Estrogen receptor beta (ERβ) expression in breast carcinomas is not correlated with estrogen receptor alpha (ERα) and prognosis: The Greek experience

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Summary

Estrogen receptors (ER) are members of the nuclear receptor superfamily of ligand-activated transcription factors and mediate the effects of estrogen on target tissues. ERα was the first estrogen receptor to be characterized, and ERβ was identified ten years later. The role of ERβ in breast cancer pathobiology is largely unknown because specific antibodies have not been available until recently. The purpose of this study was to explore the expression of ERβ in breast neoplasms and to correlate it with ERα and prognosis. ERα and ERβ expression was monitored immunohistochemically in 59 breast carcinomas. We found no correlation between ERα and ERβ expression, between ERβ expression and the known prognostic indicators such as tumor size, grade or lymph node status, or between ERβ expression and survival. Our findings contribute to the better understanding of the role of ERβ in breast cancer.

Key words: Estrogen receptor beta; Alpha; Breast; ERβ; ERα.

Introduction

Estrogens are important steroid hormones that are critical for the development, maintenance and function of many tissues such as the male and female reproductive tract, the gastrointestinal tract, the mammary tissue, the skeletal, nervous and immune system. The physiological responses of estrogen are mediated within specific tissues by at least two estrogen receptors (ERs), ERα and ERβ [1].

Estrogen receptors are members of the nuclear receptor superfamily of ligand-activated nuclear transcription factors. Estrogen binding leads to translocation of the ligand-receptor complex to the nucleus, where it binds to specific DNA recognition sequences (estrogen response elements) within the promoter region of target genes and to initiation of downstream response [2]. ERα was the first estrogen receptor to be characterized [3, 4], and ten years later, ERβ was sequenced from rodents and human testes [5-7].

Studies on the receptors' mode of action, expression patterns and tissue distribution indicate that they exert similar as well as different physiological actions, depending on the circumstances. ERα and ERβ are highly homologous, although they are products of different genes [8, 9]. Upon ligand binding ERs dimerize (ERα/ERα, ERβ/ERβ and ERα/ERβ). It has been shown that ERβ in the presence of estrogens suppresses transcriptional activation and so modulates the proliferating effects of ERα [10-14]. It is speculated that ERα and ERβ may regulate genes in different ways and their relative levels may influence cellular responses to estrogens [15].

ERα was for a long time believed to be the only estrogen receptor and its role in development as well in carcinogenesis, with a notable example being breast cancer, is fairly well characterized. The discovery of ERβ has complicated the picture of estrogen's role in cancer pathobiology. Most studies so far have focused on the expression of ERβ mRNA, since specific antibodies for the detection of ERβ expression, in formalin-fixed paraffin-embedded material have become available only recently. The first studies on the expression of ERβ protein have shown that ERβ protein expression declines in ovarian, prostatic and colon carcinomas [16-21].

The role of ERβ expression in breast cancer remains controversial. Studies with ERβ mRNA have failed to agree as to whether ERβ is a good or poor prognosticator in breast tumors [22]. The expression of ERβ protein in malignant breast lesions has only recently started to be examined and the results are not clear yet. In order to reach a definite conclusion on the role of this second estrogen receptor in breast cancer, and to a widely accepted antibody-detection technique in formalin-fixed paraffin-embedded material, which is the majority of available samples, it is necessary to search for and report ERβ protein expression in different laboratories and ethnic groups.

The aim of the present study was to investigate the expression of ERβ in breast carcinomas of various types and grades, and to correlate it with ERα expression and prognosis.

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Materials and Methods

Samples

We studied 59 breast neoplasms including 44 ductal carcinomas (1 grade 1, 25 grade 2 and 18 grade 3), 11 lobular and 4 medullary carcinomas. The median age of the patients was 58.5 years (range 31-83). Information on size and node status was available for all. In 49 patients follow-up was available.

Samples were fixed in 10% (v/v) buffered formalin and embedded in paraffin. Serial 5 μm sections were obtained for staining with hematoxylin and eosin and immunohistochemistry.

Immunohistochemistry and evaluation of ERα and ERβ expression

ERα and ERβ expression was detected by immunohistochemistry using the EnVision System (DAKO, Carpenteria, CA, USA), a mouse monoclonal antibody to ERα protein (NCL-ER-6F11, Novocastra, Newcastle upon Tyne, UK) and a monospecific polyclonal antibody to ERβ protein (Ab No. 385P, Biogenex, San Ramon, CA, USA). The latter antibody is directed against a 17-mer sequence close to the carboxyterminus of ERβ protein. Briefly 5-μm thick, histological sections were dewaxed in xylene, rehydrated through graded alcohols, immersed in 0.01 M citric buffer (pH 6.0), and microwaved two times for five minutes each. Subsequently, the sections were incubated with 0.3% H_2O_2 for 30 minutes to block endogenous peroxidase activity. The sections were then incubated for one hour at room temperature RT with the primary antibodies (dilution 1:30 for ERα and 1:500 for ERβ). Non specific binding was blocked by incubating the sections for 30 min with blocking solution (DAKO). Detection was carried out using the EnVision System kit (DAKO) with diaminobenzidine as the chromogen. Counterstaining was performed with hematoxylin Harris. Sections from normal prostate were used as controls for ERβ. Negative controls were processed by omitting the primary antibody and substituting it with non-immune serum.

Immunostained sections were assessed by a semiquantitative method estimating both the percentage of nuclei stained and the intensity of staining, as previously described [23]. Briefly the entire section was assessed and the staining intensity (i) was judged as 0 (negative), 1 (weak), 2 (weak/moderate), 3 (moderate/strong) and 4 (strong). The percentage of neoplastic cells exhibiting each staining intensity was recorded (P). An immunohistochemical score was given as follows: (i+1) X Pi. Every score > 50 was considered positive. The same method was followed for ERα evaluation and is the routine method used in our laboratory for assessing ERα expression in breast carcinomas. The evaluation of immunohistochemistry was done independently by two pathologists. In rare cases where there was disagreement, both pathologists re-examined the slides together and agreed on the final percentage/intensity.

Statistical analysis

All data were entered into a computer and were analyzed by using the Prism 4 for Windows (GraphPad Software Inc. San Diego, CA, USA).

The chi-square test was applied for the statistical analysis against clinico-pathological parameters and Pearson's r test was used for the correlation between ERα and ERβ expresssion, p values are two-tailed. Disease-free survival rates were estimated by the Kaplan-Meier method, and the statistical significance was estimated by the log-rank test.

Results

Comparison of ERα and ERβ proteins

Nuclear staining was observed with ERα and ERβ antibodies. ERα was positive in 29/59 carcinomas (49.15%) and ERβ in 25/59 (42.37%). Both receptors were detected in 20.3%, and were lacking in 28.8%. Twenty-nine percent had only ERα and not ERβ, and 22% had only ERβ and not ERα. There was no correlation between the presence of ERα and ERβ (p = 0.1662, Table 1).

The intensity of nuclear staining for ERβ within carcinomas was more variable than for ERα (Figure 1A, 1B). Besides epithelial cells, stromal cell nuclei, nuclei of endothelial cells and lymphocytes were also positive (Figure 1A).

There was a significant inverse correlation between ERα and grade, but not between ERβ and grade. There was no significant correlation between the presence of ERα or ERβ and age, location, tumor size or node status (Table 1). No correlation was found between ERβ expression and survival (Figure 2).

Table 1. — *Clinicopathological characteristics and ERβ expression.*

	ERα negative	ERα positive	ERβ negative	ERβ positive	p
Total	30	29	34	25	N.S.
Age (years, mean)	61.3	56.5	58.3	59.2	N.S.
Tumor size (cm, mean)	3	2.9	2.8	3.1	N.S.
Lymph node involvement					N.S.
+	20	20	22	18	
–	10	9	12	7	
Histological type					N.S.
Invasive ductal	22	22	28	16	
Grade 1	0	1	1	0	
Grade 2	10	15	13	12	
Grade 3	12	6	14	4	
Invasive lobular	6	5	5	6	
Medullary	1	3	2	2	
ERα					N.S.
Positive			17	12	
Negative			17	13	

N.S. = not significant.

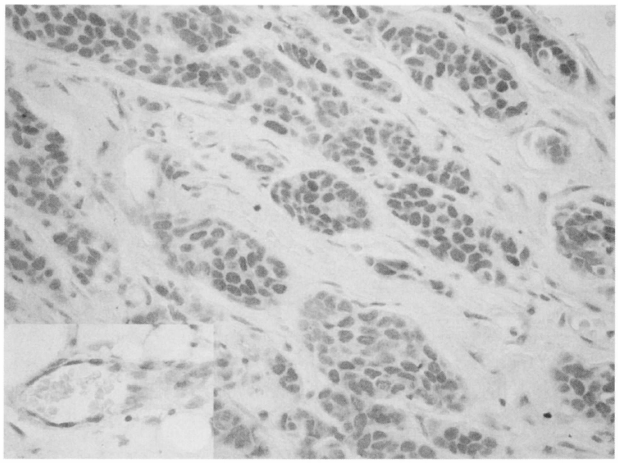
Non-neoplastic breast tissue

ERβ immunostaining was observed in non-neoplastic breast tissue in all cases where adjacent normal tissue was present. In such cases, not only epithelial, but also myoepithelial cells were positive as well (Figure 1C).

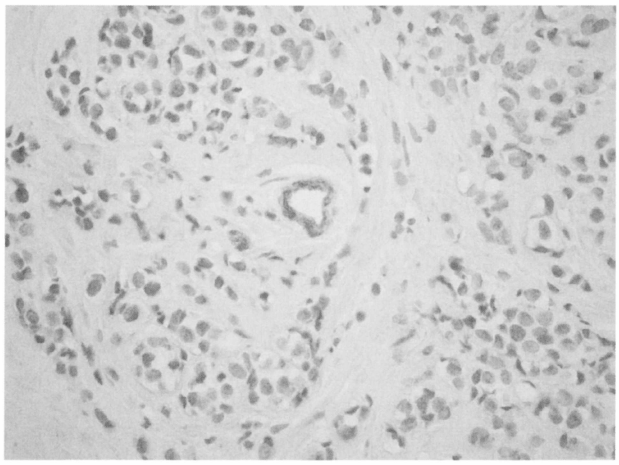
Discussion

The cloning of a second estrogen receptor, ERβ, has prompted re-evaluation of the role of ERs in carcinogenesis. Thus, in tumor types such as prostate and ovarian carcinomas, loss or reduction of ERβ expression is associated with the malignant phenotype, suggesting a potential tumor suppressing function for ERβ [16-18]. In these

1A



1B



1C

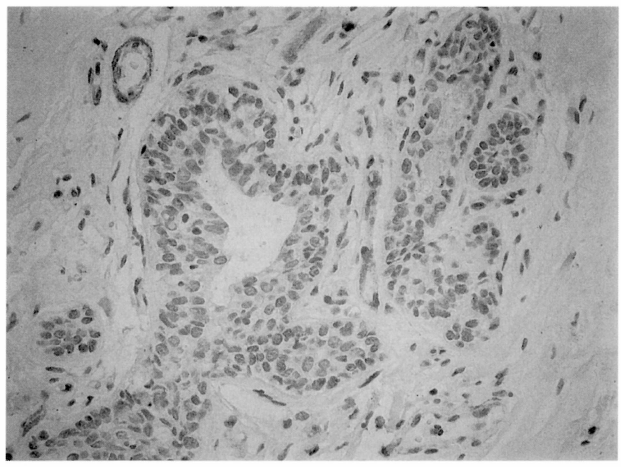


Figure 1A). — Infiltrating ductal carcinoma showing nuclear immunostaining for ERβ. In insert (lower left corner) a vessel with positive nuclear staining of endothelial cells (DAB x 400). 1B) Infiltrating lobular carcinoma showing nuclear immunostaining for ERβ (DAB x 400). 1C) Staining of epithelial and myoepithelial cells for ERβ in non-neoplastic breast (DAB x 400).

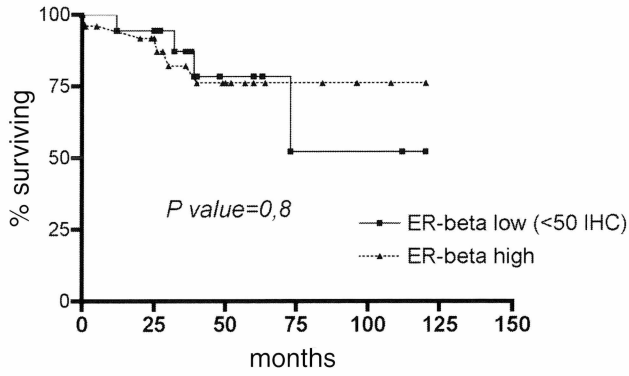


Figure 2. — Survival of patients with breast carcinoma, according to ERβ staining. Differences between patient groups with low and high ERβ expression were not statistically significant (p = 0.8).

neoplasms ERα is expressed as well, leading to the hypothesis that alterations in the ratio of ERα: ERβ may govern tumor development. In colon adenocarcinomas ERβ expression declines with tumor dedifferentiation [19-21]. Interestingly, ERα is minimally expressed in normal and neoplastic colon, so different mechanisms must be involved in tumorigenesis.

In breast cancer the situation is even more complicated. ERα is well characterized and is an established predictive marker. Its detection in breast carcinomas influences decisions on whether or not to give adjuvant therapy with anti-estrogens, such as tamoxifen. Tamoxifen, a synthetic anti-estrogen compound, has been shown to be a mixed agonist-antagonist of ERα but a pure antagonist of ERβ. Moreover, in ERα/ERβ heterodimers, when the concentrations of estradiol are low and tamoxifen is present, then the predominant suppressive effect on gene transcription is that of ERβ and not of ERα [12].

Since the first reports on ERβ, intense investigation has focused on its detection in normal and neoplastic mammary glands and its possible prognostic value. Most studies have focused on ERβ mRNA, using PCR-based techniques or in situ hybridization [24-36]. Although researchers agree that ERβ mRNA is expressed in breast cancer, there is controversy on whether it is related with other known prognostic markers or with prognosis. It has been proposed that the discrepancies might be due to the detection of different ERβ variants. The general sense is though that increased ERβ mRNA is associated with poor responsiveness to endocrine therapy and worse prognosis.

The limitations of the above-mentioned studies are the lack of information on the status of ERβ proteins, the levels and possible post-translational or post-trascriptional modifications. A functional ERβ protein is the mediator of neoplastic as well as non-neoplastic cell response to estrogens and anti-estrogens. The analysis of ERβ proteins has become possible only recently, with the development of polyclonal and monoclonal antibodies [22] and only a handful of studies have been published. Two immunohistochemical studies in frozen material have shown that ERβ protein expression is correlated with good prognostic markers and increased disease-free survival [36, 37]. The detection of ERβ protein immunoexpression in formalin-fixed paraffin-embedded tissues is

technically quite difficult [38-43]. From the literature it is speculated that ERβ is a good prognostic marker, and that loss or reduction of its expression is associated with a more malignant phenotype. So, it has been proposed that ERβ is a tumor suppressor, which could act as a dominant regulator for ER activity. However, Saji *et al.* have shown that the picture is more complicated than initially believed since expression of Erβcx, a splice variant of ERβ, in primary breast cancer correlated with poor response to tamoxifen, especially in cancers with low progesterone receptor expression [44]. Researchers agree that more archival and prospective studies are needed before definite conclusions can be drawn on the putative role of ERβ in breast carcinogenesis and its possible role as a prognostic marker in breast carcinomas [22, 45]. Skliris *et al.* in a very recent paper presented data which suggest that loss of ERβ expression is one of the hallmarks of mammary carcinogenesis and that it may be a reversible process involving methylation [46].

Although we have found no correlation between ERα and ERβ expression, or between ERβ expression and the known prognostic indicators such as tumor size, grade or lymph node status, we do believe that our data will contribute to a better understanding of the role of ERβ in breast cancer. Furthermore, we did not detect any correlation between ERβ expression and survival.

In conclusion, the discrepancy still remains between reports on the type, specificity and conditions of ERβ detection, while the list of available ERβ antibodies continues to expand.

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