Original Paper

The role of oestrogen receptor β in invasive breast cancer

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Summary

Background

The presence of estrogen receptor (ER) in breast cancer cell is a good prognostic factor. It is also a predictive factor used in hormonal therapy.

Aim

To evaluate the expression of oestrogen receptors α and β (ERα and ERβ) in the neoplastic tissues of patients with invasive breast cancer and to determine whether ERβ expression may be correlated with some clinical parameters and biological markers.

Materials/Methods

Paraffin embedded tissues from 67 patients with breast cancer were used in this study. Monoclonal antibodies against α oestrogen receptors, progesterone receptors (DakoCytomation) and polyclonal antibodies against β oestrogen receptors (CHEMICON) were used. The EnVision detection system was applied.

Results

Expression of ERα was demonstrated in 57% of all patients, while in patients over 50 years it was higher – 71%. Expression of ERβ was demonstrated in 48% of patients and this percentage was not dependent on the age of the patients. In tumours expressing ERβ, expression of p53 and Ki-67 was less common. In addition, these tumours were of a lower grade of malignancy.

Conclusions

Our results demonstrate a negative correlation between the expression of ERβ and the expression of p53 and Ki-67. The expression of ERβ may be a good prognostic indicator.

Key words

estrogen receptor β • breast cancer • immunohistochemistry

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BACKGROUND

The presence of oestrogen receptors (ER) in breast cancer cells is a good prognostic factor. It is also a predictive factor used in hormonal therapy [1].

In 1996, several laboratories independently discovered a second oestrogen receptor in rats, mice and humans. This newly discovered protein was named oestrogen receptor β (ERβ) because a protein previously found to be a receptor had been named oestrogen receptor α (ERα) [2,3]. Two separate genes on two different chromosomes code for oestrogen receptors α and β. The gene coding for oestrogen receptor β, in humans, is found on chromosome 14, while the gene coding for oestrogen receptor α is found on the long arm of chromosome 6.

The function of oestrogen receptor β has not been fully explained. There is conflicting information regarding the prognostic value of the presence of oestrogen receptor β in breast cancer [4].

Antibodies that allow the demonstration of oestrogen receptor β, by means of immunohistochemical tests, have recently become commercially available.

AIM

The purpose of the study was to assess the expression of oestrogen receptors α and β in the tumours of patients diagnosed with invasive breast cancer, and to attempt to correlate the presence of the β receptor with the expression of selected immunohistochemical markers.

MATERIALS AND METHODS

The material for the study was comprised of histological materials gathered from 67 consecutive patients, operated in the Oncology Clinic of Poznan University of Medical Sciences, in the year 2002. Immunohistochemistry and microscopy were also carried out in the University of Medical Sciences, in the Department of Tumour Pathology. Slides were produced from paraffin wax blocks containing tissues fixed in 10% neutral buffered formalin and processed to paraffin by conventional histological methods. The EnVision™ + HRP complex (DAKO) was used in immunohistochemical methods. Monoclonal antibodies against α oestrogen receptors and pro-

gesterone receptors (DakoCytomation), and polyclonal antibodies against β oestrogen receptors (CHEMICON), were used to demonstrate the presence of receptors. Also used were monoclonal antibodies against p53 protein, polyclonal antibodies against cathepsin D and monoclonal antibodies against Ki-67 (all by DAKO).

Statistical dependencies were tested for using Fisher's exact test and the Fisher-Freeman-Halton test. Findings were assumed to be significant at the level of p <0.05.

RESULTS

The clinico-histological characteristics of the studied group are shown in Table 1.

The presence of oestrogen receptor α was detected in the tumours of 57% of patients. In patients aged over 50 years this figure rose to 71%.

Oestrogen receptor β was detected in the neoplastic tissues of 48% of patients and was independent of the age of the patient. In 75% of cases where oestrogen receptor β was detected, α receptors were also found. Patients expressing oestrogen receptor β but lacking oestrogen receptor α accounted for only 12% of the studied group. Positive immunohistochemical results for the presence of progesterone receptors were obtained from the tumours of 67% of patients. Relationships between receptor content values are presented in Table 2 and in Figure 1.

In the neoplastic tissues of patients expressing oestrogen receptor β, p53 protein was found less frequently than in the group studied as a whole (Figure 2). Furthermore, expression of cathepsin D and Ki-67 were also less frequently detected in this sub-group (Figure 3).

Among such patients neoplasms were frequently of a lower grade of malignancy, histologically (Figure 4). This difference was found to be statistically significant (p=0.001). Similarly, in the case of p53 protein and Ki-67 antigen, statistically significant differences were observed (p=0.004 and p=0.02 respectively). However, in the case of cathepsin D, no statistically significant differences were found (p=0.22).

DISCUSSION

The first investigations into β receptors were performed using mRNA, with the help of the

Table 1. Patient and tumour characteristics in the studied group.

Parameter	Number of patients	%
Age		
<40 years	4	6
40–50 years	23	34
>50 years	40	60
Size of tumour		
<2 cm	42	63
>2 cm	25	37
Lymph nodes		
Uninvolved	25	37
Involved	39	58
Undetermined	3	5
Histological grade of malignancy		
G1	16	24
G2	30	45
G3	16	24
Gx	5	7
Receptors		
ERα +	38	57
ERβ +	32	48
PgR +	45	67
Positive reaction – p53	21	31
Positive reaction – Ki-67	47	70
Positive reaction – cathepsin D	40	60

Table 2. A comparison of ERα, ERβ and PgR protein expression in the tumours of the 67 patients studied (%).

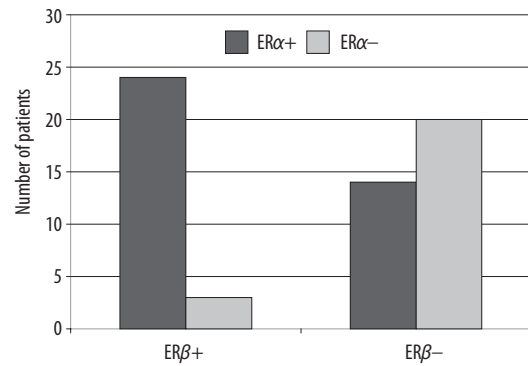| Receptors	ERβ+	ERβ–
ERα+	36	21
ERα–	12	31
PgR+	36	31
PgR–	12	21
ERα + PgR+	33	19
ERα + PgR-	3	2
ERα – PgR+	3	12
ERα – PgR–	9	19

Figure 1. The relationship between ERβ and ERα expression in 67 carcinomas (p=0.008).
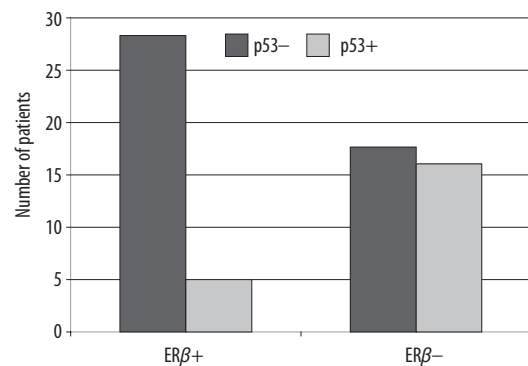

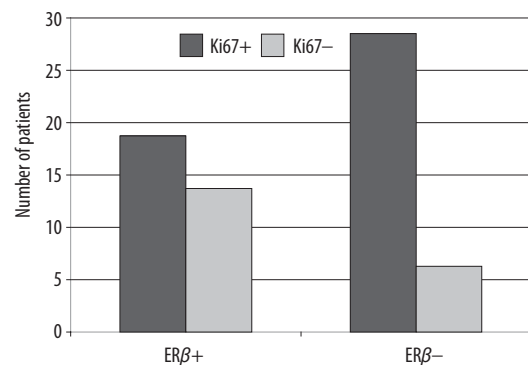**Figure 2.** The relationship between over expression of p53 protein and expression of ERβ (p=0.004).

Figure 3. Relationship between Ki-67 immunoreactivity and expression of ERβ (p=0.02).

polymerase chain reaction (PCR). Such tests were necessary because of the lack of commercially available antibodies for the detection of β receptor expression by immunological means. Our study was carried out using the immunohis-
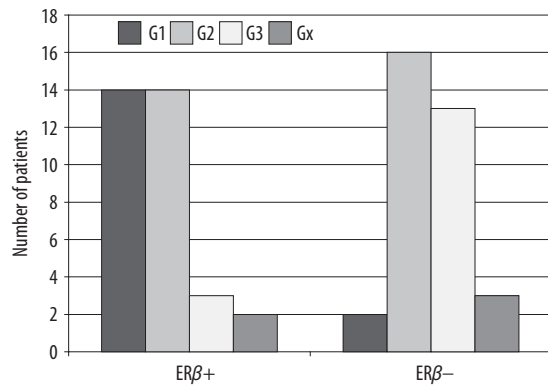
Figure 4. Relationship between expression of ERβ and the histological grade of tumours (p=0.001).

tochemistry method, which allowed us to assess archived materials, which is cheaper and more readily available. Many mono and polyclonal antibodies for the detection of β receptor expression have recently been developed. The antibodies are directed against various fragments of oestrogen receptor β. The value of the immunohistochemical method is the possibility to localize the sought antigen within cells without the danger of confusing it with non-malignant portions of the tumour. This could be of significance in the case of oestrogen receptor β as these receptors are often found, not only in normal glandular tissue, but also in blood vessels, as well as in adipose tissue and connective tissues [5].

The results of this study showed that oestrogen receptor α could be demonstrated in the tumours of 57% of patients and that this figure rose to 71% in patients older than 50 years. These findings are in agreement with those of other authors [6].

Oestrogen receptor β was detected in 48% of patients, irrespective of age. This lack of an age related link with expression of ERβ is also in agreement with the results of other studies [7]. In 75% of these patients, both ERα and ERβ were detected. Patients lacking oestrogen receptor α, but showing expression of oestrogen receptor β, account for 12% of the studied group. It is known that, in around 10% of patients with advanced breast cancer, it is possible to obtain a response to hormonal treatment and it is in these patients that no expression of ER α may be observed. Possibly this is the group of patients who are ERα negative and ERβ positive.

The expression of p53 protein was detected in 31% of tumours. The p53 gene produces p53 pro-

tein, which plays roles in the regulation of transcription, the cell cycle and apoptosis. Mutations of this gene are found in many tumours, including breast cancer. Immunohistochemistry has allowed the detection of excess p53 protein in 22–25% of breast cancer cases. While the presence of mutations in gene p53 is not included among prognostic factors of definite value, most research shows a negative relationship with improper expression of p53 [8]. In our study, we found a negative correlation between the presence of ERβ and p53. Nuclear accumulations of p53 protein were observed less frequently in patients expressing oestrogen receptor β than in the group as a whole.

Also tested was the dependence between the expression of ERβ and the proliferative antigen Ki-67. Over-expression of Ki-67 is associated with a poorer prognosis. We have found a negative correlation between the expression of Ki-67 and ERβ. Roger et al. also described this same dependency [9].

In order to better understand the purpose of β receptors in both normal and neoplastic tissues, mice were raised in which the gene coding for the receptors was inactive. In the breasts of these mice, improper growth of epithelia, multiple cysts and over-expression of Ki-67 were noted [10].

Current studies into the role of oestrogen receptor β have provided conflicting results. Speirs et al. used the RT-PCR method to assess the value of mRNA to ER α and ER β. They found that expression of oestrogen receptor β is associated with metastases to the lymph nodes [11]. A similar study found no relationships between the mRNA levels for ER α and β and the histological grade of malignancy, size of the tumour or involvement of the lymph nodes. The highest levels of mRNA for ERβ are found in tumours which are ER and PR negative, and therefore, in tumours which are insensitive to hormonal treatment [12]. Because this relationship has also been shown in other studies it has been postulated that oestrogen receptor β may be involved in resistance to tamoxifen therapy [13,14]. Mann et al. settled on opposing conclusions. Using immunohistochemistry, they assessed α and β oestrogen receptor status in a group of 118 women who took tamoxifen after surgery. Better survival times were recorded for women whose tumours had been found positive for β receptors in more than 10% of cells. This applied equally to patients with and without metastases to the lymph nodes [15]. In another study,

in which immunohistochemistry was used, a connection was studied between ERβ, ERα, PgR and selected prognostic factors. The presence of oestrogen receptor β correlated with a lack of metastases to the lymph nodes, a low histological grade of malignancy, and a low fraction of cells in the synthesis phase. Over-expression of the HER-2 receptor is seen significantly more frequently in patients whose tumours test positive for the presence of oestrogen receptor β [16].

CONCLUSIONS

In this study, we have found a negative correlation between the expression of oestrogen receptor β and the expression of p53 protein and Ki-67 antigen.

The presence of oestrogen receptor β may turn out to be a useful prognostic factor.

However, further studies are needed to assess the prognostic value of testing for the presence of oestrogen receptor β in patients with breast cancer.

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