Revealing Targeted Therapy for Human Cancer by Gene Module Maps

David J. Wong, Dimitry S.A. Nuyten, Aviv Regev, Meihong Lin, Adam S. Adler, Eran Segal, Marc J. van de Vijver, and Howard Y. Chang

Abstract

A major goal of cancer research is to match specific therapies to molecular targets in cancer. Genome-scale expression profiling has identified new subtypes of cancer based on consistent patterns of variation in gene expression, leading to improved prognostic predictions. However, how these new genetic subtypes of cancers should be treated is unknown. Here, we show that a gene module map can guide the prospective identification of targeted therapies for genetic subtypes of cancer. By visualizing genome-scale gene expression in cancer as combinations of activated and deactivated functional modules, gene module maps can reveal specific functional pathways associated with each subtype that might be susceptible to targeted therapies. We show that in human breast cancers, activation of a poor-prognosis "wound signature" is strongly associated with induction of both a mitochondria gene module and a proteasome gene module. We found that 3-bromopyruvic acid, which inhibits glycolysis, selectively killed breast cells expressing the mitochondria and wound signatures. In addition, inhibition of proteasome activity by bortezomib, a drug approved for human use in multiple myeloma, abrogated wound signature expression and selectively killed breast cells expressing the wound signature. Thus, gene module maps may enable rapid translation of complex genomic signatures in human disease to targeted therapeutic strategies. [Cancer Res 2008;68(2):369–78]

Introduction

One of the outstanding issues in treating cancer is the vexing heterogeneity in patient course and response to therapy, even in apparently similar tumors as defined by conventional criteria. Molecular genetic analyses over the last several decades have increasingly revealed the genetic heterogeneity that underlies the clinical spectrum of disease outcomes and progression. For instance, genome-wide gene expression profiling of apparently similar human tumors by traditional criteria have revealed consistent and large-scale differences in the expression pattern of hundreds of genes, which allowed definition of new molecular subtypes of cancer with distinct risks of metastasis, death, and response to therapy (1–6). However, whereas genomic technologies have improved the precision and prognostic power of cancer diagnoses, they have shed less light on how these newly recognized subtypes of cancers should be therapeutically targeted. As clinical experience with Her-2 and epidermal growth factor receptor inhibition in cancer has illustrated, identification and selection of patients who are likely to respond to molecularly targeted therapies are critical to the success of these approaches (7–10). Unfortunately, because a large number of genes with many diverse functions are identified in prognostic signatures, the laundry lists of genes may yield excellent prognostic markers without revealing much of the underlying biological mechanisms. In many instances, physicians and patients thus faced the quandary of knowledge of certain doom but little guidance for a course of action.

Previously, investigators have suggested adjusting the intensity of current chemotherapeutic regimens based on cancer subtypes as defined by gene expression signatures (11). Alternatively, other groups have suggested using gene expression signatures of oncogenic pathways (12), drug-specific gene expression signatures predictive of cell killing (13), and gene expression signatures induced by drugs (14, 15) to guide targeted therapy. Whereas these methods are promising, they require extensive gene expression profiling of in vitro cell models to define expression signatures for each chemical entity. For instance, Lamb et al. (16) carried out 564 arrays to determine the transcriptional response to 164 drugs, most of which in a single cell line.

To address this challenge, we adapted the gene module map method to identify genotype-specific therapeutic targets for human cancer (17). In a gene module map, the genome-scale expression profile of each tumor is deconvoluted into a series of activated and deactivated gene modules representing distinct functional pathways, enabling a higher-order and systematic view of the perturbed functional network in cancer cells (17). We hypothesized that by intersecting the gene module map with genotypic data, such as previously identified prognostic gene expression signatures, we can identify unique functional features that cosegregate with each genetic subtype. Genotype-specific functional pathways can then form the basis of targeted therapies that match unique molecular interventions to the subset of patients most likely to respond.

Clinically aggressive breast tumors acquire the ability to proliferate rapidly, invade through tissue planes, induce new blood vessel formation, and recruit inflammatory cells—many features that are normally reserved for the physiologic response of wound healing. We have discovered that a gene expression program reminiscent of wound healing, termed the "wound signature," is a...
powerful and independent predictor of breast cancer metastasis and survival present in 40% of primary breast tumors (11, 18). Activation of the wound signature in primary breast cancer is most prominently due to coordinate amplification of two genes on chromosome arm 8q: MYC and CSN5 (19). In a retrospective study of 295 early breast tumors (stage I and II), the wound signature was the most significant predictor of metastasis in multivariate analysis, far ahead of traditional clinical, histologic, and molecular criteria (11). Whereas patients whose tumors lack the wound signature may be more assured of their good prognosis and avoid unnecessary adjuvant systemic therapy (11, 20), how patients with an activated wound signature should be treated is unclear. Here, we use gene module map as an unbiased approach to search for therapeutic targets in wound-like breast cancers and to identify glycolysis blockade and proteasome blockade as potential therapeutic strategies for these highly aggressive tumors.

Materials and Methods

Gene module map. Gene module map was done as described with the program Genomica (17) using 1,256 gene sets derived from Gene Ontology (21) and the gene expression profiles of 295 human primary breast cancers from the Netherlands Cancer Institute (NKI) data set (11, 20). Briefly, given this collection of precompiled gene sets and this compendium of expression arrays, the method first identifies the gene sets that are induced or repressed in the breast cancers. The method then identifies the gene sets that are significantly associated with particular clinical annotations, specifically the wound signature, in the breast cancer arrays. The coactivated gene sets were then combined into higher-level modules based on their Gene Ontology relations, specifically the mitochondria and proteasome modules.
Survival analysis. We compared metastasis-free survival and overall survival of patients with breast cancers that have activated versus repressed signatures using Kaplan-Meier survival plots by the Cox-Mantel log-rank test with the program Winstat (R. Fitch Software). Overall survival was defined by death from any cause and patients were censored at last follow-up. Distant metastasis was scored only if it was the first recurrence event as previously described (11, 20). If a patient developed a local recurrence, an axillary recurrence, a contralateral breast cancer, or a second primary cancer (except for nonmelanoma skin cancer), the patient was censored at this time. This is based on the theoretical possibility that these cancers can be a source for distant metastases. An ipsilateral supravacular recurrence shortly preceded distant metastasis in all but one patient. An ipsilateral supravacular recurrence was thus considered the first clinical evidence for metastatic disease in this analysis. Therefore, these patients were not censored at time of ipsilateral supravacular recurrence.

Forty-five percent of the 295 primary breast cancers have an activated wound signature, 48% have an activated mitochondria signature, and 44% have an activated proteasome signature. Seventy-three percent of the mitochondria-activated cancers have an activated wound signature, and 80% of the mitochondria-repressed cancers have a repressed wound signature. Similarly, 81% of the proteasome-activated cancers have an activated wound signature and 83% of the proteasome-repressed cancers have a repressed wound signature.

Cell culture and drug treatment. MCF10A cell lines were stably transduced with vector only, E2F3, CSN5, MYC, or MYC and CSN5, as previously described (19). MCF10A cell lines were grown in DMEM/F-12 (Invitrogen/Cambrex) with 5% fetal bovine serum (FBS), supplemented from Millenium Pharmaceuticals. Proteasome blockade was confirmed neutralized with NaOH immediately before use. Bortezomib was purchased with 10% FBS. 3-Bromopyruvic acid (Sigma) was dissolved in water and the NKI cryo-bank. Breast cancer cells were grown in DMEM (Invitrogen) cancer cell line was treated with 1 \mu M bortezomib in low serum (0.1% FBS) for 24 h before RNA extraction. The array data are available at GEO (accession nos. GSE2824, GSE5307, and GSE8957).

Data analysis. To determine the wound, mitochondria, and proteasome signature scores, we calculated the Pearson correlation coefficient of the expression pattern of genes in the mitochondria or proteasome signature relative to the expression pattern of these genes in serum-stimulated fibroblasts, as previously described for the wound score (11). The KD50 was defined as the dose of bortezomib required to kill 50% of tumor cells. Pearson correlations between KD50 and wound or proteasome scores were calculated in Winstat.

To quantify the association of the proteasome signature with the wound signature in six independent human breast cancer data sets, we calculated the Pearson correlation of the wound signature score and the proteasome signature score across individuals in each data set. The wound scores and proteasome scores were plotted in linear regression plots, and the probability of observing the correlation by chance alone was calculated by one-sided t test (null hypothesis \( t = 0 \)). Pearson correlation and linear regression were calculated in Winstat. Genes were mapped across different platforms using Unigene identifiers for the 512 wound signatures genes and LLID for the 50 proteasome module genes. When multiple probes were mapped to one Unigene cluster or LLID, expression data of duplicate probes were averaged. For Affymetrix data, expression values were log transformed and mean centered across genes. Data from platforms that used a nonbreast cancer reference were mean centered across genes. Data from platforms that used a breast cancer reference were not centered.

Results

Mitochondria module and proteasome module are co-activated with wound signature in human breast cancer.

To identify novel therapeutic strategies for breast cancers with the poor prognosis wound signature, we used gene module map as an unbiased approach to discover new gene modules that are coactivated with the wound signature (Fig. 1A). We analyzed 1,256 gene sets defined by Gene Ontology (21) terms in the gene expression profiles of 295 human primary breast cancers from NKI (20). For each microarray profile, the algorithm first compares genes that are induced (or repressed) by 3-fold versus their membership in gene sets and identifies gene sets that show enrichment in induced (or repressed) genes more than expected by chance (\( P < 0.05 \)). Value of enrichment was determined by the hypergeometric distribution, and a false discovery rate (FDR) calculation was used to account for multiple hypothesis testing. The resulting gene module map is shown (Fig. 1B).

Second, tumors that exhibited the poor-prognosis wound signature were identified (using hierarchical clustering of 512 wound signature genes as previously described; ref. 11), and we identified enriched gene sets that were significantly coactivated with the wound signature more than expected by chance alone and in at least 15 of the breast cancers (\( P < 0.05 \), FDR<0.05, hypergeometric distribution; Fig. 1B and Supplementary Table S1).

We observed that five gene sets associated with mitochondrion were coordinately induced in tumors with an activated wound signature: the Gene Ontology terms mitochondrion, NADH dehydrogenase activity, hydrogen ion transporter activity, cation transporter activity, and oxidoreductase activity (labeled in brown in Fig. 1B). The member genes that enabled gene set hits in each of the gene sets were fused together into a single gene module, representing a refined group of coexpressed genes termed the “mitochondria module” (Fig. 2A). The resulting mitochondria module is composed of 218 unique genes consisting exclusively of genes encoded in the nuclear genome, most of which encode proteins with known localization and/or function in the mitochondria (Supplementary Table S2). The module encompasses

Figure 2. Mitochondria gene module in human breast cancers. A, gene module map highlights the association of mitochondria module with activated wound signature. Top, wound signature status and identity of gene sets derived from Gene Ontology terms contributing to the mitochondria gene module. Five gene sets related to mitochondria were coordinately induced in tumor samples that had an activated wound signature; the average expression of their member genes in each array is displayed. Shown are the subset of breast cancer samples with the strongest coordinate activation or deactivation of the mitochondria module after accounting for multiple hypothesis testing (FDR < 0.05, P < 0.05). Bottom, raw expression levels for the 218 genes that compose the mitochondria module along with their respective membership in each of the five gene sets (left). B, induction of mitochondria module in primary breast tumors predicts increased probability of death. Kaplan-Meier survival curve is shown. C, validation of the mitochondria module in an independent set of locally advanced breast cancers (2). Shown is a Kaplan-Meier relapse-free survival curve of patients in which the average expression of the mitochondria module in the primary breast tumor is above (induced) or below (repressed) zero, showing that the mitochondria module is a good predictor of survival in breast cancer in another data set.
multiple processes associated with the mitochondria, including respiration/oxidative phosphorylation, various other metabolic processes, apoptosis, mitochondrial biogenesis, and transport processes. Importantly, the genes in the mitochondria module were not simply an enriched subset of the wound signature: the overlap between the mitochondria module and wound signature was only seven genes.

We also similarly observed that gene sets associated with the ubiquitin-proteasome pathway were coordinately induced in tumors with an activated wound signature: ubiquitin cycle, proteasome complex, proteasome core complex, and protein ubiquitination (labeled in blue in Fig. 1B). The member genes that enabled gene set hits in each of the four sets were similarly fused together into a single gene module, representing the “proteasome module” (Fig. 3A). The resulting proteasome module is composed of 50 unique genes, which are listed in Supplementary Table S3. The proteasome module is strongly induced in tumors with the wound signature (P < 0.05, FDR <0.05, hypergeometric distribution; Fig. 3A). In particular, genes that encode components of the 19S regulatory and 20S proteasome core subunits are most strongly associated with activation of the wound signature (P < 10^-13, hypergeometric distribution). Only four genes of the proteasome module are part of the 512-gene wound signature; thus, this analysis also identified an expanded and coordinate activity of proteasome genes with the wound signature that we previously did not appreciate.

Mitochondria and proteasome modules associated with poor prognosis in human breast cancers. Across all 295 tumors, the primary breast tumors that had increased expression of the proteasome module had significantly worse subsequent metastasis-free and overall survival (P < 10^-3 and P < 10^-7, respectively; Fig. 3B). The mitochondria module was not significantly associated with survival across all 295 samples; however, the 50 tumors that had the highest average expression of the mitochondria module had a statistically significant association with poor survival compared with the 50 tumors that had the lowest average expression (P < 0.02, Fig. 2B). Activity of the mitochondria module was not associated with survival in the remaining 195 tumors with modest or no coregulation of the mitochondria module. We also examined the prognostic value of the mitochondria module in an additional data set of 120 locally advanced breast cancers obtained with a different microarray platform for which relapse-free survival data were available (2). We carried out the exact same analysis and identified 50 tumors with the highest and 50 tumors with the lowest average expression of the mitochondria module. Tumors with an induced mitochondria module showed a significantly increased risk of cancer relapse (P < 0.05; Fig. 2C). Applying the mitochondria module to all 120 tumors (yielding 60 tumors with an average expression level of the mitochondria module above zero and 60 tumors with an average expression level below zero) also showed a significant increased risk of relapse for tumors with induced mitochondria module (P = 0.03; data not shown). Thus, similar to the wound signature, both the mitochondria and proteasome modules are associated with poor prognosis in human breast cancers.

Cells with activated wound signature are selectively killed by 3-bromopyruvic acid. More than 70 years ago, Warburg (22) observed that cancer cells frequently induce glycolysis at the expense of mitochondrial respiration for energy production, and hypothesized that mitochondrial “respiration injury” is a fundamental defect in cancer. Our discovery of an alteration in the expression of mitochondria genes in poor prognosis breast cancers suggested that this subset of breast cancers with activated wound signature may have a mitochondrial respiratory defect and have increased dependence on glycolysis for ATP production. Thus, we examined whether cells with an activated wound signature are more susceptible to a drug that blocks glycolysis. We have previously shown that immortalized but untransformed MCF10A breast epithelial cells do not express the wound signature, but the wound signature is partially or fully induced by overexpression of MYC or MYC plus CSN5, respectively (19). Expression of CSN5 alone does not induce the wound signature. In addition, we found that the oncogene E2F3 (23) is sufficient to drive MCF10A cells into proliferation in low serum but has only a modest effect on the wound signature (Supplementary Table S4 and data not shown). We also found that the mitochondrial signature is induced by overexpression of MYC or MYC plus CSN5, but not by CSN5 alone or E2F3 (Fig. 4A). We first treated these MCF10A cells of different genotypes with 3-bromopyruvic acid, which inhibits glycolysis and has been shown in previous studies to have selective killing in animal models of hepatocellular carcinoma (24, 25). We found that the MCF10A cells expressing MYC or MYC plus CSN5 were selectively killed by 10 μmol/L 3-bromopyruvic acid compared with those expressing vector control, CSN5, or E2F3 (Fig. 4B). This selective pharmacologic effect is not simply due to increased proliferation because MCF10A cells expressing CSN5 alone or E2F3 have increased proliferation but are not killed (Fig. 4B). However, there was only a small increase in susceptibility to 3-bromopyruvic acid by MCF10A cells expressing MYC plus CSN5 at the 5 μmol/L dose and all of the MCF10A cells were equally killed at the 20 μmol/L dose (Fig. 4B), showing that the therapeutic margin of 3-bromopyruvic acid in selective killing of breast cells expressing the wound signature is narrow.

Cells with activated wound signature are selectively killed by bortezomib. Based on our discovery of the induction of the proteasome signature in poor-prognosis breast cancers with activated wound signature, we examined whether MCF10A cells with activated wound signature were more susceptible to death by drugs that inhibit the ubiquitin-proteasome pathway. MCF10A cells expressing MYC or MYC plus CSN5 were preferentially killed by the proteasome inhibitor MG132 but not by protease inhibitors of calpain (LLM) or lysosome (E64; Fig. 4C and Supplementary Fig. S1). Based on dose titration, cells engineered to express the wound signature were exquisitely sensitive to proteasome inhibition (KD50 of MG132, 1 μmol/L), whereas cells without the wound signature were not significantly affected at 50-fold the dose (Fig. 4C). Bortezomib, a boron-containing dipeptide derivative, is a potent and specific inhibitor of the proteasome with an inhibitory constant of 0.6 nM/L (26). Bortezomib is approved by the U.S. Food and Drug Administration for the treatment of refractory multiple myeloma and is currently being investigated for solid tumors (26). Cells exhibiting the wound signature were also significantly more sensitive to killing by bortezomib; the KD50 of control cells expressing E2F3 or CSN5 alone was at least higher by 100-fold (Fig. 4C). Nuclear morphology and TUNEL staining confirmed that genotype-specific cell killing occurs through apoptosis (Fig. 4D). These results show that breast
Proteasome gene module in human breast cancers. **A,** gene module map highlights the association of a proteasome module with activated wound signature. Top, wound signature status and identity of gene sets derived from Gene Ontology terms contributing to the proteasome gene module. Four gene sets related to the proteasome were coordinately induced in tumor samples that had an activated wound signature; the average expression of their member genes in each array is displayed. Shown are the subset of breast cancer samples with the strongest coordinate activation or deactivation of the proteasome module after accounting for multiple hypothesis testing (FDR < 0.05, \(P < 0.05\)). Middle, raw expression levels for the 50 genes that compose the proteasome module along with their respective membership in each of the four gene sets (left). Bottom, enrichment of high-grade histology and poor clinical outcomes in tumors with high proteasome module.

**B,** induction of proteasome module in primary breast tumors predicts increased probability of distant metastasis and death. Kaplan-Meier survival curves are shown.

Figure 3. Proteasome gene module in human breast cancers. **A,** gene module map highlights the association of a proteasome module with activated wound signature. Top, wound signature status and identity of gene sets derived from Gene Ontology terms contributing to the proteasome gene module. Four gene sets related to the proteasome were coordinately induced in tumor samples that had an activated wound signature; the average expression of their member genes in each array is displayed. Shown are the subset of breast cancer samples with the strongest coordinate activation or deactivation of the proteasome module after accounting for multiple hypothesis testing (FDR < 0.05, \(P < 0.05\)). Middle, raw expression levels for the 50 genes that compose the proteasome module along with their respective membership in each of the four gene sets (left). Bottom, enrichment of high-grade histology and poor clinical outcomes in tumors with high proteasome module. **B,** induction of proteasome module in primary breast tumors predicts increased probability of distant metastasis and death. Kaplan-Meier survival curves are shown.
Epithelial cells expressing the wound signature are selectively killed through apoptosis by the proteasome inhibitor bortezomib.

Bortezomib at both 1 and 10 µmol/L completely inhibited the ability of all of the transduced MCF10A cells to cleave a luminescent proteasome substrate (data not shown), suggesting that genotype-specific killing is not due to differential degrees of proteasome inhibition but rather due to differential susceptibility to death from proteasome inhibition.

Figure 4. Glycolysis or proteasome inhibition selectively kills breast epithelial cells exhibiting the wound signature. A, mitochondria and proteasome scores of the transduced MCF10A breast epithelial cells. The degree of activation of the wound signature in the MCF10A cells is coded. B, genotype-specific killing of MCF10A breast epithelial cells exhibiting the wound signature by a glycolysis inhibitor, 3-bromopyruvic acid (3BrPA), after 18 h. Columns, mean; bars, SE. C, genotype-specific killing of MCF10A breast epithelial cells exhibiting the wound signature by proteasome inhibitors, MG132 and bortezomib, after 24 and 48 h, respectively. Dose-response curves (mean ± SE) are shown. D, bortezomib-mediated killing induces features of apoptosis. Pyknotic nuclei (Hoechst 334) and DNA fragmentation (TUNEL) are visualized. Percent of cells positive for TUNEL staining (mean ± SE) is reported based on three independent experiments.
Wound signature predicts response to bortezomib. We next examined whether established breast cancer cell lines may also be targeted by bortezomib in a genotype-specific fashion. Breast cancer cell lines are often established from advanced breast carcinomas and have been adapted to in vitro growth. Thus, when we profiled eight breast cancer cell lines, we found that all eight had activated the wound signature, similar to MCF10A breast epithelial cells expressing MYC or MYC plus CSN5. Remarkably, the KD_{50} of bortezomib for the breast cancer lines and the transduced MCF10A cells was highly correlated with their wound signature scores as well as their proteasome signature scores (Fig. 5 and Supplementary Table S4), suggesting that the wound signature as well as the proteasome signature is a strong predictor for response to bortezomib. In addition, comparison of gene expression profiles before and after bortezomib treatment showed that bortezomib substantially decreased wound signature expression in six of eight breast cancer lines before induction of cell death (Supplementary Fig. S2A). The extent of the decrease in the wound signature after bortezomib treatment was modestly correlated with the efficacy of killing by proteasome inhibition ($R = -0.56$, $P = 0.07$; Supplementary Fig. S2B). Proteasome inhibition may be a pharmacologically tractable method to specifically kill breast tumor cells exhibiting the wound signature.

Validation of the association between proteasome module and wound signature in six independent breast cancer data sets. To test the strength of the association between the proteasome gene module and wound signature, we examined the coordinate activities of the proteasome module and wound signature in multiple patient populations representing the full spectrum of breast cancer progression. As shown in Table 1, activation of the proteasome module and the wound signature are strongly and positively correlated in six independent cohorts of human breast cancers ($P < 10^{-7}$ in all cases). These include primary stage I and II breast cancer, locally advanced disease (stage IIIb, IIIa), lymph node–positive disease, and metastatic breast carcinomas (Table 1). Thus, the activity of the proteasome module and the wound signature are intimately linked in breast cancer initiation and progression.

Discussion

The modern pharmacopeia contains a full armamentarium against diverse molecular targets; thus, the challenge in post-genomic medicine is to match the right drug with the right disease. Here, we illustrate a general prospective strategy to identify targeted therapies for molecular subtypes of cancer identified by gene expression signatures. The gene module map method is general and may be applicable to other diseases such as inflammatory or metabolic disorders. We suggest that by providing a holistic view of the cellular network of the patient’s tumors in vivo through gene module map, investigators can identify integral but previously unrecognized consequences of oncogenic transformation. These additional molecular features may be amenable to interference by a drug already available for human use, becoming
is caused by coordinate amplification of MYC in tumors. MYC has previously been shown to induce nuclearly encoded mitochondrial genes and mitochondrial biogenesis (27). We observed that breast epithelial cells that express the wound signature have only a 2-fold difference in susceptibility to 3-bromopyruvic acid. This narrow therapeutic window may be because 3-bromopyruvic acid also has some inhibitory activity on mitochondrial respiration (28), which would render cells with intact mitochondrial metabolism susceptible to this drug. Drugs that more specifically target glycolysis are being developed (29), and our findings suggest that some inhibitory activity on mitochondrial respiration (28), which would render cells with intact mitochondrial metabolism susceptible to this drug. Drugs that more specifically target glycolysis are being developed (29), and our findings suggest that this class of drugs should be examined in the future for their potential therapeutic use in breast cancers with the poor-prognosis wound signature.

The coactivation of a mitochondria gene module and the poor-prognosis wound signature is likely explained as a different facet of MYC activation in these tumors. MYC has previously been shown to induce nuclearly encoded mitochondrial genes and mitochondrial biogenesis (27). We observed that breast epithelial cells that express the wound signature have only a 2-fold difference in susceptibility to 3-bromopyruvic acid. This narrow therapeutic window may be because 3-bromopyruvic acid also has some inhibitory activity on mitochondrial respiration (28), which would render cells with intact mitochondrial metabolism susceptible to this drug. Drugs that more specifically target glycolysis are being developed (29), and our findings suggest that this class of drugs should be examined in the future for their potential therapeutic use in breast cancers with the poor-prognosis wound signature.

The association of a proteasome gene module and the wound signature was particularly intriguing and consonant with additional evidence. From an independent genetic analysis (19), we had discovered that the wound signature in human breast cancer is caused by coordinate amplification of CSN5, which encodes an activator of cullin-based E3 ubiquitin ligases, and MYC, which encodes an oncogenic transcription factor. CSN5 induces the ubiquitination of MYC, recruiting the proteasome to MYC, and increases transcriptional activation of MYC target genes. Thus, we reasoned that when MYC and CSN5 activate a metastatic gene expression program in breast epithelial cells, such cells may become uniquely dependent on abnormal protein turnover mediated by the proteasome for survival. Bortezomib has been shown to be effective in inhibiting the growth of murine mammary tumors (30), but preliminary clinical trials of bortezomib in human breast cancer patients have been disappointing (31). We suggest that a critical determinant of bortezomib response in breast cancer is the wound signature (Fig. 4C), which should be incorporated in future clinical studies of bortezomib. The inhibition of wound signature expression by bortezomib is consistent with the essential roles of the proteasome in transcription factor turnover in gene activation (32) and the recent identification of the ubiquitin ligase activator CSN5 and its substrate MYC as oncogenes for breast cancers exhibiting wound signature (19).

In summary, gene module analysis highlighted the potential role of genes with mitochondrial and proteasome function in a subset of poor prognosis breast cancers, which led to experimentally testable hypotheses for its mechanism and suggested possible therapeutic strategies. Previous gene-level analysis of the seven published breast cancer data sets did not detect the association between mitochondria-related genes or proteasome-related genes and cancer progression. As more genomic, epigenomic, and proteomic data sets are being generated, we believe that integration of these diverse streams of information via module maps will streamline studies of cancer and other diseases to provide robust biological and mechanistic insights. To facilitate such discoveries, we have implemented our method in a software tool called Genomica,9 and made it freely available for academic use, along with detailed tutorials on how to readily reproduce the results presented here and how to generate new module maps from other expression data and gene set collections. Judicious use of the gene module map may facilitate rapid translation of genomic and proteomic knowledge to specific therapies for patients.

**Table 1. Correlation between wound signature and proteasome signature in six independent breast cancer data sets**

<table>
<thead>
<tr>
<th>First author</th>
<th>Samples (n)</th>
<th>Characteristics</th>
<th>R</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Van de Vijver (20)</td>
<td>295</td>
<td>Stage I and II; lymph node negative and positive; untreated and treated adjutantly (chemo and hormonal); ER+/-</td>
<td>0.76</td>
<td>&lt;10⁻⁵⁶</td>
</tr>
<tr>
<td>Wang (33)</td>
<td>286</td>
<td>97% T1,T2,N0; 3% T3,N1; no adjuvant treatment; ER+/-</td>
<td>0.60</td>
<td>&lt;10⁻⁲⁹</td>
</tr>
<tr>
<td>Sorlie (2)</td>
<td>163</td>
<td>Stage I-IV; mostly T1,N0 or greater; treated neoadjuvantly; ER+/-</td>
<td>0.65</td>
<td>&lt;10⁻¹⁰</td>
</tr>
<tr>
<td>Minn (34)</td>
<td>99</td>
<td>T1,N0-T3,N3, no treatment data provided; ER+/-, PR+/-, Her-2/neu+/-</td>
<td>0.76</td>
<td>&lt;10⁻¹⁹</td>
</tr>
<tr>
<td>Ma (35)</td>
<td>60</td>
<td>T1,N0-T2,N2; all but four, ER+, PR+/-, mostly Her-2/neu-; adjuvant tamoxifen; no chemotherapy</td>
<td>0.63</td>
<td>&lt;10⁻¹³</td>
</tr>
<tr>
<td>Weigelt (36)</td>
<td>169</td>
<td>114 patients; 55 samples from various metastatic sites</td>
<td>0.78</td>
<td>&lt;10⁻³⁵</td>
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Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

Achilles’ heels for the cancer cell. Illustrating this concept, our observation herein that mitochondria and proteasome signatures are coactivated with the wound signature led to the discovery that the glycolysis inhibitor 3-bromopyruvic acid and the currently available proteasome inhibitor bortezomib specifically kill breast epithelial cells that exhibit the poor-prognosis wound signature.

The coactivation of a mitochondria gene module and the poor-prognosis wound signature is likely explained as a different facet of MYC activation in these tumors. MYC has previously been shown to induce nuclearly encoded mitochondrial genes and mitochondrial biogenesis (27). We observed that breast epithelial cells that express the wound signature have only a 2-fold difference in susceptibility to 3-bromopyruvic acid. This narrow therapeutic window may be because 3-bromopyruvic acid also has some inhibitory activity on mitochondrial respiration (28), which would render cells with intact mitochondrial metabolism susceptible to this drug. Drugs that more specifically target glycolysis are being developed (29), and our findings suggest that this class of drugs should be examined in the future for their potential therapeutic use in breast cancers with the poor-prognosis wound signature.

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In summary, gene module analysis highlighted the potential role of genes with mitochondrial and proteasome function in a subset of poor prognosis breast cancers, which led to experimentally testable hypotheses for its mechanism and suggested possible therapeutic strategies. Previous gene-level analysis of the seven published breast cancer data sets did not detect the association between mitochondria-related genes or proteasome-related genes and cancer progression. As more genomic, epigenomic, and proteomic data sets are being generated, we believe that integration of these diverse streams of information via module maps will streamline studies of cancer and other diseases to provide robust biological and mechanistic insights. To facilitate such discoveries, we have implemented our method in a software tool called Genomica,9 and made it freely available for academic use, along with detailed tutorials on how to readily reproduce the results presented here and how to generate new module maps from other expression data and gene set collections. Judicious use of the gene module map may facilitate rapid translation of genomic and proteomic knowledge to specific therapies for patients.

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