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Research Paper

Prediction of Anticancer Drug Potency from Expression of Genes Involved in Growth Factor Signaling

Zunyan Dai,[1] Catalin Barbacioru,[2,3] Ying Huang,[1] and Wolfgang Sadée[1,4]

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Purpose. This study develops and evaluates a systematic approach to finding biomarker genes for predicting potency of anticancer drugs against tumor cells, focusing on gene families related to growth factor signaling.
Methods. Cytotoxic potencies of 119 drugs against 60 neoplastic cell lines (NCI-60) were correlated with expression of 343 genes, including 90 growth factors and receptors, 63 metalloproteinases, and 92 ras-like GTPases as downstream signaling factors. Progressively more stringent criteria and predictive models aim at identifying the smallest subset of genes predictive of cytotoxic potency.
Results. Comparing gene expression with drug potency across the NCI-60 yielded genes with negative and positive correlations ($p < 0.001$), indicative of a role in chemoresistance and chemosensitivity, respectively. Of 17 genes with multiple negative correlations, 8 are known chemoresistance factors, validating the approach. Negatively correlated genes clustered into two main groups with distinct expression profiles and drug correlations, represented by *EGFR* and *ERBB2* (*Her-2*/*Neu*). Accordingly, no synergism was observed between EGFR and ERBB2 inhibitors. However, combinations with classical anticacer drugs were not correlated with *EGFR* and *ERBB2* expression in four cell lines tested, suggesting complex interactions in combination treatments. Finally, a subset of only 13 genes was found to be sufficient for near optimal prediction of drug potency against the NCI-60.
Conclusions. Our approach using a small subset of genes reveals known and potential biomarkers in cancer chemotherapy, providing a strategy for genome-wide analysis.

KEY WORDS: chemoresistance and chemosensitivity; correlation of gene expression and drug potency; growth factor signaling; mRNA expression array; NCI-60 cells; predictive biomarkers.

INTRODUCTION

Classification of tumors and prediction of drug response have advanced with the use of mRNA expression profiles (1–4). Pathways underlying drug response include membrane transport, drug metabolism, apoptosis, DNA repair, and cell cycle control (3,5). However, the full potential of transcriptional profiling in understanding of chemoresistance has yet to be achieved (4,6), owing to poor reproducibility between array platforms (1,7), inherent variability of gene expression *in vitro* and *in vivo*, and numerous possible approaches for analyzing vast amounts of high-dimensional data. This has resulted in different sets of candidate genes with little overlap between studies. Holleman *et al.* (8) have identified a set of 124 genes predictive of acute lymphoblastic leukemia response to four anticancer drugs, but only 3 had been previously associated with drug resistance. Because the 20–40 predictive genes for each drug differed entirely between each of the four drugs, the authors concluded that upstream mechanisms specific to each drug determine the response (8). Yet, one would have expected common downstream cell survival pathways to determine chemoresistance against multiple drugs. In this study, we have systematically evaluated a multistep approach for extracting useful information from high-dimensional datasets, with the goal of finding the minimal number of genes that are predictive of drug potency. Rather than using genome-wide data for this method development, we have focused on a limited set of gene families implicated in growth factor signaling, already implicated in chemoresistance. Moreover, our approach is based on the assumption that such genes that are generally involved in cell survival would interact with multiple drugs, regardless of the primary mechanism of action. Therefore, we focus on genes that affect cytotoxic potencies of multiple drugs. Restricting our approach to a limited set of gene families has the added advantage of reducing the number of heuristic models that can predict drug potency, thereby facilitating the search for genes as biomarkers.

[1] Program of Pharmacogenomics, Department of Pharmacology, The Ohio State University, 5078 Graves Hall, 333 West 10th Avenue, Columbus, Ohio 43210, USA.
[2] Department of Biomedical Informatics, College of Medicine and Public Health, The Ohio State University, Columbus, Ohio, USA.
[3] Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, USA.
[4] To whom correspondence should be addressed. (e-mail: wolfgang.sadee@osumc.edu)

Growth factor signaling has been implicated in chemoresistance by regulating cell proliferation, differentiation, and apoptosis (5,9). For this project, we have selected gene families encoding growth factors and their receptors, metalloproteinases, and small GTPases. Growth factors and their receptors are targeted by clinically used drugs; however, only a small subset of the genes in these two families are involved. Matrix- and membrane-associated metalloproteinases are involved in transformation, proliferation and metastasis, and growth factor release, whereas GTPases are downstream integrators of cellular signaling, with neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*) and v-Ha-ras Harvey rat sarcoma viral oncogene homolog (*HRAS*) already implicated in chemoresistance (10,11). Again, only a subset of these genes had been explored as to their role in anticancer drug response. Our study includes all members of these gene families to determine whether we can detect known chemoresistance factors and discover potential new biomarkers.

Main drug targets include the growth factor receptors EGFR (12) and ERBB2 (HER-2) (9,13). Amplification, point mutations, or chromosomal translocation can result in uncontrolled activation of growth factor signaling pathways, as exemplified by ERBB2 in breast cancer (14). Growth factor signaling also conveys chemoresistance against classical anticancer drugs (15), involving suppression of apoptosis (16) and activation of multidrug resistance genes, such as the drug efflux pump MDR1 (17). As a result, combined administration of growth factor inhibitors and conventional cytotoxic drugs can result in synergistic effects (9,18–20). However, this strategy has yielded variable results in clinical trials, possibly because of multiple parallel signaling pathways capable of bypassing the blocked signal (15). For example, signaling via type I insulin-like growth factor receptor (IGF-IR) may render anti-ERBB2 Herceptin therapy ineffective (21). Therefore, a secondary goal of this study was to assess the role of EGFR and ERBB2 in mediating chemoresistance against conventional anticancer drugs and receptor inhibitors, either given alone or in combinations. Although downstream genes, such as PI3K and AKT, play critical roles in ERBB2-mediated chemoresistance (22), we did not include these downstream signaling components in our current study because of redundancy of signaling pathway and the complexity of signaling pathway regulation (23).

To assess the role of genes involved in growth factor signaling, we exploit correlations between gene expression patterns in the NCI-60 cancer cell lines with cytotoxic drug potency. The NCI-60, a set of 60 diverse human cancer cell lines (24), has served in the screening of more than 100,000 candidate drugs. Gene–drug correlations can reveal novel drug targets or mechanisms of chemoresistance (1,25,26), whereas clustering of drug potency against the NCI-60 cells can reveal mechanisms of action (27). Moreover, mRNA expression profiles of subsets of 20–200 genes can serve as predictors of cytotoxic drug potencies against the NCI-60 (8,28). However, the underlying mechanisms remain unclear, possibly because the heuristic nature of the prediction algorithms fails to detect the critical genes. On the other hand, with a more focused approach measuring the expression of genes involved in transmembrane transport in the NCI-60 (29), we have identified, and in several cases validated, numerous new drug–transporter relationships relevant to drug targeting and potency (30) that can be extrapolated to *in vivo* studies because causal interactions are implied.

In this study, we have measured expression of 343 genes related to growth factor signaling, and other signaling pathways for comparison (See Supplementary Table I for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users), in the NCI-60 (29) and have correlated the results with cytotoxic drug potencies. Genes with positive and negative correlations potentially convey chemosensitivity and chemoresistance, respectively. Among genes with negative correlations, the known chemoresistance genes *EGFR* and *ERBB2* displayed sharply distinct drug correlation patterns. We further tested synergism and antagonism between combinations of EGFR and ERBB2 inhibitors and traditional cytotoxic anticancer drugs. Finally, we identified subsets of a minimal number of genes as predictors of cytotoxic potency for multiple drugs and as potential biomarkers and drug targets.

MATERIALS AND METHODS

Oligonucleotide Microarrays. 70-mer oligonucleotide probes (total 343 genes) were designed for 90 growth factors and their receptors (e.g., *EGF*, *IGF*, *FGF*, *PDGF*, *VEGF*, *TGF*, and *TNF* families), 63 metalloproteinases and their inhibitors (20 *MMPs*, 4 *TIMPs*, 20 *ADAMs*, and 19 *ADAMTSs*), and 92 small GTP-binding proteins (GTPases). In addition, 98 probes were targeted to GPCRs, heterotrimeric G proteins, phospholipases, etc. (See Supplementary Table I for comparison). The 70-mer oligonucleotides were designed and synthesized by Operon (Alameda, CA, USA), Qiagen (http://omad.qiagen.com/human2/index.php). Probes for these genes were added to the transporter and channel gene microarray described previously (29,31), with 25 genes overlapping with the current study. Each probe was printed four times on poly-L-lysine glass slides to permit assessment of intra-assay variability of mRNA measurements. The 25 genes analyzed in duplicate served as quality control, in addition to comparison with cDNA arrays with 144 overlapping genes (available form http://www.dtp.nci.nih.gov).

NCI-60 Cancer Cell Lines. Cell lines, purchased from the Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health (http://www.dtp.nci.nih.gov), were cultured in RPMI 1640 medium with L-glutamine, supplemented with 10% fetal bovine serum, 100 U/ml sodium penicillin G, and 100 μg/ml streptomycin. Cells were grown in tissue culture flasks at 37°C in a 5% CO_2 atmosphere.

Chemicals. EGFR inhibitor AG1478 and ERBB2 inhibitor AG825 were purchased from Calbiochem (San Diego, CA, USA). Cisplatin was obtained from Sigma (St. Louis, MO, USA). Paclitaxel and camptothecin 10-OH (CPT, 10-OH) were from the Developmental Therapeutics Program at NCI (Bethesda, MD, USA).

Microarray Hybridization. The hybridization was performed following published procedures (29,31). Total RNA was extracted from cell cultures using TriZol (Invitrogen, Carlsbad, CA, USA) and was purified by RNeasy® mini kit

(Qiagen, Valencia, CA, USA). Expression of each gene was assessed by the ratio of expression level in the sample against a pooled control sample from 12 diverse cell lines of the NCI-60 (25). Total RNA (12.5 μg) was used for cDNA synthesis and was then labeled with Cy5 or Cy3. The samples were then mixed and hybridized to the slides and were analyzed with an Affymetrix 428 scanner.

Data Analysis. Microarray data analysis was performed as previously described (29). Background subtraction and calculation of medians of pixel measurements/spot was carried out using GenePix Software 3.0 (Foster City, CA, USA). Spots were filtered out if they had both red and green intensity <500 units after subtraction of the background or if they were flagged for any visual reason (odd shapes, background noise). Data normalization was carried out using the statistical software package R (http://www.r-project.org). To correct for intensity and dye bias, we used location and scale normalization methods, which are based on robust, locally linear fits, implemented in the *SMA* R package. This method is based on transformations $R/G \rightarrow \log_2 R/G - c_j(A) = \log_2 R/k_j(A) * G \rightarrow (1/a_j) * \log_2 R/k_j(A) * G$, where $c_j(A)$ is the Lowess fit of the *M vs. A* plot for spots on the *j*th grid of each slide and a_j is the scale factor for the *j*th grid (to obtain equal variances along individual slides). After performing these transformations, the gene expression level of each probe was set to be the median of the four copies of that probe.

Correlation Analysis between Gene Expression and Drug Activity. Growth inhibition data for 119 standard anticancer drugs (25) (GI_{50} values for the 60 human tumor cell lines) were obtained from the Developmental Therapeutics Program (http://www.dtp.nci.nih.gov), expressed as the negative log of the molar concentration calculated in the NCI screen (32). Pearson's correlation coefficients were calculated for each gene–drug pair (119 × 343 pairs). Confidence intervals and unadjusted *p* values were obtained using Efron's bootstrap resampling method (33), with 10,000 bootstrap samples for each gene–drug comparison. Because controlling false discovery rate by the method of Benjamini and Hochberg (34) proved too stringent, an arbitrary cutoff of $p = 0.001$ was used for the unadjusted bootstrap *p* value. This is expected to detect more "true" gene–drug associations, at the expense of increasing the number of false positive ones, to be validated by other means.

Clustering of Cell Lines, Genes, and Drugs on the Basis of Gene Expression and Drug Potency Profile. Hierarchical clustering can be used to group cell lines and genes in terms of their patterns of gene expression (25,35). To obtain cell–cell cluster trees for 107 genes that showed distinct expression patterns across the 60 cell lines (i.e., genes that passed the filter SD ≥ 0.35), we used the programs "Cluster" and "TreeView" (36) with average linkage clustering and a correlation metric. Cells and drugs were also clustered by drug potency profiles (25), and moreover, genes and drugs were classified using correlations between each gene (expression across the NCI-60) and each drug (potency across the NCI-60) as distance measure. To reduce noise, we use stringent filters for the selection of genes included in this analysis, showing a correlation with at least one drug at $p < 0.001$.

Cytotoxicity Assay. Drug potency was tested using a proliferation assay with sulforhodamine B (SRB) (37). In each well, 3000–5000 cells were seeded in 96-well plates and incubated for 24 h. Drugs were added in a dilution series in three replicate wells. After 3 days, incubation was terminated by replacing the medium with 100 μl 10% trichloroacetic acid (Sigma), followed by incubation at 4°C for 1 h. Plates were washed with water, air-dried, and stained with 100 μl 0.4% SRB (Sigma) in 1% acetic acid for 30 min at room temperature. Unbound dye was washed off with 1% acetic acid. After air-drying and resolubilization of the protein-bound dye in 10 mM Tris–HCl (pH 8.0), absorbance was read in a microplate reader at 570 nm.

Determination of Combination Index, A Measure of Synergism or Antagonism Between Two Coadministered Agents. The combination index (CI) was calculated according to the equation $CI = d_1/D_1 + d_2/D_2$ (38,39). D_1 and D_2 represent the doses of drug 1 and drug 2 alone, required to produce *x*% effect, and d_1 and d_2 are the doses of drugs 1 and 2 in combination required to produce the same effect. The combined effect of the two drugs could be synergistic (CI < 1), additive (CI = 1), or antagonistic (CI > 1). Because the CI could differ at different levels of growth inhibition, CIs were obtained at different levels of growth inhibition, using increasing concentrations at a fixed ratio between the two drugs. The CI was plotted against the fraction affected (Fa of 0.25 would equal 75% viable cells).

Using Gene Expression Data as Predictors of Cytotoxic Potency. We developed predictive models for drug response against the 60 cancer cell lines based on gene expression profiles. Three groups of genes were used as candidate predictors: all 343 genes, genes showing strong negative correlations, and genes with strong positive correlations (each gene correlated with at least one drug at $p < 0.001$). For each compound, we separated cell lines into training and test samples. To assess the accuracy of this method, we used a leave-one-out cross-validation procedure. We first estimated the probability of the training cell lines to be resistant to a given compound by modeling drug response levels $[-\log_{10}(GI_{50})]$ as a mixture of normal distributions (Supplementary Fig. S1 is available for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users). Class assignment for a test sample is based on the predictive probabilities of class membership. This step differs from the one used in (28) by using information from all cell lines for prediction purposes and by incorporating into the analysis the bimodal behavior of growth inhibition distributions (see Supplementary Material available online for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users). Next, to select predictive genes, we sort genes by their ability to discriminate between the two classes (resistant and sensitive), using the BW measure [see Supplementary function (1) available online for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users]. In the final step, we use quadratic discriminant rule to predict resistance of the test cell line based on top-scoring genes according to the previous sorting criteria and identified the set of predictor genes capable of producing maximal percent of correct classification of the 60 cell lines for each drug. We search, stepwise, for a subset of genes that improves the percentage of correct classifications using quadratic discriminant rule by adding one new gene at each step. Once the model cannot be

improved, the final predictor genes are then selected as the smallest set of genes producing a model within 5% from the highest percentage of correct classifications obtained in the stepwise search described above. This reduces the number of predictor genes, by accepting predictor sets within 5% of the optimally observed values, which may lead to more robust predictor sets. This led to a list of genes ranked by frequency of presence in the predictor sets for a subgroup of 68 drugs (pruned from 119 to avoid redundancy between similar drugs).

In a further iteration, we compared the observed frequencies of presence in the predictor sets (see Supplementary Table VI available online for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users) with the ones one would expect if these models would be random. Only the top 9 positively and the top 12 negatively correlated genes and the top 13 genes among all 343 genes show significantly higher frequencies as predictors than expected from chance. Finally, we used only these top-scoring genes (affecting multiple drugs each) to scan all possible combinations for predicting drug response, resulting in a refined model with the least number of highly predictive genes for each individual drug (see Supplementary Table VII available online for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users).

RESULTS

Expression of Genes Involved in Growth Factor Signaling and Other Signaling Pathways

Basal mRNA expression of 343 genes was measured in the NCI-60 panel. In a previous independent experiment (29), we had measured 25 of these 343 genes with the same array method using mRNA extracts from cells grown at different times and locations. Among the 12 genes with sufficiently robust expression to permit analytical analysis, gene–gene Pearson's correlation coefficient ranged from 0.3 to 0.78. Moreover, expression data were compared with previous results obtained with a cDNA array platform (25). Based on 144 probes for genes common between the two arrays, the average Pearson's correlation coefficient between the 70-mer oligo and the cDNA array is 0.42, with 90 out of 144 probes with $r > 0.30$ ($p < 0.05$)—similar to a previous study comparing cDNA arrays (25) and Affymetrix oligonucleotide HU6800 array (7,26). These results indicate that the array results are robust, while requiring experimental validation as needed. Our array results are particularly robust for highly expressed genes, as discussed previously (40), and further analyzed in Supplementary Material. These genes are also more likely to yield significant correlation coefficients, particularly against multiple drugs, because random noise increases with less well-expressed genes.

The expression of 107 genes with differential expression across the NCI-60 (SD > 0.35) served to cluster the 60 cell lines (see Supplementary Fig. S2 available online for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users). Further validating the array results, leukemia, colon cancer, melanoma, and renal cell carcinoma clustered into groups, except for breast, ovarian, and lung cancers (25, 41).

Pearson's Correlation Coefficients of NCI-60 Gene Expression and Drug Potency

To assess the relationship between gene expression and cytotoxic potency of 119 chemotherapeutic drugs, Pearson's correlation coefficients were calculated, together with statistical significance (bootstrap p value). On the basis of previous results (29), we used $p = 0.001$ as a stringent cutoff, which yielded similar results compared to an absolute correlation coefficient value of >0.4 (Supplementary Table II is available for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users). This resulted in 49 positively and 69 negatively correlated genes, many showing correlations with multiple drugs. Among the 245 genes in the three main families studied in this report, 53 had significant correlations with at least one drug, whereas in the comparator group of 98 genes, 16 qualified, most of which with fewer drug correlations.

We first focused on chemoresistance genes with significant negative correlations (Table I; see Supplementary Table III available online for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users) because several genes have been previously implicated in chemoresistance, whereas little is known about sensitizing genes. Among the 69 genes encoding growth factors and their receptors, many showed significant negative correlations with multiple drugs—an important criterion in our analysis—while entirely lacking positive drug correlations; therefore, they are candidates as broad chemoresistance factors. Among the implicated genes, several were already known to induce chemoresistance, being involved in EGFR, ERBB2, VEGF, IGF, and PDGF signaling pathways (15). The number of significant negative drug correlations provides a crude measure of the relevance of each gene in mediating multidrug resistance, yielding a rank order of *CYR61*, *EGFR*, *IGFBP7*, *PDGFC*, and *ERBB2* (Table I). Both EGFR and ERBB2 are targets of current anticancer therapy, indicating that our approach identified known drug targets. However, other growth factors and receptors scored equally well and therefore represent interesting candidates for further study.

Among the metalloproteinases, *MMP24*, *ADAM9*, and the inhibitor *TIMP2* ranked highly, with multiple negative correlations. Furthermore, small GTPases scored strongly, with seven genes showing negative correlations with ten drugs or more, including *ARHC*, *RRAS2*, *RAB5B*, and *RALB*, consistent with their pervasive role in cellular signaling. Among the other signaling factors (98 genes involved in various signaling pathways, such as GPCRs and G protein subunits), considerably fewer genes produced multiple negative correlations (see Supplementary Table III), such as GNG10 and GNG11 with 15 and 7 negatively correlated genes, respectively. Of 29 G-protein-coupled receptors, only 4 showed strong negative correlation ($p < 0.001$) with 1 or 2 out of 119 drugs. This result suggests that these pathways are less germane to chemoresistance.

Genes with positive correlations, indicative of a possible role in chemosensitivity, are listed in Table I (and See Supplementary Table IV for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users). Representation of growth factors and associated signaling proteins in this group is consistent with the dual

Table I. Genes Negatively and Positively Correlated with Drug Response

Gene	$p < 0.001$		Representative drugs
	$r > 0$	$r < 0$	
Growth factors			
Negative			
CYR61	0	17	Tetraplatin, paclitaxel, geldanamycin
EGFR_1	0	12	Dolastatin-10, halichondrin B, paclitaxel, geldanamycin
IGFBP7	0	9	Tetraplatin, methotrexate, fluorouracil (5FU), acivicin
PDGFC	0	7	Diaminocyclohexyl-Pt-II, acivicin, taxol analogs
ERBB2	0	5	Carmustine (BCNU), cisplatin, zorubicin
Positive			
KDR	6	0	Paclitaxel, geldanamycin
Metalloproteinases			
Negative			
TIMP2	0	18	Fluorodopan, doxorubicin, fluorouracil (5FU), dichloroallyl-lawsone
MMP24	0	12	Dolastatin-10, trityl-cysteine, paclitaxel
ADAM9	0	7	Tetraplatin, taxol analogs, inosine-glycodialdehyde
Positive			
ADAMTS8	5	0	Thioguanine, thiopurine (6MP)
Small GTPases			
Negative			
ARHC	0	28	Tetraplatin, daunorubicin, Baker's soluble antifolate, geldanamycin
RAB5B	0	18	Cyclodisone, paclitaxel, vinblastine sulfate, bisantrene
ARF4	0	17	Iproplatin, doxorubicin, dichloroallyl-lawsone, taxol analogs
RRAS2	0	16	Paclitaxel, bisantrene, halichondrin B, geldanamycin, daunorubicin
RAB6A	0	15	Cisplatin, cyclodisone, hepsulfam, camptothecin, 9-MeO
RALB	0	12	Carboplatin, fluorodopan, bisantrene, deoxydoxorubicin
RAB18	0	10	Carmustine (BCNU), Baker's soluble antifolate, halichondrin B
RAB2	0	7	Daunorubicin, dichloroallyl-lawsone, paclitaxel
RHEB2	0	5	Ftorafur, taxol analogs, inosine-glycodialdehyde
Positive			
RAB37	10	0	Tetraplatin, aminopterin, hydroxyurea
RAN	9	0	Amonafide, aminopterin derivative, methotrexate, acivicin
RAC2	5	0	Chlorambucil, L-asparaginase, hydroxyurea

Only genes correlated with at least five drugs with $p < 0.001$ are included. For all genes with at least one drug at $p < 0.001$, see Supplementary Tables III and IV.

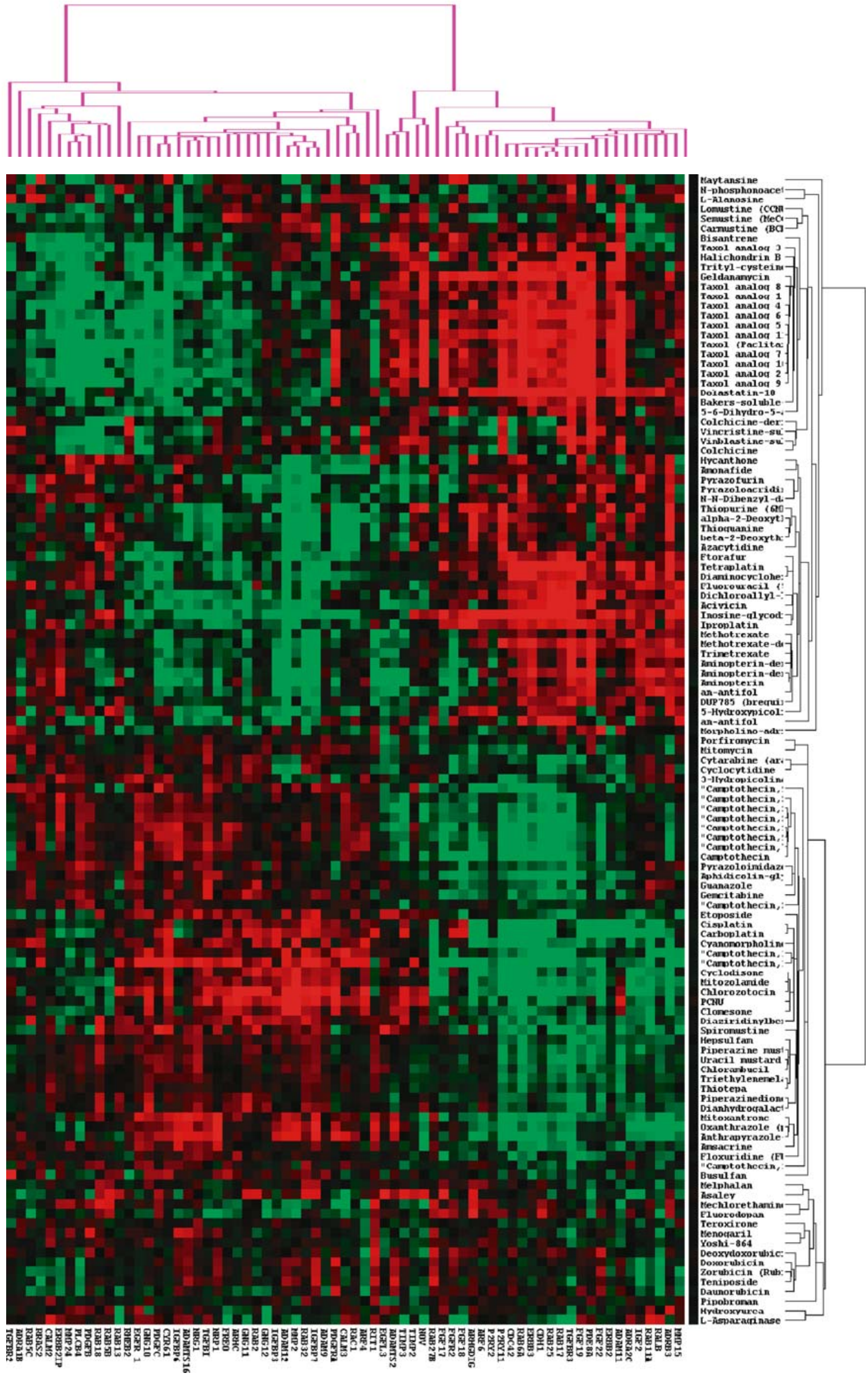
nature of growth factor signaling, supporting either cell survival or apoptosis. Positively correlated genes are less prominent, with only *RAB37* and *RAN* scoring against 9–10 drugs at $p < 0.001$. Among the other signaling pathways, phospholipases, G-protein γ subunits (GNGs), and a phosphodiesterase scored highly (See Supplementary Table IV). The mechanisms of a potential role in chemosensitivity remain uncertain, each candidate gene requiring experimental validation. Therefore, we focus mainly on chemoresistance genes in this report.

Hierarchical Cluster Analysis of Genes Negatively Correlated with at Least One Drug

Clustering the NCI-60 cells against expression of the 69 negatively correlated genes (See Supplementary Fig. S3 for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users) still resulted in the expected cell line clusters obtained from all genes with differential expression across cell lines (See Supplementary Fig. S2). Moreover, the 69 genes clustered into two main groups, suggesting some level of coexpression. To explore this further, genes were compared pairwise to each other with respect to their expression across the NCI-60. This process again resulted in the same two main groups of genes (See Supplementary Fig. S4 for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users). It remains to be determined whether the clustering of the negatively correlated genes into two main groups is biologically significant.

To determine whether the grouping of the negatively correlated genes is relevant to chemoresistance, we performed hierarchical cluster analysis of genes *vs.* drugs using gene–drug correlations as the distance measure. In this fashion, genes that affect the same set of drugs cluster together. This resulted again in the same two major groups of genes and two large clusters of drugs (Fig. 1). Moreover, drugs with similar functions tend to cluster together into

Fig. 1. Hierarchical cluster analysis of 69 negatively correlated genes against the 119 anticancer drugs using gene–drug Pearson's correlation coefficients. Genes and drugs cluster into two main groups, whereas drugs further cluster into subgroups approximately according to their mechanisms of action.


Maytansine
N-phosphonoacet
L-Alanosine
Lomustine (CCN
Semustine (MeC
Carmustine (BC
Bisantrene
Taxol analog 3
Halichondrin B
Trityl-cystein
Geldanamycin
Taxol analog 8
Taxol analog 1
Taxol analog 4
Taxol analog 6
Taxol analog 5
Taxol analog 1
Taxol (Paclita
Taxol analog 7
Taxol analog 1
Taxol analog 2
Taxol analog 9
Dolastatin-10
Bakers-soluble
5-6-Dihydro-5-
Colchicine-der
Vincristine-su
Vinblastine-su
Colchicine
Hycanthone
Amonafide
Pyrazofurin
Pyrazoloacridi
N-N-Dibenzyl-d
Thiopurine (6M
alpha-2-Deoxyt
Thioguanine
beta-2-Deoxyth
Azacytidine
Ftorafur
Tetraplatin
Diaminocyclohe
Fluorouracil (
Dichloroallyl-
Acivicin
Inosine-glycod
Iproplatin
Methotrexate
Methotrexate-d
Trimetrexate
Aminopterin-de
Aminopterin-de
Aminopterin
an-antifol
DUP785 (brequi
5-Hydroxypicol
an-antifol
Morpholino-adr
Porfiromycin
Mitomycin
Cytarabine (ar
Cyclocytidine
3-Hydropicolin
"Camptothecin,
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Camptothecin
Pyrazoloimidaz
Aphidicolin-gl
Guanazole
Gemcitabine
"Camptothecin,
Etoposide
Cisplatin
Carboplatin
Cyanomorpholin
"Camptothecin,
"Camptothecin,
Cyclodisone
Mitozolamide
Chlorozotocin
PCNU
Clomesone
Diaziridinylbe
Spiromustine
Hepsulfan
Piperazine mus
Uracil mustard
Chlorambucil
Triethylenemel
Thiotepa
Piperazinedion
Dianhydrogalac
Mitoxantrone
Oxanthrazole (
Anthrapyrazole
Amsacrine
Floxuridine (F
"Camptothecin,
Busulfan
Melphalan
Asaley
Mechlorethamin
Fluorodopan
Teroxirone
Menogaril
Yoshi-864
Deoxydoxorubic
Doxorubicin
Zorubicin (Rub
Teniposide
Daunorubicin
Pipobroman
Hydroxyurea
L-Asparaginase

smaller subgroups, such as tubulin-active antimitotic agents (taxol analogs, colchicine, vincristine sulfate, and vinblastine sulfate) and topoisomerase I inhibitors (e.g., camptothecin and its derivatives). We next grouped the genes further by performing a gene–gene cluster analysis, on the basis of gene–drug correlations. In this analysis, each gene is compared to all others, using the correlation coefficients against the 119-drug panel. This analysis revealed a sharp contrast between the two main groups of genes observed in the preceding cluster experiments (see Supplementary Fig. S5 for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users), showing that the two gene clusters are potentially related to chemoresistance.

Genes showing similar NCI-60 expression and drug potency profiles are likely to be part of the same signaling pathway. For example, *ADAM9* (a metalloproteinase mediating release of membrane-tethered growth factors such as HB-EGF), *EGFR*, and the GTPase *ARHC* (*RhoC*) clustered within the same group in all analyses and hence may represent members of a signaling pathway relevant to chemoresistance for a portion of the drug panel. Network analysis indicates that *EGFR* and *ADAM9* are close neighbors (to be published). Similarly, *ERBB2* and *RALB* clustered together implying a functional relationship. On the other hand, the close receptor homologues, *EGFR* and *ERBB2*, presumed to have similar signaling pathways, clustered at some distance in either of the two main groups, suggesting that they serve independent functions across the NCI-60 panel.

EGFR and *ERBB2*: Two Genes with Distinct Drug Correlations

The expression results of *EGFR* and *ERBB2* were reproducible between 70-mer oligonucleotide and cDNA arrays (r = 0.80 and 0.60, respectively). Each receptor correlated negatively with a number of drugs, but unsupervised cluster analysis (Fig. 1) suggested that the correlated drug sets are distinct. Figure 2 (upper panel) displays the sorted Pearson's correlation coefficients for *EGFR vs.* the 119 anticancer drugs. Keeping the same order of the 119 drugs, a clearly distinct and even opposite profile is observed for *ERBB2* correlation coefficients (Fig. 2, lower panel, correlation between *EGFR* and *ERBB2*, $r = -0.25$, $p < 0.002$, t test). This illustrates profound and unexpected differences in the interactions between *EGFR* and *ERBB2* expression and cytotoxic drugs.

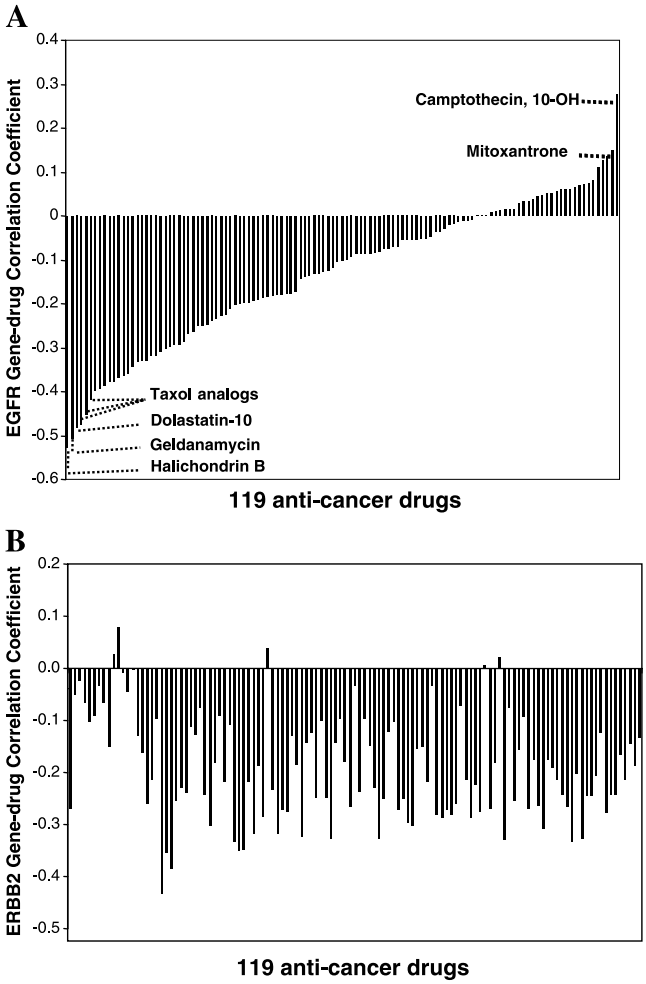

Fig. 2. Gene–drug correlation profiles for *EGFR* (A) and *ERBB2* (B) with 119 anticancer drugs. Pearson's correlation coefficients against 119 drugs are sorted for *EGFR*, whereas the order of drugs is maintained for *ERBB*.

Synergism and Antagonism Between EGFR- and ERBB2-Selective Inhibitors and Classical Cytotoxic Drugs

We tested the potency of, and interactions between, EGFR inhibitor AG1478 and ERBB2 inhibitor AG825, in cell lines with different levels of *EGFR* and *ERBB2* expression. Figure 3A shows the mRNA levels of *EGFR* and *ERBB2* in four cancer cells based on our array data, which is consistent with reported protein levels (http://dtp.nci.nih.gov/mtweb/targetinfo?moltid=MT1173&moltnbr=813) using Western hybridization (Fig. 3B). Genotyping of 37 cell lines of the NCI-60 had revealed a lack of activating mutations for *EGFR* in exons 19 and 21 (42), whereas mutational status remains unknown for *ERBB2*. In addition to AG1478 and AG825, we included the conventional anticancer drugs paclitaxel, cisplatin, and CPT, 10-OH in drug–drug interaction studies. The Pearson's correlation coefficients between *EGFR* and paclitaxel, cisplatin, and CPT, 10-OH were −0.43, −0.14, and −0.08, respectively. *ERBB2* correlated with the three drugs at −0.03, −0.33, and −0.13, respectively. Synergism or antagonism was determined by calculation of the CI *vs.* fraction affected (38). For example, AG1478 and CPT, 10-OH were synergistic in SK-MEL-2 cells (CI < 1, Fig. 4A). In contrast, antagonism was observed (CI > 1, Fig. 4B) between AG1478 and paclitaxel in SK-MEL-2 cells. AG1478 and AG825 did not act synergistically in the four cell lines tested.

Cytotoxic effects of all dual drug combinations in four cell lines are listed in Table II using the CI. For some fixed-ratio drug combinations, the CI value varied over different levels of growth inhibition (Fa). Synergistic effects were observed between AG1478 or AG825 and paclitaxel, cisplatin, or CPT, 10-OH in some cells, whereas antagonism occurred in others, with no clear relationship to *EGFR* or *ERBB2* expression. Furthermore, the gene–drug correlations for *EGFR* and *ERBB2* did not predict synergism or anta-

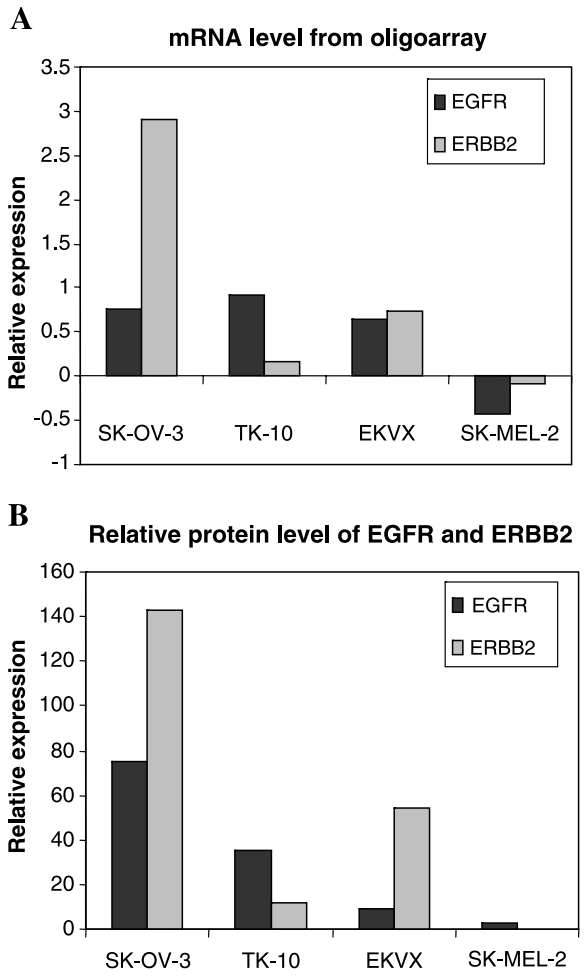

Fig. 3. Relative mRNA (A, $\log_2$ transformed from the 70-mer microarray hybridization) and protein level (B) of *EGFR* and *ERBB2* in cell lines SK-OV-3, TK-10, EKVX, and SK-MEL-2. EGFR and ERBB2 protein levels (Western blots, relative expression level compared to A431 cells) are taken from http://dtp.nci.nih.gov/mtweb/targetinfo?moltid=MT1173&moltnbr=813.

gonism when the inhibitor of EGFR (AG1478) or ERBB2 (AG825) was combined with cytotoxic anticancer drugs in the four cell lines tested. Whereas the EGFR inhibitor AG1478 potentiated CPT, 10-OH, no significant correlation was observed between *EGFR* and CPT, 10-OH ($r = -0.08$).

Prediction of Chemosensitivity Using Gene Expression Profiles and a Learning Algorithm

For this analysis, 68 drugs out of the 119-drug panel were selected to avoid redundancy and bias stemming from compounds with similar chemical structure, mechanism of action, and potency. Different predictive strategies were performed using all 343 genes, or only the 69 negatively or 49 positively correlated genes with $p < 0.001$ against at least one drug (see Supplementary Table V for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users). Drug overlap between the positively and negatively correlated genes is minimal. Using a heuristic leave-one-out strategy (see Supplementary Material and Methods for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users), we identified predictive sets of genes. For each set of predictive genes, the minimum set of genes was determined within 5% of the optimal prediction of the entire set.

Prediction accuracy ranged mainly from 0.6 to 0.9, usually with five to ten genes selected as predictors. Using all 343 genes or only the 69 negatively correlated genes yielded similar results in most cases, whereas only the 49 positively correlated genes tended to score somewhat lower. As not all possible combinations of genes can be tested in our heuristic algorithm with a large number of genes, better scoring gene sets may well exist.

Several genes recur more frequently as predictors of drug potency (see Supplementary Table VI). The top genes with negative correlations include *TGFBR3*, *RAB6A*, *RALB*, *TIMP2*, *ARHC*, *RAB17*, and *ARF4*, whereas the top positive genes are *RAB37*, *RAC2*, *PLCL2*, *PDE1B*, *PLCD4*, and *ADAM12*. It is noted that these genes are not always the highest-ranking genes sorted by number of highly correlated drugs (Table I and see Supplementary Tables III and IV).

To limit the effect of the heuristic approach on the prediction models, we reduced the number of candidate predictor genes to those appearing most frequently in predictive sets of drug potency. Only the top-scoring 9 positively and 12 negatively correlated genes and 13 genes among all 343 genes showed significantly higher frequencies as predictors than expected from random occurrence (Table IIIA). The 13 genes with both positive or negative correlations overlapped partially with those obtained from predictions using only negatively or positively correlated genes, suggesting that sampling variability is kept at a reasonable level. A small

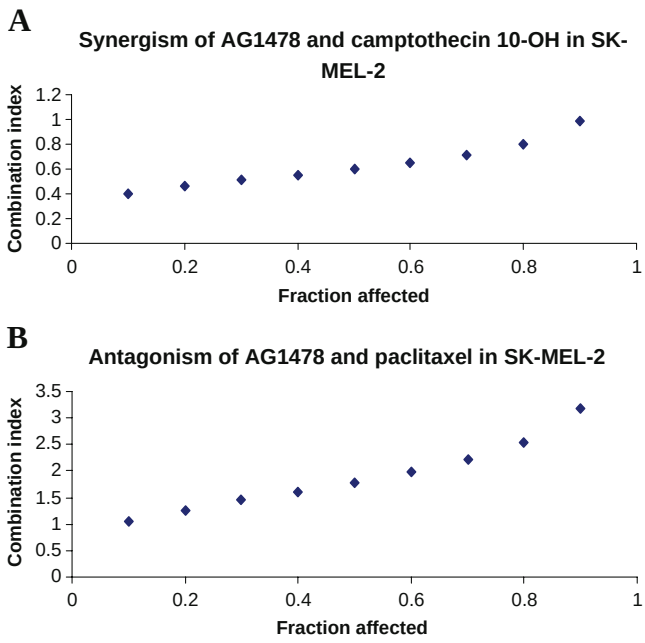

Fig. 4. The drug combination indices with respect to fraction affected (Fa) for the combination of EGFR inhibitor AG1478 with camptothecin 10-OH (A), and AG1478 with paclitaxel (B). The combination index of AG1478/camptothecin 10-OH is <1, an indication of synergism, whereas that of AG1478/paclitaxel is >1, indicative of antagonism.

Table II. Drug Combination Effects between AG1478 or AG825 and Paclitaxel, Cisplatin, or CPT, 10-OH Using the Combination Index (see Fig. 4)

	AG1478			AG825			
	Paclitaxel	Cisplatin	CPT, 10-OH	Paclitaxel	Cisplatin	CPT, 10-OH	AG1478
SK-OV-3	Additive	Additive to antagonistic	Additive to synergistic	Antagonistic	Antagonistic	Additive to antagonistic	Antagonistic
TK-10	Synergistic	Synergistic	Synergistic	Synergistic	Synergistic	Additive	Additive to antagonistic
EKVX	Synergistic	Antagonistic	Synergistic	Synergistic	Antagonistic	Additive	Additive
SK-MEL-2	Antagonistic	Antagonistic	Synergistic	Antagonistic	Antagonistic	Additive	Antagonistic

subset of 13 genes, *TGFBR3*, *FGF19*, *FGFR2*, *TIMP2*, *RAB6A*, and *ARHC* (negative correlations) and *PLCL2*, *ADAM12*, *MMPL1*, *RAB37*, *RAC2*, and *RAB39B* (positive correlations), was found to be highly predictive for many anticancer drugs (Table IIIA). *RAB39B* appeared in many predictive models because of multiple positive correlations (maximally $r = 0.47$), although the lowest p value was only 0.002. Predictive accuracy for only these highest scoring genes is listed in Table IIIB (and Supplementary Table VII), with optimal predictions derived from all possible gene combinations for each drug. This improved the prediction accuracy, especially for those drugs with previously low prediction values, which probably had resulted from the heuristic approach used to detect predictor genes. Supplementary Table VIII (available for this article at http://dx.doi.org/10.1007/s11095-005-9260-y and is accessible for authorized users) shows examples of predictive gene sets for individual compounds. Hence, starting from 343 genes in this study, we have identified a small subset of genes yielding good predictions for a majority of drugs.

Literature Validation of Chemoresistance Genes Implicated by Gene–Drug Correlations

Table I lists 17 genes showing significant negative correlations with at least five drugs. A literature survey revealed that eight of these genes are known to be involved in chemoresistance or tumor progression (Table IV). Moreover, these eight genes each have predictive power for the potency of many anticancer drugs against the NCI-60 (Table IV and Supplementary Table VI). This literature analysis supports the notion that our approach is capable of identifying known chemoresistance genes, whereas the newly suggested genes require further experimental validation. Much less is known about genes enhancing chemosensitivity so that a literature evaluation is not feasible.

DISCUSSION

We have developed an approach for evaluating the role of gene families related to growth factor signaling in chemoresistance and chemosensitivity. Comparing basal gene expression patterns with potency of 119 standard anticancer drugs in the NCI-60 panel revealed numerous significant gene–drug correlations ($p < 0.001$; $r > 0.4$). Among the 17 genes scoring strongly against multiple drugs, 8 genes were already known to be involved in chemoresistance, including growth factors and receptors, metalloproteinases, and GTPases. Members of these gene families scored more frequently and higher than genes encoding other signaling factors tested for comparison (e.g., GPCRs). In addition, novel candidate genes were revealed that scored at least as strongly as the known chemoresistance factors. It is implicitly acknowledged that basal mRNA expression profiles yield only a partial window onto all factors that determine drug responses. Moreover, gene expression profiles relevant to growth factor signaling could have been associated with tumor cell lines inherently resistant or sensitive to drugs because of other factors. Nevertheless, where significant

Table IIIA. Genes Occurring in Predictive Models More Often than Expected by Chance, Sorted by Number of Drugs

Negatively correlated genes	Number of drugs	Positively correlated genes	Number of drugs	All 343 genes	Number of drugs
TGFBR3	38	*PLCL2*	33	*RAB5B*	33
FGF19	34	*ADAM12*	33	*TGFBR3*	32
FGFR2	32	*MMPL1*	20	*PLCL2*	32
TIMP2	32	*RAB37*	18	*RAN*	24
RAB6A	28	*RAC2*	18	*RAB6A*	21
RAB17	26	*PDE1B*	18	*ARF4*	21
ARHC	23	*IGFALS*	18	*PLCD4*	21
ADRB3	21	*PLCD4*	16	*RAB37*	20
RALB	20	*PLCB2*	12	*ARHC*	18
GNG12	20			*RAHH*	17
ARF4	20			*RAC2*	15
RAB25	16			*ERBB3*	13
				RAB39B	11

Table IIIB. Comparison of Prediction Accuracy for Select Drugs, between a Heuristic Approach with All Relevant Genes (69, 49, and 343), and Using Only the Top-Scoring Genes (12, 9, and 13) Showing High Frequencies as Predictors (Table IIIA)

Drugs	Accuracy (%), negatively correlated genes		Accuracy (%), positively correlated genes		Accuracy (%), all 343 genes	
	69 genes	Top 12	49 genes	Top 9	343 genes	Top 13
Mitomycin	70	77	68	70	75	77
Lomustine (CCNU)	82	80	67	75	82	75
Cisplatin	72	78	73	73	82	75
Bisantrene	93	93	90	83	87	95
Doxorubicin	57	83	52	72	30	93
Teniposide	53	82	55	70	45	78
L-Asparaginase	62	85	42	90	32	90
Hydroxyurea	62	93	58	87	52	95
Fluorouracil (5FU)	78	82	82	83	88	90
Vinblastine sulfate	85	87	83	80	85	83
Paclitaxel	87	88	88	83	90	90

In the latter case, all possible combinations were tested, and the highest scoring set was selected. A complete list of the 68 drugs tested is available in Supplemental Table VII.

correlations are found, these can then be further validated by other means to determine whether the expression of these genes can serve as biomarker or represent a causative factor in chemoresistance or chemosensitivity. The identification of known drug resistance factors in the analyzed gene families validates our approach (Table IV).

Growth Factors and Chemoresistance

EGFR and *ERBB2* are chemoresistance factors (43–45) showing negative correlation with multiple drugs in this study. Consistent with its negative correlation with *EGFR* ($r = -0.4$), paclitaxel was shown to act synergistically with the EGFR inhibitor ZD1839 *in vitro* and against xenografts of human renal cancer SKRC-49 (46). Similarly, ERBB2 displays multiple negative correlations, with ERBB2/PI-3K/Akt signaling conveying multidrug resistance (22). Moreover, Herceptin® (ERBB2/HER2 antibody) causes chemosensitivity in animal models and clinical studies (44,45,47). Showing significant negative correlations to multiple drugs, *CYR61* is an $\alpha_v\beta_3$ integrin receptor ligand converging downstream on heregulin-ERBB2/3/4 receptor-mediated signaling (48). *CYR61* was included in the present study because of its association with breast cancer chemoresistance (49), converging on growth factor signaling through the NF-kappaB/XIAP pathway (50). Furthermore, vascular endothelial growth factor-165 receptor (*VEGF165R*), showing strong negative drug correlations, had been shown to be involved in tumor angiogenesis, progression, chemoresistance, and poor prognosis (51,52).

Our results also point to potentially novel growth factors and receptors involved in chemoresistance. *TGFBR3* scored

Table IV. Genes Previously Implicated as Chemoresistance or Tumor Progression Factors

Genes	Involvement in chemoresistance or tumor progression	Negatively correlated drugs out of 119[a] ($p < 0.001$)	Number of drugs as predictive factor (out of 68 drugs)[b]	References
Growth factor related				
EGFR	Chemoresistance and tumor progression	12	7	46, 76
ERBB2	Chemoresistance and tumor progression	5	7	22, 45
CYR61	Chemoresistance and angiogenesis	17	7	49, 50
Metalloproteinases				
ADAM9	Chemoresistance and poor prognosis	7	9	55–57
Small GTPases				
ARHC	Metastasis	28	16	66, 67
RRAS2	Transformation and chemoresistance	16	9	61–64
ARF4	EGFR signaling	17	14	77
RALB	Cancer cell survival	12	20	65

The table lists the number of negatively correlated drugs and the number of times the gene appears in a predictive subset of genes for a test set of 68 drugs.
[a] Information from Table I.
[b] Information from Supplemental Table VI.

highest as a predictor for chemoresistance. While devoid of serine/threonine kinase activity (53), *TGFBR3* appears to be a necessary component of the TGFβ receptor signaling complex (54) and therefore may represent an interesting target for cancer treatment or a predictor of treatment response. Additional growth factors implicated by negative correlations include *FGF17*, *FGF18*, *FGF19*, *IGF2*, and *NRG1*. Their relevance to chemoresistance needs to be validated in each case.

Metalloproteases and Chemoresistance

Putative chemoresistance factors include members of the matrix metalloproteinase family, such as *ADAM9* (a disintegrin and metalloproteinase domain 9), which is negatively correlated with many drugs in the NCI-60. *ADAM9* is highly expressed in hepatocellular carcinoma (55) and in pancreatic ductal adenocarcinomas where cytoplasmic expression is correlated with poor prognosis (56). Furthermore, *ADAM9* is part of the signaling cascade evading apoptosis induced by cytotoxic drugs (57), possibly by mediating release of heparin-binding EGF-like growth factor (HB-EGF) (58). Strong correlations in the expression and chemoresistance profiles in the NCI-60 support the notion that *ADAM9* and *EGFR* are functionally interacting. Several other metalloproteinases and inhibitors (Table I), such as *TIMP2* and *MMP24*, also showed strong negative correlation with multiple anticancer drugs and, moreover, served as predictors of drug potency. Their possible role in chemoresistance needs to be further validated. Negative drug correlations for a metalloproteinase inhibitor underscore their multiple biological functions, which are potentially opposite effects on drug response.

GTPases and Chemoresistance

Among the 92 GTPases tested, several were negatively correlated with multiple drugs (e.g., *ARHC*, *RAB5B*, *ARF4*, *RRAS2*/*TC21*, *RAB6A*, and *RALB*). A few GTPases showed multiple positive correlations (e.g., *RAB37*, *RAN*, *RAC2*, and *RAB39B*), indicating that signaling networks can have dual outcomes, promoting either apoptosis or survival. *RAB6A* and *RALB* (negative), and *RAB37* and *RAC2* (positive) were heavily represented in gene panels predictive of drug sensitivity.

Previous work supports a role of GTPases in cell transformation and survival (consistent with negative drug correlations). RAS couples extracellular signaling to downstream RAF/MEK/ERK and PI3-K/AKT cascades (59), activation of which via EGFR contributes to BCNU resistance in gliomas (60). Also negatively correlated with multiple drugs, *TC21*/*RRAS2* mediates transformation of cancer cells involving phosphatidylinositol 3-kinase (PI3-K) (61,62) and is activated by growth factors (63), including FGF1 and FGF2, shown to convey chemoresistance (64). Similarly implicated in our study, and a known key modulator of cell proliferation (65), *RALB* clusters together with *ERRB2* by gene expression and drug potency correlations. Lastly, *ARHC* (*RhoC*) promotes tumor metastasis (66) and seems to contribute to chemoresistance via growth factor signaling (67).

Negative correlations further point to a series of GTPases not yet directly implicated in chemoresistance, such as *ARF4*, *RHEB2*, *RAB5B*, *RAB6A*, *RAB18*, and *RAB32* (Table I). Further studies are needed to validate these genes as chemoresistance factors.

Chemosensitivity Genes

Positive gene drug correlations imply a possible role in chemosensitivity (e.g., Table I and Supplementary Table IV), e.g., *RAB37* showing multiple drug correlations. *RAB39B* is a member of the RAS oncogene family, but displayed exclusively positive correlation (highest $r = 0.47$ with iproplatin); this observation requires further study. These findings are consistent with the Janus-like role of growth factor signaling in cell survival and apoptosis. *ARHGDIA* encodes a Rho GDP dissociation inhibitor and is predictive as a chemosensitivity factor for 14 drugs, possibly by regulating Rho activity. IGFALS is an insulin-like growth factor-binding protein (acid labile subunit), which complexes IGF and IGFBP3 into a 150-kDa aggregate (see OMIM, 601489). This could account for the positive correlation and predictive power for 15 drugs. *EGFL4* and *EGFL5* encode EGF-like polypeptides containing multiple EGF repeats, and function in cell adhesion, but the physiological role remains uncertain.

Remarkably, genes with either negative or positive correlations involving many drugs are nearly exclusively either negative or positive, with no overlap between them. This reinforces the hypothesis that these genes are involved in either chemoresistance or chemosensitivity by downstream mechanisms, independent of the direct mechanism of action of each single drug. We propose that these common downstream genes will prove valuable as biomarkers or drug targets.

EGFR and ERBB2 Interactions

Our clustering results indicate that *EGFR* and *ERBB2* belong to different gene clusters with distinct expression and drug resistance patterns. This was surprising as EGFR and ERBB2 are coexpressed in some tumors and can heterodimerize (9). Although gefitinib (Iressa) in combination with trastuzumab (Herceptin) acted synergistically against human breast cancer cell growth (68), no synergism between EGFR inhibitor AG1478 and ERBB2 inhibitor AG825 was observed in the current study. Whereas members of the ERBB receptor family are mediators of cell survival, ERBB receptors might induce cell death under some circumstances (69,70). Indeed, overexpression of ERBB2 in cancer cells could result in either chemoresistance or chemosensitivity for different anticancer drugs (71). For example, multiple studies have shown that overexpression of ERBB2 can convey resistance to certain chemotherapeutic drugs. ERBB2 overexpression by transfection in MDA-MB-435 increased resistance to paclitaxel and taxotere. However, contradictory results were observed in different laboratories (45).

In addition, the combined effects of AG1478 or AG825 with cytotoxic anticancer drugs in different cell lines were not directly related to the expression level of EGFR or ERBB2. EGFR expression levels are not correlated with the potency of EGFR inhibitor gefitinib in mice bearing human tumor-

derived xenografts (72). Our results are consistent with previous findings that the synergistic inhibition of tumor growth by EGFR inhibitor gefitinib and cytotoxic agents, such as paclitaxel, did not depend on EGFR expression level (20). In addition, the antitumor effect of gefitinib in cancer patients seemed to be related to mutations in the EGFR catalytic domain, but not EGFR expression (42,73). On the other hand, no survival benefit was observed for patients with EGFR mutations (http://www.iressa-us.com/pharm.asp). The mechanism of the combined effects seems to be context dependent and is not currently predictable, confounding attempts to extrapolate to therapeutic effects *in vivo*. Another confounding factor is the lack of specificity of the inhibitors. For example, AG1478 not only inhibits EGFR, but could also inhibit ERBB4 (74), and parallel signaling pathways can bypass the block. Inhibition of growth factor signaling at different junctions might improve anticancer potency, as shown with combined inhibition of both mutated EGFR and PI3K (75).

In this study, strong negative correlation between *EGFR* and *ERBB2*, and multiple drugs, implicating chemoresistance, failed to predict synergism in some cases. The mechanisms of observed synergism and antagonism require further study to facilitate the design of effective combination treatments. Our results caution against the indiscriminate use of such combinations.

Prediction of Drug Potency

A promising avenue is the use of biomarkers to predict anticancer drug response (3,28). Here we have used expression of growth factor signaling genes for prediction of cytotoxic potency in the NCI-60 to develop an approach that can be extrapolated to genome-wide expression profiles. For each drug, we have evaluated several predictive models using only negatively or positively correlated genes or both. For example, *EGFR*, *ERBB2*, *RRAS2*, *ARHC*, *RALB*, *ARF4*, *CYR61*, and *ADAM9*, all negatively correlated with multiple anticancer drugs, have predictive power for many drugs in the NCI-60 panel (Supplementary Table VI). Using both positively and negatively correlated genes, e.g., *RAB37*, *RAB6A*, *RAC2*, *PLCL2*, and *TGFB3* (Supplementary Table VI), yields highest predictive values for multiple drugs. When we used only the top scoring genes (9 positively or 12 negatively correlated genes and 13 genes selected from all 343 genes in prediction models for all drugs, these small sets of genes were sufficient and even exceeded predictions with larger sets that yielded entirely different sets for each drug. Comparing to previous studies with reliance on the expression of many genes in each predictive model, the attained accuracy is at least as good. This result indicates that random sampling noise using heuristic models poses a problem to selecting optimally predictive gene sets. By focusing on the smallest number of genes that can be extracted from gene expression-drug potency databases, in particular, involving gene families known to function in chemoresistance, and exhaustively testing all possible combinations, we have improved prediction of drug potency in the NCI-60, although the number of predictor genes used as markers for all drugs was very small. These genes are potential key factors in chemoresistance and sensitivity and could serve as novel drug target *per se*. In future studies, we will extend this approach (progressive selection of candidate genes) to expression arrays covering the entire genome. A preliminary analysis of whole-genome expression data from the NCI-60 revealed that the genes identified in the present study are well represented among all genes showing significant gene–drug correlations. This will enable us to assemble a general set of marker genes with improved predictive potential.

CONCLUSIONS

We have developed a general approach for identifying known and novel candidate genes that could be involved in chemoresistance and chemosensitivity in the treatment of cancer patients. Whereas prediction of drug potencies *in vitro* may not be directly applicable to *in vivo* therapy, the present study guides the selection of biomarkers in cancer chemotherapy. The potential of the identified genes as novel therapeutic targets and their use as biomarkers for predicting drug responses to treatment requires further study.

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REFERENCES

1. A. Wallqvist, A. A. Rabow, R. H. Shoemaker, E. A. Sausville, and D. G. Covell. Establishing connections between microarray expression data and chemotherapeutic cancer pharmacology. *Mol. Cancer Ther.* **1**:311–320 (2002).
2. R. L. Strausberg, A. J. Simpson, L. J. Old, and G. J. Riggins. Oncogenomics and the development of new cancer therapies. *Nature* **429**:469–474 (2004).
3. N. J. Winick, W. L. Carroll, and S. P. Hunger. Childhood leukemia—new advances and challenges. *N. Engl. J. Med.* **351**:601–603 (2004).
4. C. H. Lee and P. F. Macgregor. Using microarrays to predict resistance to chemotherapy in cancer patients. *Pharmacogenomics* **5**:611–625 (2004).
5. C. Sawyers. Targeted cancer therapy. *Nature* **432**:294–297 (2004).
6. D. W. Nebert and E. S. Vesell. Advances in pharmacogenomics and individualized drug therapy: exciting challenges that lie ahead. *Eur. J. Pharmacol.* **500**:267–280 (2004).
7. J. K. Lee, K. J. Bussey, F. G. Gwadry, W. Reinhold, G. Riddick, S. L. Pelletier, S. Nishizuka, G. Szakacs, J. P. Annereau, U. Shankavaram, S. Lababidi, L. H. Smith, M. M. Gottesman, and J. N. Weinstein. Comparing cDNA and oligonucleotide array data: concordance of gene expression across platforms for the NCI-60 cancer cells. *Genome Biol.* **4**:R82 (2003).
8. A. Holleman, M. H. Cheok, M. L. Boerden, W. Yang, A. J. Veerman, K. M. Kazemier, D. Pei, C. Cheng, C. H. Pui, M. V. Relling, G. E. Janka-Schaub, R. Pieters, and W. E. Evans. Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. *N. Engl. J. Med.* **351**:533–542 (2004).

9. C. D. Britten. Targeting ErbB receptor signaling: a pan-ErbB approach to cancer. *Mol. Cancer Ther.* **3**:1335–1342 (2004).
10. B. Jansen, H. Schlagbauer-Wadl, H. G. Eichler, K. Wolff, A. van Elsas, P. I. Schrier, and H. Pehamberger. Activated N-ras contributes to the chemoresistance of human melanoma in severe combined immunodeficiency (SCID) mice by blocking apoptosis. *Cancer Res.* **57**:362–365 (1997).
11. R. Sanchez-Prieto, J. A. Vargas, A. Carnero, E. Marchetti, J. Romero, A. Durantez, J. C. Lacal, and S. Ramon y Cajal. Modulation of cellular chemoresistance in keratinocytes by activation of different oncogenes. *Int. J. Cancer* **60**:235–243 (1995).
12. R. S. Herbst, M. Fukuoka, and J. Baselga. Timeline: gefitinib—a novel targeted approach to treating cancer. *Nat. Rev., Cancer* **4**:956–965 (2004).
13. R. Nahta and F. J. Esteva. HER-2-targeted therapy: lessons learned and future directions. *Clin. Cancer Res.* **9**:5078–5084 (2003).
14. N. Dandachi, O. Dietze, and C. Hauser-Kronberger. Evaluation of the clinical significance of HER2 amplification by chromogenic *in situ* hybridisation in patients with primary breast cancer. *Anticancer Res.* **24**:2401–2406 (2004).
15. Z. Dai, Y. Huang, and W. Sadee. Growth factor signaling and resistance to cancer chemotherapy. *Curr. Top. Med. Chem.* **4**:1347–1356 (2004).
16. P. Hersey and X. D. Zhang. Overcoming resistance of cancer cells to apoptosis. *J. Cell. Physiol.* **196**:9–18 (2003).
17. J. M. Yang, A. D. Vassil, and W. N. Hait. Activation of phospholipase C induces the expression of the multidrug resistance (MDR1) gene through the Raf–MAPK pathway. *Mol. Pharmacol.* **60**:674–680 (2001).
18. M. C. Prewett, A. T. Hooper, R. Bassi, L. M. Ellis, H. W. Waksal, and D. J. Hicklin. Enhanced antitumor activity of anti-epidermal growth factor receptor monoclonal antibody IMC-C225 in combination with irinotecan (CPT-11) against human colorectal tumor xenografts. *Clin. Cancer Res.* **8**:994–1003 (2002).
19. F. Ciardiello, R. Caputo, R. Bianco, V. Damiano, G. Pomatico, S. De Placido, A. R. Bianco, and G. Tortora. Antitumor effect and potentiation of cytotoxic drugs activity in human cancer cells by ZD-1839 (Iressa), an epidermal growth factor receptor-selective tyrosine kinase inhibitor. *Clin. Cancer Res.* **6**:2053–2063 (2000).
20. F. M. Sirotnak, M. F. Zakowski, V. A. Miller, H. I. Scher, and M. G. Kris. Efficacy of cytotoxic agents against human tumor xenografts is markedly enhanced by coadministration of ZD1839 (Iressa), an inhibitor of EGFR tyrosine kinase. *Clin. Cancer Res.* **6**:4885–4892 (2000).
21. Y. Lu, X. Zi, Y. Zhao, D. Mascarenhas, and M. Pollak. Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin). *J. Natl. Cancer Inst.* **93**:1852–1857 (2001).
22. C. Knuefermann, Y. Lu, B. Liu, W. Jin, K. Liang, L. Wu, M. Schmidt, G. B. Mills, J. Mendelsohn, and Z. Fan. HER2/PI-3K/Akt activation leads to a multidrug resistance in human breast adenocarcinoma cells. *Oncogene* **22**:3205–3212 (2003).
23. I. Vivanco and C. L. Sawyers. The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat. Rev., Cancer* **2**:489–501 (2002).
24. A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, and A. Vaigro-Wolff *et al.* Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J. Natl. Cancer Inst.* **83**:757–766 (1991).
25. U. Scherf, D. T. Ross, M. Waltham, L. H. Smith, J. K. Lee, L. Tanabe, K. W. Kohn, W. C. Reinhold, T. G. Myers, D. T. Andrews, D. A. Scudiero, M. B. Eisen, E. A. Sausville, Y. Pommier, D. Botstein, P. O. Brown, and J. N. Weinstein. A gene expression database for the molecular pharmacology of cancer. *Nat. Genet.* **24**:236–244 (2000).
26. A. J. Butte, P. Tamayo, D. Slonim, T. R. Golub, and I. S. Kohane. Discovering functional relationships between RNA expression and chemotherapeutic susceptibility using relevance networks. *Proc. Natl. Acad. Sci. USA* **97**:12182–12186 (2000).
27. A. A. Rabow, R. H. Shoemaker, E. A. Sausville, and D. G. Covell. Mining the National Cancer Institute's tumor-screening database: identification of compounds with similar cellular activities. *J. Med. Chem.* **45**:818–840 (2002).
28. J. E. Staunton, D. K. Slonim, H. A. Coller, P. Tamayo, M. J. Angelo, J. Park, U. Scherf, J. K. Lee, W. O. Reinhold, J. N. Weinstein, J. P. Mesirov, E. S. Lander, and T. R. Golub. Chemosensitivity prediction by transcriptional profiling. *Proc. Natl. Acad. Sci. USA* **98**:10787–10792 (2001).
29. Y. Huang, P. Anderle, K. J. Bussey, C. Barbacioru, U. Shankavaram, Z. Dai, W. C. Reinhold, A. Papp, J. N. Weinstein, and W. Sadee. Membrane transporters and channels: role of the transportome in cancer chemosensitivity and chemoresistance. *Cancer Res.* **64**:4294–4301 (2004).
30. Y. Huang, P. E. Blower, C. Yang, C. Barbacioru, Z. Dai, Y. Zhang, J. J. Xiao, K. K. Chan, and W. Sadée. Correlating gene expression with chemical scaffolds of cytotoxic agents: ellipticines as substrates and inhibitors of MDR1 (ABCB1). *Pharmacogenomics J.* **5**:112–125 (2005).
31. P. Anderle, V. Rakhmanova, K. Woodford, N. Zerangue, and W. Sadee. Messenger RNA expression of transporter and ion channel genes in undifferentiated and differentiated Caco-2 cells compared to human intestines. *Pharm. Res.* **20**: 3–15 (2003).
32. J. N. Weinstein, K. W. Kohn, M. R. Grever, V. N. Viswanadhan, L. V. Rubinstein, A. P. Monks, D. A. Scudiero, L. Welch, A. D. Koutsoukos, and A. J. Chiausa. Neural computing in cancer drug development: predicting mechanism of action. *Science* **258**:447–451 (1992).
33. B. Efron and R. J. Tibshirani. *An Introduction to the Bootstrap*, Chapman & Hall, Inc., New York, 1993.
34. Y. Benjamini and Y. Hochberg. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc., B* **57**:289–300 (1995).
35. J. N. Weinstein, T. G. Myers, P. M. O'Connor, S. H. Friend, A. J. Fornace Jr., K. W. Kohn, T. Fojo, S. E. Bates, L. V. Rubinstein, N. L. Anderson, J. K. Buolamwini, W. W. van Osdol, A. P. Monks, D. A. Scudiero, E. A. Sausville, D. W. Zaharevitz, B. Bunow, V. N. Viswanadhan, G. S. Johnson, R. E. Wittes, and K. D. Paull. An information-intensive approach to the molecular pharmacology of cancer. *Science* **275**:343–349 (1997).
36. M. B. Eisen, P. T. Spellman, P. O. Brown, and D. Botstein. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA* **95**:14863–14868 (1998).
37. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney, and M. R. Boyd. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* **82**:1107–1112 (1990).
38. T. C. Chou and P. Talalay. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv. Enzyme Regul.* **22**:27–55 (1984).
39. J. Topaly, S. Fruehauf, A. D. Ho, and W. J. Zeller. Rationale for combination therapy of chronic myelogenous leukaemia with imatinib and irradiation or alkylating agents: implications for pretransplant conditioning. *Br. J. Cancer* **86**:1487–1493 (2002).
40. C. P. Landowski, P. Anderle, D. Sun, W. Sadee, and G. L. Amidon. Transporter and ion channel gene expression after Caco-2 cell differentiation using 2 different microarray technologies. *AAPS J.* **6**:e21 (2004).
41. S. Dan, T. Tsunoda, O. Kitahara, R. Yanagawa, H. Zembutsu, T. Katagiri, K. Yamazaki, Y. Nakamura, and T. Yamori. An integrated database of chemosensitivity to 55 anticancer drugs and gene expression profiles of 39 human cancer cell lines. *Cancer Res.* **62**:1139–1147 (2002).
42. T. J. Lynch, D. W. Bell, R. Sordella, S. Gurubhagavatula, R. A. Okimoto, B. W. Brannigan, P. L. Harris, S. M. Haserlat, J. G. Supko, F. G. Haluska, D. N. Louis, D. C. Christiani, J. Settleman, and D. A. Haber. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* **350**:2129–2139 (2004).
43. M. Schmidt and R. B. Lichtner. EGF receptor targeting in therapy-resistant human tumors. *Drug Resist. Updat.* **5**:11–18 (2002).
44. D. Yu and M. C. Hung. Role of erbB2 in breast cancer chemosensitivity. *BioEssays* **22**:673–680 (2000).

45. D. Yu. Mechanisms of ErbB2-mediated paclitaxel resistance and trastuzumab-mediated paclitaxel sensitization in ErbB2-overexpressing breast cancers. *Semin. Oncol.* **28**(5 Suppl 16):12–17 (2001).
46. M. Sumitomo, T. Asano, J. Asakuma, A. Horiguchi, and M. Hayakawa. ZD1839 modulates paclitaxel response in renal cancer by blocking paclitaxel-induced activation of the epidermal growth factor receptor-extracellular signal-regulated kinase pathway. *Clin. Cancer Res.* **10**:794–801 (2004).
47. M. D. Pegram and D. J. Slamon. Combination therapy with trastuzumab (Herceptin) and cisplatin for chemoresistant metastatic breast cancer: evidence for receptor-enhanced chemosensitivity. *Semin. Oncol.* **26**(4 Suppl 12):89–95 (1999).
48. M. S. Tsai, A. E. Hornby, J. Lakins, and R. Lupu. Expression and function of CYR61, an angiogenic factor, in breast cancer cell lines and tumor biopsies. *Cancer Res.* **60**:5603–5607 (2000).
49. J. A. Menendez, I. Mehmi, D. W. Griggs, and R. Lupu. The angiogenic factor CYR61 in breast cancer: molecular pathology and therapeutic perspectives. *Endocr. Relat. Cancer* **10**:141–152 (2003).
50. M. T. Lin, C. C. Chang, S. T. Chen, H. L. Chang, J. L. Su, Y. P. Chau, and M. L. Kuo. Cyr61 expression confers resistance to apoptosis in breast cancer MCF-7 cells by a mechanism of NF-kappaB-dependent XIAP up-regulation. *J. Biol. Chem.* **279**: 24015–24023 (2004).
51. H. Osada, T. Tokunaga, M. Nishi, H. Hatanaka, Y. Abe, A. Tsugu, H. Kijima, H. Yamazaki, Y. Ueyama, and M. Nakamura. Overexpression of the neuropilin 1 (NRP1) gene correlated with poor prognosis in human glioma. *Anticancer Res.* **24**:547–552 (2004).
52. M. Klagsbrun, S. Takashima, and R. Mamluk. The role of neuropilin in vascular and tumor biology. *Adv. Exp. Med. Biol.* **515**:33–48 (2002).
53. C. B. Brown, A. S. Boyer, R. B. Runyan, and J. V. Barnett. Requirement of type III TGF-beta receptor for endocardial cell transformation in the heart. *Science* **283**:2080–2082 (1999).
54. F. Lopez-Casillas, J. L. Wrana, and J. Massague. Betaglycan presents ligand to the TGF beta signaling receptor. *Cell* **73**: 1435–1444 (1993).
55. A. Tannapfel, K. Anhalt, P. Hausermann, F. Sommerer, M. Benicke, D. Uhlmann, H. Witzigmann, J. Hauss, and C. Wittekind. Identification of novel proteins associated with hepatocellular carcinomas using protein microarrays. *J. Pathol.* **201**:238–249 (2003).
56. R. Grutzmann, J. Luttges, B. Sipos, O. Ammerpohl, F. Dobrowolski, I. Alldinger, S. Kersting, D. Ockert, R. Koch, H. Kalthoff, H. K. Schackert, H. D. Saeger, G. Kloppel, and C. Pilarsky. ADAM9 expression in pancreatic cancer is associated with tumour type and is a prognostic factor in ductal adenocarcinoma. *Br. J. Cancer* **90**:1053–1058 (2004).
57. O. M. Fischer, S. Hart, A. Gschwind, N. Prenzel, and A. Ullrich. Oxidative and osmotic stress signaling in tumor cells is mediated by ADAM proteases and heparin-binding epidermal growth factor. *Mol. Cell. Biol.* **24**:5172–5183 (2004).
58. Y. Izumi, M. Hirata, H. Hasuwa, R. Iwamoto, T. Umata, K. Miyado, Y. Tamai, T. Kurisaki, A. Sehara-Fujisawa, S. Ohno, and E. Mekada. A metalloprotease-disintegrin, MDC9/meltrin-gamma/ADAM9 and PKCdelta are involved in TPA-induced ectodomain shedding of membrane-anchored heparin-binding EGF-like growth factor. *EMBO J.* **17**:7260–7272 (1998).
59. J. Zuber, O. I. Tchernitsa, B. Hinzmann, A. C. Schmitz, M. Grips, M. Hellriegel, C. Sers, A. Rosenthal, and R. Schafer. A genome-wide survey of RAS transformation targets. *Nat. Genet.* **24**:144–152 (2000).
60. A. Chakravarti, A. Chakladar, M. A. Delaney, D. E. Latham, and J. S. Loeffler. The epidermal growth factor receptor pathway mediates resistance to sequential administration of radiation and chemotherapy in primary human glioblastoma cells in a RAS-dependent manner. *Cancer Res.* **62**:4307–4315 (2002).
61. G. A. Murphy, S. M. Graham, S. Morita, S. E. Reks, K. Rogers-Graham, A. Vojtek, G. G. Kelley, and C. J. Der. Involvement of phosphatidylinositol 3-kinase, but not RalGDS, in TC21/R-Ras2-mediated transformation. *J. Biol. Chem.* **277**:9966–9975 (2002).
62. R. Rong, Q. He, Y. Liu, M. S. Sheikh, and Y. Huang. TC21 mediates transformation and cell survival via activation of phosphatidylinositol 3-kinase/Akt and NF-kappaB signaling pathway. *Oncogene* **21**:1062–1070 (2002).
63. D. H. Kozian and H. G. Augustin. Transcriptional regulation of the Ras-related protein TC21/R-Ras2 in endothelial cells. *FEBS Lett.* **414**:239–242 (1997).
64. S. Song, M. G. Wientjes, Y. Gan, and J. L. Au. Fibroblast growth factors: an epigenetic mechanism of broad spectrum resistance to anticancer drugs. *Proc. Natl. Acad. Sci. USA* **97**:8658–8663 (2000).
65. Y. Chien and M. A. White. RAL GTPases are linchpin modulators of human tumour-cell proliferation and survival. *EMBO Rep.* **4**:800–806 (2003).
66. E. A. Clark, T. R. Golub, E. S. Lander, and R. O. Hynes. Genomic analysis of metastasis reveals an essential role for RhoC. *Nature* **406**:532–535 (2000).
67. K. L. van Golen, L. W. Bao, Q. Pan, F. R. Miller, Z. F. Wu, and S. D. Merajver. Mitogen activated protein kinase pathway is involved in RhoC GTPase induced motility, invasion and angiogenesis in inflammatory breast cancer. *Clin. Exp. Metastasis* **19**: ?301–311 (2002).
68. N. Normanno, M. Campiglio, L. A. De, G. Somenzi, M. Maiello, F. Ciardiello, L. Gianni, D. S. Salomon, and S. Menard. Cooperative inhibitory effect of ZD1839 (Iressa) in combination with trastuzumab (Herceptin) on human breast cancer cell growth. *Ann. Oncol.* **13**:65–72 (2002).
69. A. J. Danielsen and N. J. Maihle. The EGF/ErbB receptor family and apoptosis. *Growth Factors* **20**:1–15 (2002).
70. J. P. Steinbach, A. Klumpp, H. Wolburg, and M. Weller. Inhibition of epidermal growth factor receptor signaling protects human malignant glioma cells from hypoxia-induced cell death. *Cancer Res.* **64**:1575–1578 (2004).
71. V. Smith, S. Hobbs, W. Court, S. Eccles, P. Workman, and L. R. Kelland. ErbB2 overexpression in an ovarian cancer cell line confers sensitivity to the HSP90 inhibitor geldanamycin. *Anticancer Res.* **22**:1993–1999 (2002).
72. A. E. Wakeling, S. P. Guy, J. R. Woodburn, S. E. Ashton, B. J. Curry, A. J. Barker, and K. H. Gibson. ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with otential for cancer therapy. *Cancer Res.* **62**:5749–5754 (2002).
73. J. G. Paez, P. A. Janne, J. C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F. J. Kaye, N. Lindeman, T. J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M. J. Eck, W. R. Sellers, B. E. Johnson, and M. Meyerson. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* **304**:1497–1500 (2004).
74. R. Fukazawa, T. A. Miller, Y. Kuramochi, S. Frantz, Y. D. Kim, M. A. Marchionni, R. A. Kelly, and D. B. Sawyer. Neuregulin-1 protects ventricular myocytes from anthracycline-induced apoptosis via erbB4-dependent activation of PI3-kinase/Akt. *J. Mol. Cell. Cardiol.* **35**:1473–1479 (2003).
75. Q. W. Fan, K. M. Specht, C. Zhang, D. D. Goldenberg, K. M. Shokat, and W. A. Weiss. Combinatorial efficacy achieved through two-point blockade within a signaling pathway—a chemical genetic approach. *Cancer Res.* **63**:8930–8938 (2003).
76. P. M. Navolanic, L. S. Steelman, and J. A. McCubrey. EGFR family signaling and its association with breast cancer development and resistance to chemotherapy (review). *Int. J. Oncol.* **22**:237–252 (2003).
77. S. W. Kim, M. Hayashi, J. F. Lo, Y. Yang, J. S. Yoo, and J. D. Lee. ADP-ribosylation factor 4 small GTPase mediates epidermal growth factor receptor-dependent phospholipase D2 activation. *J. Biol. Chem.* **278**:2661–2668. (2003) Epub 2002 Nov 21.