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ORIGINAL ARTICLE

Predictive value of 21-gene recurrence score assay in non-estrogen receptor-positive and lymph node-negative breast cancer

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Summary

Purpose: *The 21-gene recurrence score (RS) assay predicts relapse of estrogen receptor-positive and lymph node-negative breast cancer more accurately than traditional markers; however, whether this assay can be regarded as a molecular marker of other types of breast cancer is unclear. We aimed to identify the effect of 21-gene recurrence score assay in non-estrogen receptor-positive and lymph node-negative breast cancer.*

Methods: *We analyzed 21-gene expression by quantitative real-time PCR (qRT-PCR) in 100 cases of breast cancer tissues and followed up for 5 years to investigate the prognostic significance in non-estrogen receptor-positive and lymph node-negative breast cancer. Also, the correlation between RS and the clinicopathological features were analyzed. Adjuvant online (AOL) database was used for the analyses in the present study.*

Results: *The cases were classified as RS low (n=52), moderate (n=22) and high (n=26) risk. The RS based on the21-gene assay was not correlated with age, tumor size, histological grade, and lymph node and estrogen receptor/progesterone receptor (ER/PR) status; however, there was significant correlation with Her-2 status. The 5-year recurrence rates were 1.92%, 4.55% and 15.38% in the low, moderate and high-risk groups, respectively. In addition, there was significant difference between the low-high groups ($p<0.05$). Furthermore, the consistency of the prognosis predicted by the AOL system was 56% and 59% in the RS moderate–high risk and low risk groups, respectively.*

Conclusions: *The 21-gene RS assay was a prognostic indicator for patients with non-ER-positive and lymph node-negative breast cancer. In addition, our results coincided with those obtained using the AOL system.*

Key words: *adjuvant online, breast cancer, estrogen receptor, 21-gene, recurrence score*

Introduction

Breast cancer is the most frequent cancer and the leading cause of cancer-related deaths worldwide in women [1]. The poor prognosis of patients with breast cancer is largely due to tumor recurrence or metastasis after surgical resection. Therefore, a better method for the prediction of the early events associated with breast cancer relapse and metastasis is required to decrease mortality and improve patient quality of life. However, there is no effective marker for prognosis and drug response. The traditional assessment, which depends on the clinical and pathological features of tumors, such as age, tumor size, node infiltration and histological grade are not effective biomarkers of recurrence risk. Due to rapid advances in oncology genomics, it is now possible to tailor chemo-

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therapy and predict drug reactions/toxicity to the needs of individual patients based on specific genetic profiling of individual genes/polymorphisms [2,3].

In the B-14 study, Paik et al. used qRT-PCR to identify 21 recurrence-associated genes from 4% formalin-fixed and paraffin-embedded tumor tissue samples from node-negative and ER-positive breast cancer patients treated with tamoxifen [4]. These genes comprised proliferation-related genes (Ki-67, STK15, Survivin, CyclinB1 and MYBL2), invasion-related genes (Stromelysin3 and Cathepsin L2), Her-2-related genes (GRB7, Her-2), hormone-related genes (ER, PR, Bcl-2 and SCUBE2), GSTM1, BAG1, and CD68 and 5 reference genes [5]. The ΔCt values of the 21-genes were converted to RSs which were used to distinguish the patients suitable for chemotherapy or hormone therapy alone from those requiring hormone therapy plus chemotherapy [6]. The RS of the 21-genes can be used to predict relapse in ER-positive and lymph node-negative breast cancer more accurately than the traditional methods, which depend on patient age and tumor size [7]. However, whether RS can be regarded as a molecular marker in other types of breast cancer remains to be clarified.

The AOL is a validated global open network database of 4,083 cases with T1–2, N0–1 and M0 breast cancer following entry of data of age, tumor size, histological grade, lymph node-positive number, ER status and other disease information. The AOL system predicts the risk of recurrence and therapeutic effect in breast cancer with adjuvant treatment at 10 years based on clinicopathological features and therapy.

In this study, we performed a qRT-PCR analysis of the relative expression of the 21 genes in breast cancer tissues (100 cases) to investigate the potential of the 21-gene assay as a biomarker of recurrence in breast cancer patients. The 21-gene assay RS correlated with Her-2 status, but not with the ER and PR status. The frequencies of relapse and metastasis were lower in breast cancer patients with low RS than in patients with high RS. Our study suggests that the 21-geneRS assay is effective as a predictive biomarker of relapse in breast cancer patients and exhibits similar prognostic value to that of the AOL system.

Methods

Patients and treatment

All specimens and relevant clinical data were obtained from the Department of Oncology, the Second Hospital of Lianyungang, China, during the period from 2008 to 2010. All specimens were pathologically confirmed. Follow-up was continued to December 2014. This study was approved by the ethics committee of the Second Hospital of Lianyungang.

qRT-PCR assays

qRT-PCR assays were performed to detect mRNA expression levels of the 21 genes (7900 real-time PCR System, ABI, and HT Fast). Briefly, reactions were carried out in a total volume of 20 μL PCR reaction mixture containing 1 μL of cDNA. Relative expression levels were calculated according to the comparative Ct method using β-actin as an endogenous control and commercial RNA (Clontech) controls as calibrators. The final results were determined by the formula $2^{-\Delta\Delta Ct}$ and were analyzed with the Stratagene analysis software.

Table 1. Relationship between the 21-gene RS and pathological features in breast cancer

Characteristics	*Cases (n)*	*RS*			*p value*
		High	*Moderate*	*Low*	
Age, years					0.437
>50	56	14	16	26	
≤50	44	12	6	26	
Tumor size, cm					0.132
≤2	44	8	10	26	
>2	56	18	12	26	
Histological grade					0.967
I	32	6	10	16	
II	50	14	10	26	
III	18	6	2	10	
Lymph node metastasis					0.355
Yes	30	10	6	14	
No	70	16	16	38	

RS: Recurrence score

RS calculations

The average Ct values of the 21 genes, including 5 reference genes, were determined by qRT-PCR. The RSU values were calculated as the difference between the mean values obtained with the remaining 16 genes. The RSU was then converted to RS (0–100).

Statistics

The Statistical Package of Social Sciences (SPSS) 17.0 software (Chicago, IL, USA) was used for the statistical analysis of the experimental data. The significance of differences between groups was estimated by the Mann-Whitney U test. P values less than 0.05 were considered to indicate statistical significance.

Results

RS of 21-genes and the clinicopathologic features in breast cancer

qRT-PCR analysis of breast cancer tissues showed that the 21-genes were amplified in all samples. The final 21-gene RS results were divided into three groups: low risk group (RS<18), moderate risk group (18≤RS<31) and high risk group (RS≥31). Fifty-two cases were classified as low risk, 22 cases as moderate risk, and 26 as high risk. We also investigated the correlation between RS and clinicopathological characteristics in breast cancer. There were no significant differences in the clinicopathological parameters, such as age, tumor size, histological grade and lymph node metastasis among the three RS groups (Table 1). In addition, correlation analyses showed that there was no correlation between RS and ER/PR status. However, there was a significant correlation between RS and Her-2 status, with a higher frequency of Her-2 positive patients in the high RS group than in the low RS group (p<0.001; Table 2). In the ER/PR positive plus node-negative group and other types group there was no significant correlation with RS (Table 2).

RS was correlated with recurrence and metastasis in breast cancer

The 5-year survival data for 100 breast cancer patients were collected to further investigate

Table 2. Relationship between the 21-gene RS and the ER/PR status in breast cancer

Parameters	*Cases (n)*	*RS*			*p value*
		High	*Moderate*	*Low*	
Her-2					0.001
Negative	28	2	4	22	
Positive	72	24	18	30	
ER, PR					0.082
Negative	24	6	4	14	
Positive	76	20	18	38	
ER/PR positive node-negative					0.270
Yes	50	10	12	28	
No	50	16	10	24	

RS: recurrence score, Her-2: human epidermal growth factor receptor-2, ER: estrogen receptor, PR: progesterone receptor

Table 3. Relationship between the 21-gene RS and metastasis in breast cancer

RS Group	*5-year recurrence*		*p value*		
	Yes	*No*	*Low - Moderate*	*Moderate - High*	Low- High
Low	1	51	0.27	0.225	0.023
Moderate	1	21			
High	4	22			0.06

RS: recurrence score

Table 4. Consistency of prognosis based on RS and AOL database

Group	*Moderate–High risk in AOL and RS*	*Low risk in AOL and RS*
Consistency	56%	59%

RS: recurrence score, AOL: adjuvant online

whether the RS is correlated with tumor recurrence and metastasis. Among these patients, 74 cases were ER-positive, while the remaining were negative. With a median follow-up of 31.5 months (range 63.2–82.9), there was one case of recurrence and metastasis in the RS low risk group; the rate of relapse was 1.92%. In the RS high risk group there were 4 cases of recurrence and metastasis and the rate of relapse was 15.38% (Table 3). In addition, recurrence rate was significantly different between the low-high risk groups (p=0.023). However, there was no significant difference in the low-moderate risk groups (p=0.27) and moderate-high risk groups (p=0.225).

The consistency of RS and AOL

The prediction of consistency using the 21-gene RS and AOL systems was evaluated. The results showed that the consistency between the 21-gene RS and AOL prognoses was 56% in the high–moderate risk group and 59% in the low risk group (Table 4).

Discussion

Currently, multidisciplinary treatment models in breast cancer are usually based on the clinical and pathological features of tumors, such as histological grade and lymph node metastasis. However, these traditional methods of prediction, which depend on patient age, tumor size, lymph node invasion and histological tumor grading, do not accurately predict prognosis and treatment effect. In contrast to these methods, the 21-gene RS is better able to predict prognosis and treatment effect in breast cancer patients. Paik et al. [8] analyzed the relationship between RS and distant metastasis in 668 cases which were ER-positive, lymph node-negative and received only tamoxifen therapy for breast cancer. The results showed that the 10-year distant metastasis rates were 6.8%, 14.3% and 30.5% in the low, medium and high risk groups, respectively (p<0.001). In the NSABP B-20 study, Paik et al. [8] analyzed the relationship between CMF (cyclophosphamide, methotrexate and fluorouracil), or MF chemotherapy and RS. The results showed a significant correlation between RS and adjuvant chemotherapy (p=0.038), with RS ≥31 showing patient benefit associated with adjuvant chemotherapy. These findings are in concordance with those reported by Toi et al. showing a higher frequency of tumor recurrence and metastasis in the high RS group compared with the low RS group for ER-positive, node-negative breast cancer in Asian patients [9].

In this study, the 21-gene RS was not found to be significantly correlated with age, tumor size, histological tumor grade and lymph node metastasis. Furthermore, although RS was not correlated with ER and PR status, a significant correlation was identified with Her-2 status, with a higher frequency of Her-2 positive patients in the high RS group than in the low RS group. These results indicate that the risk of tumor recurrence and metastasis is much higher in Her-2-positive breast cancer patients than in Her-2-negative patients. Furthermore, our study also showed that, there was no significant correlation between the ER/PR positive plus node-negative group and other types and RS. This indicates that the 21-gene RS assay can be used to predict prognosis not only in node-negative patients, but also in patients with other types of breast cancer. Similarly, a recent research showed that the low RS is associated with a better prognosis after hormone therapy regardless of lymph node-positive or negative status. The prospective, multicenter, randomized ATAC [10] and SWOG 8814 studies also confirmed that RS provides better prognostic information regardless of node-positive or negative status of breast cancer patients after hormone therapy and chemotherapy [11]. The E2197 clinical trial [12] also verified the value of RS as a good marker of prognosis and clinical benefit in lymph node-negative and positive breast cancer patients after chemotherapy. In our study the recurrence and metastasis rates were 1.92%, 4.55% and 15.38% in the RS low, moderate and high risk groups, respectively. Thus, in combination, the results of these studies indicate that RS is an effective prognostic marker of clinical results in breast cancer and is suitable for popularization.

In the low risk population classified using the AOL system after tamoxifen treatment, the recurrence rates in the RS low and high risk groups were 5.6% and 12.9%, respectively. However, in high risk population by AOL the recurrence rates in the RS low and RS high risk groups were 8.9% and 30.7%, respectively. The data showed that there was a significant association between AOL and RS in the benefits from hormone therapy. However, the AOL system did not detect any relationship between chemotherapy benefit and RS. These data suggest that clinical factors and RS can be used as independent markers of prognosis. Our study also showed that there was very high consistency between RS and AOL. Thus, a combination of the two methods may provide a more accurate prognosis in breast cancer.

In conclusion, RS can be used to predict the prognosis of breast cancer patients and the results are consistent with those provided by the AOL sys-

tem. Nevertheless, this study has several limitations. For example, we could detect ERCC1, BRCA1 and TS expression on the basis of the 21-genes to facilitate individualized therapy in breast cancer. This comprehensive panel of markers provides an accurate prognosis and the theoretical basis for individualized therapy in breast cancer. Overall, the 21-gene assay can be used effectively to predict prognosis, and also to guide treatment decisions to improve the quality of life and relieve the burden on social resources. The 21-gene RS method is suitable for widespread application in the clinic.

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Conflict of interests

The authors declare no conflict of interests.

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