Distinct p53 Gene Signatures Are Needed to Predict Prognosis and Response to Chemotherapy in ER-Positive and ER-Negative Breast Cancers

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Abstract

Purpose: Estrogen receptor-positive (ER+) and -negative (ER) breast cancers are molecularly distinct diseases. We hypothesized that p53 mutations may lead to different transcriptional changes and carry different prognostic value in these two different types of cancers.

Experimental Design: We developed a 39-gene p53 signature derived from 213 ER+ and a separate 30-gene signature from 38 ER– cancers with known mutation status and tested their prognostic and chemotherapy response predictive values in ER+ and ER– cancers, respectively.

Results: External validation to predict p53 status (n = 103) showed sensitivity and specificity of 89% and 54% for the 39-gene signature, and 82% and 61% for the 30-gene signature. The 39-gene signature was predictive of worse distant metastasis free survival in ER+ cancers in two separate prognostic data sets (n = 255, HR = 2.3, P = 0.005 and n = 198, HR = 2.17, P = 0.09). It also predicted for poor prognosis even with adjuvant tamoxifen therapy (n = 277, HR = 2.43, P < 0.0001) but it was not prognostic in ER– cancers. It was also associated with higher chemotherapy sensitivity in ER+ but not in ER– cancers. The prognostic and predictive values remained significant in multivariate analysis. The 30-gene, ER–, p53 signature showed no prognostic or predictive values in ER+ cancers but it was associated with better prognosis in ER– cancers. It also had no chemotherapy response predictive value in ER– or ER+ cancers.

Conclusions: P53 dysfunction is prognostically most relevant in ER+ cancers and supports the hypothesis that different predictive or prognostic markers will be needed for different molecular subsets of breast cancer. Clin Cancer Res; 17(8); 2591–601. ©2011 AACR.

Introduction

Altered function of the p53 protein due to mutation or other causes leads to a cascade of transcriptional changes that play an important role in cancer development. p53 functional status can be assessed by direct DNA sequencing, yeast functional complementation assay or by transcriptional read out of p53 activity (i.e., p53 gene signature; refs. 1–5). The prognostic, predictive, and therapeutic relevance of altered p53 status detected by any of these methods remains uncertain in breast cancer (1, 2, 6). Most previous studies examined the clinical value of p53 abnormalities across all breast cancers and p53 transcriptional signatures were invariably derived from the entire study population including all of the different breast cancer molecular subtypes. Miller and colleagues published a seminal work to determine a p53 transcriptional signature using both U133A and U133B chips, they have shown that patients with p53 mutant cancers had a worse prognosis and most importantly a 32-gene p53 signature could identify a subset of aggressive tumors that showed transcriptional hallmarks of p53 dysfunction even in the absence of detectable p53 mutation. Developing gene signatures across all breast cancers when the genomic abnormality to be predicted by a gene expression signature is not distributed evenly across the major molecular types of breast cancer will inevitably include some molecular-phenotype related probe sets that will reduce the specificity and sensitivity of the resulting predictor. Breast cancer is no longer considered to be a single disease but a collection of molecularly and clinically distinct neoplastic diseases of the breast (7, 8). The most extensive molecular and clinical
Translational Relevance

We hypothesized that p53 gene mutations may lead to different transcriptional changes in distinct molecular subtypes of breast cancer and these may translate into different prognostic values. To test this hypothesis, we developed gene expression-based predictors of p53 status separately for estrogen receptor-positive (ER+) and -negative (ER−) cancers and tested their prognostic and chemotherapy predictive values. A 39-gene p53 signature for ER+ cancers was predictive of poor prognosis with or without adjuvant tamoxifen therapy and higher chemotherapy sensitivity in ER+ cancers. The same signature had no prognostic or predictive values in ER− cancers. A 30-gene ER− p53 signature could identify p53 status but had little predictive or prognostic value in either ER− or ER+ disease. These observations support the hypothesis that p53 dysfunction leads to partly different transcriptional consequences in ER− and ER+ cancers. p53 dysfunction is more predictive of clinical outcome in ER+ cancers.

Differences exist between ER+ and ER− breast cancers (9, 10). We therefore examined the hypothesis that p53 dysfunction may lead to at least partly different transcriptional changes in ER+ and ER− cancers and these may be associated with different prognostic and chemotherapy response predictive values. Even if some p53-regulated genes are shared between ER− and ER+ breast cancers, different p53 signatures may be optimal to characterize p53 status in these different cancers (2, 11–13).

It is important to note that the frequency of p53 mutations (or functional alterations) is significantly higher in ER− compared with ER+ breast cancers. This imbalance in p53 status by hormone receptor status has important consequences for the development and interpretation of p53 gene signatures. A simple comparison of p53 mutant and wild-type cases implies comparing a mostly ER− breast cancers. A 30-gene ER− p53 signature could identify p53 status but had little predictive or prognostic value in either ER− or ER+ disease. These observations support the hypothesis that p53 dysfunction leads to partly different transcriptional consequences in ER− and ER+ cancers. p53 dysfunction is more predictive of clinical outcome in ER+ cancers.

Materials and Methods

Patients and materials

The discovery set was composed of 251 breast cancers from a publicly available data set by Miller and colleagues with known p53 mutation status including 213 ER+ and 38 ER− cancers (12). In this study, p53 mutation was assessed by cDNA sequencing of exons 2 to 11 of the p53 gene and ER status was determined by routine clinical ER immunohistochemistry (IHC). Among the ER+ patients, 39 (18.3%) had p53 mutations and among the ER− breast cancers, 19 (50%) were p53 mutated.

We tested the ability of our own p53-gene signatures derived from the Miller and colleagues data to predict p53 functional status on an independent cohort of 98 breast cancers (MDACC/IGR cohort n = 42 ER+, n = 56 ER−). In this validation cohort, p53 functional status was assessed by a yeast complementary assay and ER by routine IHC (5). Among the 42 ER+ patients, 24 (57%) had p53 functional tumors and 18 (43%) had p53 dysfunction. Among the 56 ER− cancers, 18 (32%) showed intact p53 function and 38 (68%) showed p53 dysfunction.

We assessed the pure prognostic values of each of the 2 different, “ER-specific” p53 signatures in 2 separate breast cancer data sets that included cancers from patients who did not receive any systemic adjuvant treatment (Wang and colleagues n = 255, Transbig n = 198; refs. and 16 and 17). We also tested the prognostic/endocrine predictive value in 277 ER+ breast cancers that received adjuvant tamoxifen without any chemotherapy (Institut Jules Bordet, IJB; ref. 18). In 2 additional data sets that included patients who received neoadjuvant (preoperative) chemotherapy (MDACC/MAQC n = 233, MDACC/IGR n = 103) we directly tested the chemotherapy response predictive values of the signatures. In the MDACC/MAQC data set, patients received 12 courses of weekly Paclitaxel (T) followed by 4 courses of 5-fluorouracil, doxorubicin, and cyclophosphamide (FAC). In the MDACC/IGR data set, patients received 6 courses of FAC (or FE[epirubicine]C) chemotherapy. A subset (n = 98) of the MDACC/IGR cohort was also used to test how well our gene signatures predicted p53 functional status because these cancers had p53 function evaluation by the yeast assay. Pathologic complete response was defined as the absence of any invasive cancer in the breast or lymph nodes in the MDACC/MAQC cohort and no invasive cancer or only isolated tumor cells in the MDACC/IGR cohort. Clinical and pathological characteristics of all patients included in the various data sets used in these analyses are summarized in Table 1 for ER+ patients and in Supplementary Table S1 for ER− patients.

Microarray data processing

The raw data and clinical annotations for the Miller (12), Wang (17), TRANSBIG (16), IJB (18), and MDACC/MAQC data sets were downloaded from the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo) with accession numbers GSE 3494, GSE 2034, GSE 7390, GSE 9195, and GSE 16716, respectively. All the gene expression data were generated by using Affymetrix U133 oligonucleotide microarrays. Gene expressions were normalized by the mas5 method. Probe-set signal values were natural log transformed and scaled by adjusting the mean intensity to a target signal value of log 600. Statistical programming language R (http://cran.r-project.org/) and Bioconductor packages (www.bioconductor.org) were used for higher-level analysis.

We identified differentially expressed genes (i.e., probe sets) between p53 mutant and wild-type cases using
unequal variance $t$ test and assessed the false discovery rate using $\beta$ uniform mixture analysis of $P$ values. We used the rank-ordered differentially expressed probe set list to train multivariate models using diagonal linear discriminant analysis (DLDA) to predict p53 mutation status (14). Increasing numbers of features were explored in the model building process, and the nominally most accurate prediction model on this training data was selected for validation.
in independent samples. Functional evaluation of the genes included in the prediction model was performed using the Ingenuity Systems pathway software. We attributed molecular classes according to the method reported by Parker and colleagues (19) and the genomic grade according to the method reported by Liedtke and colleagues (20).

**Statistical analysis**

Dichotomous variables including histological grade, node status (clinical and histological), tumor size (clinical and histological), HER2 receptor status, p53 mutation status were analyzed using the Pearson $\chi^2$ test or Fisher’s exact test (if the number of events was $<5$ in any category). Continuous variables including age and histological tumor size were analyzed using the Student’s $t$ test for variables with normal distribution, or the Wilcoxon test if not.

In the discovery set (Miller and colleagues) and in the p53 status validation set (MDACC/IGR), the accuracy, sensitivity, specificity, predictive positive value (PPV), and predictive negative value (NPV) of the 2 distinct p53 signatures were calculated with a 95% CI. To assess the predictive value of p53 signature on pathological complete response (pCR) after preoperative chemotherapy, univariate and multivariate logistic regression analysis was used to test the association of p53 signature and several other variables including age (continuous variable), clinical (pre-chemotherapy) tumor size (continuous variable), clinical node status (N0 vs. N1–2), nuclear grade (grade 1/2 vs. grade 3), HER2 status (normal vs. overexpressed), and p53 signature status (negative vs. positive) to response to chemotherapy.

For survival analysis, we used end points that were available from the original studies. These included time from diagnosis to distant metastasis (i.e., distant metastasis-free survival or DMFS), overall survival (OS), and recurrence free survival (RFS). The Kaplan–Meier estimate was used to compute distant DMFS curves, and the log-rank test for variables with normal distribution, or the Wilcoxon test if not. The Kaplan–Meier estimate was used to test the association of p53 signature and several other variables including age (continuous variable), clinical (pre-chemotherapy) tumor size (continuous variable), clinical node status (N0 vs. N1–2), nuclear grade (grade 1/2 vs. grade 3), HER2 status (normal vs. overexpressed), and p53 signature status (negative vs. positive) to response to chemotherapy.

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correctly assigned p53 functional status to 29 of 42 cases (accuracy 69%, 95% CI: 55–76). The sensitivity was 89% (95% CI: 73–97), specificity was 54% (95% CI: 42–60), the positive (PPV) and negative (NPV) predictive values were 59% (95% CI: 48–65), and 87% (95% CI: 67–96), respectively (Table 2). When a modified version of the original Miller and colleagues predictor, using only the 17 probe sets that are present on the U133A chips, was applied to these 42 independent cases, its overall accuracy was 69% (95% CI: 54–81), sensitivity 61% (95% CI: 44–75), specificity 75% (95% CI: 62–85), PPV 59% (95% CI: 46–79), and NPV 72% (95% CI: 60–82). This suggests that both predictors capture p53 functional status but the signature derived from ER− cancers only had a greater sensitivity, however an important limitation of this observation is that the ER-specific signature is compared with an abbreviated (17 probe sets only) and platform modified (U133A arrays only) version of the original signature.

Gene expression based methods may capture the transcriptional consequences of p53 pathway dysregulation more broadly than other methods that only identify defects in the p53 gene itself. We hypothesized that if the 39-gene signature captures p53 pathway status more accurately than sequencing, we would observe lower expression of known transcriptional targets of p53 in the p53 signature-positive (i.e., dysfunctional) cases even if the gene was wild type. We compared mRNA expression levels of the p53 and 10 other canonical p53 pathway genes that were not included in our signature (SEMA3B, PMAIP1/NOXA, FDXR, CCNG1, LRDD, CHEK1, CHEK2, PERP, BAX, and SFN) between p53 signature-positive (n = 33) and p53 signature-negative (n = 141) but p53 wild-type cases in the Miller data set. All of these genes showed numerically lower expression in the p53 signature-positive cases compared to p53 signature-negative cases and of these differences (64%) reached statistical significance (P < 0.05). This supports the idea that the transcriptional signature reflects a broad p53 pathway dysfunction. To further gain confidence in our signature, we plotted OS for the 174 ER-positive and p53 wild-type patients stratified by the 39-gene p53 signature in the Miller data set. OS was significantly worse in patients with positive 39-gene p53-signature (n = 33) compared with the signature-negative cases (HR = 4.55, 95% CI: 0.83–5.56, log-rank P = 0.03). This suggests that ER+ cancers with a transcriptional signature of p53 dysfunction have worse prognosis than cases with normal p53 signature even if the gene is wild type by sequencing.

**Table 2. Performance of the 39-gene p53 signature derived from ER+ cancers to predict p53 status in the discovery and validation sets**

<table>
<thead>
<tr>
<th>Data sets</th>
<th>Discovery set</th>
<th>Validation set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller (n = 213, ER+ p53 mutation)</td>
<td>p53 wild type</td>
<td>p53 mutated</td>
</tr>
<tr>
<td>p53 negative</td>
<td>141</td>
<td>6</td>
</tr>
<tr>
<td>p53 positive</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Accuracy (95% CI)</td>
<td>81.7% (77.1–84.6)</td>
<td>69% (55.1–75.8)</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>23.5 (9.3–59.1)</td>
<td>9.5 (1.93–44.4)</td>
</tr>
<tr>
<td>Sensitivity (95% CI)</td>
<td>84.6% (72.1–92.5)</td>
<td>88.9% (72.7–96.7)</td>
</tr>
<tr>
<td>Specificity (95% CI)</td>
<td>81% (78.2–82.8)</td>
<td>54.2% (42–60)</td>
</tr>
<tr>
<td>PPV (95% CI)</td>
<td>50% (42.6–54.6)</td>
<td>59.3% (48.4–64.5)</td>
</tr>
<tr>
<td>NPV (95% CI)</td>
<td>95.9% (92.6–98)</td>
<td>86.7% (67.2–96.1)</td>
</tr>
</tbody>
</table>

**Abbreviations:** GR, Institut Gustave Roussy; IPPV, positive predictive value; MDACC, M.D. Anderson Cancer Center; NPV, negative predictive value; OR, odds ratio.

**Prognostic value of the 39-gene p53 gene signature in ER+ cancers**

To further evaluate the true prognostic value of the 39-gene p53 signature, we tested it on 2 separate cohorts of ER+ breast cancers that received no systemic adjuvant therapy (Wang and colleagues n = 192 and TRANSBIG n = 134; refs. 16 and 17). In the Wang data set, the model assigned 101 patients to p53 dysfunctional status and these patients had significantly worse distant metastasis-free survival (DMFS) than patients (n = 90) with p53 normal signature (HR: 2.3, 95% CI: 1.25–4.23, log-rank P = 0.005). Kaplan–Meier survival curves are shown in Figure 1. In the TRANSBIG data set, 49 patients were assigned to p53 mutant status and they had a trend for worse DMFS.
with an HR of 2.17 (95% CI: 0.85–5.56, log-rank \( P = 0.09 \)). Recurrence free survival (RFS) and OS were also available for the TRANSBIG data and these were significantly worse for the p53 mutant group (HR = 2.25; 95% CI: 1.08–4.68, log-rank \( P = 0.002 \) for RFS and HR = 2.43; 95% CI: 0.96–6.15, \( P = 0.05 \) for OS; Supplementary Fig. S1). In Cox multivariate analysis including age, histological grade, tumor-size, and p53 signature score only the p53 signature was significantly associated with OS (\( P = 0.036 \); Table 3 and Supplementary Figs. S2–S4). These same clinical variables or OS data were not available for the Wang data set and therefore similar analysis could not be performed. Overall, these results from 2 independent data sets indicate that ER\(^+\) cancers with p53 dysfunction measured by our 39-gene signature have poorer prognosis in the absence of systemic adjuvant therapy than ER\(^+\) cancers with normal p53 signature.

Today, almost all ER\(^+\) early stage breast cancers receive adjuvant endocrine therapy. It is therefore important to also examine the performance of any putative prognostic marker for ER\(^+\) cancer in patients treated with adjuvant endocrine therapy. We tested our predictor on 277 ER\(^+\) patients treated with adjuvant tamoxifen therapy (IJB data set; ref. 18). The 123 cases that were assigned to p53 mutant status by the 39-gene signature had significantly worse DMFS than the p53 normal cases (HR = 2.43; 95% CI: 1.35–4.38, log-rank \( P < 0.0001 \); Fig. 1). In Cox multivariate analysis including age, histological grade, nodal status, tumor size, and p53 prediction score only the p53 signature was significantly associated with worse DMFS (\( P = 0.001 \); Table 3). When we plotted Kaplan–Meier survival curves by histological grade and nodal status with or without stratification by p53 status we noted that neither grade or nodal status was significantly associated with DMFS but p53 status was (Supplementary Fig. S5). Similar analysis could not be performed for OS because this information was not available. These results indicated that p53 dysfunction remains a marker of poor prognosis in ER\(^+\) cancers even after adjuvant endocrine therapy.

### Chemotherapy response predictive value of the 39-gene p53 gene signature in ER\(^+\) cancers

We next examined whether p53 status influenced chemotherapy sensitivity in ER\(^+\) cancers in 2 separate neoadjuvant data sets (MDACC/MAQC \( n = 142 \) and MDACC/IGR \( n = 42 \)). We correlated p53 status assessed by the 39-gene signature with pCR to preoperative chemotherapy. In the MDACC/MAQC data set, 9 patients had pCR (6%) and 8 of these were p53-positive by the gene signature. The overall accuracy, sensitivity, specificity, PPV, and NPV of the p53 signature to predict pCR were 68%
Table 3. Multivariate analyses of routine clinical variables and the 39-gene p53 signature as predictors of clinical outcome in ER+ cancers in 3 different data sets

<table>
<thead>
<tr>
<th>Variables</th>
<th>DMFS (HR (95% CI), P)</th>
<th>OS (HR (95% CI), P)</th>
<th>RFS (HR (95% CI), P)</th>
<th>IJB (HR (95% CI), P)</th>
<th>MDACC/MAQC (HR (95% CI), P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.02 (0.96–1.08) 0.5</td>
<td>1.03 (0.97–1.09) 0.4</td>
<td>0.99 (0.96–1.04) 0.92</td>
<td>0.99 (0.97–1.03) 0.84</td>
<td>1 (0.93–1.06) 0.88</td>
</tr>
<tr>
<td>Tumor size</td>
<td>2.20 (0.95–5.11) 0.06</td>
<td>1.62 (0.62–4.23) 0.32</td>
<td>1.14 (0.61–2.11) 0.68</td>
<td>1.73 (0.97–3.06) 0.06</td>
<td>1.03 (0.45–2.35) 0.94</td>
</tr>
<tr>
<td>Node status</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1 (0.55–1.81)* 0.99</td>
<td>0.84 (0.18–3.81)** 0.82</td>
</tr>
<tr>
<td>Histological grade</td>
<td>1.51 (0.71–3.21) 0.28</td>
<td>0.85 (0.44–1.63) 0.85</td>
<td>1.11 (0.70–1.76) 0.65</td>
<td>1.28 (0.77–2.13) 0.34</td>
<td>1.04 (0.48–2.25) 0.92</td>
</tr>
<tr>
<td>HER2 status</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.04 (0.27–9.47) 0.6</td>
<td></td>
</tr>
<tr>
<td>HER2 status by genea</td>
<td>0.72 (0.15–3.49) 0.68</td>
<td>0.69 (0.15–3.23) 0.64</td>
<td>0.82 (0.27–2.50) 0.72</td>
<td>1.69 (0.76–3.75) 0.20</td>
<td>NA</td>
</tr>
<tr>
<td>p53 signature</td>
<td>1.61 (0.6–4.27) 0.3</td>
<td>2.67 (1.06–6.69) 0.036</td>
<td>1.83 (0.94–3.54) 0.07</td>
<td>2.62 (1.47–4.68) 0.001</td>
<td>15.26 (1.71–135.8) 0.01</td>
</tr>
</tbody>
</table>

Abbreviations: DMFS, distant metastasis free survival; IJB, Institut Jules Bordet; MAQC, MicroArray Quality Control project; OS MDACC, M.D. Anderson Cancer Center; overall survival; pCR, pathological complete response; RFS, relapse free survival; *; pathologic nodal status; **, clinical nodal status; HER2 status was determined by immunohistochemistry or FISH.

aHER2 status was determined based on mRNA expression of HER2 as described in Bianchini and colleagues (24).
(95% CI: 64–70), 89% (95% CI: 58–98), 67% (95% CI: 65–68), 15% (95% CI: 10–17), and 99% (95% CI: 96–100), respectively, with an odds ratio (OR) of 16.2 (P = 0.001). In the MDACC/IGR data set, 10 patients had pCR (24%) and 9 of these were p53-mutant by the signature. The accuracy, sensitivity, specificity, PPV, and NPV of the p53 signature to predict pCR were 55% (95% CI: 42–59), 90% (95% CI: 64–98), 44% (95% CI: 36–46), 33% (95% CI: 24–36), and 93% (95% CI: 76–99), respectively, with an OR of 7 (P = 0.07). **The different rates of pCR in the 2 separate patient cohorts reflect differences in patient characteristics, for example, the MDACC/MAQC cohort included fewer high-grade cases (34% vs. 47%) and fewer patients with highly proliferative tumors which are among the most chemotherapeutic sensitive subset of ERþ cancers. We also performed multivariate logistic regression analysis on the MDACC/MAQC data set including the p53 signature, histological grade, pretreatment node status, tumor size, and HER2 status as variables. The p53 signature was the only variable significantly associated with pCR (OR = 15.26, 95% CI: 1.71–135.8, P = 0.01; Table 3). The same analysis could not be performed on the MDACC/IGR data set because of missing values and small sample size. These results show that ERþ breast cancers with p53 dysfunction, measured by a transcriptional signature, are more chemotherapy sensitive than p53 normal cases. In both data sets, almost all of the complete responses (17 of 19) occurred in the p53 mutant cases.

Performance of the 39-gene p53 signature in ERþ cancers

Finally, we examined if the p53 signature developed from the ERþ cases was also predictive of p53 status or predicted prognosis and chemotherapy sensitivity in ERþ cancers. The Miller data set included 38 ERþ patients and the 39-gene signature was able to distinguish p53 mutant (n = 19) from p53 normal cases with an accuracy of 79% (95% CI: 65–83), sensitivity 95% (95% CI: 81–99), and specificity 63% (95% CI: 49–68), P = 0.0004 (Supplementary Table S4). The MDACC/IGR data set included 56 ERþ patients with known p53 functional status assessed by the yeast assay. The 39-gene signature could again significantly discriminate between p53 dysfunctional (n = 38) and normal cases [accuracy 68% (95% CI: 56–76), sensitivity 63% (95% CI: 54–69), and specificity 78% (95% CI: 59–90), P = 0.009]. This shows that the signature derived from the ERþ cases remained informative to identify 53 dysfunctional cases even among ERþ cancers. The transcriptional changes induced by p53 mutation are partly overlapping among ERþ and ERÁ cancers. We next examined the prognostic value of this signature among the ERÁ cancers in the 2 prognostic data sets (Wang and colleagues, n = 63 and TRANSBIG n = 64). Surprisingly, in the Wang data set, all 63 ERÁ patients were classified as p53-normal by the 39-gene signature therefore survival analysis could not be performed. In the TRANSBIG data set, 41 patients (64%) were p53-mutant by gene signature and these patients had a better DMFS than patients with p53-normal cancers (log-rank P = 0.004); this is the opposite of what has been observed in the ERÁ cohort in this data set. We also tested the association between chemotherapy sensitivity of ERÁ cancers and the 39-gene signature status in the MDACC/IGR (n = 61, ERÁ) and MDACC/MAQC (n = 91, ERÁ) neoadjuvant data sets. The p53 signature was not associated with pCR in either of these data sets. Theses results suggest that unlike in ERÁ cancers, the 39-gene p53 signature is not informative of sensitivity to these chemotherapy regimens in ERÁ cancers.

p53 signature in ERÁ breast cancer

To complete a symmetrical analysis, we also developed a predictor of p53 status from the ERÁ cohort of the Miller data set using the same strategy as for the ERÁ cases. A 30-gene, ERÁ p53 signature showed 92% accuracy (95% CI: 79–96), 90% sensitivity (95% CI: 77–94), and 95% specificity (95% CI: 82–99) to identify p53 mutant cases in the discovery data (P < 0.0001). It also correctly assigned 53 functional status to 42 of the 56 ERÁ cases in the MDACC/IGR validation cohort (accuracy 75% (95% CI: 63–84), sensitivity 82% (95% CI: 73–88), specificity 61% (95% CI: 43–76), P = 0.002; Supplementary Table S5). When this predictor was applied to the ERÁ cohort of the Miller data sets to predict p53 mutation status it showed significant predictive value [accuracy 78% (95% CI: 73–82), sensitivity 74% (95% CI: 61–85), and specificity 79% (95% CI: 76–82); P = 0.002]. There were 22 discordant pairs between Miller signature and ER pos signature. Between ERÁ and ERÁ signature, we observed 20 discordant pairs. Interestingly, among these 20 discordant pairs, using the ERÁ signature correctly classified 14 tumors. However, when tested on the ERÁ cases of the MDACC/IGR data with known p53 functional status, it had no significant discriminating ability: 55% accuracy (95% CI: 43–59), 94% sensitivity (95% CI: 81–99), and 25% specificity (95% CI: 15–28), P < 0.21). This suggests only partial overlap in the p53-induced transcriptional changes in ERÁ and ERÁ cancers.

In the ERÁ cohort of the TRANSBIG data, the 30-gene signature was associated with better RFS (P = 0.03); a similar but nonsignificant trend was seen for DMFS (P = 0.32) and OS (P = 0.27; Fig. 2). These results are in contrast with the findings in ERÁ cancers where p53 dysfunction was significantly and consistently predictive of poor survival. The 30-gene ERÁ p53 signature was not prognostic in the ERÁ cancers with adjuvant endocrine therapy (IJB data set; ref. 18) in Cox multivariate analysis including age (P = 0.78), histological grade (P = 0.2), nodal status (P = 0.9), tumor stage (P = 0.05), and p53 prediction score (P = 0.11) for DMFS; whereas the 30-gene ERÁ p53 signature was prognostic in the ERÁ cancers without adjuvant endocrine therapy (TRANSBIG data set; ref. 16) in Cox multivariate analysis including age (P = 0.42), histological grade (P = 0.68), tumor stage (P = 0.56), tumor size (P = 0.1), and p53 prediction score (P = 0.02) for DMFS. It was also not predictive of pCR in the ERÁ or ERÁ cancers in the 2 neoadjuvant data sets.

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Different diseases that arise from different cell types can represent very different molecular contexts in which a particular genomic abnormality may occur (7, 8). For example, the molecular machineries of ER+ and ER-/C0 breast cancers differ in the expression of thousands of genes (9, 10). It is reasonable to assume that p53 mutation may lead to partly different transcriptional consequences and may also have different prognostic or predictive values in these 2 different types of breast cancers (1, 2, 11, 12). We explored this hypothesis by reanalyzing a public gene expression data set with known p53 mutation status (12). p53 signatures derived separately from ER+ and ER-/C0 cancers were indeed different with no overlap between the genes included in the signatures. However, despite the lack of common genes, both signatures could predict p53 dysfunction in both types of cancers, although with different sensitivity. This suggests that the most informative genes may be different by ER-status but the broader transcriptional changes are partly overlapping. The information contained on the U133B chip may also improve accuracy of p53 status. This, however, cannot be assessed in the absence of available breast cancer data sets profiled using both U133A and U133B chips.

We next assessed the prognostic and chemotherapy response predictive values of the 2 different p53 signatures in ER-/C0 and ER+ cancers, respectively in 5 distinct clinical data sets (12, 16–18). The 39-gene ER+ p53 signature consistently identified ER+ patients with poor prognosis with or without endocrine therapy and also defined a group that had greater chemotherapy sensitivity. This is consistent with findings from a smaller pilot study that used the yeast assay to determine p53 function and also reported greater chemotherapy sensitivity among p53 dysfunctional cancers (2). The same signature had no consistent prognostic or chemotherapy response predictive value in ER-/C0 cancers. The 30-gene, ER-/C0 p53 signature was associated with better recurrence free survival in ER-/C0 cancers and showed limited and inconsistent prognostic value in ER+ cancers. This signature also had no chemotherapy response predictive value in either ER- or ER+ cancers. These observations suggest that dysfunction in the p53 gene may have more important clinical consequences in ER+ cancers where it confers worse prognosis in the absence of systemic therapy or with adjuvant endocrine therapy alone and at the same time, it also defines a subset of cancers that are more sensitive to chemotherapy compared to p53 normal ER+ cancers. On the other hand, p53 dysfunction does not appear to affect the sensitivity of ER- breast cancers to standard anthracycline and paclitaxel-based chemotherapy and has lesser prognostic value in untreated ER- patients. Jordan and colleagues have recently showed that the functional and nonfunctional missense mutations may distinguish tumors, implying that heterogeneity in the functionality of specific p53 mutations could affect clinical behavior and outcome (21). This chemosensitivity performance may someday need to be put in context of another signature that predicts outcome and chemosensitivity in ER+ breast cancer.

This ER-status–dependent interaction between p53 function and chemotherapy response and prognosis may explain the large number of controversial results on this subject in the literature. Troester and colleagues investigated the p53-dependent gene expression signatures of cell types.
lines excluded genes that were associated with subtype but not downstream of p53 signaling, and identified a signature for p53 loss that is shared across breast cancer subtypes (22). The prognostic information provided by their signature was not specifically studied in ER+ tumors. Depending on the composition of cases (i.e., proportion of ER+ cancers) and the type of therapy that patients received in a particular study, p53 may or may not be significantly associated with survival. In studies that included primarily ER+ cancers and most patients were treated with adjuvant endocrine therapy alone, p53 is likely to be a significant predictor of poor outcome. In other studies that included a large number of ER- cancers and many of the ER+ patients received adjuvant chemotherapy, the association with survival may be blunted. Differential prognostic and predictive effect by ER status has been observed for other markers as well (23). In particular, proliferation markers and histological grade of breast cancer show similar ER-dependent interaction with prognosis and chemotherapy response (24). These results suggest that in the future predictive or prognostic biomarkers may be best developed separately for different clinical and molecular subsets of breast cancers.

Disclosure of Potential Conflicts of Interest

The authors declared no conflicts of interest. The corresponding author certify that all authors have agreed to all the content in the manuscript, including the data are presented.

Author Contributions

All authors took part in the design and implementation of the study, and read and approved the final report. The corresponding author has had access to all data in this study. C. Coutant collected and analyzed all the data, performed the statistical analysis and wrote the article. R. Rouzier supervised the statistical analysis and wrote the article. Y. Qi supervised the statistical analysis. J. Lehmann-Ché collected the local data and corrected the article. C. Bianchini corrected the article. T. Iwamoto corrected the article. G.N. Hortobagyi corrected the article. W.F. Symmans collected the local data and corrected the article. S. Uzan corrected the article. F. Andre collected the local data and corrected the article. H. de The collected the local data and corrected the article. L. Pusztai designed and supervised this work and wrote the article.

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