 1. Incidence of Expression of ER-α and ER-β and Aromatase Activity in Breast Tumors with Different Phenotype

PCR results	ER^+PR^+ tumors			ER^+PR^-, ER^-PR^+, and ER^-PR^- tumors		
	ER-α expression, number of cases	ER-β expression, number of cases	aromatase activity, fmol/mg protein/h	ER-α expression, number of cases	ER-β expression, number of cases	aromatase activity, fmol/mg protein/h
Positive	4	3	5.4±1.07	2	1	
Weakly positive	4	3		3	0	10.6±2.6
Negative	3	5		6	10	

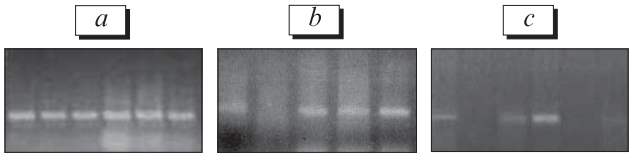

Fig. 1. Positive and negative results of PCR used for evaluating the expression of estrogen a- (*b*) and b-receptors (*c*) in breast cancer tissue. *a*) expression of glyceraldehyde-3-phosphate dehydrogenase gene (control).

absence of estrogen ER-α in these tumors, while the role of ER-β in PR biosynthesis is less important than that of ER-α [10].

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