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ORIGINAL PAPER

EGFR expression correlates with decreased disease-free survival in triple-negative breast cancer: a retrospective analysis based on a tissue microarray

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Abstract The aim of this study was to analyze the prognostic value of EGFR expression for patients with triple-negative breast cancer (TNBC). Clinical data of these patients were collected and analyzed, and immunohistochemical staining for EGFR was performed on tissue microarrays of operable breast cancer from 287 patients with TNBC, who were treated at Sun Yat-sen University Cancer Center from January 1995 to December 2008. EGFR expression was found in 36.2% of the cases with TNBC. A significant correlation was found between EGFR expression and disease-free survival (DFS). Univariated analysis indicated that EGFR expression had a significant prognostic value in TNBC patients, whereas multivariate analysis indicated that EGFR was a significant independent prognostic factor of DFS ($P = 0.011$) in all patients. Our results suggested that EGFR was an independent prognostic factor of DFS in patients with TNBC. Therefore, EGFR could become a good therapeutic target in the treatment of TNBC.

Keywords Breast carcinoma · Triple-negative · EGFR · Prognosis · Tissue microarray

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Introduction

Triple-negative breast cancers (TNBC) are characterized by the lack of expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2/neu) expression. TNBC accounts for about 15–20% of all breast cancers [1–4]. It is associated with poor overall prognosis, a high probability of early relapse after diagnosis, and increased risk of death after relapse. These clinical characteristics represent a major challenge to physicians in optimizing patient management [5–8].

Epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors. Its role in the development of many human malignant tumors has been investigated and it is now regarded as a promising target for cancer therapy [9]. In breast cancer, EGFR expression is found mainly in TNBC, and it could be a target for specific inhibitors [10]. Nevertheless, the prognostic role of EGFR in breast cancer remains controversial [11–16], and few studies have been made in regarding TNBC. The purpose of this study is to evaluate the expression of EGFR in TNBC, and to demonstrate its correlation with the prognosis of patients with TNBC.

Materials and methods

Patients and tissues

This retrospective study comprised 287 female patients with TNBC diagnosed without any evidence of distant

metastasis at the time of surgery between 1995 and 2008 at Sun Yat-sen University Cancer Center. Tissue samples were obtained from the patients through curative surgical resection. All specimens were formalin-fixed paraffin embedded. Clinical data, including patient age at diagnosis, menstrual status, tumor size, lymph node status, pathologic stage, treatment (surgery, chemotherapy, and radiotherapy), tumor recurrence, and follow-up status were retrospectively obtained from hospital medical records. The data analysis was approved by our cancer center review board.

Tissue microarray

Tissue microarrays were constructed as described previously [17]. Briefly, 287 formalin-fixed, paraffin-embedded tissue blocks containing breast cancer specimens were retrieved from the archives of the Department of Pathology. Representative areas of each invasive carcinoma were identified on the corresponding slides stained with hematoxylin and eosin. Tissue cylinders with 1-mm diameter were punched from each donor tissue block and entered into a recipient paraffin block using a tissue microarrayer. The recipient paraffin block was subsequently cut and the slices were transferred with adhesive tape onto coated slides. Then, the slides were dipped in paraffin to prevent oxidation. Each sample was arrayed in triplicate to minimize tissue loss and to overcome tumor heterogeneity.

Immunohistochemistry

Tissue microarray sections were immunohistochemically stained for EGFR. Briefly, tissue microarray slides generated from the paraffin-embedded tissue blocks were deparaffinized and rehydrated for 5 min. After microwave pretreatment in citrate buffer (pH 6.0) for antigen retrieval, the slides were immersed in 0.3% (vol/vol) hydrogen peroxide for 20 min to block endogenous peroxidase activity. The slides were then washed and incubated overnight at 4°C with primary antibodies against EGFR (1:250 dilution; DAKO). After a second incubation with a biotinylated anti-goat antibodies, the slides were incubated with peroxidase-labeled streptavidin. The reaction products were visualized by immersing the slides in diaminobenzidine tetrachloride and counterstaining with Harris hematoxylin. Staining for EGFR was considered positive only if a minimum of 10% of definite tumor cells show positive reaction.

Statistical analysis

Statistical analysis was performed using SPSS16.0 software. A chi-square test was used to investigate the significance of the relationship between EGFR and the individual variables. The relationship between EGFR expression and the clinical outcomes was estimated though both univariate and multivariate analyses. The disease-free survival (DFS) and overall survival (OS) curves were estimated using the Kaplan–Meier method, and the difference in the survival curves were compared using the log-rank test. A multivariate analysis was performed using Cox's regression model. The P values ≤ 0.05 denote statistical significance.

Results

Patient and tumor characteristics

A total of 287 TNBC patients were grouped according to their EGFR status (positive or negative). Among the 287 tumor specimens, 104 (36.2%) were positive for EGFR (Fig. 1). The association of EGFR expression with various clinicopathological parameters is listed in Table 1. All clinicopathologic parameters, including tumor size, lymph

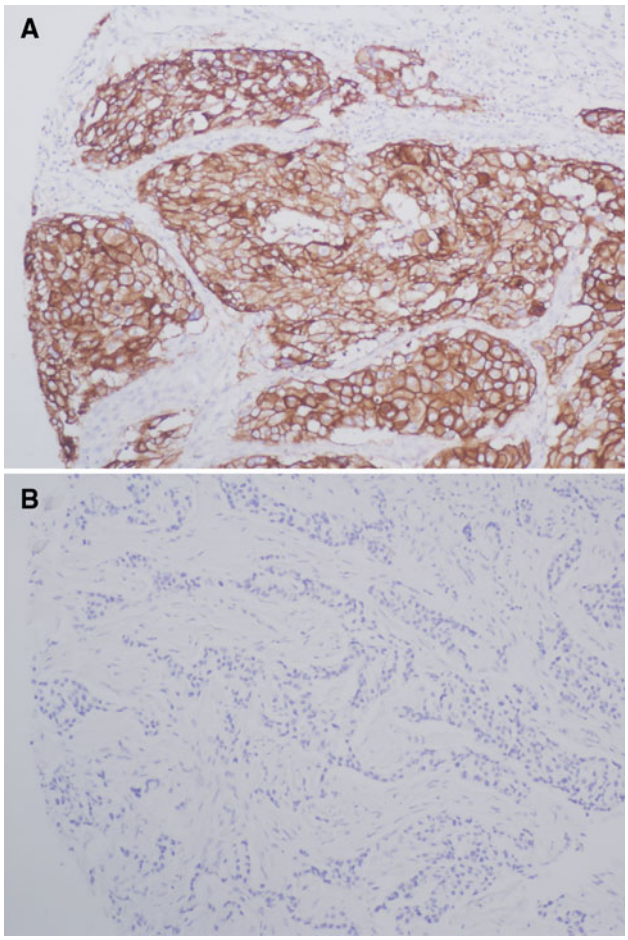

Fig. 1 Representative immunohistochemical staining results for EGFR (×100). **a** EGFR positive; **b** EGFR negative

Table 1 Clinicopathologic characteristics of patients according to EGFR expression

Characteristics	EGFR negative		EGFR positive		*P* value
	No.	%	No.	%	
Number	183	63.8	104	36.2	–
Age, years					
≤40	19	10.3	15	14.4	0.344
>40	164	89.7	89	85.6	
Tumor size, cm					
≤2	44	24.0	23	22.1	0.773
>2	139	76.0	81	77.9	
Node status					
Negative	96	52.5	50	48.1	0.539
Positive	87	47.5	54	51.9	
Stage					
I/II	135	73.8	72	69.2	0.980
III	48	26.2	32	30.8	
Differential grade					
I	8	4.4	3	2.9	0.906
II	32	17.5	19	18.3	
III	30	16.4	19	18.3	
Unknown	113	61.7	63	60.6	
Primary surgery					
Mastectomy	180	98.4	102	98.1	0.262
Breast conservation surgery	3	1.6	2	1.9	
Adjuvant chemotherapy					
Do	176	96.2	99	95.2	0.762
Undo	7	3.8	5	4.8	
Adjuvant radiotherapy					
Do	35	19.1	27	26.0	0.183
Undo	148	80.9	77	74.0	

node status, pathologic stage, histopathology, adjuvant chemotherapy, and adjuvant radiotherapy were not significantly different between the two groups (Table 1).

Survival

As of December 2009, the median follow-up time was 72 months (range, 8–182 months). Of all 287 patients, 67 relapsed and 48 died. The 5-year DFS and OS of all patients were 84.9 and 87.8%, respectively. When the patients were stratified in terms of EGFR status, the 5-year DFS for EGFR-positive and EGFR-negative patients were 69.0 and 83.8%, respectively. DFS was significantly poorer for the EGFR-positive patients (HR = 2.11, $P = 0.011$) (Fig. 2, Table 2). The overall survival rates of EGFR-positive and EGFR-negative patients were 79.5 and 88.9%, respectively. A similar trend was seen although it did not reach statistical significance (HR = 1.65; $P = 0.090$, data not shown).

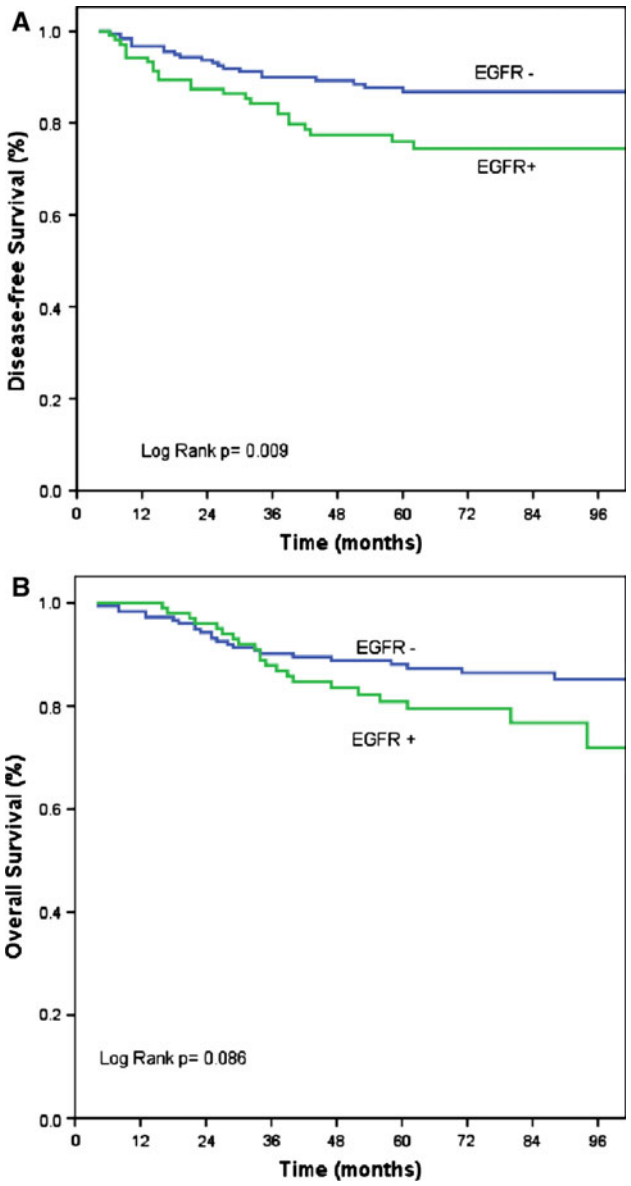

Fig. 2 Disease-free survival (**a**) and overall survival (**b**) curves according to EGFR status

Univariate analysis and multivariate analysis

Statistically significant predictors of DFS within the univariate analysis are listed in Table 2. Positive lymph nodes, higher stage, radiotherapy, and positive EGFR were correlated with shorter DFS. Patients with tumor cells positive for EGFR expression had significantly worse outcomes in terms of DFS ($P < 0.001$) than patients with negative EGFR expression. In the multivariate analysis, positive EGFR remained a significant predictor of DFS when

Table 2 Univariate and multivariate analysis of disease-free survival in all population

Variables	Univariate			Multivariate		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Age, years (≤40 vs. >40)	0.63	0.29–1.34	0.228	0.72	0.34–1.57	0.412
Tumor size, cm (≤2 vs. >2)	1.23	0.59–2.54	0.582	0.95	0.45–2.00	0.886
Node status (Neg. vs. Pos.)	4.39	2.186–8.83	<0.001	3.42	1.53–7.66	0.003
Stage (I/II vs. III)	2.59	1.45–4.61	0.001	0.98	0.48–2.01	0.962
Differential grade (I/II vs. III)	1.39	0.65–301	0.398	1.07	0.49-2.34	0.866
Chemotherapy (Do vs. Undo)	2.35	0.32–17.04	0.399	0.99	0.13–7.76	0.997
Radiotherapy (Do vs. Undo)	3.24	1.81–5.79	<0.001	2.11	1.07–4.14	0.030
EGFR status (Neg. vs. Pos.)	2.11	1.19–3.74	0.011	1.97	1.10–3.51	0.022

entered into a model containing all clinicopathologic variables ($P = 0.022$) (Table 2).

Discussion

Our retrospective study demonstrated that the positive rate of EGFR was 36.2% in TNBC. EGFR expression was reported in more than 50% of TNBC patients [18]. The differences reported may be due to the differences in the methods using in detecting EGFR, in selecting the patient population, and in the number of cases analyzed. The present study consisted of a large series of patients at a single center and the method for detecting EGFR was the same for all patients. Furthermore, the criterion for positivity in our study was a minimum of 10% of definite tumor cells.

There have been many studies analyzing the prognostic value of EGFR for breast cancer [11–16]. However, the role of EGFR as a prognostic marker in breast cancer remains unclear. Our data suggest that patients with EGFR-positive TNBC have significantly less favorable prognoses than patients with EGFR-negative TNBC, although the two groups are not significantly different in terms of various clinicopathological parameters. These results were consistent with those of published studies [19, 20].

The univariate analysis shows a significant relationship between EGFR expression and DFS. There have been a few studies analyzing the prognostic value of EGFR in the subgroups. The multivariate analysis indicated that EGFR had a prognostic significance for DFS. Our findings show that EGFR-positive patients with TNBC had worse clinical outcomes compared with EGFR-negative patients. For OS, a similar trend was seen although it did not reach statistical significance. The type of disease management the patients received might have caused this difference.

Epidermal growth factor receptor is considered an attractive therapeutic target in many human malignancies. However, its role in breast cancer treatment is still poorly understood. Applying EGFR-targeted therapy in the treatment of TNBCs, therefore, offers promising new approaches.

In conclusion, our study indicates that EGFR is an independent prognostic factor for DFS in a large series of patients with TNBC. Hence, EGFR-targeted therapy can potentially improve survival of patients with TNBC.

Conflict of interest All authors have no conflicts of interest.

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