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Estrogen receptor positive breast cancer identified by 95-gene classifier as at high risk for relapse shows better response to neoadjuvant chemotherapy

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ABSTRACT

A 95-gene classifier (95-GC) recently developed by us can predict the risk of relapse for ER-positive and node-negative breast cancer patients with high accuracy. This study investigated association of risk classification by 95-GC with response to neoadjuvant chemotherapy (NAC). Tumor biopsy samples obtained preoperatively from 72 patients with ER-positive breast cancer were classified by 95-GC into high-risk and low-risk for relapse. Pathological complete response (pCR) rate was numerically higher for high-risk (15.8%) than low-risk patients (8.8%) although the difference was not statistically significant. Pathological response evaluated in terms of the pathological partial response (pPR) rate (loss of tumor cells in more than two-thirds of the primary tumor) showed a significant association ($P = 0.005$) between the high-risk patients and a high pPR rate. Besides, external validation study using the public data base (GSE25066) showed that the pCR rate (16.4%) for high-risk patients ($n = 128$) was significantly ($P = 0.003$) higher than for low-risk patients (5.7%) ($n = 159$). These results demonstrate that the high-risk patients for relapse show a higher sensitivity to chemotherapy and thus are likely to benefit more from adjuvant chemotherapy.

1. Introduction

One of the most urgent needs for breast cancer treatment practice is the development of a prognostic classifier for ER-positive and node-negative breast cancer patients which is more accurate than the conventional pathological prognostic factors. Recent advances in molecular technology has enabled the development of the classifiers based on multigene expression, such as Oncotype DX [1,2], MammaPrint [3,4], and genomic grade index (GGI) [5], which can provide valuable information on prognosis which is not obtainable with conventional pathological examination. Oncotype DX in particular is most widely used in practice for prediction of prognosis of ER-positive and node-negative patients. Very recently, however, we have also been able to develop a 95-gene classifier (95-GC) which can classify ER-positive and node-negative breast cancer patients into high-risk and low-risk with high accuracy [6].

Oncotype DX was originally developed as a predictor of prognosis for ER-positive and node-negative breast cancer patients, but interestingly, later studies have shown that the patients identified as high risk by Oncotype DX are more likely to benefit from adjuvant chemotherapy. It is reported that there was a significant improvement in disease-free survival in high-risk patients treated with adjuvant chemo-hormonal therapy than those with adjuvant hormonal therapy alone, but such a significant improvement was not observed in the low- and intermediate-risk patients [7]. A more direct association between Oncotype DX and sensitivity to chemotherapy has been investigated for breast cancer patients treated with neoadjuvant chemotherapy and it was reported that high-risk patients determined by Oncotype DX showed a significantly higher pathological complete response (pCR) to neoadjuvant chemotherapy than low-risk patients [8]. Similar results have also been reported for MammaPrint [9–11] and GGI [12,13], namely, that high-risk breast cancer patients identified by these classifiers are more sensitive to neoadjuvant chemotherapy.

The reason for this association between high-risk patients determined by these classifiers and sensitivity to neoadjuvant chemotherapy is thought to be attributable, at least in part, to the fact that all these classifiers include expression of various genes related to cell proliferation, and significant association between high-risk patients and cell proliferation verified with the Ki67 marker has been reported [6]. Since the 95-GC classifier also includes several genes related to cell proliferation, it is speculated that high-risk

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patients identified by 95-GC are also more sensitive to chemotherapy. In order to investigate this possibility, we conducted the study reported here, in which the association between risk classification by 95-GC and response to chemotherapy was compared in the neoadjuvant setting.

2. Materials and methods

2.1. Patients and tumor samples

Seventy-two patients with stage II and III primary breast cancer who were treated with neoadjuvant chemotherapy and subsequent surgery (mastectomy or breast conserving surgery) between 2003 and 2010 were retrospectively recruited for this study. Neoadjuvant chemotherapy consisted of paclitaxel 80 mg/m^2 weekly for 12 cycles followed by a combination of 5-FU [500 mg/m^2], epirubicin [75 mg/m^2], and cyclophosphamide [500 mg/m^2] every 3 weeks for 4 cycles [P-FEC]. Before neoadjuvant chemotherapy, all the patients underwent tumor biopsy with a vacuum-assisted core-biopsy instrument (Mammotome 8G HH; Ethicon Endosurgery Inc., Cincinnati, OH) under ultrasonographic guidance for histological examination and gene expression analysis. Tumor samples for histological examination were fixed in 10% buffered formaldehyde, and tumor samples for gene expression analysis were snap frozen in liquid nitrogen and stored at −80 °C until use. Prior to the tumor biopsy, informed consent regarding the study was obtained from each patient.

As postoperative adjuvant therapy, tamoxifen (20 mg/day), goserelin (3.75 mg every 4 weeks) plus tamoxifen (20 mg/day), leuprorelin (3.75 mg every 4 weeks) plus tamoxifen (20 mg/day), anastrozole (1 mg/day), and letrozole (2.5 mg/day) were given to 12, 3, 7, 16 and 9 patients, respectively. Tamoxifen, anastrozole, or letrozole was administered typically for 5 years or until recurrence within 5 years, and goserelin or leuprorolin for 2 years or until recurrence within 2 years. Trastsuzumab (6 mg/kg every three weeks for one year) was given to 10 patients with HER2-positive tumors. Patient characteristics are listed in Table 1.

Table 1
Relationships between clinicopathological parameters[a] and risk category classified by 95-GC.

	95-gene classifier		*P*-value
	High-risk	Low-risk	
No.	38	34	
Menopausal status			
Pre-	22	19	0.86
Post-	16	15	
Histological type			
Infiltrating ductal carcinoma	37	26	0.011[b]
Infiltrating lobular carcinoma	1	8	
T category (clinical)			
*T*1	2	3	0.95
*T*2	27	23	
*T*3	7	6	
*T*4	2	2	
Nodal status (clinical)			
Positive	25	23	0.87
Negative	13	11	
Histological grade			
1	4	11	0.023
2/3	34	23	
PR			
Positive	21	25	0.11
Negative	17	9	
HER2			
Positive	11	6	0.26
Negative	27	28	
Ki67			
Positive	21	8	0.008
Negative	17	25	
Recurrence score (Oncotype DX)			
High risk	35	6	<0.0001
Intermediate risk	2	6	
Low risk	1	22	

Abbreviations: PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.
[a] Determined in tumor biopsy samples before neoadjuvant chemotherapy.
[b] Fisher's exact test.

2.2. RNA extraction and DNA microarray analysis

Trizol (Invitrogen, Carlsbad, CA) or Qiagen RNeasy mini kit (QIAGEN Sciences, Germantown, MD) was used to extract RNA from tumor biopsy samples obtained before neoadjuvant chemotherapy. The presence of tumor cells in the biopsy samples was estimated by histological confirmation of their presence in the adjacent tumor biopsy samples. RNA (50 ng) was subjected to gene expression analysis using a DNA microarray (Human Genome U133 Plus 2.0 Array; Affymetrix, Santa Clara, CA) according to a previously described method [14].

2.3. Histological evaluation of response to chemotherapy

The pathological response to P-FEC was evaluated by using the surgical specimens obtained at surgery. The surgical specimens were cut into 5-mm slices, and hematoxylin- and eosin-stained sections (3-μm) were prepared to determine the presence or absence of tumor cells. A complete loss of invasive tumor cells and lymph node-negative status were defined as pCR irrespective of the presence or absence of ductal carcinoma in situ components. In addition, we defined pPR (pathological partial response) as loss of tumor cells in more than two-thirds of the primary tumor. pPR thus was equal to the histologically determined effect of grade IIa + IIb + III according to the General Rules for Clinical and Pathological Recording of Breast Cancer 2005 [15].

2.4. Immunohistologic examination

ER, PR, and Ki67 levels in tumor biopsy samples obtained before neoadjuvant chemotherapy were determined by immunohistochemistry according to a previously described method [14]. Cut-off values for ER, PR, and Ki67 were 10%, 10%, and 20%, respectively. HER2 amplification was determined by fluorescence in situ hybridization (FISH) using the PathVysion HER-2 DNA Probe Kit (Vysis/Abbott Molecular Inc., Chicago, IL). A tumor was determined as HER2-amplified if the FISH ratio was >2.0.

2.5. Statistics

DNA microarray data were used for analysis with the 95-GC classifier of tumors from 72 ER-positive patients into high-risk and low-risk according to a previously described method [6]. The median follow-up was 3 years. In addition, we used public databases for the following external validation studies. The gene expression data of ER-positive tumors (n = 299) treated with neoadjuvant chemotherapy consisting of weekly paclitaxel × 12 cycles or triweekly docetaxel x 4 cycles followed by FAC (5-FU/doxorubicin/cyclophosphamide) × 4 cycles were extracted from the public data base GSE25066 [16], and the patients were classified into high-risk and low-risk with 95-GC. In addition, the gene expression data of ER-positive and node-positive tumors treated with adjuvant hormonal therapy alone were extracted from public data bases GSE2990 [5], GSE4922 [17], GSE6532 [18], and GSE9195 [19], followed by classification into high-risk and low-risk patients by 95-GC. Intrinsic subtyping of breast tumors was done as previously described [20]. Hierarchical clustering analysis combined with Spearman's rank correlation coefficient and Ward's method was performed for visualization by means of Partek Genomics Suite 6.5 (Partek Inc., St. Louis, MO).

Patients were also classified into the high-, intermediate-, and low-risk groups by recurrence score (≧31, 18–30, and <18, respectively) of Oncotype DX 21-gene classifier (21-GC), which was determined using "Recurrence Online" (http://www.recurrenceonline.com/) that was developed by Győrffy et al. [21]. "Recurrence Online" can compute 21-GC-based recurrence score using expression data obtained by Affymetrix microarray.

Distant relapse-free survival (DRFS) was calculated with the Kaplan–Meier method and evaluated with the log-rank test. Association of the 95-GC-classified high- or low-risk group with the various clinicopathological parameters was determined with the chi-square test or Fisher's exact test. All statistical analyses were two-sided and $P < 0.05$ was judged to be significant.

3. Results

3.1. Relationship between 95-GC-based risk groups and various clinicopathological parameters

Patients (n = 72) with ER-positive tumors were classified into high-risk (n = 38) and low-risk (n = 34) by means of 95-GC and the associations of their classification with the various clinicopathological parameters determined in tumor biopsy samples before neoadjuvant chemotherapy is shown in Table 1. Results of