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Original Article

Expression and prognostic value of estrogen receptor beta in breast cancer patients

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Abstract: This study is to determine the expression of estrogen receptor beta (ERβ) in breast cancer patients and to evaluate its relationship with clinicopathological parameters of breast cancer and its effects on the prognosis of breast cancer patients. Paraffin-embedded primary tumor tissue sections from 490 breast cancer patients were collected consecutively from January 2000 to December 2010. They had complete clinical data and follow-up records. Immunohistochemical staining was conducted to determine ERβ expression. The Kaplan-Meier method was used for survival analysis. Difference in survival was analyzed by the Log-Rank test. The Cox proportional hazard model was performed to evaluate the prognostic value of ERβ expression in breast cancer patients. The ERβ high and over expression rate in 490 breast patients was 22.4% (110/490). ERβ expression was not associated with clinicopathological parameters of breast cancer. The mean survival time in patients with ERβ negative expression, ERβ low expression, ERβ high expression and ERβ over expression was 9.9 years, 9.2 years, 8.6 years and 5.6 years. Statistically, patients with ERβ high and over expression had significantly shorter disease-free survival (DFS) time compared with the patients with ERβ negative and low expression. The Cox multivariate analysis revealed that ERβ high and over expression, the pathologic stages of tumor and chemotherapy were the independent predictors for poor DFS in breast cancer patients. ERβ expression is an independent prognostic factor of breast cancer patients and its high and over expression indicates poor prognosis of breast cancer. There was no correlation between ERβ expression and clinicopathological parameters in breast cancer.

Keywords: ERβ, breast cancer, prognosis

Introduction

The expression levels of hormone receptors in breast cancer tissue could affect the prognosis of breast cancer patients [1]. Traditionally, breast cancer estrogen receptor (ER) refers to ERα, the main subtype of ER. Many clinical studies showed that ERα positive breast cancer patients had better prognosis and longer disease-free survival (DFS) and overall survival than ERα negative breast cancer patients. In 1996, Kuiper et al found that ERβ was another subtype of ER in breast cancer tissues [2]. Studies have shown that the ERα and ERβ had different structures and functions, suggesting that they may play different roles in the development of breast cancer. However, whether ERβ could be used as a prognostic indicator in breast cancer is unclear. Current studies on the role of ERβ in the prognosis of breast cancer are in discrepancy. Some studies indicate that ERβ expression may be an indicator of poor prognosis of breast cancer. For example, Hou et al found that ERβ overexpression promoted the proliferation of breast cancer cells [3]. The positive expression of vascular endothelial growth factor in breast cancer tissues indicates the rapid tumor growth, the strong tumor invasive ability and the poor prognosis of patients. Knowlden et al reported that ERβ over expression was correlated with the positive expression of vascular epithelial growth factor receptor in breast cancer tissue [4]. Wu et al [5] detected the expression of ERβ and vascular endothelial growth factor in 35 cases of breast cancer tissues by semi-quantitative reverse transcription polymerase chain reaction. Then they analyzed the relationship between ERβ expression and vascular endothelial growth factor expression. They also analyzed the relation-

ship between expression of ERβ and vascular endothelial growth factor and the pathological parameters of breast cancer. They found that ERβ over expression was positively correlated with vascular endothelial growth factor expression, suggesting that angiogenesis in breast tumors may be affected by ERβ and that high expression levels of ERβ may promote tumor metastasis through promoting tumor angiogenesis. However, other studies suggest that ERβ expression may be an indicator of good prognosis of breast cancer. For example, it is reported that ERβ expression could inhibit proliferation of breast cancer cells [6-8]. Nakopoulou et al [9] found that breast cancer patients with ERβ positive tumors had an improved long term survival, indicating that ERβ expression might be used as an indicator of good prognosis in breast cancer. Therefore, the role of ERβ in the prognosis of breast cancer still needs further investigation. In this study, we detected the expression levels of ERβ in breast cancer tissues using immunohistochemical staining. Then the relationship between ERβ expression and clinicopathological parameters of breast cancer was investigated. The effect of ERβ expression on the prognosis of breast cancer was also analyzed.

Materials and methods

Clinical data

A total of 520 cases of pathologically confirmed breast cancer patients, who underwent surgery in the First Affiliated Hospital of Xinjiang Medical University from January 2000 to December 2010, were enrolled in this study. Their clinical data were complete. Paraffin-embedded primary tumor tissue sections from these breast cancer patients were collected. All patients were female. The onset age was 32-72 years old, with a median age of 49 years old. All patients underwent standardized comprehensive treatment, including surgery, anthracycline/Taxol-based chemotherapy and radiotherapy. The patients were followed up for 2 to 12 years, with the median follow-up time of 5 years. The follow-up was cut off on December 31, 2012. During the follow-up time, 30 patients were censored. Patients died of other causes, lost to follow-up at the time of last contact or before study cut-off were censored. At the end of the follow-up, there were 393 cases of disease-free survival (DFS) patients and 97 cases of survival patients with cancer recurrence and metastasis.

Clinical data of patients were obtained from patient medical records and by re-examination or telephone follow-up.

The DFS referred to the period timing from tumor diagnosis to the end of the follow-up in patients without cancer recurrence and metastasis, or to the time of first distant metastasis.

Prior written and informed consent was obtained from every patient and the study was approved by the ethics review board of Xinjiang Medical University.

Immunohistochemistry

Expression levels of ERβ in breast cancer tissue were detected by SP immunohistochemical method. Briefly, specimens were fixed in formaldehyde and embedded in paraffin. Tissue sections were dewaxed, rehydrated in graded alcohols and processed before incubation with antibodies. ERβ positive sample was used as a positive control. In the negative control, the primary antibody was replaced with PBS. The anti-ERβ antibody and the working solution were purchased from Fuzhou Maixin Biotechnology Development Co., Ltd (Fuzhou, China).

Determination of ERβ expression levels

The expression levels of ERβ were determined using Single-blind method. The two observers did not know anything about the patient's clinical data. Cells with brown particles in the nucleus were ERβ positive cells. Ten fields at high-magnification were randomly selected and at least 100 cells were counted in each field. ERβ positive expression rate was defined as the ratio of ERβ positive cell number to total cell count. Cancer cells with no nucleus staining or with a positive rate of < 1% were defined as ERβ negative (ERβ (-)) cells. Cancer cells with a positive rate between 1% and 10% were defined as low expression (ERβ (+)) cells. Cancer cells with a positive rate between 10% and 50% were defined as ERβ high expression cells (ERβ (++)) and cells with a positive rate of > 50% were defined as over expression cells (ERβ (+++)).

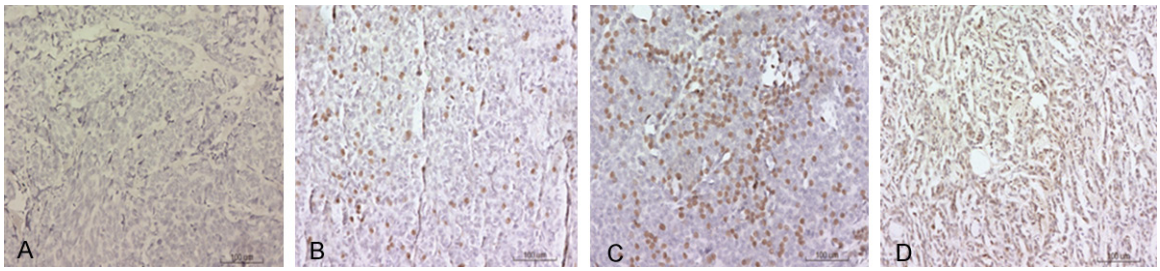

Figure 1. Expression of ERβ in breast cancer tissue. ERβ expression was detected by immunohistochemistry. Representative results were shown. Cells with brown particles in the nucleus were ERβ positive cells. ERβ positive expression rate was defined as the ratio of ERβ positive cell number to total cell count. Scale bar, 100 μm. A: ERβ negative cells. ERβ (-), cancer cells with no nucleus staining or with a positive rate of < 1%. B: ERβ low expression cells. ERβ (+), cancer cells with a positive rate between 1% and 10%. C: ERβ high expression cells. ERβ (++), cancer cells with a positive rate between 10% and 50%. D: ERβ overexpression cells. ERβ (+++), cancer cells with a positive rate of > 50%.

Table 1. Correlation between ERβ expression and clinicopathological parameters in 490 patients with breast cancer

Clinical pathological parameters	Number of cases (490)	ERβ high and over expression (n = 110)	ERβ negative and low expression (n = 380)	*P* value
Tumor size				0.7
≤ 2 cm	161	34 (21.1)	127 (78.9)	
> 2 cm~≤ 5 cm	264	63 (23.9)	201 (76.1)	
> 5 cm	65	13 (20.0)	52 (80.0)	
Lymphatic metastasis				0.4
0	251	59 (23.5)	192 (76.5)	
1~4	125	32 (25.6)	93 (74.4)	
5~9	58	10 (17.2)	48 (82.8)	
> 9	56	9 (16.1)	47 (83.9)	
Histological grades				0.5
Grade I	72	19 (26.4)	53 (73.6)	
Grade II	290	66 (22.8)	224 (77.2)	
Grade III	128	25 (19.5)	103 (80.5)	
Pathologic stages				0.9
Stage I	111	24 (21.6)	87 (78.4)	
Stage II	294	65 (22.1)	229 (77.9)	
Stage III	85	21 (24.7)	64 (75.3)	

Statistical analysis

Data were analyzed by SPSS17.0 software. Chi-square analysis was performed to analyze the relationship between ERβ expression and clinicopathological parameters of breast cancer. The Kaplan-Meier method was used for survival analysis. The Log-Rank test was conducted for difference analysis in survival. And the Cox test was performed for multivariate analysis. The statistical tests were two-sided probability tests. Statistically significant level was considered as "alpha = 0.05".

Results

Expression of ERβ in breast cancer

ERβ expression in breast cancer was detected by immunohistochemistry. Representative immunohistochemical staining results were shown in **Figure 1**. Cells with brown particles in the nucleus were ERβ positive cells. No cells were stained positive in **Figure 1A**, suggesting that ERβ expression was negative (ERβ (-)). Cells were positively stained in **Figure 1B-D**, indicating a positive expression of ERβ. The positive expression cells were counted and the positive expression rate was calculated. Low expression of ERβ (ERβ (+)) with a positive rate between 1% and 10% was shown in **Figure 1B**. High expression of ERβ (ERβ (++)) with a positive rate between 10% and 50% were shown in **Figure 1C** and over expression of ERβ (ERβ (+++)) with a positive rate of > 50% were shown in **Figure 1D**, respectively.

Statistically, there were 380 cases with negative and low ERβ expression, accounting for 77.6% of the total 490 breast cancer cases. And the number of cases with ERβ high and

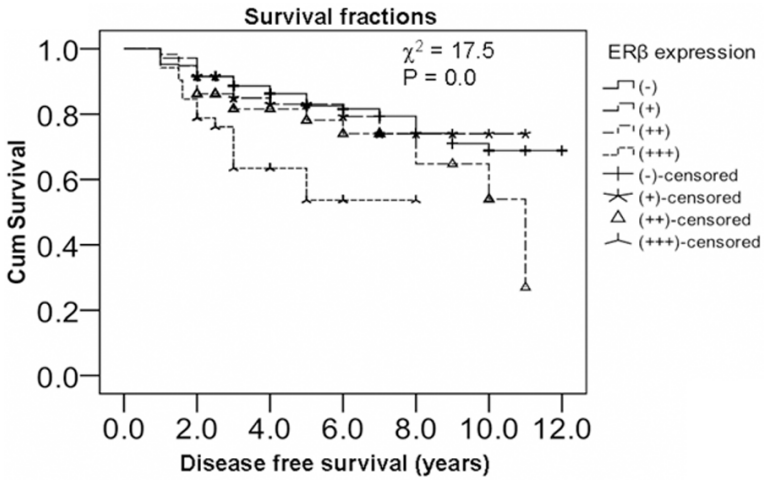

Figure 2. Effect of ERβ expression on disease-free survival of breast cancer patients. Kaplan-Meier survival curve of patients with ERβ negative expression (ERβ (-)), low expression ((+)), high expression (ERβ (++)) and over expression (ERβ (+++)) was displayed. Log-Rank test was performed to compare the difference in survival. Patients with negative and low expression of ERβ had an increased disease free survival compared to patients with high and over expression of ERβ.

over expression was 110, accounting for 22.4% of the total 490 breast cancer cases.

ERβ expression is not associated with clinicopathological parameters of breast cancer

To investigate the relationship between ERβ expression and clinicopathological parameters of breast cancer, we did correlation analysis by using Chi-square analysis. ERβ expression was divided into ERβ negative and low expression and ERβ high and over expression. The analyzed clinicopathological parameters included tumor size, lymph node metastasis, histological grade and pathologic stages of tumors. As shown in **Table 1**, there was no significant difference in ERβ expression among the different clinicopathological parameters of breast cancer ($P > 0.05$), indicating that ERβ expression may not be associated with clinicopathological parameters of breast cancer.

Higher and over ERβ expression shortens survival of breast cancer patients

To determine the relationship between ERβ expression and survival of breast cancer patients, we did survival analysis. The Kaplan-Meier curve for DFS was shown in **Figure 2** and the statistical results were shown in **Table 2**. The mean survival time in patients with ERβ negative expression, ERβ low expression, ERβ high expression and ERβ over expression was 9.9 years, 9.2 years, 8.6 years and 5.6 years, respectively. Thus the DFS in patients with ERβ negative and low expression was longer than that in patients with ERβ high and over expression. As revealed by the Log-Rank test, the DFS in patients with ERβ high and over expression was significantly lower than that in patients with ERβ negative and low expression ($\chi^2 = 17.5$, $P = 0.0$). This data suggest that high and over ERβ expression may decrease the survival of breast cancer patients.

Analysis of prognostic indicators of breast cancer

To further analyze the prognostic indicators of breast cancer, we performed Cox multivariate analysis. The analyzed variables were ERβ high and over expression, pathologic stages of tumors, endocrine therapy, chemotherapy and radiotherapy. As shown in **Table 3**, the factors of ERβ high and over expression, pathologic stages of tumors and chemotherapy could affect the DFS of breast cancer patients. This result suggests that these factors may be used as independent prognostic indicators of breast cancer.

Discussion

ERα is a member of nuclear receptor super family. Its expression level in breast cancer is closely related to treatment options and prognosis evaluation of breast cancer [10]. Besides ERα, ERβ is another newly discovered ER subtype. Several studies have shown that the expression of ERβ was also related to the clinicopathological parameters. However the results were controversial. Speirs et al [11] reported that ERβ expression in primary breast cancer tissue was positively correlated with axillary lymph node metastasis of breast cancer. Miyoshi et al [12] showed that ERβ expression in breast cancer was positively correlated with the high histological grade of breast cancer. In contrast, using immunohistochemistry and in situ hybridization, Jarvinen et al [13] detected the expression of ERβ in 92 cases of

Table 2. Disease free survival analysis of breast cancer patients with different levels of ERβ expression

ER-β expression	Mean survival time (year)				Median survival time (year)			
	Estimate	Standard Error	95% Confidence Interval		Estimate	Standard Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
ER-β (-)	9.9	0.3	9.3	10.4	-	-	-	-
ER-β (+)	9.2	0.4	8.3	10.0	—	-	-	-
ER-β (++)	8.6	0.6	7.4	9.7	11.0	1.6	7.9	14.1
ER-β (+++)	5.6	0.5	4.7	6.4	-	-	-	-
Overall	9.5	0.2	9.1	9.9	-	-	-	-

Note: "-" stands for null.

breast cancer tissue. And they did correlation analysis between expression levels of ERβ and clinicopathological parameters. They found that the ERβ positive expression rate in the group without axillary lymph node metastasis was higher than that in the group with axillary lymph node metastasis. And ERβ positive expression was negatively correlated with the histological grade of breast cancer. Skliris et al [14] suggest that the expression levels of ERβ in breast cancer decrease with tumor progression. High and over expression of ERβ may be the cause of lymph node metastasis but not the result of lymph node metastasis in breast cancer. However, Vinayagam et al [15] found that although ERβ2 mRNA levels were significantly associated with better outcome in the ERα positive breast cancer patients, this association was independent of grade, size, nodal status and progesterone receptor status. In this study, the percentage of ERβ negative and low expression was 77.6% (380 cases out of 490 cases) while the percentage of ERβ high and over expression was 22.4% (110 cases out of 490 cases). There were no significant difference in ERβ expression levels among the clinicopathological parameters of tumor size, lymph node metastasis, pathologic stage and histological grade. Our data were consistent with the results reported by Vinayagam [15], suggesting that ERβ expression may not be associated with clinicopathological parameters of breast cancer. The relationship between ERβ expression and the clinicopathological parameters of breast cancer is not conclusive and needs further investigation.

Although there was no significant difference in ERβ expression level among tumor size, lymph node metastasis, pathologic stage and histological grade, the survival of breast cancer patients were affected by ERβ expression. The mean survival time of patients with negative ERβ expression (ERβ (-)), low ERβ expression (ERβ (+)), high ERβ expression (ERβ (++)) and over ERβ expression (ERβ (+++)) was 9.9 years, 9.2 years, 8.6 years and 5.6 years. This showed that the mean survival time was gradually decreased along with the increase in ERβ expression level. Statistically, patients with high and over ERβ expression had significantly shorter survival time than those with negative and low ERβ expression, indicating that ERβ expression may be an indicator for poor prognosis. As mentioned previously, the prognostic value of ERβ in breast cancer is controversial. Our results were consistent with previous reports by Hou et al [3], Knowlden et al [4] and Wu et al [5]. However, our results were contrary to previous reports by Speirs et al [6], Ström et al [7], Nilsson et al [8] and Nakopoulou et al [9]. We suppose that this inconsistency might be caused by different subjects used and different sample size. Further studies are needed to clarify the role of ERβ in the prognosis of breast cancer.

Multivariate analysis by Cox test showed that, in breast cancer patients, ERβ high and over expression, pathologic stages of tumor and chemotherapy were independent prognostic risk factors for poor prognosis, whereas, endocrine therapy and radiotherapy were not independent prognostic factors. This may be caused by the short follow-up time of this study. The values of endocrine therapy and radiotherapy as prognostic factors were not fully presented during this short follow-up time.

Table 3. Cox multivariate analysis of the prognostic factors for the patients with breast cancer

Risk factors	Regression coefficient	Standard error	Wald value	*P* value	Odds ratio	95% Confidence Interval	
ERβ high and over expression	0.7	0.3	7.1	0.0	2.0	1.2	3.2
Tumor Stage			28.0	0.0			
Tumor Stage I/II	1.0	0.5	4.1	0.0	2.6	1.0	6.6
Tumor Stage III	2.1	0.5	18.3	0.0	8.4	3.2	22.4
Chemotherapy	1.0	0.3	8.4	0.0	2.6	1.4	5.1
Radiotherapy	0.5	0.3	3.4	0.1	1.6	1.0	2.7
Endocrine therapy	-0.1	0.2	0.3	0.6	0.9	0.5	1.4

In summary, ERβ expression was not related with the clinicopathological parameters of breast cancer. However, high and over expression of ERβ significantly affected the DFS of breast cancer patients. Multivariate analysis showed that ERβ could be used as an independent prognostic factor. High and over expression of ERβ were prognostic risk factors and implied poor prognosis. Collectively, our results suggest that ERβ may be used as a new prognostic indicator for breast cancer.

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Disclosure of conflict of interest

None.

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