72-Gene Classifier for Predicting Prognosis of Estrogen Receptor–Positive and Node-Negative Breast Cancer Patients Using Formalin-Fixed, Paraffin-Embedded Tumor Tissues

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Abstract

The 72-gene classifier (72-GC) was developed for recurrence-risk prediction for patients with estrogen receptor–positive and node-negative breast cancer. 72-GC could differentiate the high-risk from the low-risk patients with a high statistical significance, and is considered to be applicable to formalin-fixed, paraffin-embedded (FFPE) tumor tissues because the results of 72-GC on fresh-frozen tissues and FFPE tissues showed a high concordance.

Background: The 95-gene classifier (95-GC) can classify patients with estrogen receptor (ER)–positive and node-negative breast cancer into those with low and high risk of relapse with an accuracy similar to that of 21-GC (Oncotype DX). Because 95-GC uses RNA from fresh-frozen (FF) tumor tissues, we herein attempted to develop a gene classifier that is applicable to RNA from formalin-fixed paraffin-embedded (FFPE) tumor tissues. **Patients and Methods:** 25 paired FF and FFPE tumor tissues were subjected to DNA microarray for gene-expression analysis. Of the 95 probes included in the 95-GC, 72 were selected for construction of the gene classifier for FFPE tumor tissues, because the gene expression detected by these 72 probes was well preserved in the FFPE tumor tissues. **Results:** The 72-GC was constructed with these 72 probes for the training set comprising 549 FF tumor tissues and validated with 434 FF tumor tissues (relapse-free survival at 10 years was 91% for the low-risk and 74% for the high-risk group ($P = 3.74 \times 10^{-7}$). The predictive capability of 72-GC for prognosis was found to be comparable to that of 95-GC. The 25 paired FF and FFPE tumor tissues from each of 25 patients were classified into the same risk group by 72-GC for 23 patients (92% concordance). 72-GC using the FFPE tumor tissues showed that the prognosis for the low-risk group was significantly ($P = .007$) better than for the high-risk group. **Conclusion:** 72-GC is comparable to 95-GC in terms of accuracy of prognosis prediction, and may be effective for FFPE tumor tissues.

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Introduction

Patients with estrogen receptor (ER)–positive and node-negative breast cancer have a relatively favorable prognosis when treated with adjuvant hormonal therapy alone. However, about 20% of them ultimately develop recurrence by 10 years after surgery. This recurrence rate is high enough for the majority of patients to opt for adjuvant chemotherapy in addition to hormonal therapy, in order to reduce the risk of recurrence. As a result, it is thought that unnecessary adjuvant chemotherapy is currently administered to many ER-positive (ER^+) and node-negative patients, so that an urgent need has arisen for an accurate prognosis predictor to avoid such needless adjuvant chemotherapy. In response to this need, multigene classifiers, including Oncotype DX and MammaPrint, have been developed and are used in medical practice in some countries.[1-8] Furthermore, we have succeeded in developing a

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95-gene classifier (95-GC) using RNA derived from fresh-frozen (FF) tumor tissues,[9] and very recently, we have been able to demonstrate that 95-GC is as accurate as 21-GC in prognosis prediction, which is thought to be equivalent to Oncotype DX.[10] From a practical point of view, however, there is also a need for a prognosis predictor that can be used for RNA derived from formalin-fixed, paraffin-embedded (FFPE) tumor tissues, because such tissues are routinely prepared in medical practice. Moreover, tissue samples should be easy to store and ship for multigene assays.

RNA derived from FFPE tumor tissues is significantly degraded, and thus it has been thought that such RNA is not suitable for DNA microarray analysis of gene expression. Recently, however, Mittempergher and colleagues[11] reported that gene expression data of the paired FF and FFPE tumor tissues obtained with the complementary DNA (cDNA)–mediated annealing, selection, expression and ligatation (DASL) assay showed a high correlation (Pearson correlation > 0.7) and that the classification results obtained with multigene classifiers, including intrinsic subtype,[12] genomic grade index (GGI),[13] and 70-gene classifier[14] showed a high concordance between the results for paired FF and FFPE tumor tissues. In addition, 3 studies have been reported on gene-expression analysis that uses RNA from FFPE tissues and DNA microarray (Affymetrix U133), a more widely used method for gene-expression analysis than DASL. First, Scicchitano et al.[15] directly compared DNA microarray data obtained from paired FF and FFPE bone marrow stromal cell pellets, and found there was a good correlation between them. Second, Linton et al.[16] developed a gene-expression signature that can predict the prognosis for soft-tissue sarcoma using the DNA microarray data obtained from 34 FFPE tumor tissues. And third, Lassmann et al.[17] compared the DNA microarray data of 6 paired FF and FFPE colon tumor tissues and used an unsupervised hierarchical cluster analysis to show that all the paired samples were clustered next to each other. These reports thus indicate that DNA microarray analysis of gene expression using RNA from FFPE tumor tissues is feasible.

These results have prompted us to study the possibility of applying 95-GC, which was developed for FF tumor tissues, to FFPE tumor tissues by using a DNA microarray (Affymetrix U133). The gene expression data in public data sets are obtained most often with this microarray, and their easy availability is crucial for the construction of a robust multigene classifier. This means that, if the applicability of 95-GC for FFPE tumor tissues can be verified, its clinical utility would be substantially enhanced.

Patients and Methods

Patients Whose Tumor Tissues Were Used for Comparison of Gene Expression by DNA Microarray Between FF and FFPE Tumor Tissues

This study comprised 27 female patients with ER^+ and node-negative invasive breast cancers whose FF tumor tissues were stored at −80°C. These patients underwent breast-conserving surgery followed by radiation therapy or mastectomy between 2003 and 2008 and were treated with adjuvant hormonal therapy alone at Osaka University Hospital. The median follow-up period was 95 months with a range of 12 to 105 months. Of these patients, 16 were treated postoperatively with anastrozole (1 mg/d) for a median of 60 months (range, 14-60 mo), 2 with tamoxifen (20 mg/d) for a median of 32 months (range, 4-60 mo), 9 with goserelin (3.75 mg/4 wk) for a median of 24 months (range, 10-24 mo) months plus tamoxifen (20 mg/d) for a median of 60 months (10-60 mo). In all, 5 patients developed recurrence. Patient characteristics are shown in Table 1. Immediately after the surgery, a tumor tissue sample (about 5 × 5 mm) was macroscopically dissected from the tumor and cut into half. One half was fixed in 10% neutral formalin buffer at room temperature for 48- to 72 hours and embedded in paraffin, and the other half was snap frozen in liquid nitrogen and kept at −80°C until use. Informed consent regarding the study was obtained from each patient before surgery.

Patients And Public Data Sets for Construction of a Prognosis Prediction Model

From the public data sets in which gene expression data obtained by DNA microarray using FF tumor tissues are deposited (GSE2034, GSE2990, GSE4922, GSE6532, GSE7390, GSE9195), we selected the data for 549 ER^+ and node-negative breast cancer patients who received adjuvant hormonal therapy

Table 1 Clinicopathological Characteristics of 25 Patients Whose FFPE and FF Tumor Samples Were Used for Analyses

	Number of Patients
Median age, years	56
(range, years)	(34-81)
Post-menopausal	16
T grade	
T1	13
T2	12
Histological grade	
1	11
2	12
3	2
ER status	
Positive[a]	25
Negative	0
PR status	
Positive[b]	21
Negative	4
HER2 status	
Positive[c]	0
Negative	25
Ki67 status	
Positive[d]	6
Negative	17
Prognosis	
With recurrence	5
Without recurrence	20

Abbreviations: ER = estrogen receptor; FFPE = formalin-fixed, paraffin-embedded; HER2 = human epidermal growth factor receptor 2; Ki67 = Kiel 67; FF = fresh-frozen; PR = progesterone receptor.
[a]Immunohistochemistry, cutoff: 10%.
[b]Immunohistochemistry, cutoff: 10%.
[c]Immunohistochemistry (+3) or fluorescence in situ hybridization result (ratio ≥ 2.0).
[d]Immunohistochemistry, cutoff: 20%.