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ORIGINAL ARTICLE

Association of insulin-like growth factor 1 receptor and estrogen receptor with pathological complete response to neoadjuvant chemotherapy in HER2-negative breast cancer

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Abstract

Purpose To investigate the association of insulin like growth factor 1 receptor (IGF-1R) and estrogen receptor (ER) with pathological complete response (pCR) secondary to neoadjuvant chemotherapy in human epidermal growth factor receptor-2 (HER2)-negative breast cancer.

Materials and methods The immunohistochemical expressions of IGF-1R and ER were detected in puncture specimens from 273 patients who received neoadjuvant chemotherapy. Association of IGF-1R and ER expression with pCR to neoadjuvant chemotherapy was evaluated.

Results In 273 cases of breast cancer, the high expression rate of IGF-1R was 42.1% (115/273) and the ER-positivity rate was 63.0% (172/273). The positive rate of IGF-1R in ER-positive patients (51.2%) was significantly higher than that in ER-negative patients (26.7%; $p < 0.01$). Multivariate analysis showed that the numbers of chemotherapy circles, TNM stage and ER status were independent factors for pCR rate. The pCR rate in ER-negative patients (14.9%) was significantly higher than that in ER-positive patients (6.4%; $p < 0.05$). The pCR rate in patients with low and high expression of IGF-1R was 8.9% and 10.4%, respectively, which was not statistically significant ($p > 0.05$). Further analysis of the impact of IGF-1R expression status on the pCR rate in ER-positive subgroup did not show any significant associations. Similar results were found in the ER-negative subgroup ($p > 0.05$). In the high-IGF1R subgroup, the pCR rate was higher for ER-negative than that for ER-positive (25.9% vs. 5.7%) patients, which was statistically significant ($p < 0.01$), while in the low-IGF1R subgroup, there was no significant difference between ER-negative and ER-positive (10.8% vs. 7.1%, $p = 0.42$) patients. The pCR rate of ER-negative and high-IGF-1R expression (25.9%) patients was significantly higher than the overall pCR rate ($p < 0.01$).

Conclusion ER-negative patients were more sensitive to neoadjuvant chemotherapy, and the pCR rate was significantly higher in ER-negative than in ER-positive patients in the high-IGF1R subgroup.

Keywords Insulin-like growth factor type 1 receptor · Estrogen receptor · Breast cancer · Neoadjuvant chemotherapy

Introduction

Neoadjuvant chemotherapy is the standard treatment for locally advanced breast cancer [1]. It is increasingly used for patients with operable breast cancer who are desirous of breast-conserving surgery [2]. A combination of docetaxel with epirubicin (TE) has been shown to be most effective in reducing the risk of postoperative tumor recurrence and metastasis, and in improving remission rates and prolonging the survival of patients with recurrent metastatic breast cancer [3]. Breast cancer is a highly heterogeneous malignancy at the molecular level, and different subtypes exhibit variable sensitivity to a particular regimen [4]. Identification of subtypes that are highly sensitive to a particular regimen facilitates

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the implementation of targeted regimens and individualized treatment.

Pathological complete response (pCR) to neonadjuvant chemotherapy was shown to be an independent predictor of prognosis and to significantly prolong the disease-free survival and overall survival of patients [5]. Response to chemotherapy is influenced by several factors including those related to the breast tumor itself, type of chemotherapy used and the number of treatment cycles [6, 7]. No doubt, the tumor characteristics including tumor size, histological grade, number of involved lymph nodes, and receptor status are equally important. The expression status of several receptors, such as hormone receptors and type 1 insulin-like growth factor receptor (IGF-1R), has been shown to correlate with chemotherapeutic response [8, 9].

IGF-1R is a tyrosine kinase protein that was shown to play an important role in the proliferation, invasion, and metastatic behavior of various cancers including breast cancer [10–13]. IGF-1R expression was shown to be strongly associated with higher mitotic scores and shorter disease-free survival in patients with triple-negative breast cancer [14]. IGF-1R is activated by type 1 insulin-like growth factor (IGF-1) and is related to resistance to chemotherapy and hormonal therapy [15, 16]. Estrogen receptor (ER) is a classical hormone receptor which is a crucial mediator of endocrine therapy for breast cancer. The interaction between IGF-1R and ER has increasingly evoked the interest of researchers. Studies have shown that positive expression of IGF-1R is a favorable prognostic factor in patients with ER-positive tumors; however, this effect is lost in the case of ER-negative invasive ductal breast carcinoma [17]. Zhang et al. [18] demonstrated that IGF1/IGF-1R signaling axis may play a causal role in the resistance of breast cancer cells to anti-estrogen therapy. Indeed, IGF1 is considered a new target for treatment of ER-positive breast cancer [19]. In vitro and in vivo studies have shown that IGF1 signaling pathway induces transcriptional activation of ER target genes and plays an important role in inducing resistance of ER-positive breast cancer cells to tamoxifen. Moreover, ER can also be activated by the downstream gene of IGF-1R [20, 21].

Therefore, a comprehensive exploration of the correlation of IGF-1R and ER expression status with pCR to neoadjuvant chemotherapy may help in early prediction of the response and to direct the next therapy. However, few studies have investigated this correlation. In this study, we examined the expression levels of IGF-1R and ER proteins in patients with breast cancer to investigate their association with response to TE regimen therapy.

Materials and methods

Patients

This study was approved by the ethical committee of Tianjin Medical University Cancer Institute and Hospital. A retrospective analysis of 273 female patients (age range, 22–69 years) with breast cancer in our hospital from January 2008 to January 2016 was performed. The inclusion criteria were: (1) patients diagnosed with invasive breast cancer by invasive needle biopsy; HER2 evaluated according to immunohistochemical (IHC) examination results [22] with 0–1(+) identified as negative expression; if the level of HER2 was 2 (+), the patient would have to carry on fluorescence in situ hybridization (FISH) detection with HER2 expression identified negative when no amplification was detected; (2) patients with clinical stage (TNM stage) IIB, III, IIB and IIIA (T3N1M0), who desired breast conservation, locally advanced disease, including IIIA (T2N2M0, T1N2M0, T2N2M0, T3N2M0), IIIB (T4N0M0, T4N1M0, T4N2M0), and IIIC (TN3M0); (3) no history of radiotherapy, chemotherapy or biotherapy; (4) presence of measurable lesions based on the Response Evaluation Criteria In Solid Tumors (RECIST); (5) availability of well-preserved paraffin-embedded specimens for immunohistochemical examination; (6) availability of clinical and imaging data for each cycle of neoadjuvant chemotherapy; (7) availability of pathological data of puncture and postoperative specimens. The clinical and pathological data of 273 breast cancer patients are shown in Table 1.

Treatment

All patients received TE neoadjuvant chemotherapy with docetaxel 75 mg/m^2 plus epirubicin 90 mg/m^2. The responses were evaluated after 2 cycles of chemotherapy to determine the follow-up treatment options. Seventeen patients received 2 cycles, 39 cases received 3 cycles, 199 cases received 4 cycles, 10 cases received 5 cycles, and 8 cases received 6 cycles of chemotherapy. Patients underwent surgery 10–14 days after the end of chemotherapy (6 patients underwent breast-conserving surgery and 267 patients underwent modified radical mastectomy).

Response evaluation

Clinical response to neoadjuvant chemotherapy was evaluated according to RECIST solid tumor assessment criteria (version 1.1) [23]. pCR was defined as absence of invasive

Table 1 Patient and tumor characteristics

	N = 473	Percentage (%)
Age at diagnosis (years)		
Mean, range	45 (22–69)	
Menopausal status at diagnosis		
Pre	134	49.1
Peri	37	13.6
Post	102	37.3
Tumor size (cm)		
2–5	189	69.2
>5	84	30.8
Axillary nodal status		
Positive	201	73.6
Negative	72	26.4
Histological grade		
Grade 1	2	0.7
Grade 2	239	85.9
Grade 3	32	11.8
TNM stage		
IIA	30	11.1
IIB	79	28.9
IIIA	103	37.7
IIIB	43	15.8
IIIC	18	6.5

carcinoma in the post-therapy resection specimen and in the regional lymph nodes.

Immunohistochemical examination for IGF-1R and ER expressions

IGF-1R expression is mainly located in the cell membrane or cytoplasm. The following methodology was used for assessment: (1) scores were awarded according to the percentage of positive cells (0 = no positive cells; 1 = 10–25%; 2 = 26–50%; 3 = more than 50% cells); (2) the cell staining intensity score was awarded as follows: 0 = no-coloration; 1 = yellow; 2 = brown; 3 = deep brown. The sum of the above two scores was used to determine the staining intensity level [0–1 = negative (−), 2–3 = weakly positive (+), 4–5 = moderately positive (++), 6 = strong positive (+++)]. The sum of positive cell percentage score and cell staining intensity score more than 4 was classified as high IGF-1R expression and the score less than 4 was classified as low IGF-1R expression. ER-positive standard was at least 10% positive tumor nuclei in the breast samples according to Dutch guidelines (http://www.oncoline.nl).

Statistical analysis

Statistical analysis was performed with SPSS software 22.0 (SPSS, Chicago, IL, USA). pCR was compared among different IGF-1R and ER expression classes using Chi-squared test. Multivariate analysis was done using logistic regression. A p value < 0.05 was considered statistically significant.

Results

ER expression in breast cancer cells is localized in the nucleus. As shown in Fig. 1, nuclear staining present in more than 10% of the cells was considered ER positive. Among 273 breast cancer patients, the positivity rate of ER was 63.0% (Table 2). IGF-1R expression in breast cancer was predominantly seen on the membrane and minimal expression was observed in the cytoplasm (Fig. 2). The high expression rate of IGF-1R was 42.1%. The high expression rate of IGF-1R in ER-positive and ER-negative patients was

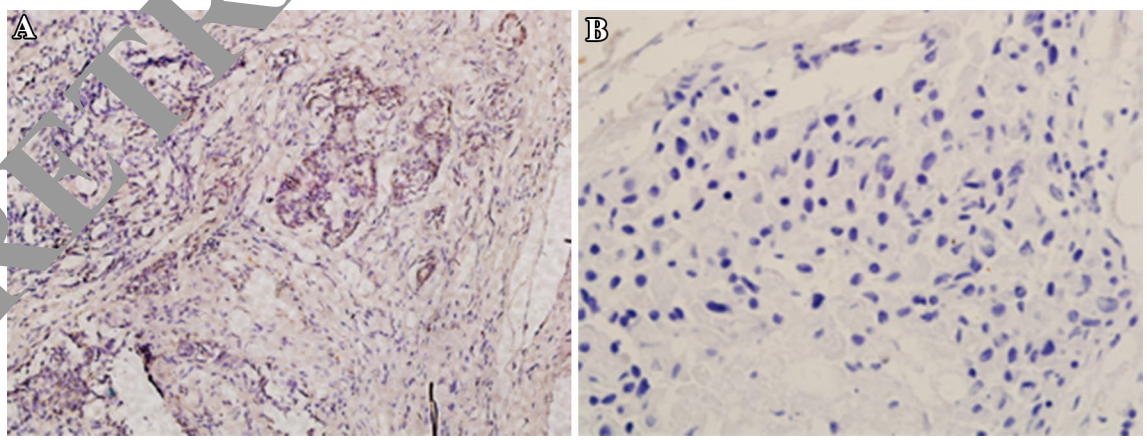

Fig. 1 Representative images of immunohistochemical staining showing **a** ER-positive and **b** ER-negative breast cancer (magnification ×20). *ER* estrogen receptor

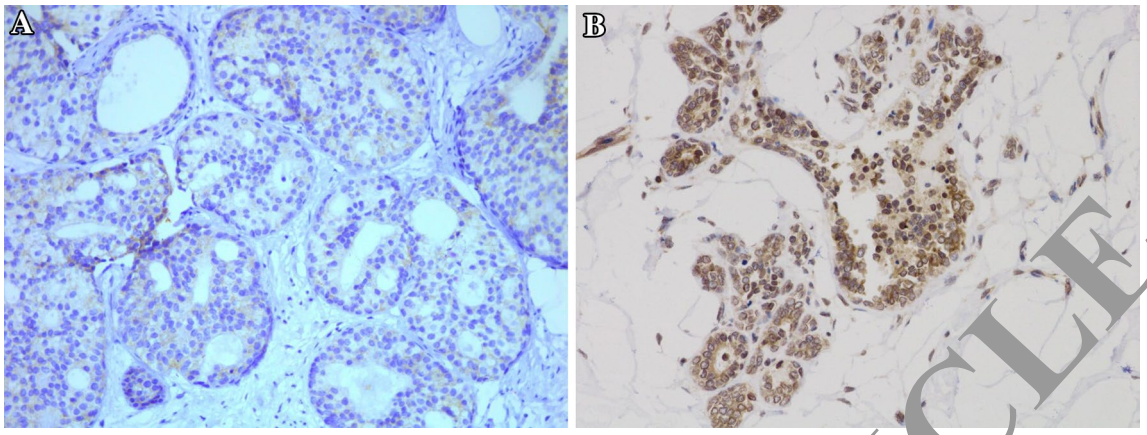

Fig. 2 Representative images of immunohistochemical staining showing a IGF-1R low expression (magnification ×20) and b IGF-1R high expression (magnification ×40). IGF-1R are stained deep brown. IGF-1R, type 1 insulin-like growth factor receptor

Table 2 ER and IGF-1R expression in 273 breast cancer lesions

ER	IGF-1R (%)		Total	Statistical significance (*P*)
	High expression	Low expression		
Positive	88 (51.2)	84 (48.8)	172 (63.0)	< 0.001
Negative	27 (26.7)	74 (73.3)	101 (37.0)	
Total	115 (42.1)	158 (57.9)	273	

ER estrogen receptor, *IGF-1R* insulin-like growth factor receptor 1

51.2% and 26.7%, respectively; the between-group difference was statistically significant ($p < 0.01$, Table 2).

After receiving TE regimen neoadjuvant chemotherapy, 26 out of the 273 (9.5%) patients showed pCR. The description of different factors is shown in Table S1 and univariate analysis results showed that the number of chemotherapy circles, axillary nodal status, TNM stage and ER status were significant factors for pCR rate. Multivariate analysis showed that the numbers of chemotherapy circles, TNM stage and ER status were independent factors for pCR rate (Table S2).

Considering ER alone, pCR rate was 14.9% and 6.4% in ER-negative and ER-positive patients, respectively; the between-group difference was statistically significant ($p < 0.05$). Considering IGF-1R alone, the pCR rate in patients with high and low expression levels of IGF-1R was 10.4% and 9%, respectively; the between-group difference was not statistically significant ($p > 0.05$).

In the ER-negative subgroup, the pCR rate was numerically higher in high-IGF1R patients than that in low-IGF1R (25.9% vs. 10.8%) patients. However, there was no statistical significance ($p = 0.11$). In the ER-positive subgroup, the PCR rate of high-IGF1R and low-IGF1R patients was 5.7% and 7.1%, respectively. There was no significant difference ($p > 0.05$). In the high-IGF1R subgroup, the pCR rate was higher in ER-negative than in ER-positive (25.9% vs. 5.7%) patients, which was statistically significant ($p < 0.01$). While in the low-IGF1R subgroup, there was no significant difference between ER-negative and ER-positive (10.8% vs. 7.1%, $p = 0.42$) patients. The PCR rate of ER-negative/high-IGF1R was significantly higher than that of the overall PCR rate ($p < 0.05$) (Table 3).

Discussion

In this study, we analyzed the expressions of ER and IGF-1R in 273 breast cancer patients and found that high expression of IGF-1R was commonly observed in ER-positive cancers and low expression was observed in ER-negative cancers. Our findings are consistent with the results reported by Rohit Bhargava and colleagues [24], and we believe that the expression patterns observed in the present study support the close link between the expressions of IGF-1R and ER. It is evident that IGFs pathways can activate ER, and the activated ER complexes bind to the target genes including IGF-1R and regulate their transcription. An increasing body of evidence from preclinical studies shows that the effects of ER activation may be amplified by IGF-1R with or without estradiol. Therefore, co-targeting ER and IGF-1R may offer benefit to breast cancer patients [25].

Subsequently, we analyzed the association of IGF-1R and ER expression status with pCR to TE regimen neoadjuvant chemotherapy. We found that regardless of IGF-1R

Table 3 Association of ER and IGF-1R with pCR to neoadjuvant chemotherapy in 273 breast cancer patients

	pCR (%)	Not pCR (%)	Statistical significance (*P*)
ER^+	11 (6.4)	161 (93.6)	0.02
ER^-	15 (14.9)	86 (85.1)	
IGF-$1R^+$	12 (10.4)	103 (89.6)	0.66
IGF-$1R^-$	14 (8.9)	144(91.1)	
ER^+, IGF-$1R^+$	5 (5.7)	83 (94.3)	0.94
ER^+, IGF-$1R^-$	6 (7.1)	78 (92.9)	
ER^-, IGF-$1R^+$	7 (25.9)	20 (74.1)	0.11
ER^-, IGF-$1R^-$	8 (10.8)	66 (89.2)	
IGF-$1R^+$, ER^+	5 (5.7)	83 (94.3)	0.007
IGF-$1R^+$, ER^-	7 (25.9)	20 (74.1)	
IGF-$1R^-$, ER^+	6 (7.1)	78 (92.9)	0.42
IGF-$1R^-$, ER^-	8 (10.8)	66 (89.2)	
ER^-, IGF-$1R^+$	7 (25.9)	20 (74.1)	0.02
All patients	26 (9.5)	247 (90.5)	

ER+ estrogen receptor positive, *ER−* estrogen receptor negative, *pCR* pathological complete response, *IGF-1R*$^+$ IGF-1R high expression, *IGF-1R*$^-$ IGF-1R low expression

expression, the pCR rate in ER-negative patients was signi-cantly higher than that in ER-positive patients. This resu is similar to other published data [26]. Considering IGF-1R expression alone, no significant differences with respect to the pCR rate were observed between patients with lo and high expressions of IGF-1R. There is a dearth studies that have investigated the association betwe IGF-1 expression alone and the prognosis of breast cancer. Mostly, studies have revealed the association of GF-1R with shorter disease-free survival in patients with ent molecular subtypes of breast cancer, espe in the context of ER-positive and triple-negative breast cancer [15, 17].

We next evaluated the ect of IGF-1R on the pCR of ER-positive and ER-ne ents separately. The results showed that among all the oups, ER-negative and IGF-1R high expressio nts exhibited the highest (25.9%) pCR. This result i consist with the previous literature. Rohit Bhargava et al. [24] analyzed 86 breast cancer patients who received n adjuv nt chemotherapy and found that patients wit -nega e and high IGF-1R expression had the high-s ensi ity to the TE regimen (pCR rate: 40%). Further anal s of the impact of IGF-1R expression on the pCR rate in ER-positive and ER-negative subgroups separately showed no significant differences. But Rohit Bhargava et al. [24] found that IGF-1R could be predictive of pCR in the ER-positive subgroup and provided no useful information in the ER-negative subgroup. This difference is likely attributable to differences with respect to the analytic method for immunohistochemistry staining of IGF-1R, sample size and the diversity of the study population between the two studies. In their study, only membranous immunoreactivity was taken into account for scoring of IGF-1R, while in the present study, both membranous and cytoplasmic staining were considered.

There are several limitations of the present study. A previous study found that cytoplasmic IGF-1R was associated with a more favorable prognosis in ER-po ive breast cancer; however, this effect was not observed wit membranous or overall IGF-1R expression [17, 24]. In the present study, we just analyzed the overall expressio including the membranous and cytoplasmic IGF-1R expre ion; the impact of membranous and cytoplas ic IGF-1R on pCR of breast cancers needs to be separ tely aluated in a future study. In addition, the sample size is o small with a lot of deviation and more cases are eeded in ture work.

In conclusion, patie with ER-negative breast cancer are more sensitiv neoadj vant chemotherapy with TE regimen, and the pC rate of ER-negative patients was significantly higher an that of ER-positive patients in the high-IGF1R subgroup. Concomitant assessment of biomarkers of ER and IG may help predict the efficacy of neoadjuvant chemotherapy and can facilitate selection of personalized chemotherapy regimens for specific populations.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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