RESEARCH ARTICLE

Prognostic Significance of Interactions Between ER Alpha and ER Beta and Lymph Node Status in Breast Cancer Cases

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Abstract

Objective: Both estrogen receptors, ER alpha (ERα) and ER beta (ERβ), are expressed in 50-70% of breast cancer cases. The role of ERα as a prognostic marker in breast cancer has been well established as its expression is negative correlated with tumor size and lymph node metastasis. ERβ is also a favorable prognostic predictor although this is less well documented than for ERα. Materials and Methods: To explore whether ERs independently or together might influence clinical outcome in breast cancer, the correlation between the ERs with the clinicopathological features was analyzed in 84 patients. Results: ERα expression negatively correlated with tumor stage (r=-0.246, *p*=0.028) and tended to be negatively correlated with lymph node status (r=-0.156, *p*=0.168) and tumor size (r=-0.246, *p*=0.099). Also, ERβ was negatively correlated with nodal status (r=-0.243, *p*=0.028), as was coexpression of ERα and ERβ (*p*=0.043, OR=0.194, 95% CI= 0.040- 0.953). Conclusion: Coexpression of ERs might serve as an indicator of good prognosis in breast cancer patients.

Keywords: Breast cancer - ER alpha - ER beta - interaction - lymph node - metastasis

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Introduction

Breast cancer is the second leading cancer related deaths worldwide (Lacey et al., 2002), and in China it is predicted that breast cancer incidence will increase to 85 per 100,000 women by 2021 (Ziegler et al., 2008). However, the etiology of breast cancer is not completely understood. For example, estrogen, a steroid hormone, is critically important for regulation of the growth, proliferation and differentiation of normal breast epithelial tissue (Williams et al., 1991), while estrogen hormone signaling pathways also play critical role in the onset and progression of breast cancer (Robertson et al., 1996; Kirschner et al., 1977).

Estrogen exerts its biological response via binding to two estrogen receptor subtypes, ERα and ERβ. ERs are transcription factors that when bound by ligands, can bind as either a hetero- or homodimer to the promoter of target genes containing estrogen response elements. Targets of ERs, especially ERα, are involved in cell-cycle regulation, proliferation (Lin et al., 2007; Williams et al., 2008) and cell-cell adhesion (Rochefort et al., 1998; Jordan et al., 2007). Although ERα mediates the effect of estrogen in the onset and progression of breast cancer, ERα positive tumors usually show less invasiveness and have a more favorable prognosis (Platet et al., 2004). ERα expression is negatively correlated with tumor grade and lymph node metastasis (Jarvinen et al., 2000; Fuqua et al., 2003; O'Neill et al., 2004). ERβ expression is also negatively correlated with nodal status (Fleming et al., 2004; Koda et al., 2004; Sugiura et al., 2007) and tumor grade (Jarvinen et al., 2000; Omoto et al., 2002; Sugiura et al., 2007) independent of the expression of ERα. However, many cell-based studies have shown that ERβ acts as a negative modulator of ERα actions, as ERβ inhibits ERα transcriptional activity and suppresses the sensitivity of the cell to estrogen (Pettersson et al., 2000; Nilsson et al., 2001). An unanswered question is whether co-expression of both ERs exerts a favorable or unfavorable influence on specific clinical features of breast cancer.

In the current report we addressed this question by conducting a population-based study to determine if interactions between ERα and ERβ are correlated with a set of well-known clinicopathological features of breast cancer.

Materials and Methods

Specimens

The case-control study including a total of 84 primary

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breast cancers, all from the Department of Surgery, Xijing Hospital, Xi'an, that were diagnosed as invasive duct carcinoma according to the WHO classification (Umemura et al., 2006). Specimens were obtained from women undergoing mastectomy or quadrantectomy for early breast cancer. Lymph nodes status was determined through biopsy. Tumor stage was determined according to the AJCC TNM criteria (Page et al., 2002).

This study was approved by the Ethics Committees of Capital Medical University and the Beijing People Hospital, and was conducted in accordance with the principles of the Helsinki Declaration II. Informed consents were obtained from all participants.

Immunohistochemistry and Assessment

Specimens were fixed in 10% neutral buffered formalin for 24 to 48 h and embedded in paraffin. Tissue cores (0.6 mm) were taken from representative areas from each cancer using a manual arraying device. Slides (4 um) were deparaffinized in xylene and rehydrated in a graded series of ethanol/water rinses, then antigen retrieval was performed by autoclaving sections in a 10mM citrate buffer (pH6.0) for 10 minutes. After cooling to room temperature, the sections were treated with 3% hydrogen peroxide for 5 min followed by primary antibody for 30 min at room temperature. A monoclonal mouse anti-human ERα antibody (Novocastra) that recognizes the full-length ERα protein was applied at a dilution of 1/40. A monoclonal mouse anti-human ERβ antibody (Novocastra) that recognizes the C-terminal region was applied at a dilution of 1/50. ER proteins were visualized with 3,3'-Diaminobenzidine. Non-immune serum instead of the primary antibody was used for negative controls. ERα positive was defined as nuclear staining in more than 10% of cancer cells regardless of staining intensity (Umemura et al., 2006). For ERβ, the presence of nuclear-stained cells was considered as positive regardless of the number or staining intensity. All staining were evaluated by two pathologists independently, and in case of discrepancy, a third examination was performed to reach consensus. In the 84 specimens, the success rates were 96.43% (81/84) and 97.62% (82/84) for detection of ERα and ERβ antibody respectively.

Statistical analysis

All statistical analyses were performed using SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA). Spearman's rank correlation coefficient was used to evaluate the correlation between the expression of ERs in the cancer tissue and clinicopathological features, including size and stage of tumor, age at operation and lymph node status. A multivariate regression analysis model was employed to examine the correlation of the co-expression of two ER with the clinicopathological features, with multivariate logistic regression used for assessment the correlation of the interaction with tumor stage and nodal status, and multiple linear regression for that of interaction with tumor size and age at operation. ERα, ERβ, age at surgery and tumor size were considered as confounding factors, and were adjusted in all the multivariate regression models. Two sided significance tests were used throughout, $P \leq 0.05$ was considered as of statistically significance.

Table 1. Clinicopathological Features of the 84 Breast Cancer Patients

Clinical parameter	N (%)
Mean age(range)	50.7 (29-84)
Tumor size(range)	3.6 (2.5-6.0)
Tumor stage	
I	1 (1.2)
II	60 (71.4)
III	23 (27.4)
Lymph node status	
Positive	13 (15.5)
Negative	71 (84.5)

Table 2. ERα and ERβ Correlatively Expressed in the Breast Cancer Tissue

ERα expression	ERβ expression			
	positive	negative	r	*p*
Positive	44	16	0.322	0.003
Negative	8	13		

*significant at the level of $p<0.05$; r, correlation coefficient

Table 3. Correlation Between ERα/ERβ and Clinicopathological Features in Breast Cancer

Variables	ERα		ERβ	
	r	*p*	r	*p*
Age at operation	-0.123	0.274	-0.155	0.521
Nodal status	-0.156	0.168	-0.243	0.028*
Tumor stage	-0.246	0.028*	-0.123	0.274
Tumor size	-0.246	0.099	0.044	0.768

*significant at the level of $p<0.05$; r, correlation coefficient

Results

Expression of ERα and ERβ in breast cancer tissue

From July to December of 2009, we recruited 84 patients from the Xijing Hospital (Xi'an, China) for the study. The age of patients ranged from 29 to 84 years old (mean: 50.69). Among these patients, 36 cases had tumors located in the left breast, while 48 cases had tumors located in the right breast (Table 1).

In excised breast cancer tissues from the 84 patients, positive expression of ERα and ERβ were 74.1% (60/81) and 63.4% (52/82), respectively. We observed a co-expression of ERα and ERβ as 54.3% (44/81) were positive for both ERα and ERβ, and 16.0% (13/81) were negative for both ERα and ERβ expression (r=0.332, p=0.003) (Table 2). This result was consistent with the findings from several other studies.

ERα/ERβ expression correlated with some clinicopathological features of breast cancer

As shown in Table 3, there was a correlation between the expression of ERα/ERβ and some clinicopathological features of breast cancer. ERα negatively correlated with the tumor stage (r =-0.246, p=0.028) and showed a trend to be negatively correlated with nodal status (r=-0.156, p=0.168) and tumor size (r=-0.246, p=0.099). However,

Table 4. Correlations Between ERs Coexpression and Lymph Node Metastasis

Variable	coefficen	standard error	wald χ^2	*p*	odds ratio	95%CI
ERα	-0.642	0.82	0.612	0.434	0.526	0.105- 2.626
ERβ	-2.052	1.072	3.661	0.056	0.129	0.016-1.051
ERα*ERβ	-1.638	0.811	4.08	0.043*	0.194	0.040-0.953
Age	0.014	0.031	0.221	0.638	1.015	0.955-1.077
Tumor size	0.77	0.516	2.232	0.135	2.16	0.787-5.934

*significant at the level of $p<0.05$, OR=exp *(b)*

ERβ expression was negatively correlated with nodal status (r=-0.243, *p*=0.028) only.

Coexpression of ERα and ERβ correlated with enhancement of each ERs' protective effect on lymph node metastasis in breast cancer

As we have shown, both ERs negatively correlated or shown a trend towards negative correlation with lymph node metastasis. In order to examine whether there is an co-expression between the ERs and how the co-expression influences breast cancer clinicopathological features, a multivariate logistic or linear regression analysis model was employed to analyze the correlations. In these models, the correlation of co-expression between ERs (ERα*ERβ) with the clinicopathological features of breast cancer (including tumor size, stage of tumor, lymph node status and age at surgery), was examined.

The result indicated that co-expression of ERα and ERβ with lymph node status (*p*=0.043; OR=0.194, 95% CI=0.040-0.953), suggesting that patients who co-expressed ERα and ERβ were associated with a reduced the risk of lymph node metastasis, which was 0.194 fold to the other patients (Table 4). The interaction was not correlated with tumor size (b=-0.282, *p*=0.139, 95% CI=-0.657-0.093), age at surgery (b=-1.836, *p*=0.431, 95% CI=-0.645- 2.778), but for tumor stage, interactions approached significance (*p*=0.074; odds ratio=0.393, 95% CI=0.141-1.095).

Discussion

In the present study, we observed that the co-expression of ERα and ER beta is correlated with an enhancement of each ERs' ability to prevent lymph node metastasis. To our knowledge, there are no previous population-based published studies describing how ERα and ERβ co-expression interact to influence the clinicopathological features of breast cancer.

In normal resting mammary glands, 10-20% of breast epithelial cells are ERα positive, whereas in breast cancer ERα expression is observed in 50-80% of cells (McGuire et al., 1978; Osborne et al., 1998). This indicates that an elevated receptivity to estrogens in these tissues is involved in a higher risk of tumorigenesis. However, several population-based studies demonstrated that in mammary carcinogenesis, the expression of ERα is associated with less tumor invasiveness and a more favorable prognosis (Platet et al., 2004). Particularly, ERα expression is associated with low tumor grade and negative lymph node status (Pettersson et al., 2000; Fuqua et al., 2003; O'Neill et al., 2004). In the present study, we observe that ERα expression is negatively correlated with tumor stage (r =-0.246, p=0.028) and shows a trend to be negatively correlated with nodal status (r=-0.156, *p*=0.168) and tumor size (r=-0.246, *p*=0.099). The correlation between ERβ and invasiveness is not well established as that of ERα, although several studies had shown that ERβ expression correlated with negative axillary lymph node metastasis (Pettersson et al., 2000; Fleming et al., 2004; Koda et al., 2004; Sugiura et al., 2007), which is consistent with our results shown in the present study (r=-0.243, *p*=0.028).

Since our results indicated that expression of both ERs is negatively correlated with lymph node status, and many cell model based studies have suggested that ERβ acts as a negative modulator of ERα action (Pettersson et al., 2000; Nilsson et al., 2001), we examined whether the co-expression between ERα and ERβ correlated with lymph node status in a population -based study. By employing a multivariate logistic regression analysis model, we observed that there was a correlation between the co-expression of ERα and ERβ (ERα* ERβ) (*p*=0.043; OR=0.194; 95% CI: 0.040-0.953), which indicated that the co-expression of ERα and ERβ was associated with further enhancing each of their individual actions on protecting axillary lymph node from metastasis. Some studies have suggested that the patients who express both ERαand ERβin their breast cancer tissues have a better prognosis (Platet et al., 2004; Lin et al., 2007), and our results might partly account for it. Although it has been known that when both ERs co-expressed in cell lines, ERβ inhibits ERα transcriptional activity and reduces the sensitivity of the cells to estrogen (Pettersson et al., 2000; Younes et al., 2011), the precise mechanism underlying the protective effect of ERs interaction on lymph node metastasis remain to be elucidated in further cell- and population-based studies. Since more then 50% of breast cancer co-express ERαand ERβprotein (Speirs et al., 1999; Fuqua et al., 2000; Murphy et al., 2003), and further as we have shown in the present study that the expression of ERs was correlated (r=0.322, *p*=0.003), it is important to define the nature and effect of co-expression of ERα and ERβ on tumor progression and disease prognosis. The present study suggests that co-expression of both ERs in breast cancer tissue is a good predictor for disease prognosis since it enhances each ER's protective effect on lymph node metastasis.

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References

Fleming FJ, Hill AD, McDermott EW, O'Higgins NJ, Young LS (2004). Differential recruitment of coregulator

proteins steroid receptor coactivator-1 and silencing mediator for retinoid and thyroid receptors to the estrogen receptor-estrogen response element by beta-estradiol and 4-hydroxytamoxifen in human breast cancer. *J Clin Endocrinol Metab*, **89**, 375-83.

Fuqua SA, Schiff R, Parra I, et al (2003). Estrogen receptor beta protein in human breast cancer: correlation with clinical tumor parameters. *Cancer Res*, **63**, 2434-9

Jarvinen TA, Pelto-Huikko M, Holli K, Isola J (2000). Estrogen receptor beta is coexpressed with ER alpha and PR and associated with nodal status, grade, and proliferation rate in breast cancer. *Am J Pathol*, **156**, 29-35.

Jordan VC, Brodie AM (2007).Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer. *Steroids*, **72**, 7-25.

Kirschner MA (1977). The role of hormones in the etiology of human breast cancer. *Cancer*, **39**, 2716-26.

Koda M, Sulkowski S, Kanczuga-Koda L, Surmacz E, Sulkowska M (2004). Expression of ER alpha, ER beta and Ki-67 in primary tumors and lymph node metastases in breast cancer. *Oncol Rep*, **11**, 753-9.

Lacey JV, Jr., Devesa SS, Brinton LA (2002). Recent trends in breast cancer incidence and mortality. *Environ Mol Mutagen*, **39**, 82-8.

Lin CY, Strom A, Li Kong S, et al (2007). Inhibitory effects of estrogen receptor beta on specific hormone-responsive gene expression and association with disease outcome in primary breast cancer. *Breast Cancer Res*, **9**, R25.

McGuire WL (1978). Hormone receptors: their role in predicting prognosis and response to endocrine therapy. *Semin Oncol*, **5**, 428-33.

Murphy L, Cherlet T, Lewis A, Banu Y, Watson P (2003). New insights into estrogen receptor function in human breast cancer. *Ann Med*, 35, 614-31.

Nilsson S, Makela S, Treuter E, et al (2001). Mechanisms of estrogen action. *Physiol Rev*, 81, 1535-65.

Omoto Y, Kobayashi S, Inoue S, et al (2002). Evaluation of oestrogen receptor beta wild-type and variant protein expression, and relationship with clinicopathological factors in breast cancers. *Eur J Cancer*, 38, 380-6.

O'Neill PA, Davies MP, Shaaban AM, et al (2004). Wild-type oestrogen receptor beta (ERbeta1) mRNA and protein expression in Tamoxifen-treated post-menopausal breast cancers. *Br J Cancer*, **91**, 1694-702.

Osborne CK (1998). Steroid hormone receptors in breast cancer management. *Breast Cancer Res Treat*, **51**, 227-38.

Page DL, Fleming ID, Fritz A (2002). AJCC Cancer Staging Manual 6th edition. breast cancer. Lippincott-Raven, Philadelphia, Pa, USA.

Pettersson K, Delaunay F, Gustafsson JA (2000). Estrogen receptor beta acts as a dominant regulator of estrogen signaling. *Oncogene*, **19**, 4970-8.

Platet N, Cathiard AM, Gleizes M, Garcia M (2004). Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. *Crit Rev Oncol Hematol*, **51**, 55-67 .

Reisenbichler ES, Lester SC, Richardson AL, et al (2013). Interobserver Concordance in Implementing the 2010 ASCO/CAP Recommendations for Reporting ER in Breast Carcinomas: A Demonstration of the Difficulties of Consistently Reporting Low Levels of ER Expression by Manual Quantification. *Am J Clin Pathol*, **140**, 487-94

Robertson JF (1996). Oestrogen receptor: a stable phenotype in breast cancer. *Br J Cancer*, **73**, 5-12.

Rochefort H, Platet N, Hayashido Y, et al (1998).Estrogen receptor mediated inhibition of cancer cell invasion and motility: an overview. *J Steroid Biochem Mol Biol*, **65**, 163-8.

Sofi GN, Sofi JN, Nadeem R (2012). Estrogen receptor and progesterone receptor status in breast cancer in relation to age, histological grade, size of lesion and lymph node involvement. *Asian Pac J Cancer Prev*, **13**, 5047-52 .

Speirs V, Parkes AT, Kerin MJ, et al (1999).Coexpression of estrogen receptor alpha and beta: poor prognostic factors in human breast cancer? *Cancer Res*, **59**, 525-8.

Sugiura H, Toyama T, Hara Y, et al (2007).Expression of estrogen receptor beta wild-type and its variant ERbetacx/beta2 is correlated with better prognosis in breast cancer. *Jpn J Clin Oncol*, **37**, 820-8 .

Umemura S, Kurosumi M, Moriya T, et al (2006). Immunohistochemical evaluation for hormone receptors in breast cancer: a practically useful evaluation system and handling protocol. *Breast Cancer*, **13**, 232-5.

Williams C, Edvardsson K, Lewandowski SA, Strom A, Gustafsson JA (2008). A genome-wide study of the repressive effects of estrogen receptor beta on estrogen receptor alpha signaling in breast cancer cells. *Oncogene*, **27**, 1019-32.

Williams G, Anderson E, Howell A, et al (1991) .Oral contraceptive (OCP) use increases proliferation and decreases oestrogen receptor content of epithelial cells in the normal human breast. *Int J Cancer*, **48**, 206-10.

Younes M, Honma N (2011): Estrogen receptor beta. *Arch Pathol Lab Med,* **135**, 63-6.

Ziegler RG, Anderson WF, Gail MH (2008).Increasing breast cancer incidence in China: the numbers add up. *J Natl Cancer Inst*, **100**, 1339-41.