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Associations between clinical-pathological parameters and biomarkers, HER-2, TYMS, RRMI, and 21-gene recurrence score in breast cancer

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ABSTRACT

Background: Chemotherapy is the predominant treatment option for patients with breast cancer. Selection of patients according to biomarker will improve chemotherapy efficacy. In the present study, we examined the relations of the expression of candidate genes and 21-recurrence score (RS) results to patients' demographic characteristics, histopathological factors, and outcomes.

Methods: A total of 146 patients were enrolled in this study. The patients underwent 21-gene RS testing. In addition, expressions of candidate genes, TYMS, RRM1, TUBB3, TOP2A, PTEN, were detected. Information was obtained on age, tumor size, TMN stage, tumor grade, and status of Ki-67, HER2, ER and PR. The treatment information on the type of endocrine therapy was also obtained.

Results: Results clearly showed that the 21-gene RS significantly correlated with the TNM stage of breast cancer ($P = 0.047$). The RS also correlated with the number of sentinel lymph node ($P = 0.038$). The pathological type of tumors was strongly associated with the expression of RRM1 ($P < 0.015$), and slightly correlated with TYMS ($P = 0.095$) and tumor size ($P = 0.061$). Further analysis showed that TYMS and RRM1 were two independent factors affecting the disease progression of patients. Besides, for HER-2 stain, staining of grade 2 or above significantly increased the risk of disease progression.

Conclusion: Our studies showed that TNM stage and sentinel lymph node were important clinical parameters correlated with 21-gene RS results. Also, RRM1, TYMS and HER2 expressions were independent factors associated with disease progression in breast cancer patients. Future study is warranted to investigate the usefulness of these genes in treatment efficacy.

1. Introduction

Breast cancer is the most commonly diagnosed cancer and the leading cause of cancer death in women [1]. It was estimated that 2.1 million new cases of breast cancer were diagnosed in 2018, accounting for 1 in 4 cancer cases among women [2]. In China, breast cancer is also the most common cancer among women [3]. Primary breast cancer has heterogeneous clinical features, and a rational personalized treatment is needed. To date, overexpression of human epidermal growth factor receptor 2 (HER2) protein is used to identify patients who benefit from adjuvant HER-2directed therapy [4]; while the expressions of estrogen receptor and the progesterone receptor are used to identify patients who benefit from adjuvant endocrine therapy [5]. The use of adjuvant chemotherapy reduces the risk of recurrence, and the widespread use of adjuvant chemotherapy has contributed to the reduced incidences of breast-cancer mortality [6]. However, it was appeared that many of the patients may receive chemotherapy unnecessarily [7]. Efforts have been made to identify molecular signature to improve treatment decision.

The 21-gene recurrence score (RS) test (Oncotype DX, Genomic Health) is a reverse-transcription polymerase chain reaction-based assay that detects mRNA expression levels of 5 reference genes and 16 cancer-related genes [8]. Majority of the genes in the RS test are related to estrogen receptor (*ESR1, PGR, BCL2, SCUBE2*) and proliferation (*Ki67, STK15, Survivin, MYBL2, CCNB1*); and there are other genes (*BACG1, CD68, GSTM1, CTSL2, MMP11, GRB7, HE-2*). The test became commercially available in 2004 to predict the 10-year cancer recurrence risk and benefits of adjuvant chemotherapy for patients with

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estrogen receptor (ER)-positive breast cancer and negative axillary nodes [7]. The RS score uses a continuous scale from 0 to 100, of which the RS < 10 predicts a very low rate of distant recurrence at 10 years and is not likely to be affected by adjuvant chemotherapy [7,9]; while the RS > 30 indicates the benefits of chemotherapy surpass the risks of side effects [9,10]. The benefits of chemotherapy in patients with intermediate RS is uncertain. Later, several studies also demonstrated the prognostic utility of the test in patients with ER-positive and node-positive breast cancer [11,12].

In addition to 21-gene RS, other potential molecular markers have been suggested to guide treatment decision and/or provide prognostic information, such as thymidylate synthase (TYMS) for Capecitabine and fluorouracil [13], ribonucleotide reductase large submit (RRM1) for Gemcitabine [14], topoisomerase II alpha (TOP2A) [15], Phosphatase and tensin homolog (PTEN) for Trastuzumab [16], and class III beta-tubulin (TUBB3) [17]. In the present study, we examined the relations of the expression of these genes and 21-RS to patients' demographic characteristics, histopathological factors, and outcomes.

2. Patients and methods

2.1. Study population

This study was approved by the institutional review boards of our hospital. A total of 146 patients were enrolled in this study, and all of them provided signed informed consent. All of the patients were HER-2 negative and ER positive. The patients underwent 21-gene RS testing at between June, 2013 and September, 2017. In addition, expressions of TYMS, RRM1, TUBB3, TOP2A, PTEN were detected. Information was obtained on age, tumor size, TMN stage, tumor grade, and status of Ki-67, HER2, ER and PR. The treatment information on the type of endocrine therapy (i.e. Anastrozole, Letrozole, Tamoxifen, Toremifene, and Exemestane) was also obtained.

2.2. Statistical analysis

All analyses were performed using statistical software SAS version 9.3. Student's *t*-test and Wilcoxon two sample test was used to analyze continuous variables with and without normal distribution, respectively. Comparisons between counting variables were done using chi-square or Fisher's exact test. Patients' PFS were studied using Kaplan-Meier curve with log-rank test, with the differences between survival curves evaluated using Tukey-Kramer corrected P values. Risk factors and their associated hazard ration (HR) for disease progression were identified using COX multivariate regression model. Statistical significance was indicated by *P* value < 0.05.

3. Results

3.1. Overall characteristics of the study population

The present study enrolled a total of 146 patients undergoing 21-gene RS testing in addition to the standardized diagnosis. The average age of patients was 50.8 years (Table 1). Invasive ductal carcinoma (IDC) represented the most prevalent form (74%), with the mixed IDC and ductal carcinoma in situ (DCIS) and invasive lobular carcinoma (ILC) being the second (10.3%) and the third (8.2%) reported, respectively. About two-third of the tumors were grade 2. For the 21-gene RS, 59.6%, 33.6% and 6.9% of the tumor were at low, intermediate and high risk, respectively. For the endocrine therapy, most patients received Letrozole (47.3%) and Tamoxifen (47.3%).

3.2. Clinical correlation of 21-gene RS

Analysis was done to identify clinical correlates of the 21-gene RS in our study cohort (Table 2). Results clearly showed that the RS

Table 1
Baseline characteristics of patients and tumors.

Clinical parameters		Number of patients, n (%)
Age, years	< 50	65 (44.5)
	≥50	81 (55.5)
Tumor size, cm	< 20	63 (45.6)
	≥20	75 (54.4)
Sentinel lymph node, n	< 5	64 (46.4)
	≥5	74 (53.6)
Pathological types	IDC	108 (74.0)
	Mixed IDC and DCIS	15 (10.3)
	ILC	12 (8.20)
Pathological grade	Grade 1-2	71 (65.7)
	Grade 3	37 (34.3)
TNM stage	T1N0M0	63 (45.6)
	T2N0M0	75 (54.4)
Clinical risk assessment	Low	60 (55.0)
	Intermediate	49 (45.0)
Ki-67 category (%)	< 10	32 (22.9)
	> 10, < 20	47 (33.6)
	≥20	61 (43.6)
PR stain	Grade 0-1	52 (36.6)
	Grade 2	46 (32.4)
	Grade 3	44 (31.0)
AR stain	Grade 0	17 (46.0)
	Grade 1	20 (54.0)
TYMS expression	Low	45 (35.2)
	Intermediate	65 (50.8)
	High	18 (14.0)
RRM1 expression	Low	43 (33.6)
	Intermediate	58 (45.3)
	High	27 (21.1)
TUBB3 expression	Low	31 (24.2)
	Intermediate	76 (59.4)
	High	21 (16.4)
TOP2A	Low	37 (28.9)
	Intermediate	59 (46.1)
	High	32 (25.0)
HER2 expression	Low	24 (18.8)
	Intermediate	69 (53.9)
	High	35 (27.3)
PTEN expression	Low	21 (16.4)
	Intermediate	55 (43.0)
	High	52 (40.6)
Endocrine therapy	Anastrozole	3 (2.1)
	Letrozole	69 (47.3)
	Tamoxifen	69 (47.3)
	Toremifene	1 (0.7)
	Exemestane	1(0.7)
	No endocrine therapy	3 (2.1)
21-gene RS	Low risk	87 (59.6)
	Media risk	49 (33.6)
	High risk	10 (6.9)

Table 2
Clinical correlation of the 21-gene RS in 146 patients with breast cancer.

Clinical parameters	21-gene RS, n			Total	*P*
	Low	Intermediate	High		
Age, years					
< 50	38	23	4	65	0.894
≥50	49	26	6	81	
TNM stage					
T1N0M0	45	16	2	63	**0.047**
T2N0M0	39	29	7	75	
Pathological type					
IDC	62	37	9	108	0.422
Other	25	12	1	38	
Grade					
1-2	44	24	3	71	0.084
3	16	16	5	37	
Sentinel lymph node, n					
< 5	41	23	0	64	**0.038**
≥5	43	24	7	74	

Table 3
Risk factors for disease progression in patients with breast cancer.

Clinical variables	Hazard ratio (HR)	95% Confidence interval (CI)		*P* value
		Lower limit	Upper limit	
TYMS percentage	0.213	0.068	0.667	0.008
RRM1 percentage	2.048	0.976	4.297	0.058
HER-2 stain				
Grade 2 or above vs Grade 0	1.882	1.014	3.492	0.045

significantly correlated with the TNM stage of breast cancer ($P = 0.047$). The RS also correlated with the number of sentinel lymph node ($P = 0.038$). The association between RS and tumor grade was barely significant with a *P* value of 0.084. Of note, the RS was not associated with patients' age ($P = 0.894$) and pathological type ($P = 0.422$).

3.3. Clinical correlation of age, pathological type and grade, and sentinel lymph node

A significant association between patients' age and ER stain was observed ($P < 0.001$). Patients' age was also fairly associated with AR stain with a *P* value of 0.092. The pathological type of tumors was strongly associated with the expression of RRM1 ($P < 0.015$), and slightly correlated with TYMS ($P = 0.095$) and tumor size ($P = 0.061$). For the pathological grade, the evaluated clinical risk was identified as a correlate ($P < 0.001$). The number of sentinel lymph node was strongly associated with the evaluated clinical risk ($P = 0.015$), and slightly correlated with tumor size ($P = 0.065$), pathological grade ($P = 0.077$), and TOP2A expression ($P = 0.059$).

3.4. Risk factors for disease progression

The clinical parameters potentially associated with the risk of disease progression were identified using Cox regression analysis (Table 3). Analysis showed that TYMS (HR, 0.213; 95% CI, 0.068 to 0.667; *P*, 0.008) and RRM1 (HR, 2.048; 95% CI, 0.976 to 4.297; *P*, 0.058) were two independent factors affecting the disease progression of patients. Besides, for HER-2 stain, staining of grade 2 or above significantly increased the risk of disease progression by nearly 2 folds when compared to grade 0 (HR, 1.882; 95% CI, 1.014 to 3.492; *P*, 0.045).

3.5. Discussion

Gene expression differences contribute to chemotherapeutic response variability between individuals. Selection of patients according to biomarker will improve chemotherapy efficacy. Many studies have shown the usefulness of 21-gene RS in making treatment decision. However, the use of 21-gene testing is expensive, especially for those patients from rural area; therefore, the RS testing is not widely adapted in China. Many physicians still need to rely on clinicopathological information to make treatment decision. The present findings showed that the 21-gene RS results significantly correlated with TNM stage and sentinel lymph node, which are important parameters in guiding breast cancer treatment. A study investigating the use of 21-gene RS in Chinese population showed that the 21-gene RS could increase oncologists' confidence level in treatment recommendation, and the use of adjuvant chemotherapy was reduced [18].

In this study, TYMS expression was a risk factor significantly associated with disease progression. TYMS has a role in DNA synthesis and is a target for 5-fluorouracil. Its expression has a strong prognostic feature in prostate cancer, independent of Gleason grade, pT stage, preoperative PSA, pN stage, or resection margins [19]. The expression of TYME was predictive of treatment response. In lung adenocarcinoma and gastric cancer, the efficacy of pemetrexed chemotherapy was associated with the expression of TYMS [20,21]. A recent study also reported that TYMS expression predicted therapeutic sensitivity of pemetrexed chemotherapy in advanced breast cancer patients [21], indicating TYMS expression analysis alone or with other molecular parameters may provide clinical useful information in breast cancer.

In addition to TYMS, RRM1 was identified as a potential risk factor for disease progression, although the statistical analysis was barely significant with a *P* value of 0.058. RRM1 is a structural subunit of ribonucleoside-diphosphate reductase, an enzyme that produces deoxyribonucleotides prior to DNA synthesis in the S phase of dividing cells. The cytoplasmic staining of RRM1 was substantially higher in breast cancer tissues when compared to the non-tumor tissues adjacent to tumors, and the expression of RRM1 was significantly associated with the lymph node involvement, pathological classification and ER status [22]. RRM1 aberrations, examined by fluorescence in situ hybridization, were revealed as a risk factor for decreased overall survival in patients with advanced breast cancer receiving docetaxel/gemcitabine treatment [23]. RRM1 was identified as a molecular determinant of gemcitabine efficacy in lung and pancreatic cancers [24,25]. In breast cancer, the combined expression of RRM1 and 6 other genes could predict gemcitabine response with 85% accuracy [26]. A study on patients with metastatic breast cancer (MBC) also showed that RRM1 was highly associated with DNA repair enzymes ERCC1 and BRCA1, implicating the use of RRM1 as markers for clinical decision making [14].

In conclusion, our studies showed that TNM stage and sentinel lymph node were important clinical parameters correlated with 21-gene RS results in HER-2 negative and ER positive breast cancer patients. Also, RRM1 and TYMS expressions were associated with disease progression in these patients, suggesting the possibility of the utility of the gene expression results of these genes in clinical setting. In the last decade, it has been recognized that tumor-specific genomic data could provide important information for treatment decision-making. Further prospective study may be conducted to accumulate evidence of these genes in association with specific treatment efficacy.

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