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ORIGINAL ARTICLE

ERCC1 expression in triple negative breast cancer

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Summary

Purpose: *Excision repair cross-complementation group 1 (ERCC1), which is a component of nucleotide excision repair (NER) pathway, removes platinum-induced DNA adducts. Overexpression of ERCC1 has been associated with resistance to platinum-based chemotherapy in ovarian and lung cancers. Detecting ERCC1 overexpression is important in considering treatment options for triple negative breast cancer (TNBC), and in conducting and interpreting trials that search to find specific chemotherapy regimens for TNBC. In this study we aimed to study ERCC1 overexpression in patients with TNBC.*

Methods: *A monoclonal antibody against ERCC1 was used for immunohistochemical (IHC) analysis of tumor samples. Tumor samples from 45 patients were evaluated by two experienced pathologists who were blinded to clinical data. A semi-quantitative H score (intensity staining scale ranging from no staining/0 to very intense staining/3^{+}) was calculated by multiplying staining intensity with extent score. Tumors with H score ≥ 1 were classified as ERCC1-positive.*

Results: *ERCC1 expression was positive in 73.3% of the tumor samples with an H score ≥ 1 and 26.7% of the tumor samples stained negative with an H score < 1. Of the tumor samples 15.5% stained diffusely and intensively.*

Conclusion: *Our study demonstrated that about two thirds of the TNBC showed positive expression of ERCC1, which may be predictive of a poor response to platinum-based chemotherapy.*

Key words: ERCC1, platinum-based chemotherapy, triple negative breast cancer

Introduction

TNBC is characterized by lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER-2) [1]. TNBC comprises 15% of sporadic breast cancer cases [2,3]. It is more prevalent among premenopausal Afro-American women and it is seen in younger populations. The median age of TNBC patients at the time of diagnosis was 53 years compared to 58 years in other subtypes of breast cancer [4,5].

TNBC has more aggressive course compared to non-TNBC. Although it is more aggressive, treatment options for TNBC are limited due to hormone receptor (HR) and HER-2 negative status [6]. Up to know no specific chemotherapeutic regimen exists, and standard cytotoxic chemotherapeutic regimens are used as for other sporadic breast cancers [6,7]. A number of ongoing studies try to identify regimens according to the sensitivity of this tumor subtype to specific chemotherapeutics [8].

Both preclinical and clinical studies have shown that tumors with BRCA1 dysfunction are more susceptible to chemotherapeutics that create defects in DNA repair pathways such as platin analogs [9,10]. The relationship between TNBC and BRCA1 dysfunction has led to many studies that try to identify the importance of platin analogs in the treatment of TNBC [11-13]. Garber et al. reported that neoadjuvant single-agent cisplatin yielded a 23% pathological complete response (pCR) rate in patients with TNBC [11]. In another study 50% of patients with TNBC experienced pCR with neoadjuvant docetaxel and carboplatin, compared with 31% of patients with HER-2 (+) disease with docetaxel/carboplatin and trastuzumab [14]. Torrisi et al. reported overall response rate of 86% and pCR rate of 40% with 4 cycles of neoadjuvant epirubicin, cisplatin, and fluorouracil (ECF) chemotherapy followed by 3 cycles of weekly paclitaxel [15]. Platinum compounds seem effective in metastatic

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disease as well. Yi et al. reported 106 metastatic breast cancer patients of whom 36 had TNBC and received platinum-containing chemotherapy as first or second-line treatment; overall response rate was 39% and disease control rate 67%, similar to the rate seen in other phenotypes [16]. Uhm et al. reported similar overall response rates to platinum and taxane combination as first- or second-line treatment in metastatic breast cancer [17].

As mentioned before, there are many ongoing studies trying to identify the importance and place of platinum analogs in the treatment of TNBC. Platinum analogs have been used for years in the treatment of many other tumors such as lung, head and neck, ovarian, bladder and testicular cancers. Cisplatin leads to cell death by binding to DNA and forming DNA adducts, disrupting the structure of DNA molecule and creating changes in the helix [18]. Nucleotide excision repair pathway repairs these DNA lesions that alter the structure of DNA molecule [18,19].

Resistance to platinum-based chemotherapy limits its efficacy in many malignant diseases [19]. DNA repair mechanisms are important in resistance to cisplatin. As mentioned earlier, nucleotide excision repair pathway seems to be the key pathway involved in mediating resistance to platinum compounds [18]. The ERCC1 protein plays a key role in nucleotide excision repair. ERCC1 forms a dimer with xeroderma pigmentosum complementation group F, and this complex excises the cisplatin-induced DNA adducts [19]. The relation between ERCC1 expression and resistance to platinum compounds has been reported in testicular, ovarian, gastric, esophageal and non-small cell lung cancer [20-31]. Sidoni et al. reported that ERCC1 expression was positive in 26 out of 81 (32%) TNBC [32].

Since there are many ongoing studies to identify a specific chemotherapeutic regimen for TNBC and platinum compounds seem promising in this setting, we aimed to detect ERCC1 expression in our patient population who had TNBC.

Methods

Patients

Tumor tissue samples of 45 patients diagnosed with TNBC were obtained from the Hacettepe University Hospital, Department of Pathology archive. Patient clinical characteristics were assessed from the patients hospital records. Slides stained for ER, PR, and HER-2 were re-evaluated by two pathologists experienced in breast cancer pathology, who revised the tumor types and grades, and confirmed that tumor samples were ER, PR and HER-2 negative. ERCC1 immunostaining was performed and evaluated at the Hacettepe University Department of Pathology.

Immunohistochemical evaluation of ER, PR, and HER-2

ER and PR staining results were characterized as negative when < 1% of the tumor cells were positive. For HER-2 HIC staining ASCO/CAP recommendations for breast cancer were used [33]. Cases were defined as 3+ when IHC staining was uniform and intense in >30% of tumor cells. No staining or membrane staining in <30% of tumor cells were defined as 0. Faint membrane staining in > 30% of tumor cells or only part of the membrane was defined as 1+. Weak/moderate complete membrane staining in >30% of tumor cells was defined as 2+, and for definite characterization of HER-2 status FISH was performed in these cases. Each 0 and 1+ cases were characterized as HER-2 negative. Also 2+ cases that were FISH-negative were also characterized as HER-2 negative.

Immunohistochemical analysis of ERCC1

Four-microns tissue sections prepared from formalin-fixed and paraffin-embedded representative tumor samples were used. After deparaffinization, rehydration and blockage of peroxidase activity were carried out, using 0.3% solution of hydrogen peroxidase in phosphate-buffered saline (PBS) (0.01 mol/L, pH 7.5) at room temperature for 10 min. The sections were immersed in 0.01 M sodium citrate buffer (pH 6.0) for 3 min for epitope retrieval. Then, the primary antibody was allowed to react at room temperature for 40 min in ERCC1 dilutions of 1/25 (mouse, clone 8F1, Novus Biologicals, Littleton, CO, USA). After washing in PBS, secondary antibody was applied for 10 min, followed by streptavidin-peroxidase complex (ScyTek Laboratories, Logan, Utah, USA). Peroxidase was visualized by diaminobenzidine tetrahydrochloride containing 0.3% H_2O_2. After rinsing in deionized water and counterstaining in Harris' hematoxylin, the slides were dehydrated and mounted. Sections of normal tonsil tissues were included as external positive controls and stromal cells around the tumor area as internal positive controls. *Tumor* slides were evaluated independently by two *pathologists* who were unaware of the clinical patient characteristics. The samples were analyzed using standard *light microscopy*. Tumor sections were evaluated by a semiquantitative scoring system [34]. The staining intensity was graded on a scale of 0 to 3, higher number indicating higher intensity. The percentage of positive tumor nuclei was calculated for each tumor sample and a proportion score was assessed; 0 if 0%, 0.1 if 1-9%, 0.5 if 10-49%, and 1.0 if 50% or more. This score was multiplied by the staining intensity of tumor samples to obtain a final semiquantitative H score. Tumor samples were considered positive for ERCC1 when the H score was ≥ 1.

Statistical analysis

A computer program package SPSS version 15.0 was used for all statistical analyses. Baseline characteristics of ERCC1 positive cases were compared with ERCC1 negative ones by x^2 test (for categorical variables) or by two-sample t-test for continuous variables. All p values were two-tailed, and the 0.05 level was considered statistically significant.

Results

Patient and disease characteristics

The median age of 45 patients included in this study was 49.8 years (range 30-81). Twenty-two

(48.9%) of them were premenopausal, 3 (6.7%) perimenopausal and 20 (44.4%) postmenopausal. T status was T1 in 15 (33.3%) patients, T2 in 23 (44.4%), T3 in 3 (6.7%) and T4 in 4 (8.9%). N status was determined as N0 in 23 (51.1%) patients, N1 in 11 (24.4%), N2 in 1 (2.2%), N3 in 7 (15.6%), while in 3 (6.7%) patients N status was not known. Only one patient (2.2%) had metastatic disease. Forty-two (93.4%) of 45 patients had infiltrative ductal carcinoma (IDC), 1 (2.2%) infiltrative lobular carcinoma (ILC) and 2 (4.4%) patients had mixed IDC and ILC. The histological grade of 13 (28.9%) patients was 2 and 29 (64.4%) patients had grade 3. Nine (20%) patients had stage 1 disease, 20 (44.4%) stage 2, stage 3 had 12 (26.7%) patients and stage 4 1 (2.2%). Only one patient (2.2%) had history of another non-breast cancer. Eighteen (40%) patients had history of cancer in the family and 6 (13.3%) had family history of breast cancer. Table 1 summarizes the clinicopathological characteristics of our patient population.

Table 1. Patient and disease characteristics

Characteristics	*N (%)*
Age (years)	Median 49.8 Range 30-81
Menopausal status	
Premenopausal	22 (48.9)
Perimenopausal	3 (6.7)
Postmenopausal	20 (44.4)
T stage	
T1	15 (33.3)
T2	23 (51.1)
T3	3 (6.7)
T4	4 (8.9)
N stage	
0	23 (51.1)
1	11 (24.4)
2	1 (2.2)
3	7 (15.6)
Unknown	3 (6.7)
TNM stage	
I	9 (20.0)
II	20 (44.4)
III	12 (26.7)
IV	1 (2.2)
Unknown	3 (6.7)
Grade	
1	–
2	13 (28.9)
3	29 (64.4)
Unknown	3 (6.7)
Histologic type	
IDC	42 (93.4)
ILC	1 (2.2)
IDC+ILC	2 (4.4)

IDC: invasive ductal carcinoma, ILC: invasive lobular carcinoma

Immunohistochemical assessment of ERCC1 expression

Thirty-three (73.3%) out of 45 TNBC patients' tumor samples stained positive for ERCC1 (Figure 1). Tumor samples of 7 patients (15.5%) stained intensively and diffusely positive for ERCC1. Figure 2 shows the staining intensity patterns of tumor samples.

Clinicopathological characteristics of the patients and ERCC1 expression

ERCC1 expression was not correlated with any of the following clinical parameters: age at diagnosis, menopausal status, tumor size, nodal status, metastasis, stage of breast cancer, history of cancer. There was a trend for higher ERCC1 expression with tumor grade (Pearson's correlation coefficient, 0.22; p=0.14) and family history of cancer (Pearson's correlation coefficient, 0.29; p=0.053).

Discussion

TNBC composes 10-15% of all breast cancer patients [2,3]. Carey et al. reported that TNBC was more prevalent among premenopausal Afro-American women [4]. Ihemendalu et al. reported that the ratio of patients who were diagnosed before the age of 35 was 57.1% for basal-like/TNBC and 25% for luminal A breast cancer subtype [35]. One study has reported that mean age at diagnosis was 53 for TNBC and 58 for oth-

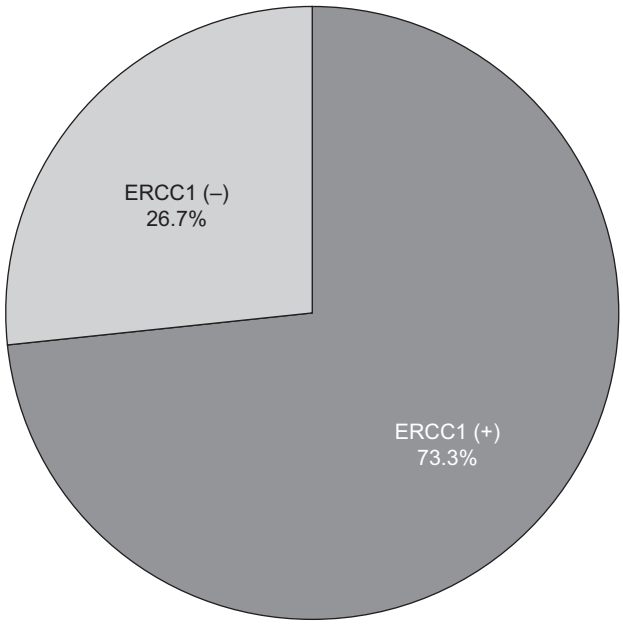

Figure 1. ERCC1 expression in tumor samples of TNBC patients.

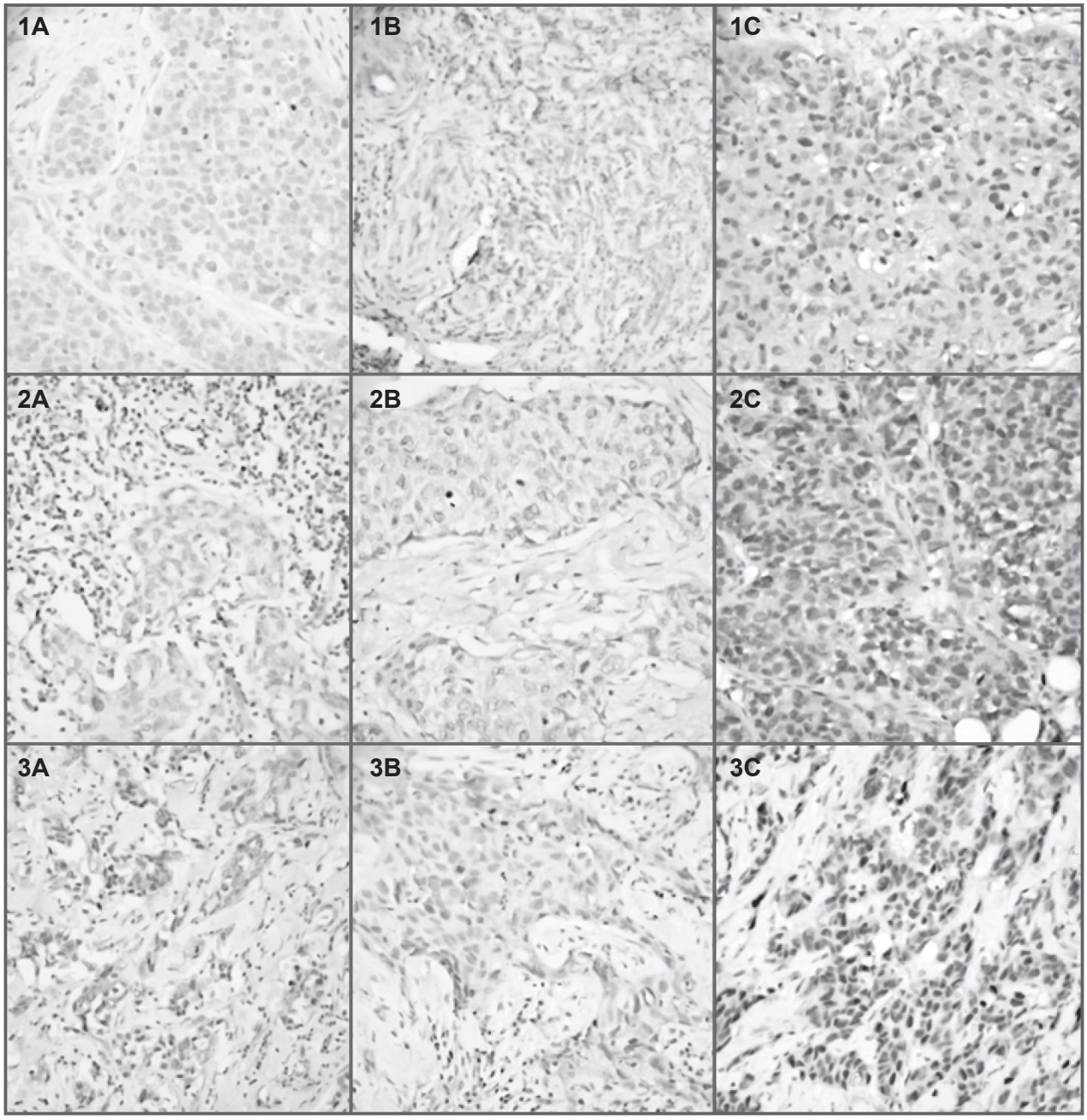

Figure 2. ERCC1 staining intensity scores of tumor samples according to stages. **1A:** Stage 1 case with 1(+) staining; **1B:** Stage 1 case with 2(+) staining; **1C:** Stage 1 case with 3(+) staining; **2A:** Stage 2 case with 1(+) staining; **2B:** Stage 2 case with 2(+) staining; **2C:** Stage 2 case with 3(+) staining; **3A:** Stage 3 case with 1(+) staining; **3B:** Stage 3 case with 2(+) staining; **3C:** Stage 3 with 3(+) staining.

er subtypes of breast cancer [5]. In our study the mean age at the time of diagnosis was 49.8 years and 17.8% of our study population was diagnosed before the age of 40. In a study by Dirier et al. 10.7% of all breast cancer patients were diagnosed before 40 years of age [36]. In another study by Abraham et al., 20% of TNBC patients were diagnosed before 40 years of age [37].

Triple negative tumors are less differentiated, high grade tumors and they are diagnosed at a later stage [38]. Stark et al. reported that the possibility of a tumor to be triple negative is 16-fold higher for a high grade and advanced stage tumor and 31-fold higher for high grade (grade 3) tumors compared to grade 1 and grade 2 tumors [39]. One study revealed that 77% of TNBCs were high grade [40]. In our study 64.4% of TNBCs were high grade, a result consistent with the literature.

Although TNBC is more sensitive to chemotherapy, the risk of metastasis and relapse is higher compared to non-TNBC cases; this constitutes a good reason for defining specific chemotherapy regimens for TNBC.

There are many ongoing studies trying to identify the importance and place of platinum analogs in the treatment of TNBC. In our study ERCC1 expression was positive in 73.3% of the cases. Sidoni et al. reported positive ERCC1 expression in 32% of TNBCs and the authors reported that there was no statistically significant relationship between clinicopathologic characteristics and ERCC1 expression [32]. Similarly, in our study we didn't find any statistically significant relationship between clinicopathologic characteristics and ERCC1 expression. The results of our study seem to be of value for the interpretation of the results of ongoing trials and the determination of the patient population who may derive benefit from cisplatin-based regimens.

To our knowledge the present study is the second one that aimed to determine ERCC1 expression in TNBCs. Prospective studies with lung and ovarian cancers in which platinum-based regimens were used showed that ERCC1 expression was a predictor of treatment outcome [23,24,26].

Response to platinum compounds in TNBC may be related to factors other than ERCC1. BRCA1 mutation and p63/73 pathway were found to be related to cisplatin sensitivity [41]. In our study we didn't evaluate tumor samples for BRCA1 mutation and p63/73 pathway inhibition. Conducting randomized prospective trials to evaluate ERCC1, BRCA1 and p63/73 pathway in patients to be treated with platinum analogs might help determine those patients who may derive benefit from these compounds.

The present study has some limitations; it was retrospective and included a small number of patients.

Also, another important limitation of this study was the lack of a control group. Evaluation of ERCC1 expression in hormone-positive tumors could possibly help interpret the results of our study more accurately.

Large prospective randomized trials are needed to define the exact role of ERCC1 in the molecular biology and the treatment of TNBC.

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