Prognostic and Therapeutic Implications of Distinct Kinase Expression Patterns in Different Subtypes of Breast Cancer

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Abstract

Different kinases are expressed in different clinical subsets of breast cancer. In this study, we assessed kinase expression patterns in different clinical subtypes of breast cancer, evaluated the prognostic and predictive values of kinase metagenes, and investigated their functions in vitro. Four hundred twenty-eight protein kinases in gene expression data were examined from 684 cases of breast cancer and 51 breast cancer cell lines to identify kinase expression patterns. We tested the prognostic value of kinase metagenes in 684 node-negative patients who received no adjuvant therapy and the predictive value in 233 patients who received uniform neoadjuvant chemotherapy. Twelve kinases were overexpressed in estrogen receptor (ER)–positive/human epidermal growth factor receptor 2 (HER2)–negative, 7 in HER2+, and 28 in ER+/HER2− cancers, respectively. We examined the functional role of 22 kinases overexpressed in ER+/HER2− cancers using siRNA. Downregulation of these kinases caused significant subtype-specific inhibition of cell growth in vitro. Two robust kinase clusters, including an immune kinase cluster and a mitosis kinase cluster, were present in all clinical subgroups. High mitosis kinase score was associated with worse prognosis but higher pathologic complete response (pCR) in ER+/HER2− cancers, but not in ER+/HER2+ cancers, in univariate and multivariate analyses including other genomic predictors (MammaPrint, genomic grade index, and the 76-gene signature). Conversely, higher immune kinase score was associated with better survival in ER+/HER2+ and HER2+ tumors and also predicted higher probability of pCR in HER2+ cancers. Taken together, our results indicate that kinases regulating mitosis and immune functions convey distinct prognostic information that varies by clinical subtype. Cancer Res; 70(21); 8852–62. ©2010 AACR.

Introduction

Protein kinases are key regulatory proteins and also provide attractive targets for drug discovery (1). The complete sequencing of the human genome allowed the identification of a full repertoire of human protein kinases (2). We hypothesized that different clinical subsets of breast cancers may have distinct kinase expression patterns, and kinases themselves may form coexpression clusters that represent biologically important functions. These kinase clusters, or metagenes, could have prognostic and predictive value and may represent breast cancer subtype–specific targets (3). The goal of this study was to identify kinases that are differentially expressed in the three clinically most important subsets of breast cancers defined by estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) expression. We assessed the consistency of kinase expression patterns across multiple different human data sets and in breast cancer cell lines. We examined the prognostic and chemotheraphy response predictive values of kinase metagenes (i.e., consistently coexpressed kinases) in patient cohorts that did not receive any systemic therapy and in patients who received neoadjuvant chemotherapy, respectively. Because kinases represent readily druggable therapeutic targets, we also studied the functional consequences of downregulating the expression of 22 different kinases using siRNA in a panel of 15 breast cancer cell lines. For these functional experiments, we selected kinases that were overexpressed in ER-negative cancers and were not previously systematically studied in the context of breast cancer.

Materials and Methods

Microarray data sets

Two separate data sets including the M.D. Anderson Cancer Center/Microarray Quality Control Consortium (n = 233, Gene Expression Omnibus accession no. GSE16716) and Miller and
colleagues \( n = 251 \), GSE3494; ref. 4) data sets were used as discovery sets to identify differentially expressed genes between (a) ER-positive/HER2-negative, (b) ER- and HER2-negative, and (c) HER2-positive (with any ER) tumors, respectively. These data sets were also used to define kinases that were consistently and highly coexpressed with one another and therefore could be combined into metagenes. To assess the prognostic value of these metagenes, we studied three other data sets including Wang and colleagues \( n = 286 \), GSE2034; ref. 5), TRANSBIG \( n = 198 \), GSE7390; ref. 6), and Mainz \( n = 200 \), GSE11121; ref. 7) corresponding to node-negative breast cancers that received no systemic adjuvant therapy. We examined the prognostic value separately in each data set to assess consistency and also in the pooled data set to improve the power of the analysis. We assessed the chemotherapeutic response predictive value of the metagenes in the MDACC/MAQC data set that included patients who received sequential paclitaxel and 5-fluorouracil, doxorubicin, and cyclophosphamide (T/FAC) preoperative chemotherapy. The clinical characteristics of the patients included in the various data sets used in this study are presented in Supplementary Table S1. We also studied gene expression data from two breast cancer cell line cohorts including a 51-cell line public data set (ArrayExpress accession no. E-TABM-157; ref. 8) and another 15 cell lines from MDACC that were used in the functional siRNA experiments (http://bioinformatics.mdanderson.org/pubdata.html; ref. 9).

Normalization of gene expression data and genomic signatures

All of the gene expression data used in this study were generated with Affymetrix HG-U133A gene chips. To have uniform normalization across data sets, we used MAS5 normalization with a median target array intensity of 600, and expression values were transformed to log 2 using the Bioconductor software. This method was chosen because only normalized data were available for the Wang and colleagues data set. The cell lines were scaled to a target array intensity of 1,500. Uniform MAS5 normalization was necessary to compare the newly derived kinase scores with previously reported prognostic predictors (10); we followed the same methods as described in the original articles or used the annotation provided by the investigators (6, 11, 12). To calculate the genomic grade index (GGI), we followed the method that does not require knowledge of the histologic grade of the samples. This method requires robust scaling with setting of the interquartile range to 1 and of the median to 0 within each data set (13). We used the GGI threshold defined in the Wang and colleagues data set, and we applied this threshold to the TRANSBIG and Mainz data sets for uniformity.

Identification of kinase genes and assessment of hormone receptor and HER2 status

Human protein kinases were identified online (http://kinase.com/human/kinome; ref. 14). We could map 428 of the 529 known kinases to 783 probe sets on the Affymetrix HG-U133A chip using Gene ID annotation (http://www.affymetrix.com/Auth/analysis/downloads/na27/ivt/HG-U133A.na27.annot.csv.zip, version November 30, 2008). When multiple probe sets targeted the same gene, we retained only the probe set with the highest average expression value and greatest variance. When more than one probe set met these criteria (i.e., showed very similar values), we retained each of these sets and considered them as separate measurements. This yielded a final list of 540 probe sets corresponding to the 428 kinases (Supplementary Table S2). To calculate metagene scores, each gene was represented by a single probe set that had the nominally highest variance.

ER and HER2 receptor status was determined based on mRNA expression levels as described previously (15). However, positivity threshold values had to be recalculated for this analysis because we used MAS5 normalization that is different from the dChip normalization used in the original publication. The cutoff points were established from the MDACC/MAQC data, and log 2 normalized values >10.18 of the probe set “205225_at” were considered as ER positive and values >12.54 for the probe set “216836_s_at” were considered as HER2 positive. Progesterone receptor (PGR) and Ki67 expression were measured by probe sets “208305_at” and “212022_s_at,” respectively, and were used as continuous variables in univariate and multivariate analyses.

Functional evaluation of kinases as potential therapeutic targets by siRNA screen

We selected 22 kinases for functional evaluation that were all overexpressed in ER-negative and HER2-negative breast cancers and have not been systematically studied previously. Each gene was targeted with four distinct siRNAs obtained from Dharmacon, Inc., which were applied to 15 different breast cancer cell lines (9 ER/HER2 negative and 6 receptor positive). Twelve cell lines were obtained directly from the American Type Culture Collection (ATCC) within 2 months of culture before being used in this study. All the cell lines were authenticated. Three cell lines (MDA-MB-231, MDA-MB-435, and MDA-MB-468) were obtained from the ATCC within more than 6 months of culture before being used in this study. For authentication, we performed a gene expression profiling for each cell line as described elsewhere (9). MDA-MB-468 resulted to be a luminal cell line in this cohort rather than a triple-negative cell line as described by Neve and colleagues (8). This cell line was accordingly considered as a probably mislabeled cell line and considered in the non–triple-negative group in the current analysis.

We optimized the transfection conditions for each cell line separately using three different types of negative controls including (a) cells grown in regular OptiMEM medium (Invitrogen, Inc.); (b) cells grown in the presence of transfection reagents only; and (c) cells transfected with control siRNA including five different siGENOME-1, siGENOME-2, siGENOME-3, siGENOME-4 constructs and four different ON-TARGETplus siRNA constructs (Dharmacon). Four different positive controls (40 nmol/L final concentration) were used to define optimal transfection efficiency conditions, including anti-PLK1 (polo-like kinase 1), anti-KIFF11 (kinesine family member 11), anti-COPB2 (coatamer protein β2), and...
Table 1. Differentially expressed kinases between ER-positive and ER-negative cancers (adjusted for HER2 status) and between HER2-positive and HER2-negative cancers (adjusted for ER status) in the three clinical validation data sets and a cohort of 51 breast cancer cell lines

<table>
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<tr>
<th>Probe set</th>
<th>Gene symbol</th>
<th>Primary tumors (validation data sets)</th>
<th>Cell lines</th>
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<td></td>
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<td>Ratio,* Wang and colleagues</td>
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<td>Plausible</td>
<td>51 cells</td>
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isiCONTROL TOX constructs. We examined eight different transfection reagents (DharmaFect-1, -2, -3, and -4; Xtreme Gene; HiPerect; RNAiMax; and siPORT NeoFX) to find the most efficient and least toxic for each cell line. Cell viability was determined 96 hours after transfection using the CellTiter Blue cell viability assay (Promega). The optimal transfection conditions were established separately for each cell line, defined as the most effective transfection reagent and the best negative and positive siRNA controls that yielded the highest Z-factor. The Z-factor was calculated as 
\[ Z = 1 - \frac{3 \times SSD}{R} \]
where \( R \) is the dynamic range of the assay (i.e., the absolute difference between mean cell viability for a given negative control and positive control) and SSD is the sum of the SDs for the positive and negative controls. The full siRNA screen was performed for all target genes in three replicates in 384-well plates using the optimal transfection conditions. Cells were seeded at a density that yielded about 70% to 90% confluence in the control wells at 96 hours. Each plate included wells with target siRNAs (40 nmol/L per well in 50 μL of total volume) and negative and positive siRNA controls; the plates were incubated at 37°C for 96 hours. Percent cell viability was calculated as follows: First, the median value of absorbance of wells that contained medium alone was subtracted from the absorbance readings of all other wells; next, the absorbance readings were normalized to the negative control wells (i.e., cells transfected with noncoding control siRNA) by dividing the test well values with the median absorbance obtained from the negative siRNA controls and multiplied by 100. The average percent viability across the three replicate plates was reported for each siRNA. A cell viability score of 100 indicates no inhibitory effect compared with negative control wells, and 0 indicates complete lack of viable cells. Supplementary Table S3 presents the siRNA target sequences and Dharmacon product IDs and product names for each of the 22 kinases that were targeted. Unequal variance \( t \) test was used to assess significant decrease in viability compared with controls.

### Statistical analysis

Statistical analyses were performed using the BRB-ArrayTools v 3.7.0 Patch_1 (http://linus.nci.nih.gov/BRB-ArrayTools.html) and the R software (http://www.r-project.org). All statistical tests were two-sided. Differentially expressed genes between ER-positive and ER-negative breast cancers were identified by \( t \) test with adjustments for HER2 status. The multivariate permutation test implemented by this software package to estimate false discovery due to multiple comparisons is based on a study by Korn and colleagues (16). This method allows for a probabilistic control of the false discovery proportion by allowing setting the maximum acceptable percent false-positive findings (e.g., 10%) at a pre-defined confidence interval (e.g., 90%) for a given set of \( n \) genes. To control for the potentially confounding effects of HER2 and ER status during ANOVA, these were used as blocking variables. We used the randomized block design implemented in BRB-ArrayTools that fits two linear models to the expression data for each gene. The full model included the ER class variable and the block variable (i.e., HER2), and the reduced model included only the block variable. The likelihood ratio statistics was used to test the significance of the difference between the classes (i.e., ER+ versus ER-). A similar analysis was performed for HER2-positive and HER2-negative cases when ER status was used as a blocking variable.

We defined kinase metagenes based on coexpression. To be included in a metagene group, a gene had to show a minimum Pearson correlation coefficient of ≥0.4 with the other members of the gene cluster. This was based on the empirical assessment of between-gene correlations in data sets and represents a value that defines genes that frequently cluster together using hierarchical clustering methods. A metagene also had to contain a minimum of 15 genes in a given data set to avoid defining a large number of small unstable gene groups. For the final metagenes that were tested for prognostic or predictive values, we included only those probe sets that were consistently present in a given kinase coexpression cluster in both discovery data sets (MDACC/MAQC and Miller...
and colleagues). The metagene score was defined as the arithmetic mean of the log 2 expression values of all gene members.

For survival analysis, we used the R-Design package (http://www.r-project.org). Distant metastasis-free survival was the primary end point that was available in all data sets, and it was defined as the time from the diagnosis to distant metastasis. To make the results comparable across data sets, survival was right censored at 10 years. To avoid the "optimal cutoff selection bias" (17), univariate and multivariate Cox regression analyses were carried out by using metagene scores as continuous variables in various clinical subgroups. During analysis of the pooled data sets, sample source was considered as a stratification variable. The likelihood ratio test was used to compare multivariate models with or without metagene scores of interest. Patients with missing variables were excluded in the multivariate analysis. To plot Kaplan-Meier survival curves, three metagene score categories were corresponding to low-, intermediate-, and high-score groups, which were compared by the log-rank test. We also tested if our metagene scores could further risk-stratify high-risk ER-positive patients defined by other previously reported genomic signatures. For plotting purposes, we defined the lowest 30% and the highest 25% percent values as low and high categories. These distributions were defined in the Wang and colleagues data set and corresponded to scores of 7.737 and 8.54, respectively, for the mitotic kinase metagene score (MKS). The same cutoffs were used in the other two prognostic data sets to plot survival curves.

Univariate linear logistic regression was used to evaluate the likelihood of pathologic complete response (pCR) according to kinase metagene scores. To avoid overfitting of the data due to the small number of events in each molecular subgroup, a multivariate logistic regression analysis was performed including only the variables that showed a P value of ≤0.5 in the univariate analysis.

Results

Differentially expressed kinases between different breast cancer clinical subtypes

In the two separate discovery data sets (Miller and colleagues and MDACC/MAQC), 118 and 154 probe sets were differentially expressed between ER-positive and ER-negative tumors after adjustment for HER2 status with a false discovery proportion ≤1% (Supplementary Table S4). Fewer probe sets, 31 and 14, were differentially expressed between HER2-positive and HER2-negative tumors. To define the most robust and consistent differentially expressed kinases, we introduced two further filters and removed genes that had a P value of >0.01 or had a ratio of <1.5 of the geometric mean expression between the two classes of comparison; these criteria had to be met in both data sets. This left 16 genes defined as consistently overexpressed in ER+, 24 in ER+, 7 in HER2+, and 1 in HER2− cancers in the two discovery data sets. These same genes were tested for significant differential expression in the remaining three human data sets and in the 51-cell line data set (i.e., validation cohorts). The results are presented in Table 1. Supplementary Fig. S1 shows the distribution of expression values for each individual genes in the four receptor subsets in each data set. The kinases overexpressed in ER-positive cancers included several growth factor receptors (ERBB3, ERBB4, FGFR3, IGFRI, and BMPR1B) and kinases overexpressed in ER-negative tumors including mostly intracellular kinases that regulate cell proliferation (AURKA, AURKB, CDC2, TTK, PLK1, NEK2, BUB1, MELK, IRAKI, and LYN). Among the HER2-associated kinases, only CRKBS is located on chromosome 17 within the ERBB2 amplicon. Supplementary Tables S5 and S6 provide further results of various group-wise comparisons.

Kinase metagene are predictive of prognosis and response to chemotherapy

We observed only two distinct groups of kinases that were strongly and consistently coexpressed in the two discovery data sets (Supplementary Table S7). One cluster included 12 kinases involved in mitosis, spindle checkpoint, and G2-M transition; therefore, we refer to these as the mitosis kinase metagene cluster (MKS). The second cluster included 15 kinases involved in the regulation of immune cells (Supplementary Table S8); this is referred to as the immune kinase metagene cluster (IKS).

We assessed the prognostic value of these two metagenes in node-negative patients who received no systemic adjuvant therapy and were also not included in the definition of these metagenes. We examined the prognostic value separately in the three main clinical subsets of cancers. The mitosis kinase score (MKS) was not prognostic in ER-negative/HER2-negative or in HER2-positive cancers. However, it showed strong and statistically significant association with worse survival in ER-positive/HER2-negative cancers in each of the three data sets (Table 2). In pooled analysis, the hazard ratio (HR) was 3.04 [95% confidence interval (95% CI), 2.26–4.11; P = 3.00E−13] for a 1-unit increase of this score. In Cox multivariate analyses performed separately in all three prognostic cohorts including Ki67, PGR expression, age, and the Nottingham Prognostic Index, all considered as continuous variables, the MKS remained significant (Table 3).

To plot Kaplan-Meier survival curves, we defined two cutoff points in the Wang and colleagues data set corresponding to the top 30% and bottom 25% scores and applied these to the Mainz and TRANSBIG data sets. Results for the individuals data sets and for the pooled data are shown in Fig. 1. In the pooled analysis, the 10-year distant metastasis-free survival rates for high, intermediate, and low MKS were 0.88 (95% CI, 0.83–0.93), 0.74 (95% CI, 0.68–0.82), and 0.45 (95% CI, 0.36–0.57), respectively (log-rank test P < 1.00E−10). We also tested if the MKS can further risk-stratify patients who are assigned to the high-risk category by other prognostic scores. The MKS was able to identify a low-risk group based on low or intermediate MKS among the patients who were considered high risk by the 70-gene MammaPrint, the GGI, or the 76-gene Veridex signature (Fig. 2; Supplementary Fig. S2). We also performed a Cox multivariate analysis using the MKS as a continuous
The MKS showed a significant and independent prognostic value ($P < 0.05$) in four of five cases with a significant likelihood ratio test when comparing the multivariate models with and without the MKS (Supplementary Table S9).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Wang and colleagues data set (178 pts, 66 events)</th>
<th>TRANSBIG data set (124 pts, 20 events)</th>
<th>Mainz data set (155 pts, 24 events)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multivariate* †</td>
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<tr>
<td></td>
<td>HR (95% CI)</td>
<td>$P$</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>MKS</td>
<td>2.28 (1.32–3.94)</td>
<td>0.003</td>
<td>5.22 (1.73–15.7)</td>
</tr>
<tr>
<td>PGR</td>
<td>0.85 (0.76–0.95)</td>
<td>0.005</td>
<td>0.83 (0.68–1.02)</td>
</tr>
<tr>
<td>MKI67</td>
<td>1.03 (0.75–1.41)</td>
<td>0.85</td>
<td>1.11 (0.64–1.93)</td>
</tr>
<tr>
<td>IKS</td>
<td>0.61 (0.39–0.94)</td>
<td>0.03</td>
<td>0.44 (0.18–1.07)</td>
</tr>
<tr>
<td>Age</td>
<td>NA</td>
<td></td>
<td>1.03 (0.97–1.09)</td>
</tr>
<tr>
<td>NPI</td>
<td>NA</td>
<td></td>
<td>1.22 (0.63–2.37)</td>
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NOTE: All variables are used as continuous variables.
Abbreviations: MKI67, Ki67 mRNA expression measured by probe set 212022_s_at; PGR, progesterone receptor mRNA expression measured by probe set 208305_at; NPI, Nottingham prognostic index; NA, not available; pts, patients.

* $P = 0.003$ (Wang), $P = 0.002$ (TRANSBIG), and $P = 0.04$ (MAINZ) for the comparison of the multivariate models with and without the MKS using the likelihood ratio test.
† $P = 0.02$ (Wang), $P = 0.06$ (TRANSBIG), and $P = 0.02$ (MAINZ) for the comparison of the multivariate models with and without the IKS using the likelihood ratio test.

The immune kinase score (IKS), just like the MKS, was not prognostic in ER$^-$/HER2$^-$ cancers. However, it was significantly associated with better survival in ER$^+$/HER2$^-$ and also in HER2$^+$ tumors in two of the three prognostic data sets. In the third data set, a similar but statistically
not significant trend was observed. In the pooled analysis, the HR was 0.55 (95% CI, 0.38–0.79; \( P = 0.001 \)) in the ER\(^+\)/HER2\(^-\) subgroup and 0.33 (95% CI, 0.17–0.65; \( P = 0.001 \)) in HER2\(^+\) tumors (Table 2). In the Cox multivariate analysis, the IKS remained statistically significant in two of three data sets [Wang and colleagues: HR, 0.61 (95% CI, 0.39–0.94; \( P = 0.03 \)); TRANSBIG: HR, 0.44 (95% CI, 0.18–1.07; \( P = 0.07 \)); Mainz: HR, 0.39 (95% CI, 0.16–0.94; \( P = 0.03 \))], including MKS, PGR, and Ki67 as continuous variables.

We also examined the association between pCR and the MKS and IKS in the MDACC/MAQC data set. In the univariate but not in the multivariate analysis, higher MKS was significantly associated with a higher chance of pCR in ER-positive/HER2-negative cancers with an odds ratio (OR) of 2.6 (95% CI, 1.12–7.09, \( P = 0.04 \); Supplementary Table S10). The MKS was not associated with pCR in any of the other clinical subsets. In the univariate and multivariate analyses, higher IKS was also significantly associated with higher probability of pCR in the HER2-positive subgroup, with an OR of 9.94 (95% CI, 2.17–94.1; \( P = 0.01 \)), but not in other clinical subsets (Supplementary Table S9).

**Functional evaluation of 22 kinases overexpressed in ER-negative/HER2-negative breast cancers**

We assessed the functional relevance of 22 kinases overexpressed in ER- and HER2-negative cancers using an siRNA screen including 15 different breast cancer cell lines. Figure 3B shows the expression of the 22 genes in each of the cell lines and the effect of the siRNAs on cell viability. The expression levels of these genes were generally high in triple-negative cell lines compared with the average expression of all genes (Supplementary Fig. S3), and the expression values tended to be higher in triple-negative breast cancer cell lines compared with others (Supplementary Table S11), suggesting that these cell lines may be appropriate models to study the

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**Figure 1.** Kaplan-Maier estimates of distant metastasis-free survival for low-, intermediate-, and high-MKS groups in ER-positive/HER2-negative and node-negative patients who received no systemic therapy. A to C, results for the three separate prognostic cohorts corresponding to the Wang and colleagues, TRANSBIG, and Mainz data sets. D, results for the pooled data set.
functional importance of these genes. Downregulation of the expression of these genes with siRNA resulted in an overall greater degree of growth inhibition in ER- and HER2-negative cells compared with receptor-positive cell lines over a broad range of cell viability values. Supplementary Table S11 shows the results for individual genes; 12 genes had statistically significantly higher ($P < 0.05$) growth inhibition in ER- and HER2-negative cell lines compared with receptor-positive cells, and 6 other genes were borderline significant, with $P$ values between 0.05 and 0.10. The greatest absolute growth inhibition, a 65% decrease in cell viability, in ER- and HER2-negative cells was seen with PTK7 (protein tyrosine kinase 7). The greatest relative growth inhibition compared with the effects on receptor-positive cells was seen with EPHB3 (ephrin receptor B3). Supplementary Figs. S4 and S5 show the most significant correlation plots between kinase mRNA expression and decrease in cell viability after siRNA knockdown in both triple-negative and non–triple-negative cell lines.

**Discussion**

Different clinical subtypes of breast cancers are characterized by different gene expression patterns, distinct DNA copy number alterations, and differential distribution of various mutations (18–20). In this study, we examined the expression distribution of 428 protein kinases in the different clinical subtypes of human breast cancers. Two recent articles suggested that kinase expression patterns may be different between molecular classes of breast cancer. Finetti and colleagues (21) identified 16 kinases involved in mitosis that distinguished luminal from basal cancers and could also define prognostic subgroups within luminal A cancers. Speers and colleagues (22) reported novel kinase targets for ER-negative cancers and also found that proliferation-related kinases predict for poor prognosis.

We focused our comparisons on ER-negative and ER-positive cancers after adjusting for HER2 status and on HER2-negative and HER2-positive cancers after adjusting...
for ER status as a confounder. We selected these receptor-based clinical categories because they reflect the current clinical approach to breast cancer, and more complex molecular classification methods have not yet been standardized (23). Molecular class assignment of individual cases with current methods is more variable than classification based on ER and HER2 (24). We found that 16 protein kinases were consistently overexpressed in ER-positive tumors across five different data sets. This suggests that these could provide some important biological function to sustain the growth of ER-positive cancers. Several of these kinases were cell surface growth factor receptors (FGFR3, ERBB3, ERBB4, IGF1R, and BMPR1B) and readily targetable with experimental drugs. Laboratory research also supports the existence of cross talk between ER signaling and the various epidermal and insulin-like growth factor receptor pathways (25–27).

Our data raise the possibility that a combined blockade of IGFR, FGFR3, and ERBB3 or ERBB4 may enhance endocrine sensitivity. We also observed high expression of the bone morphogenetic protein receptor 1B (BMPR1B) in ER-positive cancer, which is particularly intriguing because these cancers have a predilection for metastasis to the bone.

We also attempted to identify kinases that are frequently coexpressed in a consistent manner. Such coexpression clusters could indicate a functional kinase unit or reflect the presence of a particular cell type that is characterized by these kinases. Only two robust and consistent clusters were observed. One of these corresponded to mitosis kinases, and the other to kinases that are mostly expressed in lymphocytes. These kinase coexpression clusters were present in all clinical subtypes to variable extents. We evaluated their prognostic role and found that higher expression of the mitosis-related kinases (MKS) was strongly and significantly associated with poor prognosis in ER-positive/HER2-negative cancers, but not in the other subtypes. This is consistent with numerous previous reports that indicated a major prognostic value for proliferation markers in ER-positive/HER2-negative cancers (29–31). However, our MKS remained significant in the multivariate analysis even after adjustment for other proliferation markers including Ki67 and was able to restratify patients who were considered high risk by other prognostic gene signatures (the 76-gene signature, the GGI, and the 70-gene MammaPrint). These results suggest that the prognostic information provided by proliferation may not be optimally

Figure 3. Functional evaluation of 22 kinases using siRNA in 15 cell lines in vitro. TN, ER- and HER2-negative cell lines; non-TN, receptor-positive cells. A, the heatmap shows the cell viability score results (red, maximum effect; green, no effect) for each kinase siRNA in each cell line. B, the heatmap displays the expression value of each kinase in each cell line (red, higher expression; green, lower expression). C, the fraction of siRNAs inhibiting cell growth is greater in TN compared with non-TN cell lines over a broad range of cell viability thresholds.
Kinase Expression in Breast Cancer

captured by the empirically developed first-generation prognostic signatures. Indeed, the performance of some previously published prognostic signatures even improved after removing non–proliferation-related genes (31).

The IKS also provided consistent prognostic information on multiple data sets independent of the MKS. Higher IKS was associated with better prognosis in ER-positive/HER2-negative and HER2-positive tumors (but not in ER- and HER2-negative cancers). Previous studies that examined the association between immune cell infiltration of breast cancer and prognosis yielded contradictory results. Several recent articles described various immune-related gene signatures that were prognostic in different subsets of breast cancers and generally supported the hypothesis that the presence of immune cell heralds better prognosis (7, 32–34). Which arm of the immune system, humoral immunity or T-cell– or natural killer cell–mediated effects, if any, is responsible for the better outcome is yet to be established and may vary from tumor subtype to subtype or even from patient to patient.

In one homogeneously treated neoadjuvant patient cohort, we also examined the association between these kinase scores and pathologic response to neoadjuvant chemotherapy. Both high MKS and IKS were associated with significantly higher probability of pCR in ER-positive/HER2-negative and HER2-positive cancers, respectively, but not in ER/HER2-negative tumors. The association between higher proliferation rate and better response to neoadjuvant chemotherapy, particularly in ER-positive cancers, is well known (35). However, the importance of the immunologic parameters in predicting treatment sensitivity is just emerging. Two recent reports showed that a CD40 gene signature (corresponding to B cells) and tumor-associated lymphocyte infiltration were both predictive of better response to neoadjuvant chemotherapy (36, 37).

In summary, these results support the hypothesis that in addition to the strong prognostic effect of proliferation, immunologic variables also predict prognosis and chemotherapy response particularly among ER-positive and HER2-positive cancers. We also described a core set of consistently differentially expressed kinases between various clinical subtypes of breast cancer. These results provide a roadmap to systematically explore the therapeutic relevance of these cancer subtype–specific kinases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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4. Schmitt M, Bohm D, von Torne C, et al. The humoral immune system (corresponding to B cells) and tumor-associated lymphocyte infiltration were both predictive of better response to neoadjuvant chemotherapy (36, 37).


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