Research Paper

An Eighteen-Gene Classifier Predicts Locoregional Recurrence in Post-Mastectomy Breast Cancer Patients

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ABSTRACT

We previously identified 34 genes of interest (GOI) in 2006 to aid the oncologists to determine whether post-mastectomy radiotherapy (PMRT) is indicated for certain patients with breast cancer. At this time, an independent cohort of 135 patients having DNA microarray study available from the primary tumor tissue samples was chosen. Inclusion criteria were 1) mastectomy as the first treatment, 2) pathology stages I-III, 3) any locoregional recurrence (LRR) and 4) no PMRT. After inter-platform data integration of Affymetrix U95 and U133 Plus 2.0 arrays and quantile normalization, in this paper we used 18 of 34 GOI to divide the mastectomy patients into high and low risk groups. The 5-year rate of freedom from LRR in the high-risk group was 30%. In contrast, in the low-risk group it was 99% (p < 0.0001). Multivariate analysis revealed that the 18-gene classifier independently predicts rates of LRR regardless of nodal status or cancer subtype.

1. Introduction

The conventional method in determining the indication for post-mastectomy radiotherapy (PMRT) is largely based on clinical variables, such as tumor size, axillary lymph node involvement, hormone receptor status, age at diagnosis, lymphovascular invasion (LVI), etc. These factors are known risk factors associated with locoregional recurrence (LRR). They are, however, imperfect in predicting recurrence (Cheng et al., 2006a; Taghian et al., 2004). More reliable biological markers are being sought. Sporadic reports have attempted to show that some isolated genes could link to LRR (van der Hage et al., 2004; Zellars et al., 2000).

Patients with four or more axillary lymph-node involvement (N2 or N3 disease) generally would be given PMRT (Recht et al., 2001). However, there is controversy concerning patients with 1–3 positive nodes (N1 disease), even though NCCN guidelines "strongly consider" giving PMRT based on large meta-analyses from many randomized control trials

(Clarke et al., 2005; Early Breast Cancer Trialists' Collaborative, 2002; EBCTCG et al., 2014). In fact, the likelihood of LRR after 10 years in node negative patients is reported to be approximately 2–8%, N1 patients 20%, and N2 patients 32% (Clarke et al., 2005; EBCTCG et al., 2014). Therefore, if we rely only on clinical parameters, around 70–80% of the node-positive patients could potentially undergo overtreatment, whereas those at risk in node negative disease could potentially be undertreated.

Recent progress in genomic analyses for evaluating tumor biology show significant agreement in the outcome predictions for individual patients who are probably sharing a common set of biologic phenotypes. This opened a new possibility to improve risk stratification that led to more personalized prognostication for breast cancer patients (Fan et al., 2006; Sorlie et al., 2001). Studies from gene expression profiling have shown a greater capability of determining prognosis and predicting response to adjuvant chemotherapy in Tamoxifen-treated patients (Paik et al., 2004a, 2004b, 2006). In 2006, we reported 34 and 258 gene sets that could partition the LRR high risk patients from the LRR low risk patients after mastectomy. The low risk group determined by the gene expression profiling had a 3-year LRR rate of less than 3%, and the high risk group had an LRR rate of more than 50% (Cheng et al., 2006b). In this study, we evaluated 34 genes of interest (GOI) in the prediction of LRR using a completely different patient population

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and aimed to identify individuals with low risk of recurrence for whom PMRT could be avoided.

2. Material and Methods

2.1. Patients

Patients with invasive breast cancer were collected in a study of "*Precision Medicine in Oncology for Taiwan*" at Koo Foundation Sun Yat-Sen Cancer Center in an attempt to develop a new taxonomy for breast cancer, which was approved by the Bio-bank Ethics Committee and Institutional Review Board. All 135 patients eligible for this study had had the approval of these two review bodies.

The present study focused on validating the gene expression profiles that are related to LRR following mastectomy. Patients are eligible if they satisfied the inclusion criteria set in our 2006 study: i.e. 1) mastectomy as first treatment, 2) frozen fresh tissue available, 3) pathology stages I-III disease, 4) any LRR, 5) no PMRT, 6) minimal follow up of 2 years, and 7) an informed consent (Fig. 1). One hundred thirty five (135) patients were enrolled in this study.

We hypothesize that if the 18-gene classifier were capable of identifying locally aggressiveness of tumor biology and predicting LRR for

mastectomy patients, it would also be effective to predict LRR after BCS even though they had adjuvant radiotherapy. We then used the breast-conserving surgery (BCS) patients who were treated in the same study period (n = 87) as a confirmatory cohort.

2.2. Samples and Microarray Analysis

A total of 135 frozen tissue samples came from surgical specimens of the primary tumors taken from patients prior to any treatment between 2005 and 2012. Tumor RNA was extracted from primary tumor tissues with Trizol (Invitrogen, Carlsbad, CA) and purified with the RNeasy Mini Kit (Qiagen, Valencia, CA), and assessed for quality with an Agilent 2100 Bioanalyzer. According to the Affymetrix protocol, hybridization targets were prepared from the total RNA and hybridized to U133 Plus 2.0 arrays. The details of the study method have been reported previously by investigators of our institution (Kao et al., 2011).

2.3. Statistical Analysis to Identify Gene Expression Profiles of LRR

In this study, the microarray platform was shifted from Affymetrix U95 to U133 Plus 2.0 array (Fig. 1). There were 30 of 34 GOI identified in this new platform, where they were distributed in 84 probes. An
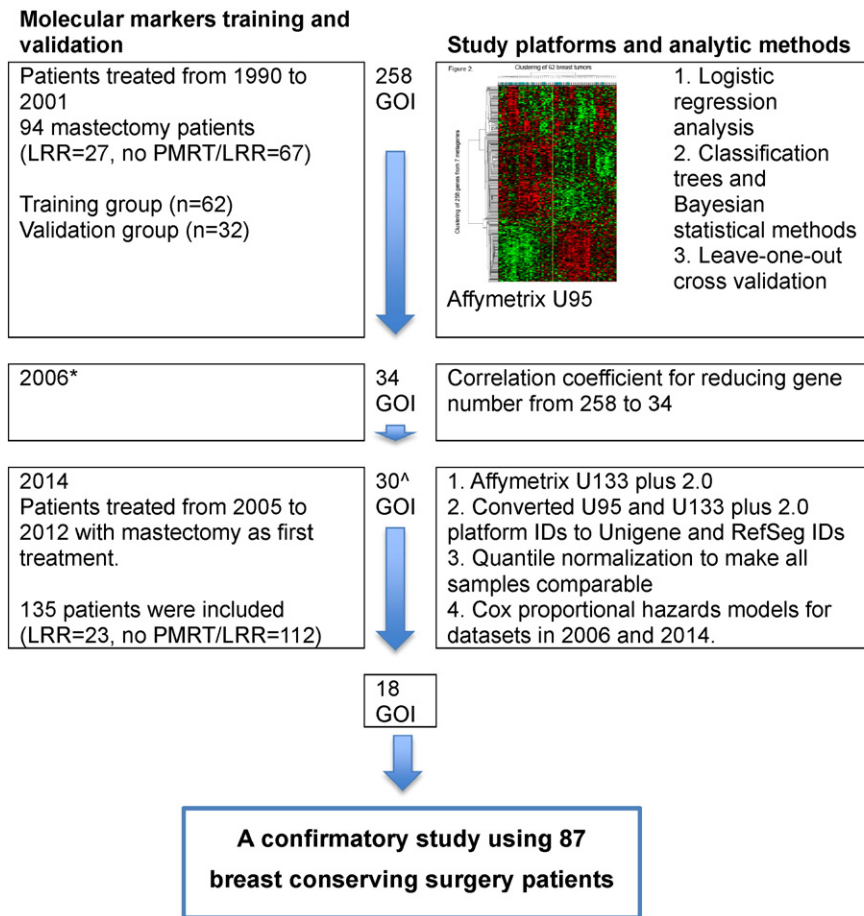
Fig. 1. Diagram to develop gene expression profiling that predicts locoregional recurrence in mastectomy patients. The genes of interest (GOI) reduced from 258 to 18 and maintained a similar, or greater ability to partition the low and high risk patients after mastectomy.