Estrogen-Regulated Genes Predict Survival in Hormone Receptor–Positive Breast Cancers

Daniel S. Oh, Melissa A. Troester, Jerry Usary, Zhiyuan Hu, Xiaping He, Cheng Fan, Junyuan Wu, Lisa A. Carey, and Charles M. Perou

Abstract

Purpose
The prognosis of a patient with estrogen receptor (ER) and/or progesterone receptor (PR)–positive breast cancer can be highly variable. Therefore, we developed a gene expression–based outcome predictor for ER+ and/or PR+ (ie, luminal) breast cancer patients using biologic differences among these tumors.

Materials and Methods
The ER+ MCF-7 breast cancer cell line was treated with 17β-estradiol to identify estrogen-regulated genes. These genes were used to develop an outcome predictor on a training set of 65 luminal epithelial primary breast carcinomas. The outcome predictor was then validated on three independent published data sets.

Results
The estrogen-induced gene set identified in MCF-7 cells was used to hierarchically cluster a 65 tumor training set into two groups, which showed significant differences in survival (P = .0004). Supervised analyses identified 822 genes that optimally defined these two groups, with the poor-prognosis group IIE showing high expression of cell proliferation and antiapoptosis genes. The good prognosis group IE showed high expression of estrogen- and GATA3-regulated genes. Mean expression profiles (ie, centroids) created for each group were applied to ER+ and/or PR+ tumors from three published data sets. For all data sets, Kaplan-Meier survival analyses showed significant differences in relapse-free and overall survival between group IE and IIE tumors. Multivariate Cox analysis of the largest test data set showed that this predictor added significant prognostic information independent of standard clinical predictors and other gene expression–based predictors.

Conclusion
This study provides new biologic information concerning differences within hormone receptor–positive breast cancers and a means of predicting long-term outcomes in tamoxifen-treated patients.

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estrogen signaling in breast epithelial cell biology, we hypothesized that differential expression of estrogen-regulated genes would be useful in predicting outcome.

**Materials and Methods**

**Cell Collection and Collection of mRNA**

MCF-7 cells were maintained as described previously. Cells were plated in 150-mm dishes and grown until 50% confluence. Media was changed and cells maintained for 48 hours in estrogen-free medium (phenol red-free RPMI-1640 with 10% charcoal-dextran-stripped fetal bovine serum) before treating for 2, 4, 8, or 24 hours with $10^{-10}$ M 17β-estradiol (Sigma-Aldrich Inc, St Louis, MO). Cells were harvested, and mRNA isolated using a Micro-FastTrack kit (Invitrogen Corp, Carlsbad, CA). A reference mRNA sample was collected from cells maintained for 48 hours in estrogen-free medium (ie, estrogen-starved cells).

**Microarray Experiments**

Human whole-genome microarrays (Agilent Technologies Inc, Palo Alto, CA) were hybridized according to manufacturer’s protocol with Cy3-CTP–labeled cRNA from estrogen-starved cells (2 μg/sample) and Cy5-CTP–labeled cRNA from 17β-estradiol-treated cells (2 μg/sample), with dye-flip replicates for each time point. Microarrays were scanned and image files analyzed as described previously. All primary microarray data are available via the University of North Carolina (Chapel Hill, NC; UNC-CH) Microarray Database (https://genome.ucsc.edu/) and the Gene Expression Omnibus (GEO; National Center for Biotechnology Information [NCBI], http://www.ncbi.nlm.nih.gov/geo/) with series number GSE2740 (GSM52882-GSM52909, GSM34423-GSM34568).

**Analysis of Microarray Data to Identify GATA3 and Estrogen-Regulated Genes**

Data from microarray experiments were calculated as described. Genes were excluded from data analysis if they did not have signal intensity of at least 30 in both channels for at least 70% of the experiments. To identify estrogen-regulated genes, we used one-class significance analysis of microarrays (SAM) to identify genes that changed in all estrogen-treated time points (as a single class) relative to the estrogen-starved cells. In our SAM analyses, we did not use the fold-change cutoff option to avoid the fold-change associated complications/pitfalls described by Larsson et al. Using a false discovery rate (FDR) of 0.04%, SAM identified 383 estrogen-induced and 574 estrogen-repressed genes; for subsequent estrogen-SAM analyses, only the 383 induced genes were used. Average linkage hierarchical cluster analysis was conducted and the results visualized in TreeView (http://taxonomy.zoology.gla.ac.uk/rod/ /treview.html). GATA3-induced genes were identified by microarray experiments on 293T cells transfected with GATA3 gene constructs, as detailed in Usary et al. One-class SAM analysis (0.58% FDR) identified 407 genes that were induced in the GATA3 samples (as a single class) relative to empty vector controls.

**Analysis of Primary Breast Tumor Data Using the Estrogen-Induced Gene Set**

The primary breast tumor samples (collected with patient consent and UNC-CH Human Investigations Review Committee approval) used in the training data set are described in Hu et al (submitted for publication), except for 14 new tumor samples. A total of 118 fresh frozen breast tumors and nine nontumor breast samples represented by 160 microarray experiments were analyzed using the 1,300-gene “breast intrinsic” gene set developed by Hu et al (submitted), which identified 65 tumors as belonging to the luminal subtype. These luminal tumors included 61 ER+ and/or PR+ tumors according to immunohistochemistry, three ER– and PR–, and one not determined.

The 383-gene MCF-7 estrogen-induced gene list was used to hierarchically cluster the 65 luminal tumors resulting in two groups, which we called groups I and II. We used a two-class, unpaired SAM analysis (with 1% FDR) to identify 822 genes (referred to as the estrogen-SAM list) that optimally differentiated group I versus group II tumors.
Genes regulated by estrogen and/or GATA3 in vitro are present in the primary tumor luminal epithelial/estrogen receptor–positive (ER+/H11001) gene cluster. (A) Scaled-down representation of 118 tumors hierarchically clustered using the 1,300-gene intrinsic list developed by Hu et al (submitted for publication). (B) Luminal/ER+/H11001 gene cluster. Blue, luminal epithelial subtype; pink, HER2+/ER–; red, basal-like; green, nontumor breast–like.

Fig 1. Genes colored as follows: red = estrogen-induced only; green = estrogen-repressed only; pink = GATA3–induced only; orange = induced by both estrogen and GATA3; blue = repressed by estrogen but induced by GATA3.

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Fig 2. Hierarchical cluster analysis of the 65 luminal tumors (identified in Fig 1) using the 822-gene estrogen–significance analysis of microarrays–derived list. (A) Scaled-down representation of the complete cluster diagram. Group IE and IIE tumors are indicated by blue and orange, respectively. Gene clusters containing (B) XBP1, (C) ribosomal genes, (D) progesterone receptor, (E) FOXA1, (F) MAGE genes, (G) proliferation signature, and (H) apoptosis and interferon-response genes.
near these transcription factors in vivo and help to define an expression pattern seen in many studies.23,30,32,34

**Analysis of Luminal Tumors Using Estrogen-Induced Genes**

We hypothesized that expression differences of estrogen-induced genes may define clinically relevant subgroups within clinically defined ER+ and/or PR+ tumors. To test this hypothesis, we clustered the 65 tumors identified as luminal in Figure 1 (blue dendrogram branch) using the 383 MCF-7 estrogen-induced genes. Two main groups resulted. Group I had higher expression of XBP1, PR, and TFF, which are all known ER targets. Group II had higher expression of a cluster of estrogen-induced genes that included CTPS, E2F6, and FANCA. Kaplan-Meier survival analysis showed that group I patients had significantly better relapse-free survival (RFS) outcomes than group II ($P = .0004$).

To further characterize the differences between group I and II tumors, we performed a supervised analysis (two-class SAM with 1% FDR) using the major dendrogram branch division to define the two supervising groups. This analysis identified 822 genes for which group I and II tumors showed significant differential expression. This gene set, the estrogen-SAM list, was then used to hierarchically cluster the 65 luminal tumors (Fig 2), which as expected, resulted in a very similar grouping of samples when compared with that using the 383 estrogen-induced genes. Kaplan-Meier analysis showed that using the estrogen-SAM list grouped the tumors into two groups (referred to as group IE and IIE) that predicted RFS ($P = .019$; Fig 3A).

Group IE tumors showed high expression of XBP1, FOXA1, PR, and many ribosomal genes (Fig 2B-E). According to EASE, the GO categories “transcriptional regulation,” “DNA binding,” and “extracellular” were over-represented relative to chance in group IE tumors. Group IIE tumors showed the high expression of a prominent proliferation signature including Ki-67, MYBL2, Survivin, STK6, and CCNB2 (Fig 2G); these first four genes plus CCNB1 form the basis for the proliferation portion of the Paik et al recurrence score predictor,13 which is a gene expression–based outcome predictor for ER+/node-negative, tamoxifen-treated patients. Recently, Dai et al10 performed a supervised analysis for genes that correlated with outcomes in patients with high ER expression relative to age, and identified this same proliferation signature as the main determinant for predicting patient outcomes; however, they identified few genes associated with good outcomes.

Group IIE tumors also showed high expression of a cluster of MAGE-A genes (Fig 2F), which have been associated with an increased recurrence risk and poor tumor differentiation.37 Figure 2H shows

![Graphs A, B, C, D](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAfIAAAAIeCAYAAAAwzT0gAAAgAElEQVR42mOwzDgAAAAABCAAAAAC...)
that group IIE tumors have high expression of genes with functions in the interferon pathway and apoptosis, such as FLIP/CFLAR, which is an inhibitor of tumor necrosis factor receptor–mediated apoptosis.38 Several antiapoptosis genes including FLIP, AVEN, Survivin, and BCL2A1 showed high expression in group IIE, suggesting an impaired ability to undergo cell death. Recent reports have shown that high expression of FLIP39 or BCL2A140,41 can directly contribute to chemoresistance, suggesting that functional inhibition of these proteins may provide a therapeutic target for group IIE patients. According to EASE, the GO categories “cell cycle/mitosis,” “antiapoptosis,” and “MHC-I” were over-represented relative to chance in group IIE.

**Group IE-IIE Classification Predicts Outcome in ER+ and/or PR+ Tumors**

To test the group IE-IIE classification as a clinically relevant outcome predictor, we analyzed ER+ and/or PR+ tumors from three published breast tumor microarray data sets.9,12,22 We used a single-sample prediction algorithm to classify tumors in each test data set, which involved creating group IE and IIE centroids/average profiles from the training data set (described in Analysis of Primary Breast Tumor Data Using the Estrogen-Induced Gene Set, under Materials and Methods). Kaplan-Meier analysis (Fig 3B-D) showed that group IE tumors had significantly better RFS in all test data sets. The group IE-IIE classification was also a significant predictor of overall survival (OS) for the test data sets in which OS data was available.9,22 Furthermore, by decreasing the FDR in SAM, we were able to define groups IE and IIE using a reduced estrogen-SAM list of 113 genes without any loss of predictive ability (Appendix A1, online only).

**Multivariate Analysis**

Multivariate Cox proportional hazards analysis was performed on the Chang et al data set (Table 1). Using RFS and OS as the end points, multivariate analysis showed that classifying tumors as group IE or IIE provided significant prognostic power independent of standard clinical factors (RFS P < .0001; OS P = .001). The group IE-IIE designation had the strongest association of all variables with RFS and OS.

In multivariate analyses that included Chang et al wound-response signature and van’t Veer et al’s23 70-gene signature along with the clinical variables, the group IE-IIE classification continued to

### Table 1. Multivariate Cox Proportional Hazards Analysis of Various Prognostic Factors in Relation to Relapse-Free Survival and Overall Survival for ER+ and/or PR+ Tumors in the Chang et al Data Set

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relapse-Free Survival</th>
<th>Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Group IE v IE*</td>
<td>2.90</td>
<td>1.71 to 4.92</td>
</tr>
<tr>
<td>Age†</td>
<td>0.48</td>
<td>0.31 to 0.74</td>
</tr>
<tr>
<td>Size‡</td>
<td>1.59</td>
<td>1.01 to 2.48</td>
</tr>
<tr>
<td>Tumor grade 2 or 3 v 1</td>
<td>1.80</td>
<td>0.99 to 3.3</td>
</tr>
<tr>
<td>Node status§</td>
<td>2.11</td>
<td>1.08 to 4.11</td>
</tr>
<tr>
<td>Hormonal or chemotherapy v no adjuvant therapy</td>
<td>0.36</td>
<td>0.18 to 0.71</td>
</tr>
</tbody>
</table>

NOTE. Boldfacing indicates variables found to be significant (P < .05) in the Cox proportional hazards model.

†Tumors were classified as group IE or IIE using the estrogen–significance analysis of microarrays–derived list.

‡Age was a continuous variable formatted as decade-years.

§Size was a binary variable: 0 = diameter < 2 cm; 1 = diameter > 2 cm.

$Node status was a binary variable: 0 = no positive nodes; 1 = one or more positive nodes.

### Table 2. Multivariate Cox Proportional Hazards Analysis for ER+ and/or PR+ Tumors in the Chang et al Data Set Using Various Prognostic Factors Including the Group IE-IIE Classification, the van’t Veer et al 70-Gene Signature, and the Chang et al Wound-Response Signature

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relapse-Free Survival</th>
<th>Overall Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hazard Ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Group IE v IE*</td>
<td>2.01</td>
<td>1.15 to 3.49</td>
</tr>
<tr>
<td>70-gene signature (poor v good)</td>
<td>2.76</td>
<td>1.50 to 5.06</td>
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<tr>
<td>Wound-response signature (activated v quiescent)</td>
<td>2.30</td>
<td>1.09 to 4.85</td>
</tr>
<tr>
<td>Age†</td>
<td>0.56</td>
<td>0.36 to 0.87</td>
</tr>
<tr>
<td>Size‡</td>
<td>1.45</td>
<td>0.93 to 2.28</td>
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<tr>
<td>Tumor grade 2 or 3 v 1</td>
<td>0.93</td>
<td>0.47 to 1.82</td>
</tr>
<tr>
<td>Node status§</td>
<td>1.72</td>
<td>0.89 to 3.33</td>
</tr>
<tr>
<td>Hormonal or chemotherapy v no adjuvant therapy</td>
<td>0.37</td>
<td>0.19 to 0.74</td>
</tr>
</tbody>
</table>

NOTE. Boldfacing indicates variables found to be significant (P < .05) in the Cox proportional hazards model. The 70-gene signature and the wound-response signature classifications were taken exactly as calculated in Chang et al27 and their performances in multivariate analysis may be optimistic.

†Tumors were classified as group IE or IIE using the estrogen–significance analysis of microarrays–derived list.

‡Age was a continuous variable formatted as decade-years.

§Size was a binary variable: 0 = diameter < 2 cm; 1 = diameter > 2 cm.

$Node status was a binary variable: 0 = no positive nodes; 1 = one or more positive nodes.
provide significant prognostic power independent of other variables in the model (RFS \( P = .014; \) OS \( P = .042; \) Table 2). The performance of the 70-gene and wound-response signatures in this multivariate analysis may be optimistically high because a subset of the patients in the Chang et al data set was used to train/optimise these two signatures; therefore, the ability of the group IE-IIE classification to show independent prognostic power in a model containing these two predictors indicates its usefulness in predicting outcomes.

**Group IE-IIE Associations With Clinical and Biologic Parameters**

To examine the hypothesis that group IE may be more differentiated than group IIE tumors, we determined whether an association existed between this classification and histologic grade. Two-way contingency table analysis showed significant association between grade and group IE-IIE class (Appendix Table A2, online only), with grade 1 and 3 tumors more likely to be classified as group IE and IIE, respectively. Cramer’s \( V \) statistic, which measures the strength of association between two variables in a contingency table, indicated a substantial association (Cramer’s \( V > 0.36 \)) between grade and group IE-IIE class for all data sets. For the Sorlie et al data set, p53 mutation data was available, and a two-way contingency table analysis showed a significant association between p53 status and group IE-IIE class, with group IIE more likely to be p53 mutant (\( P = .0019; \) Cramer’s \( V = 0.44 \)).

**Comparison of Group IE-IIE Classification to Luminal A/B classification**

We compared group IE-IIE classification to the luminal A/B classification.\(^8,9\) To identify Luminal A and B tumors in the three test data sets, we used the single-sample predictor developed in Hu et al (submitted), which employs centroids for each of the five breast tumor “intrinsic subtypes.” We then reclassified luminal A and B tumors from each data set as group IE or IIE. Kaplan-Meier analyses showed that the group IE-IIE classification did equally well or slightly better compared with the luminal A/B classification in separating luminal tumors into two groups with different survival outcomes (Appendix Table A3, online only).

**DISCUSSION**

The search for markers that predict long-term outcomes in hormone receptor-positive tamoxifen-treated patients has been an intense area of study. Genomic analyses have contributed to this area, with the development of several predictive gene sets and assays based on the selection of genes that directly correlate with patient/tumor outcomes.\(^10,13,23\) We took a different approach and selected genes on the basis of regulation by estrogen and their natural patterns of expression in primary tumors. The 822-gene estrogen-SAM list identified many genes that may help explain the outcome differences seen in ER+ and/or PR+ patients. Good-outcome group IE tumors tended to be more differentiated, and highly expressed a subset of estrogen- and GATA3-regulated genes. Conversely, poor-outcome group IIE tumors were more likely to be poorly differentiated. Association of the group IE-IIE profile with grade is expected because grade includes a measure of proliferation, which is an important determinant of outcomes in ER+ and/or PR+ patients.\(^8,10,13,19\) However, because the group IE-IIE distinction was significant in a multivariate analysis with grade included, this distinction adds prognostic information beyond what grade provides.

We used three published data sets as test sets and confirmed that the Group IE-IIE classification was a significant predictor in ER+ and/or PR+ patients. We note, however, that the relapse rates differed between data sets, and that the Group IE tumors showed 7% to 40% relapse rates depending on the data set (Fig 3). This underscores the fact that relapse rates are dependent on the characteristics of the patient set used. For example, comparing relapse rates in the Chang et al\(^2\) data set to those observed in Paik et al\(^13\) may not be valid because the Paik et al data set comprised tamoxifen-treated, node-negative patients, whereas the majority of the Chang et al patients received no adjuvant therapy and many were node positive. However, the multivariate analysis we performed on the Chang et al data set indicated that our predictor had significant prognostic value independent of standard clinical factors and other gene expression–based predictors, and a hazard ratio of 2.90 for group IIE versus IE indicates that our predictor has potential clinical utility. By limiting the Chang et al data set to those patients who received adjuvant therapy and were stage I-II, we observed a relapse rate for group IE patients of 12% (\( P = .007 \)) and significance for overall survival outcomes (data not shown). This indicates that given a patient population similar to Paik et al, our predictor’s “good group” can achieve outcomes similar to the Paik et al low-risk group.

An important unanswered question is whether the group IE-IIE distinction predicts pure prognosis, responsiveness to endocrine therapy, or both. From analyses of patient subsets in the Chang et al data set, it is clear that the group IE-IIE distinction predicts outcome in ER+ and/or PR+ patient subsets either receiving or not receiving adjuvant hormone therapy (data not shown). Paik et al observed similar results for their predictor.\(^13\) This is not surprising because half (eight of 16) of the Paik et al genes were present in the estrogen-SAM gene set. However, an advantage of our analysis is that it provides additional biologic information (eg, antiapoptosis genes) that the Paik et al and other predictors did not. Paik et al’s finding that their predictor also predicts benefit of chemotherapy\(^42\) may also apply to ours. Thus, the most pressing questions remaining regarding the group IE-IIE classification are (1) whether group IE and IIE gain similar benefits from chemotherapy, and (2) because group IIE tumors do poorly in the presence of tamoxifen, might they do better if administered alternative endocrine therapies.

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Estrogen-Regulated Genes in ER+/-PR+ Breast Cancer


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Appendix
The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Authors’ Disclosures of Potential Conflicts of Interest
The authors indicated no potential conflicts of interest.
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Provision of study materials or patients: Lisa A. Carey, Charles M. Perou
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Data analysis and interpretation: Daniel S. Oh, Melissa A. Troester, Jerry Usary, Cheng Fan, Charles M. Perou
Manuscript writing: Daniel S. Oh, Melissa A. Troester, Jerry Usary, Lisa A. Carey, Charles M. Perou
Final approval of manuscript: Daniel S. Oh, Zhiyuan Hu, Lisa A. Carey, Charles M. Perou

GLOSSARY

Centroid: Mean expression profile of a group of samples for a given set of genes.

Cramer’s V statistic: This statistic provides a quantitative measure of the strength of association between the two variables in a contingency table (which cannot be obtained from the P value). Cramer’s V values range from 0 to 1, with 0 indicating no relationship and 1 indicating perfect association. Traditionally, values between 0.36 and 0.49 indicate a substantial relationship, and values greater than 0.50 indicate a very strong relationship. The V statistic is a generalization of the more familiar $\phi$ statistic to non-$2 \times 2$ contingency tables, and for $2 \times 2$ tables the V statistic is equal to the $\phi$ statistic.

DWD (distance-weighted discrimination): A method to identify and adjust systematic biases that are present within microarray datasets. These systematic biases are a result of many different experimental features including different microarray platforms and different production lots of microarrays. DWD is useful in merging/comparing two tumor microarray datasets completed on different microarray platforms.

EASE (Expression Analysis Systematic Explorer): A computer program used for rapid biologic interpretation of gene lists that result from the analysis of microarray or other high-throughput genomic data. The main function of EASE is to perform theme discovery for a given list of genes.

Gene ontology: Allows for annotating genes and their products with a limited set of attributes, with the three organizing principles being molecular function, biological process, and cellular component. The development of structured, controlled vocabularies (ontologies) that describe gene products in terms of these organizing principles in a species-independent manner is a constantly evolving process.

Luminal epithelial tumors: Human breast tumors that are characterized by the expression of genes typically expressed in the cells that line the ducts of normal mammary glands; these genes include the estrogen receptor (ER), GATA3, X-box binding protein 1, FOXA1, and cytokeratins 8 and 18.

SAM (significance analysis of microarrays): A statistical technique using established software that determines the significance in changes of gene expression seen in microarray analysis (eg, cDNA and oligonucleotide microarrays), which measures the expression of thousands of genes and identifies changes in expression between different biologic states. On the basis of changes in gene expression relative to the standard deviation of repeated measurements, SAM assigns a score to each gene. When scores are greater than an adjustable threshold, permutations of repeated measurements are used by SAM to estimate the percentage of such genes identified by chance, the false discovery rate (FDR). In addition, SAM correlates gene expression data to a wide range of clinical parameters, including treatment, diagnosis categories, and survival time.

Supervised analysis: Analysis of gene expression profiling data in which external information such as survival status is used as a guide to select genes from microarray data.

Two-way contingency table: A tabular representation of categorical data, it shows the frequencies for particular combinations of values of two discrete variables X and Y. Each cell in the table represents a mutually exclusive combination of X-Y values. A $\chi^2$-based P value can be calculated for a contingency table and is used to determine whether there is a statistically significant association between the two variables in the contingency table. A $2 \times 2$ contingency table refers to a table in which X and Y are binary variables.
Functional Analysis of the Breast Cancer Genome

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Clinicians have long recognized that a diagnosis of breast cancer encompasses different tumor types with very different clinical outcomes. The first critical step toward a biomarker-based subclassification schema to aid disease management occurred with the widespread introduction of tumor estrogen-receptor (ER) measurement three decades ago. It is worth revisiting the literature of the time, because the introduction of ER testing was very controversial. In particular, investigators worried that a benefit from endocrine therapy in ER– disease could not be excluded.1

It took the weight of a meta-analysis to resolve this issue,2 but even today we worry about excluding a patient from appropriate endocrine therapy because of false-negative ER results. The single-sample prediction problem continues to be central to the current debate regarding new and more complex biomarker approaches. Apparently robust group predictions typical of these analyses does not necessarily require great measurement accuracy. Prospective clinical testing, on the other hand, needs to be highly precise to avoid the potentially tragic consequences of tumor misclassification.

Further subclassification for ER+ disease to prospectively identify endocrine therapy–resistant ER+ tumors is of longstanding interest. The introduction of HER2 testing has already taken us some way down this road. It is clear that the activation of HER2 by gene amplification drives endocrine therapy resistance,3 and the introduction of the HER2-targeting monoclonal antibody trastuzumab into the adjuvant setting improves outcomes for patients with ER+ HER2+ tumors.4 HER2 gene amplification does not, however, explain all or even most cases of endocrine therapy failure, and new insights and predictive models are needed. One longstanding idea is to consider the ER in the context of an entire pathway; as our insight into the role of estrogen in tumorigenesis deepens, we will be able to move beyond reliance on progesterone receptor (PgR) measurement for ER activity assessment. The late William McGuire, who championed progesterone receptor measurement in breast cancer,5 would therefore have been very interested in the paper by Oh et al6 in this issue of the Journal of Clinical Oncology. The University of North Carolina–Chapel Hill (Chapel Hill, NC) team assessed the biomarker potential of hundreds of genes, their subsequent work in the paper focused on only upregulated genes. As an interesting side issue in the article, Oh et al compared this list of genes with a list induced by transfection of the GATA3 transcription factor into a GATA3 null cell line and found considerable overlap. Thus GATA3 and ER must work in concert to regulate this large gene repertoire. There was also a good deal of overlap with genes found to be expressed in ER+ breast cancers in earlier studies (referred to as “luminal” in keeping with Oh et al’s earlier work on breast cancer classification) as well as genes represented in the only clinically available multigene test that predicts tamoxifen resistance.7

To ascertain the role these estrogen-induced genes might play in clinical outcomes, Oh et al first performed hierarchical clustering of the estrogen-induced genes in a 64–luminal tumor training set and identified two major groups, groups I and II. All of the gene information available from these cases was subsequently compared between groups I and II (a supervised analysis), with a 1% false-discovery rate, to produce an 822-gene list. The tumors were then reclustered with the 822 differentially expressed genes to reproduce two groups (called IE and IIE). Information on as many of the 822 genes as possible was then sought from three publicly available, clinically annotated breast cancer microarray databases. After data manipulation to remove “platform and source systemic bias” an average expression index or “centroid” was used to classify clinically ER+ and/or PgR+ tumors into two groups with significant differences in survival. From a functional genomics standpoint, it is interesting to note that the favorable-prognosis group IE gene list was largely populated by transcription factors XBP1, FOXA1, and PgR and ribosomal genes. Unfavorable Group IIE tumors were rich in proliferation genes (Ki-67, MYBL2, Survivin, STK6, and CCNB2), as well as apoptosis regulators FLIP/CFLAR, AVEN, and BCL2A1. These gene signatures imply, not surprisingly, that endocrine therapy resistance is associated with a deregulated cell cycle and resistance to programmed cell death.

So what are we to make of these data? The first issue, of course, is that Oh et al’s extensive gene list is far from a clinical test. Although the signature is robust, and survives the data averaging algorithms necessary for cross-platform assessment, clinical testing needs to be formulated into a reproducible technology that can be repeated thousands of times in a cost-effective manner. Here, we are faced with a dilemma that is becoming more acute every day. Although the systematic evaluation of expression from 822 genes in a clinical environment sounds expensive, actually, by using smaller customized microarrays, it could be achieved for a couple hundred dollars with a turnaround of 1 or 2 days. However, the starting material for RNA extraction (at least currently) must be a...
snap-frozen tumor biopsy whose quality and tumor content have been assessed by histopathology. On the other hand, the measurement of 822 genes by quantitative polymerase chain reaction from RNA extracted from a paraffin block is impractical at this stage, and a step to reduce the gene number closer to 100 or so without loss of predictive abilities would be required for the clinical implementation of Oh et al’s signature. We may have to face the fact that, like our colleagues who treat lymphoma, breast cancer physicians will have to insist on frozen tumor acquisition in the near future. We achieved fresh tissue acquisition for biochemical ER testing before and I predict that, despite the barriers, we will be doing it again. An obvious move in this direction is the prospective evaluation of the Amsterdam 70-gene prognostic signature that requires frozen tissue RNA analysis.8

One of the most interesting aspects of the Oh et al article concerns the glimpses of the molecular complexities of the biologic system that we are trying to understand. Certain genes, such as STK6, keep surfacing in breast cancer profiling studies. This gene is present in the National Surgical Adjuvant Breast and Bowel Project (NSABP) recurrence score published by Paik et al.7 As a side note, gene nomenclature is extremely frustrating; STK6 is actually assigned to the mouse gene, whereas the human homolog is Aurora-A/AURKA/STK15/BTAK. STK15 is deregulated in a wide spectrum of poor-prognosis cancers9-11 and of interest, Aurora kinase inhibitors are in development.12 The power of the HER2-trastuzumab paradigm is that we have successfully paired a somatic mutation with a targeted therapy. It seems likely that the final formulation of a biologically based classification of breast cancer will have a strong bias toward genetic abnormalities that create opportunities for targeted treatments. The functional annotation of the breast cancer genome required for this vision has only just begun, and the excitement in the breast cancer research community is palpable.

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