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Construction of multi-gene classifier for prediction of response to and prognosis after neoadjuvant chemotherapy for estrogen receptor positive breast cancers

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ABSTRACT

The aims of this study were to develop a multi-gene expression-based prediction model for pathological complete response (pCR) to neoadjuvant chemotherapy (NAC) and to evaluate its prognosis prediction for estrogen receptor (ER) positive breast cancers. The training set included the NAC-treated patients (n = 104) with ER+ breast tumors in our hospital and the validation set included the NAC-treated patients (n = 259) with ER+/HER2– breast tumors in the public database (GSE25066). Gene expression in the tumor biopsy specimens obtained before NAC was analyzed with DNA microarray, and the prediction model (MPCP155) for pCR was constructed for the training set by using the genes (155 probes) involved in the metabolic pathways which the pathway analysis identified as being significantly associated with pathological response. With MPCP155, the tumors in the validation set could be classified into low chemo-sensitive (low-CS) (pCR rate = 2.6%) and high-CS (pCR rate = 15.3%; $P = 0.0006$) groups. Furthermore, the low-CS group showed a significantly better prognosis than the high-CS group ($P = 2.0E{-}6$). Moreover, prognosis prediction by MPCP155 was independent of the residual cancer burden score. MPCP155 may be helpful for decision making regarding the indication for neoadjuvant chemotherapy. In addition, MPCP155 was found to be useful for prognosis prediction for NAC-treated patients with ER+/HER2– tumors.

Introduction

Currently breast cancer patients are often treated with neoadjuvant chemotherapy (NAC) to improve the operability of those with locally advanced inoperable breast tumors as well as to enhance the feasibility of breast conserving surgery for those with relatively large breast tumors. NAC has another advantage in that chemo-sensitivity can be assessed histologically in the surgical specimens after NAC, and pathological complete response (pCR) to NAC is generally accepted as a surrogate marker for excellent prognosis [1]. However, only 20–30% of all breast tumors attain pCR [2], so that numerous attempts have been made to develop predictors for pCR which would facilitate decision making regarding the indication of NAC. The multi-gene classifier has been attracting major attention as a pCR predictor because it has been very successful in improving prediction for patient prognosis [3–7]. From the practical point of view, the aim of these predictors is to identify patients who are very unlikely to respond to NAC and thus to avoid unnecessary NAC in the same manner as ER for hormonal therapy and human epidermal growth factor receptor 2 (HER2) for anti-HER2 therapies [8–10].

The multi-gene classifiers for prediction of response to NAC have been developed by incorporating into the model the genes with expressions that are significantly different for pCR and non-pCR tumors. Although such prediction models can increase the positive predictive value (PPV) of pCR up to around 30–40%, the negative predictive value (NPV) remains around 80–90% [10], indicating that 10–20% of breast tumors can still attain pCR in spite of the negative prediction. From the practical point of view, NPV of 10–20% is too high for NAC to be avoided. Instead, it should be less than 5% if the prediction model is to be used for identifying patients for whom NAC is not indicated.

Although the multi-gene classifiers are based on the difference in gene expression in pCR and non-pCR tumors, breast tumors cannot be so simply divided into chemo-sensitive (pCR) and chemo-resistant (non-pCR) tumors since a significant proportion of breast tumors show intermediate chemo-sensitivity. The criteria for histological evaluation of response to NAC are used to classify response into three major categories, i.e., grade I (poor response), grade II

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; DMFS, distant metastasis-free survival; pCR, pathological complete response; NAC, neoadjuvant chemotherapy; NAH, neoadjuvant hormonal therapy; NPV, negative predictive value; MPCP155, metabolic process-associated chemo-sensitivity predictor 155; CS, chemo-sensitive; RCB, residual cancer burden; RD, residual disease; KEGG, Kyoto Encyclopedia of Genes and Genomes.

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(intermediate response), and grade III (good response (pCR)) [11,12]. Thus, the multi-gene classifiers so far developed seem to be intended to identify highly chemo-sensitive tumors which are very likely to attain pCR but not chemo-resistant tumors which are very unlikely to attain this because non-pCR tumors comprise both grade I and grade II tumors. We therefore believe that, to develop a prediction model for chemo-resistant tumors, the gene selection should be based on differentiation between grade I tumors on the one hand and grade II and III tumors on the other hand.

Estrogen receptor (ER) positive tumors generally show a lower pCR rate (around 10–20%) than ER negative tumors (around 30–40%) [1,13], and treatment with neoaduvant hormonal therapy (NAH) is an option for the former [14,15]. Thus, it seems to be easier and clinically more important to develop a prediction model with a much higher NPV for ER positive than for ER negative tumors. Such a prediction model might well be useful for decision making as to whether NAC or NAH should be used for the treatment of ER positive tumors. In the study reported here, we therefore attempted to develop a multi-gene classifier for ER positive tumors using the new strategy described above. In addition, the impact of such a classifier on patient prognosis was also investigated on the assumption that the level of chemo-sensitivity could be translated into a more accurate prognosis.

Materials and methods

Patients recruited for development of the prediction model for NAC

For this study 148 patients with stage II or III breast cancer who had been treated with NAC and subsequent surgery (mastectomy or breast conserving surgery) between 2003 and 2012 at Osaka University Hospital were retrospectively recruited. NAC 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 NAC, all 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 [9,12]. 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 every patient. The median follow-up time was 49 months with a range of 1–97 months. Patient characteristics are shown in Table 1. For postoperative adjuvant therapy, 12 were treated with tamoxifen, 22 with tamoxifen plus LH-RH agonist, 64 with aromatase inhibitor, and 14 with trastuzumab.

We planned to include only ER positive tumors in our study and to develop a prediction model by using not only our cohort but also the public databases. Unfortunately, different methodologies and cut-off values were used to determine ER status for these two cohorts. Thus, we decided to use the ESR1 (probe "205225_at") expression level "500" determined by DNA microarray as the cut-off value as previously described by Gong et al. [16,17]. Of the above-mentioned 148 patients, 104 met this inclusion criterion and were included in the training set (Table 1). For the independent validation set, 259 ER positive and HER2 negative tumors of patients who were treated with neoadjuvant sequential taxane and (F)AC (218 were treated with 12 cycles of weekly paclitaxel followed by 4 cycles of fluorouracil, doxorubicin and cyclophosphamide and 41 were with 4 cycles of doxorubicin and cyclophosphamide followed by 4 cycles of paclitaxel or docetaxel) were selected from the database of GSE25066 (Table 1) [18]. Since only a small number (n = 3) of ER positive and HER2 positive tumors were included in this database, they were not included in the validation set.

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 biopsy samples of tumors included in the training set and obtained before NAC. 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 [9,12].

Histological evaluation of response to NAC

The pathological response to NAC was evaluated by using surgical specimens from the training set obtained at surgery. The surgical specimens were cut into 5-mm slices, and hematoxylin- and eosin-stained sections were prepared to determine the presence or absence of tumor cells. A complete loss of invasive tumor cells in the breast and lymph nodes was defined as pCR irrespective of the presence or absence of non-invasive components. In addition, pathological response to NAC was also evaluated according to the Japanese Breast Cancer Society (JBCS) criteria for evaluation of the chemotherapeutic response to NAC [11]. On the basis of the JBCS criteria, this response is classified into three categories, i.e., grade I (poor response; number of tumor cells eliminated less than 2/3 of that in the original tumor area), grade II (intermediate response; elimination of tumor cells in equal to or more than 2/3 of the original tumor area but no complete elimination of invasive tumor cells), and grade III (good response; complete elimination of invasive tumor cells irrespective of the presence or absence of non-invasive tumor cells).

We also used RCB (residual cancer burden) data for the validation set. Symmans et al. reported that RCB, which represents the quantity of residual tumors after NAC, is useful for the prediction of prognosis for patients treated with NAC [19]. RCB consists of four categories, i.e., RCB-0 (pCR), RCB-I (near pCR), RCB-II (moderate residual disease (RD)), and RCB-III (extensive RD). It has been reported that the prognosis is excellent for the RCB-0 and RCB-I groups but becomes worse as the tumor burden increases for the RCB-II and RCB-III groups.

Table 1
Clinicopathological parameters for the training set and the validation set.

	Training set		Validation set	
	No.	%	No.	%
No.	104	100	259	100
Menopausal status				
Premenopausal	56	54		
Postmenopausal	48	46		
Age				
<50	54	52	123	47
≥50	50	48	136	53
cT				
T1/2	83	80	156	60
T3/4	21	20	103	40
cN				
Positive	70	67	172	66
Negative	34	33	87	34
Histological grade				
1	23	22	24	9
2/3	81	78	219	85
Unknown			16	6
PR				
Positive	62	60	165	64
Negative	42	40	92	36
Unknown			2	0
HER2				
Positive	24	23	0	0
Negative	80	77	259	100
Unknown			0	0
Ki67				
Positive	48	46		
Negative	54	52		
Unknown	2	2		
Subtype				
Luminal A	41	39	121	47
Others	63	61	138	53
GGI				
Low-GGI			125	48
High-GGI			134	52
Genomic predictor				
Insensitive			161	62
Sensitive			98	38

cT: clinical tumor size; cN: clinical nodal status; PR: progesterone receptor; HER2: human epidermal growth factor receptor 2; GGI: genomic grade index.

Immunohistologic examination

ER, PR, and Ki67 levels in tumor biopsy samples obtained from the training set before NAC were determined immunohistochemically according to a previously described method [9]. The cut-off values were 10% for ER, 10% for PR, and 20% for Ki67. HER2 amplification was determined by means of fluorescence in situ hybridization (FISH) using the PathVysion HER-2 DNA Probe Kit (Vysis/Abbott Molecular Inc., Chicago, IL). A tumor was identified as HER2-amplified if the FISH ratio was ≥2.0.

Statistics

After MAS5 and log 2 conversion, as a pre-filter probes that showed a coefficient of variation (CV) value of less than 0.05 and more than 0.25 were excluded beforehand from the training set (n = 104) (Supplementary Method 1). The gene expression of the tumors with grade I and grade II + III response to NAC were compared