Gene Expression Profiling-Based Prediction of Response of Colon Carcinoma Cells to 5-Fluorouracil and Camptothecin

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ABSTRACT

5-Fluorouracil (5-FU) is the most common chemotherapy agent used in the treatment of colorectal cancer, yet objective response rates are low. Recently, camptothecin (CPT) has emerged as an effective alternative therapy. Decisive means to determine treatment, based on the likelihood of response to each of these agents, could greatly enhance the management of this disease. Here, the ability of cDNA microarray-generated basal gene expression profiles to predict apoptotic response to 5-FU and CPT was determined in a panel of 30 colon carcinoma cell lines. Genes whose basal level of expression correlated significantly with 5-FU- and CPT-induced apoptosis were selected, and their predictive power was assessed using a “leave one out” jackknife cross-validation strategy. Selection of the 50 genes best correlated with 5-FU-induced apoptosis, but not 50 randomly selected genes, significantly predicted response to this agent. Importantly, this gene expression profiling approach predicted response more effectively than four previously established determinants of 5-FU response: mismatch repair (MMR) status and expression of dipyrimidine dehydrogenase, dipyrimidine phosphorylase, and dipyrimidine phosphorylase activity; and p53 and mismatch repair status. Furthermore, reanalysis of the database demonstrated that selection of the 149 genes best correlated with CPT-induced apoptosis maximally and significantly predicted response to this agent. These studies demonstrate that the basal gene expression profile of colon cancer cells can be used to predict and distinguish response to multiple chemotherapeutic agents and establish the potential of this methodology as a means by which rational decisions regarding choice of therapy can be approached.

INTRODUCTION

5-Fluorouracil (5-FU) has been the treatment of choice for both advanced colon cancer and adjuvant therapy for earlier disease, yet it is far from uniformly effective. Objective response rates for late-stage patients are approximately 20–30%, whereas only 20% of stage III patients who receive 5-FU-based adjuvant therapy show improved disease-free and overall survival (1–4). Moreover, other drugs such as camptothecin (CPT) and oxaliplatin are effective alternative treatments (5–8). The ability to predict response based on objective and quantifiable markers is therefore of importance for several reasons. First, patients unlikely to respond to a given therapy can be spared the toxicity, time, and expense associated with these treatment regimens and, more importantly, can be placed on alternate therapies. Second, because several chemotherapeutic agents induce the acquisition of drug resistance, administration of the agent likely to induce maximal response in the first course of treatment is critical to enhance treatment success. Finally, identification of markers that predict response may provide significant insight into the differences among tumors that establish different relative sensitivities to alternative therapies.

The identification of markers capable of predicting 5-FU response has been a subject of considerable interest (9). There is significant literature to suggest that the target of 5-FU, thymidylate synthase (TS), is an important predictor of response (9). For example, lower TS expression was associated with improved response to 5-FU in colorectal cancer patients with stage III and IV tumors (9, 10). In addition to TS, it has been reported that measurement of enzymes that affect the metabolism of 5-FU, including thymidine phosphorylase (TP) and dipyrimidine dehydrogenase, can also predict response (11, 12).

Several studies suggest p53 status is an important determinant of 5-FU sensitivity, with improved response and prolonged survival observed in patients with tumors wild-type (WT) for p53 (13–15). Similarly, it was recently demonstrated that tumors which retained heterozygosity at either 17p or 18q showed improved response to 5-FU-based adjuvant therapy (16). Other predictors of improved response include mismatch repair (MMR) status (17, 18) and the ratio of antiapoptotic/proapoptotic bcl-2 family members (19). In our own investigations, we have established that low-level amplification of c-myc was associated with longer overall survival in response to 5-FU-based adjuvant therapy (20). More recently, these findings were extended to demonstrate that tumors with amplification of c-myc, which also retained WT p53 function, had significantly improved response to 5-FU both in vitro and in vivo (21). A likely explanation for these findings was recently offered by Seoane et al. (22), who demonstrated that c-myc represses p53 induction of p21WAF1/Cip1 after DNA damage, promoting the induction of apoptosis over cell cycle arrest, an observation we have recently confirmed in terms of response to CPT (23).

However, two major limitations exist in the utility of these limited numbers of markers for predicting chemotherapeutic response. First, for several of the markers described, conflicting reports also exist. For example, a number of studies have reported that TS levels fail to distinguish between patient groups with differential response to 5-FU (24–27). Likewise, whereas TS is often overexpressed in 5-FU-resistant cells in vitro (28), studies of unsellected panels of cell lines have failed to consistently show a correlation between intracellular TS levels and response (29–31). Contrasting findings have also been observed for p53 status (24) and for TP expression, with both high and low levels of TP linked to 5-FU response (11, 32, 33).

Second, an approach that measures the ability of single markers to predict response to a specific agent generally fails to identify alternative treatment options.

The advent of high-throughput methodologies such as microarray-based gene expression profiling enables the transcriptional profile of a tumor sample to be determined on a global scale. A number of years ago, we suggested that such gene expression profiling could be fundamental in characterizing the phenotype of cells, including relative drug sensitivity of cancer cells (34, 35). Such gene expression profiling has the potential to probe more deeply into the factors that determine response to multiple drugs than a single assay. This in turn could reveal subtleties of mechanism that may be useful in identifying new drug targets, in discriminating among patients who show varying...
sensitivity to drugs, and in defining new treatment strategies, such as drug interactions that may be synergistic or antagonistic on a molecular level. The potential for gene expression profiling as a means toward prediction of response to chemotherapeutic agents is highlighted by its recent success in class discovery and prognosis in several cancer types (36, 37).

In this report, we approach this for colon cancer by defining 5-FU sensitivity for 30 colon carcinoma cell lines based on three different assays of response (growth inhibition, apoptosis, and clonogenicity) and linking this to the basal expression profile of >9000 sequences using a cDNA microarray approach. Gene sets were identified that show significant correlation with 5-FU sensitivity, and a formal statistical analysis (“leave one out” or jackknifing) was used to demonstrate that these genes are predictive for response. Importantly, this approach had greater power to predict response than four previously reported determinants of 5-FU response: TS and TP activities; and p53 and MMR status. The analysis was then repeated for sensitivity of the cell lines to CPT, a topoisomerase I inhibitor now commonly used in the treatment of colon cancer, and a second gene set, predictive for sensitivity to CPT, was identified. These experiments demonstrate that the basal gene expression profile of colon cancer cells can be used to predict response to chemotherapeutic agents and establish the potential of this approach as a means by which rational decisions regarding treatment of colon cancer can be approached.

MATERIALS AND METHODS

Cell Lines and Cell Culture

The panel of colorectal cancer cell lines used were: Caco-2, Colo205, Colo320, DLD-1, HCT116, HCT-15, HCT-8, HT29, LoVo, LS174T, RKO, SK-CO-1, SW1116, SW403, SW48, SW480, SW620, SW837, SW948, T84, and WiDr (all from American Type Culture Collection, Manassas, VA); HT29-C116E and HT29-C119A (from Dr. Laboisse; Institut National de la Recherche Medicaule U539, Nantes, France (38)); LIM1215 and LIM2405 (from Dr. Bob Whitehead; Ludwig Institute for Melbourne and Vanderbilt University, Nashville, TN (39, 40)); HCC2998 and KM12 (from the National Cancer Institute-Frederick Cancer Tumor Repository); and RW2982 and RW7213 (41). All cells were maintained in MEM supplemented with 10% fetal bovine serum (FBS), 1% antibiotics/antimycotic (100 units/ml streptomycin, 100 units/ml penicillin, and 0.25 µg/ml amphotericin B), 100 µM nonessential amino acids, and 10 mM HEPES buffer solution (all from Invitrogen Corp., Carlsbad, CA).

Determination of p53 and MMR Status

The p53 status of Caco-2, Colo205, Colo320, HT29, KM12, SW620, SW480, DLD-1, LoVo, SK-CO-1, LS174T, WiDr, SW837, RKO, HCT8, HCT116, HCT15, HCC2998, and SW1116 has been reported previously (Table 3). The p53 status of LIM1215, LIM2405, RW2982, RW7213, SW403, SW498, and T84 was determined by polymerase chain reaction (PCR) amplification and sequencing of exons 5–8 of the p53 gene, the location of the majority of p53 mutations (42), and confirmed by measurement of p53 protein levels by Western blot analysis. Mutations in the p53 gene are often associated with increased levels of p53 protein due to conformational changes in the p53 polypeptide that result in increased stability (43). DNA from each cell line was isolated using the DNeasy kit (Qiagen, Valencia, CA) and used as the template for two different PCR reactions, one amplifying exons 5 and 6, and the other amplifying exons 7 and 8. The sequences of the primers used were as follows: Exon 5 Forward, GGAATCTGGTCACTGGCCTGAGTATCTAC; Exon 6 Reverse, AGGGCGTACGAACACCCCTTAC; Exon 7 Forward, ACAGGGTCCCAAGAGGCAGCTGG; and Exon 8 Reverse, GGAATTCT-GAGGGCATACGTACCCCTTTGCT. The LIM1215, LIM2405, SW498, SW403, SK-CO-1, SW48, and T84 cell lines were classified as p53 WT because no mutations were identified in the DNA sequence analysis of exons 5–8. For each of these cell lines, very low to undetectable p53 expression was detected by Western blotting, in comparison with known p53 mutant cell lines (data not shown). Mutations in the R2982 (9-bp repeat in exon 5) and R27213 (T to G substitution in codon 257, exon 7) cell lines were identified, and Western blotting revealed the presence of a prominent p53 band in these cell lines.

The MMR status of 27 of the 30 cell lines was derived from the literature (Table 1). The MMR status of LIM1215, LIM2405, and HCC2998 cells was assessed using five fluorescence-labeled microsatellite markers (The Bethesda Panel: BAT25; BAT26; D2S123; D5S346; and D17S250). Primer sequences have been reported previously (44). PCR reactions were carried out in a 10-µl reaction volume containing 50–100 ng of genomic DNA, 1X PCR buffer (Applied Biosystems, Foster City, CA), 250 µM each deoxynucleotide triphosphate, 0.5 µM each primer, and 1 unit of AmpliTaQ Gold polymerase (Applied Biosystems). The MgCl2 concentration was 2.5 mM for BAT25 and 2.75 mM for BAT26, D2S123, D5S346, and D17S250. Predenaturation was performed at 95°C for 10 min, and final extension was performed at 72°C for 10 min in all reactions. PCR products were loaded on a 5% Long Ranger 6 x urea gel (FMC BioProducts, Rockland, ME) and run in an ABI PRISM 377 DNA Sequencer (Applied Biosystems) according to the manufacturer’s instructions. The data were collected automatically and analyzed by GeneScan 3.1 software (Applied Biosystems).

Measurement of Apoptosis

For analysis of apoptosis, cells were seeded in triplicate in 6-well plates. Seeding densities varied between 5 x 104 and 7.5 x 104 cells/well and were calculated such that control cell density approximated 80% confluence at the completion of the experimental period. Forty-eight h after seeding, cells were treated with 5, 50, or 500 µM 5-FU (Sigma, St. Louis, MO) or 1 µM CPT (Calbiochem, La Jolla, CA), for 72 h. Both attached and floating cells were harvested, washed in cold PBS, and resuspended in 50 µg/ml propidium iodide, 0.1% sodium citrate, and 0.1% Triton X-100. Cells were stained overnight at 4°C, and 10,000 cells were analyzed for DNA content using a Becton Dickinson FACScan (Becton Dickinson Immunocytometry Systems, San Jose, CA). The percentage of cells with a subdiploid DNA content was quantified using WinList 2.0 (Verity Software House, Topsham, ME).

Growth Inhibition Assay

The concentration of 5-FU that induced 50% inhibition of control cell growth (GI50) was determined by staining cells with sulforhodamine B, according to the protocol used in the National Cancer Institute in vitro Anticancer Drug Discovery Screen Program (45, 46). Cells were seeded in 96-well plates at plating densities ranging from 5 x 103 to 5 x 104 cells/well. As for the apoptosis assay, seeding density was assessed for each cell line before experiment to ensure control cell density did not exceed 80% confluence at the completion of the 72-h experimental period. Twenty-four h after plating, one plate of each cell line was fixed in situ with 10% trichloroacetic acid to measure the cell population at the time of drug addition (T0). Cells in a parallel plate were treated with 0, 0.01, 0.1, 0.5, 1, 2.5, 5, 10, 25, 50, 100, and 500 µM 5-FU for 72 h. Cells were fixed and stained with sulforhodamine B [0.4% (w/v)] for 30 min, and GI50, which is the drug concentration that results in a 50% inhibition in the net protein increase relative to control cell growth, was calculated as described previously (45, 46).

Clonogenic Assay

Each cell line cultured in the growing phase was treated with 5, 50, or 500 µM 5-FU (Sigma) for 9 h. Medium was removed, and cells were harvested in trypsin, counted, and resuspended in triplicate in 6-well plates at a density of 500 cells/well. Colony formation was monitored over the following 3 weeks, depending on the cell line. When colonies were of sufficient size to enable clear visualization, cells were stained with 1% crystal violet for 30 min, washed with distilled water, air-dried, and scanned using a Perfection 1250 flatbed scanner (Epson America Inc., Long Beach, CA). Colony formation was quantified by analysis of TIFF images using TotalLab 1.1 software (Nonlinear Dynamics, Durham, NC). Each cell line was assayed three times, each time in triplicate.

RNA Isolation and Preparation of Reference RNA

For isolation of RNA for cDNA microarray experiments, each cell line in the exponentially growing phase (60–80% confluence) was harvested in PBS,
and pellets were snap frozen in liquid nitrogen. In each case, medium was changed 12 h before harvesting cells. RNA was isolated using the RNeasy kit (Qiagen). For preparation of the reference RNA, equal amounts of RNA were pooled from 12 cell lines (Caco-2, HT29, HT29 cl.19A, HT29 cl.16E, SW620, SW480, RKO, HCT116, LS174T, Dld-1, LoVo, and WiDr) grown to confluence.

Microarray Analysis

For all microarray hybridizations, 100 μg of RNA isolated from each cell line were labeled with Cy5 dUTP, and 100 μg of reference RNA were labeled with Cy3 dUTP. Probe preparation, hybridization conditions, and array scanning procedures were as described previously (47, 48). Arrays used in this report, encompassing 9216 sequences, were prepared by the microarray facility at the Albert Einstein College of Medicine (49). Signal and background intensities for each channel, at each spot on the microarray, were determined using Genechip Pro software (Axon Instruments, Union City, CA). Each spot was normalized by division of the ratio of red/green signal by the median ratio for the entire array and log transformed. For each cell line, microarrays were performed in duplicate using RNA isolated from two independent cell passages. For each set of replicates, the mean value for each spot was determined and entered into a final database for further analyses.

Statistical Analyses

Normality Tests, Correlation Analyses, Comparison of Subgroups. All 5-FU and CPT sensitivity data and TS and TP activity were tested for normality using a Shapiro-Wilk test (Proc univariate; SAS Procedures Guide Version 8; SAS Institute Inc., Cary, NC). Raw data not normally distributed were log (LN) transformed and reanalyzed for normality. Correlations between two normally distributed data sets were compared using a Pearson’s correlation analysis; otherwise, data were compared by Spearman’s correlation analysis.

Comparisons between cell lines separated according to p53 or MMR status were made using a Mann-Whitney test.

Microarray Data. Unsupervised cluster analysis of the cell lines was performed and displayed using the Cluster and Treeview programs of Eisen et al. (50). For functional Group analysis, named genes on the microarray were categorized into 1 or more of 50 functional categories, and functional group analysis was performed as described previously (48, 51).

"Leave One Out" or Jackknife Analysis. The following text describes the stepwise procedure for the jackknife statistical analysis (52). All jackknife analyses were performed using genes that showed a significant level of expression above background in each of the 30 cell lines (3725 of the 9216 genes on the arrays). First, from the 30 cell lines, cell line 1 was removed from consideration, leaving 29 cell lines for analysis. For these 29 cell lines, the Pearson correlation between the level of expression of each of the 3725 genes and apoptosis induced by 5-FU or CPT was computed, and the N highest absolute value correlations (i.e., corresponding to N genes) were selected. N was varied from the 10–200 best-correlated genes. As a control, N randomly selected genes were also analyzed. To reduce the number of genes to a smaller set of variables, Principal Components Analysis (PCA) was performed. PCA enables a large number of variables to be reduced to linear combinations of variables that can be used to predict an outcome. From the PCA, the principal components (PCs) having the 10 largest eigenvalues were selected. In general, these 10 PCs accounted for approximately 60% of the variance in the selected genes. Next a multiple regression model was developed using the 10 PCs to predict apoptosis, based on the 29 cell lines in the analysis. Once the regression equation was derived, the 10 PCs corresponding to the “left out” cell line were computed and substituted into the derived regression equation to yield a prediction of apoptosis in the left out cell line. Thus, the final results for this first jackknife procedure were the predicted value of apoptosis for the left out cell line (y1*) and the observed value (y1).

After this first jackknife procedure was completed, the left out cell line was...
replaced in the dataset, and cell line 2 was removed, once again leaving 29 cell lines in the dataset with 1 cell line left out. The entire procedure was repeated, and this entire sequence of procedures was repeated for all 30 cell lines so that the final result was a set of predicted apoptosis values for each cell line that had been left out and the corresponding observed value. Each of these 30 jackknife procedures yielded 30 pairs of predicted and observed apoptosis values: \( y_i^p, y_i, y_i^o, y_i^*, \ldots, y_{30}^n, y_{30}^o \).

To determine how well a given regression model predicted observed apoptosis in the left out cell line, the natural log of observed apoptosis [\( \ln(y_i) \)] was plotted as a function of the natural log of the predicted value [\( \ln(y_i^*) \)], and a simple linear regression was constructed. The purpose of this regression analysis was to determine whether the predicted and observed values obeyed simple linear regression was constructed. The purpose of this regression analysis was to determine whether the predicted and observed values obeyed the equation \( y_i = y_i^* \) (i.e., whether the points fall on the line of equality). If the prediction rule is true, then observed and predicted values would be equal or nearly equal. The measure of linear fit was \( r \), and the hypothesis of falling on the line of equality was tested by comparing the slope to unity and \( y \) intercept to zero.

**Quantitative Real-Time PCR**

The expression levels of 10 genes significantly correlated with 5-FU response were selected for further confirmation using quantitative real-time PCR. In addition to significant correlation with 5-FU response, the 10 genes selected were those with the greatest expression range across the panel of 30 cell lines. RNA aliquots (5 \( \mu \)g) from each cell line were reverse-transcribed using SuperScript II (Invitrogen). PCR primers for specific target genes were designed using Primer Express software (Applied Biosystems). cDNA (10 ng) from each cell line was amplified with specific primers using the SYBR green Core Reagents Kit and a 7900HT real-time PCR instrument (Applied Biosystems). Expression of each gene was standardized using glyceraldehyde-3-phosphate dehydrogenase as a reference, and relative levels of expression across the panel of cell lines were quantified by calculating \( 2^{-\Delta\Delta C_t} \), where \( \Delta C_t \) is the difference in \( C_t \) (cycle number at which the amount of amplified target reaches a fixed threshold) between target and reference.

**Measurement of TS and TP Activity**

For both TS and TP activity, cell extracts were prepared by brief homogenization of cells on ice in Tris-mannitol buffer [50 mm Tris-mannitol, 2 mm Trizma base (pH 7.4), and 0.1% Triton X-100].

**TS Activity.** TS was measured in cell extracts by measurement of \([^{14}C]\) thymidylate release from [5'-\( ^{3}H \)] dUMP in the presence of 5,10-methylenetetrahydrofolate (53). Each 150-ml assay contained 5–50 \( \mu \)g of protein extract, 50 mm Tris-HCl (pH 7.4), 10 \( \mu \)M [5'-\( ^{3}H \)] dUMP (0.33 Ci/mmol), and 250 \( \mu \)M 5,10-methylenetetrahydrofolate and was incubated for 10 min at 37°C. Reactions were stopped by the addition of 0.8 ml of ice-cold 5% acid charcoal; after 10 min on ice, the samples were centrifuged (10 min at 10,000 rpm), and a 0.5-ml aliquot of the supernatant was assayed for radioactivity in a liquid scintillation spectrometer.

Reactions were linear with respect to time and protein concentration and were dependent on reduced folate for activity.

**TP Activity.** TP activity was measured in the supernatants of cell extracts (10–50 \( \mu \)g of protein) by incubation in 0.2 \( \mu \)l KH 2PO 4 (pH 7.8) containing 0.2 mm [5'-\( ^{3}H \)] thymidine (Moravek), as described recently (54). In all cases, results were expressed relative to total protein.

**Immunofluorescence**

For immunofluorescence detection, cells were treated with 5 or 50 \( \mu \)M 5-FU for 24 h and fixed, prepared, and visualized as described previously (55). To detect mitochondria, a mouse monoclonal HSP60 antibody was used (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), and binding was detected using a goat antimouse FITC-conjugated secondary antibody (Roche Diagnostics/Boehringer Mannheim Corp., Indianapolis, IN). Cytochrome c was detected using a mouse monoclonal anti-cytochrome c IgG (1:200 dilution; PharMingen, San Diego, CA), followed by exposure to a goat antimouse Cy5-conjugated secondary antibody (Amersham Biosciences, Piscataway, NJ). Bak was detected with a rabbit polyclonal IgG (1:100 dilution; Upstate Biotechnology, Lake Placid, NY) followed by exposure to a goat Cy3-conjugated antirabbit secondary antibody (Amersham). All secondary antibodies were used at a dilution of 1:200 with incubation for 1 h. The number of cells exhibiting Bak localization to the mitochondrial membrane and concurrent cytochrome c release, with and without exposure to 5 or 50 \( \mu \)M 5-FU, was quantified by examination of 200 cells in each of three independent experiments.

**RESULTS**

**Microarray Database of 30 Colon Cancer Cell Lines.** To determine the efficacy with which the basal gene expression profile of colon cancer cells predicts response to chemotherapeutic agents, we assembled a panel of 30 established colon carcinoma cell lines. The basal gene expression profile of each cell line in the exponentially growing phase was determined in duplicate, by comparison with a reference RNA, using 9216-member cDNA microarrays.

To evaluate the reproducibility of our microarray database, the data for the 60 resulting arrays (each cell line in duplicate) were analyzed by unsupervised clustering, using the Cluster and Treeview programs (50). For 27 of the 30 cell lines, the duplicates (from independent experiments and different passages for the same cell lines) clustered together, illustrating the high degree of reproducibility of the microarray data (data not shown). For each cell line, the mean of the two replicates was computed and used in subsequent analyses. First, we selected genes that showed a significant level of expression above background in each of the 30 cell lines. A total of 3725 genes satisfied these criteria, which were used for subsequent analyses. Unsupervised hierarchical clustering of the 30 cell lines based on the expression levels of these 3725 genes revealed several important observations that emphasize the robust nature of this database (Fig. 1). First, the Colo201 and Colo205 cell lines, which were derived from the same patient, clustered together. Second, the HT29 cell line and three of its
derivatives, HT29 cl.19A, HT29 cl.16E, and WiDr, clustered together. Third, the Dld-1 and HCT15 cell lines, which were derived from the same colon carcinoma by two independent researchers (56), clustered closely together. Finally, the SW480 and SW620 cell lines, which were generated from a primary and metastatic cancer from the same patient, respectively, also clustered together (Fig. 1). Previous gene expression profiling studies using large panels of cell lines have consistently demonstrated clustering of cell lines according to tissue of origin (31, 57, 58). In this study, the clustering of cell lines derived from the same patient demonstrates an additional degree of sensitivity of gene expression profiling and illustrates the ability of this technique to recognize the unique signatures that exist among individual patients, despite the common tissue origin of these tumors. In turn, should heterogeneity in gene expression be the basis for differences in response to 5-FU, it establishes the potential that these differences may be distinguishable by gene expression profiling.

**Sensitivity of Cell Lines to 5-FU.** In parallel, the panel of 30 colon carcinoma cell lines was characterized for response to 5-FU-induced apoptosis by measurement of the percentage of cells with a subdiploid DNA content. This was done at three concentrations of 5-FU (5, 50, and 500 μM) and for a treatment period of 72 h. The data for 5 μM 5-FU are presented in Fig. 2A, in which the 30 cell lines are rank-ordered according to sensitivity (also see Table 1). Resistance to 5-FU-induced apoptosis may be overestimated by this assay because exposure to this agent, particularly at higher doses, could result in nonspecific toxicity and thus in the failure of cells to undergo apoptosis. Therefore, the clonogenic potential of each cell line after 5-FU treatment was also assessed. As for apoptosis, a continuum of response was observed across the panel of cell lines. Fig. 2B illustrates two representative cell lines showing high sensitivity to 5-FU (HCC2998 and HCT116) by this assay and two representative cell lines showing low sensitivity to 5-FU (SW620 and SW1116) by this assay (a summary of these data for the 30 cell lines is presented in Table 1). As a final measure of sensitivity, the effect of 5-FU on cell growth was assessed. Fig. 2C illustrates the response of eight representative cell lines to varying concentrations of 5-FU, measured in two separate experiments, each time in quadruplicate. Two of the cell lines shown were relatively sensitive (HCT116 and HCC2998), two of these eight cell lines were relatively resistant (SW620 and SW1116), and the remaining four cell lines exhibited intermediate sensitivity to 5-FU (HCT8, HCT15, LS174T, and Caco-2). The data for the 30 cell lines, reflected as the GLso, are presented in Table 1. Importantly, significant correlations among 5-FU-induced apoptosis and GLso (r = −0.39; P = 0.037), apoptosis and clonogenicity (r = −0.40; P = 0.028), and clonogenicity and GLso (r = 0.42; P = 0.029) were observed among the three assays (values shown are Spearman’s correlation coefficient for the 5 μM 5-FU dose), illustrating that these assays identify closely related, but not necessarily identical, responses to 5-FU.

**Identification of Genes Correlated with 5-FU Response.** To investigate the ability of the basal gene expression data to predict relative sensitivity to 5-FU-induced apoptosis, the correlation between the basal level of expression of each gene (3725 in total) and apoptotic response to 5 μM 5-FU was calculated for the 30 cell lines. Apoptosis induced by 5 μM 5-FU was selected because it was the closest concentration tested to the mean GLso for the drug across the panel of cell lines (4.1 μM; Table 1) and is a concentration of 5-FU achievable in vivo (59, 60). Rank ordering of the absolute value of the correlation coefficients identified 420 genes whose expression was significantly correlated with 5 μM 5-FU-induced apoptosis (P < 0.05; Table 2). One hundred and sixty five of these correlated positively (higher expression in 5-FU-sensitive cells) with 5-FU-induced apoptosis, and 255 correlated negatively (higher expression in 5-FU-resistant cells) with 5-FU-induced apoptosis. To confirm the microarray data, the 10 most differentially expressed sequences in the gene list were selected, and their difference in expression across the panel of 30 cell lines was confirmed by quantitative real-time PCR. Significant correlation (r > 0.65; P < 0.005) between the microarray and RT-PCR data were observed for 9 of these 10 sequences (Table 2).

To determine whether this list was significantly enriched for genes with a role in specific biological processes, we performed a functional group analysis as described previously (48). Genes involved in two biological processes, DNA replication and repair (P = 0.02) and protein processing/targeting (P = 0.02), were significantly enriched for expression on the list of 420 genes significantly correlated with 5-FU response. This analysis was further confirmed using the MappFinder software (Gladstone Institute, University of California San Francisco), which enables the visualization and estimation of enrichment of functionally related genes by linking microarray data to the Gene Ontology hierarchy (61, 62). MappFinder also identified significant enrichment in genes involved in DNA replication (z-score, 2.22), protein targeting (z-score, 2.35), and protein folding (chaperones; z-score, 2.30).

Genes involved in DNA replication and repair included MLH1, PCNA, replication factor C, nuclease assembly protein 1, origin recognition complex, and topoisomerase II. Importantly, each gene in this category was negatively correlated with 5-FU response, indicating higher expression levels in 5-FU resistant cells. Increased expression of topoisomerase II in 5-FU-resistant cells is consistent with a previous report in vivo (63). The second functionally related group of genes enriched for expression were those involved in protein processing and trafficking, including several chaperones. As for DNA replication and repair, the majority of these sequences were negatively correlated with 5-FU-induced apoptosis. Genes in this category included chaperonin containing TCP1 subunits 4 and 8; lectin mannos-binding 1, heat shock 70kDa protein 8, nucleophosmin, and hypoxia up-regulated 1. Chaperones protect cells from environmental stress by binding denatured proteins, dissociating protein aggregates, and regulating the correct folding and intracellular translocation of newly synthesized polypeptides (64). High basal levels of expression of these genes may enhance a cell’s ability to survive after 5-FU-induced genotoxic stress. Consistent with this role, nucleophosmin is up-regulated in colorectal carcinoma (65), is translocated from the nucleolus to the nucleoplasm after treatment with anticancer drugs (66), and has been associated with resistance to UV radiation-induced apoptosis (67).

We also identified three proapoptotic genes (Bak, TSSC2, and DAPK1) whose expression was positively correlated with 5-FU response, suggesting that their respective gene products may play a role in 5-FU-induced apoptosis. We chose to further explore the role played by Bak for two reasons. First, it is well established that proapoptotic members of the bcl-2 family, such as Bak, translocate from a predominantly cytoplasmic localization to mitochondria, where they trigger apoptosis through a mechanism dependent on release of cytochrome c (68). Second, Bak has previously been shown to be up-regulated in colon cancer cell lines treated with 5-FU (69).

Subcellular localization of Bak was examined with and without 5-FU treatment in four cell lines (RKO, HCT116, RW2982, and HCC2998) by immunofluorescence. Representative photomicrographs for the RKO cell line are shown in Fig. 3. In all cell lines examined, basal Bak expression was low and diffusely distributed. For the RKO cell line, treatment with 5 μM 5-FU for 24 h resulted in intense punctate staining for Bak in approximately 5% of cells (Fig. 3, B and D, white arrows). This was associated with its localization to the mitochondrion, as indicated by the overlap of Bak staining with the mitochondrial marker HSP60 (Fig. 3B, yellow arrow). Co-staining of 5-FU-treated cells for Bak and cytochrome c demonstrated that
Fig. 2. A, response of panel of 30 cell lines to 5-FU-induced apoptosis. Cells were treated with 5 μM 5-FU for 72 h, and apoptosis was determined by propidium iodide staining and fluorescence-activated cell-sorting analysis. B, clonogenicity of colon carcinoma cell lines after 5-FU treatment. Cell lines were treated with 5, 50, or 500 μM 5-FU for 9 h and reseeded in fresh medium, and colony formation was determined. Examples of four cell lines are shown. C, response of colon carcinoma cell lines to 5-FU-induced growth inhibition (GI50). Examples of response of 8 of the 30 cell lines are shown.
Table 2a  
Genes correlated with 5\(\mu\)M 5-FU-induced apoptosis

<table>
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<th>Accession</th>
<th>Correl</th>
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<td>EST*</td>
<td>Unknown</td>
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<td>AA022679</td>
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<td>0.58</td>
<td>TSSC3 (tumor supp. subtrans. cand 3)*</td>
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Basal gene expression ratios were correlated with 5 \(\mu\)M 5-FU-induced apoptosis across the panel of 30 colon carcinoma cell lines, and 420 significantly correlated genes identified. Values shown are the Pearson’s correlation coefficient (Correl) between basal gene expression and apoptosis induced by 5 \(\mu\)M 5-FU. *Gene also significantly correlated (in the same orientation) with 1 \(\mu\)M CPT-induced apoptosis. *Microarray data validated by Real-Time PCR (\(r > 0.65, P < 0.005\) for correlation of microarray and RT-PCR data).
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Basal gene expression ratios were correlated with 5 μM 5-FU-induced apoptosis across the panel of 30 colon carcinoma cell lines, and 420 significantly correlated genes identified. Values shown are the Pearson's correlation coefficient (Correl) between basal gene expression and apoptosis induced by 5 μM 5-FU. *Gene also significantly correlated (in the same orientation) with 1 μM CPT-induced apoptosis. +Microarray data validated by Real-Time PCR (r > 0.65, P < 0.005 for correlation of microarray and RT-PCR data).
mitochondrial Bak translocation was linked to diffuse cytoplasmic localization of cytochrome c, indicative of its release from the mitochondrion (Fig. 3D, cytochrome c, white arrows). In contrast, in untreated cells, cytochrome c staining was always punctate; co-staining with HSP60 indicated that this was due to its mitochondrial localization (data not shown). Quantitation of this event demonstrated that a 24-h exposure to 5-FU induced a concentration-dependent increase in the number of RKO cells demonstrating simultaneous Bak translocation and cytochrome c release, compared with untreated cells ([0.8 ± 0.4, 3.3 ± 0.6, and 8.7 ± 2.1/200 cells counted for 0, 5, and 50 μM 5-FU, respectively (mean ± SD); P < 0.005 for both 5 and 50 μM 5-FU compared with control (paired t test)]). Similar results were obtained for the HCT116, RW2982, and HCC2998 cell lines (data not shown). These results clearly indicate a role for Bak in mediating 5-FU-induced apoptosis and serve as validation for the array data.

Finally, it is noteworthy that expression of methylenetetrahydrofolate dehydrogenase, a gene involved in folate metabolism, was negatively correlated with the induction of apoptosis after 5-FU treatment (r = -0.46; Table 2). Methylenetetrahydrofolate dehydrogenase converts 5,10-methylene tetrahydrofolic acid (CH₂-FH₄) to 5,10-methyl methyl tetrahydrofolate. Because CH₂-FH₄ is required for the formation of the TS ternary complex by 5-fluoro-dUMP (an active metabolite of 5-FU), it follows that lower levels of an enzyme that could reduce the levels of CH₂-FH₄ would enhance the cytotoxic actions of 5-FU. Increased methylenetetrahydrofolate dehydrogenase has also been reported in 5-FU-resistant gastric tumor cell lines (70), and it is also of interest that genetic polymorphisms that reduced the activity of methylenetetrahydrofolate reductase, an enzyme that also utilizes CH₂-FH₄ as a substrate, were linked to improved response to 5-FU among patients with advanced colorectal cancer (71).

**Predictive Value of Genes Correlated with 5-FU Response.** The concept behind gene profiling is that expression levels of multiple genes considered together may better predict phenotype than measurement of single markers. We hypothesized that gene expression profiling would therefore be a more effective means of predicting response to 5-FU than conventional single marker approaches. To determine whether this was the case for apoptotic response to 5 μM 5-FU, a “leave one out” or jackknife cross-validation approach was...
GENE PROFILING-BASED PREDICTION OF CHEMOSensitivity

Fig. 4. Gene expression profiling-based prediction of 5-FU-induced apoptosis. A, Pearson’s correlation between observed and predicted apoptotic response for a given cell line versus the predicted value for the 30 jackknife calculations. For this analysis, the Pearson’s correlation coefficient between observed and predicted apoptosis was 0.47 (P = 0.008), demonstrating that selection of the 50 genes best correlated with 5 μM 5-FU response had excellent predictive value. In contrast, derivation of a predictor based on 50 randomly selected genes resulted in poor correlation between observed and predicted apoptosis (r = 0.099; P = 0.601; Fig. 4B).

Whereas selection of the 50 most highly correlated genes was highly predictive for 5-FU response, we wished to determine the effect of varying the number of input genes (N) on the predictive power. To determine the optimum value of N, we repeated this analysis, varying N from the 10 to the 200 best-correlated genes for 5 μM 5-FU-induced apoptosis, for each jackknife calculation (Fig. 4C). This analysis demonstrated that selection of anywhere from the 40–160 best-correlated genes resulted in significant correlation between observed and predicted apoptosis, with maximal prediction observed for 50 genes. In contrast, 10–200 randomly selected genes in each case failed to predict response to 5-FU (Fig. 4D).

Repition of these analyses using apoptosis induction by 5-FU at concentrations of 50 and 500 μM failed to identify gene sets capable of predicting response. However, at these higher concentrations, the continuum of apoptotic response across the panel of 30 cell lines is less pronounced because the majority of cell lines undergo significant apoptosis. In parallel, genes significantly correlated with apoptotic response tended to have less variation in expression range across the 30 cell lines and thus are less robust predictors of apoptotic response. Furthermore, except for brief periods of time after bolus administration, the 50 and 500 μM concentrations of 5-FU are 1–2 orders of magnitude greater than those achievable in vivo and may indicate that, due to toxicity, these concentrations of drug do not stimulate a complete biological response, thus decreasing the influence of a specific gene program on cellular response to this agent at these higher concentration.

Predictive Efficacy of TS and TP Activity and of p53 and MMR Status. Having demonstrated the ability of the basal gene expression profile of colon carcinoma cells to predict response to 5-FU, we compared the efficacy of this approach with four previously established determinants of 5-FU response: TS and TP activity; and p53 (72) and MMR status (73, 74).

Levels of TS and TP have previously been linked to 5-FU response, used, in which the predictive power of genes significantly correlated with 5-FU-induced apoptosis (described above) was tested. The primary objective of this statistical analysis was to develop a model that would predict level of apoptosis as a function of gene expression for multiple genes. The method used to develop this model utilized the jackknife technique (52), and its predictive value was validated on an independent observation.

In this approach, one cell line is omitted from the analysis, and a rule that predicts 5-FU response is derived based on the basal gene expression profile of the remaining 29 cell lines (see “Materials and Methods” for rule derivation). The predictive power of this rule is then tested on the cell line omitted at the start of the analysis. This process is repeated iteratively, on 30 separate occasions, with a different cell line omitted from each analysis.

Fig. 4A illustrates the result of an analysis in which the 10 PCs of the 50 genes with the highest absolute correlation with 5 μM 5-FU-induced apoptosis were used to derive the predictor. The 30 data points in the figure are the observed apoptotic response for a given cell line versus the predicted value for the 30 jackknife calculations. For this analysis, the Pearson’s correlation coefficient between observed and predicted apoptosis was 0.47 (P = 0.008), formally demonstrating that selection of the 50 genes best correlated with 5 μM 5-FU response had excellent predictive value. In contrast, derivation of a predictor based on 50 randomly selected genes resulted in poor correlation between observed and predicted apoptosis (r = 0.099; P = 0.601; Fig. 4B).

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Levels of TS and TP have previously been linked to 5-FU response,
with high and low activity of TS and TP, respectively, associated with 5-FU resistance. Measurement of TS and TP activities in the panel of 30 cell lines demonstrated that TS activity was negatively correlated with 5-FU-induced apoptosis, and TP activity was positively correlated with 5-FU-induced apoptosis, although this was not statistically significant for all concentrations of 5-FU tested (Fig. 5; Table 3). This link between low TS/high TP activity and enhanced 5-FU response is consistent with some (31), but not all, previous reports in which basal TS and TP levels in a panel of unselected cell lines have been correlated with 5-FU response (29, 30, 32). To determine the predictive efficacy of these markers on 5-FU-induced apoptosis, we used a jackknife approach similar to that used for the gene expression data.

For these analyses, however, only a single marker, basal TS or TP activity, was used to derive the rule. Prediction of apoptotic response using basal TS activity resulted in a weak correlation between observed and predicted 5-FU-induced apoptosis that was not statistically significant (r = 0.21 and P = 0.28, r = 0.07 and P = 0.70, and r = 0.23 and P = 0.23 for apoptosis induction at 5, 50, and 500 μM 5-FU, respectively; all values are log transformed). Likewise TP activity failed to predict response, except for apoptosis induction at the highest concentration of 5-FU tested (r = 0.11 and P = 0.56 and r = 0.06 and P = 0.77 for 5 and 50 μM 5-FU-induced apoptosis, respectively; and r = 0.45 and P = 0.01 for apoptosis induced at 500 μM 5-FU). Analyses for 5 μM 5-FU are shown in Fig. 6, A and B.

The relationship between p53 status of colon tumors and response to 5-FU has been examined extensively, both in vitro and in vivo, with conflicting findings reported (14, 24). The p53 status of the panel of 30 cell lines, some not reported previously, is shown in Table 1. As illustrated in Fig. 6C, no significant difference in sensitivity to 5-FU-induced apoptosis was observed between p53 WT and mutant cell lines, despite a tendency of p53 WT cell lines to be more sensitive (P = 0.12, P = 0.14, and P = 0.12 for 5, 50, and 500 μM 5-FU, respectively).

Similar to p53, conflicting reports also exist regarding the effect of tumor MMR status on 5-FU response (17, 75, 76). The MMR status of the 30 cell lines is shown in Table 1. Comparison of the effect of 5-FU-induced apoptosis in 21 MMR-proficient and 9 MMR-deficient cell lines revealed no significant difference in 5-FU-induced apoptosis at any of the concentrations of 5-FU tested (Fig. 6D).

Therefore, in summary, for the clinically relevant concentration of 5-FU (5 μM), gene expression profiling had greater predictive power than four previously reported determinants of 5-FU response.

Extension of Analysis to CPT. A limitation of using single markers to predict response to specific agents is that they do not necessarily identify sensitivity to alternate treatment options. An assay capable of determining the treatment likely to be most effective for a particular tumor, therefore, would clearly have greater clinical benefit. To test this, we extended our analyses to the topoisomerase I inhibitor CPT, an alternative chemotherapeutic agent with proven efficacy in the treatment of colon tumors nonresponsive to 5-FU (77, 78), and determined whether the microarray database could be reanalyzed to predict relative response to CPT.

As described for 5-FU, the panel of 30 cell lines was characterized for response to CPT-induced apoptosis (Fig. 7). Fig. 7 illustrates the continuum of response of the panel of 30 cell lines to 1 μM CPT-induced apoptosis. No significant differences in CPT-induced apoptosis were observed when cell lines were separated according to p53 or MMR status (data not shown). Importantly, several cell lines relatively resistant to 5-FU exhibited sensitivity to CPT, and the converse was also true. These included Colo205 (rank order of apoptotic response, 7 versus 27 for 5-FU and CPT, respectively), HT29 cl.16E (rank order of apoptotic response, 14 versus 30 for 5-FU and CPT, respectively), and LIM1215 (rank order of apoptotic response, 23 versus 2 for 5-FU and CPT, respectively).

Correlation of gene expression with sensitivity to 1 μM CPT-induced apoptosis for the 30 cell lines identified 308 significantly correlated genes. Of these, 130 correlated positively and 178 correlated negatively with CPT-induced apoptosis (Table 4). Functional group analysis revealed that this gene list was significantly enriched for genes involved in the formation of membrane channels and in drug metabolism and resistance. Five of the seven genes involved in drug metabolism and resistance were negatively correlated with CPT response or more highly expressed in resistant cell lines. These included glutathione S-transferase M1 (P = 0.078, P = 0.109, P = 0.004), ATP-binding cassette, subfamily B (MDR/TAP), member 1 (P = 0.47), heparin sulfate (P = 0.38), glutaredoxin (P = 0.47), and 3'-phosphoadenosine 5'-phosphosulfate synthase 1 (P = 0.40).

### Table 3 Correlation among TS and TP activities and 5-FU-induced apoptosis

<table>
<thead>
<tr>
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<th>5 μM-induced apoptosis (LN)</th>
<th>50 μM</th>
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<tr>
<td>TS activity (LN)</td>
<td>r = 0.327*</td>
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<td>0.072</td>
</tr>
<tr>
<td>TP activity (LN)</td>
<td>r = 0.078</td>
<td>0.109</td>
<td>0.004</td>
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</table>

 Pearson’s correlation coefficient was used when both enzyme activity and apoptosis data were normally distributed (*). Otherwise, comparisons were made using a Spearman’s correlation coefficient.
The same jackknife approach used for 5-FU was then applied to the CPT data to determine whether profiles of gene expression capable of predicting response to this agent could be identified. The results of the analyses are summarized in Fig. 8, in which selection of the 10–200 genes best correlated with CPT-induced apoptosis revealed that selection of the 149 best-correlated sequences maximally predicted response to CPT (Fig. 8A). As observed for 5-FU, 10–200 randomly selected genes failed to predict CPT response (Fig. 8B). These results clearly demonstrate that the basal gene expression profile of a cell line can be used to predict differential response to multiple chemotherapeutic agents.

Importantly, whereas notable individual variations were identified in the response of the panel of 30 cell lines to 5 μM 5-FU and 1 μM CPT, the overall continuum of response to both agents was significantly correlated (r = 0.46; P < 0.01). Therefore, despite the two agents having different mechanisms of action (antimetabolite versus topoisomerase I inhibitor for 5-FU and CPT respectively), the overall response of cell lines to these mechanistically different agents was similar. Driven by this similarity, 32% and 24% of genes significantly correlated with CPT and 5-FU response, respectively, overlapped with the other agent. This finding suggests that whereas the activity of pathways specific to the mechanism of action of individual agents is undoubtedly important in determining response to a given agent, it is the activity of these pathways in the overall context of the cells ability to undergo apoptosis that is a major determinant of sensitivity.

DISCUSSION

Objective response rates to 5-FU-based chemotherapy, administered either in an adjuvant setting or to patients with late-stage colorectal cancer, are approximately 20–30%, yet this remains the treatment of choice as initial therapy. Significant attempts have been made to identify markers that predict response to 5-FU, with particular attention paid to enzymes involved in the actions of 5-FU, including...
Table 4 Genes correlated with 1 μM CPT-induced apoptosis

<table>
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TS, TP, and dipyrimidine dehydrogenase, as well as p53 and MMR status (10–12, 15, 18). Whereas several studies have demonstrated significant predictive efficacy for these markers, other studies have contradicted these findings (24–26). In the present study, we demonstrate the ability of basal gene expression profiling to predict response to 5-FU, using a panel of 30 colon carcinoma cell lines. This study demonstrated several advantages of a gene expression profiling approach for prediction of 5-FU response. First, gene expression profiling outperformed four previously reported markers (TS and TP activity; p53 and MMR status) in predicting apoptotic response to 5-FU. Low TS and high TP expression, respectively, have previously been linked with improved sensitivity to 5-FU in vitro (31, 32). Consistent with these studies, in general, basal TS activity was negatively correlated with 5-FU-induced apoptosis, and basal TP activity was positively correlated with 5-FU-induced apoptosis. However, a jackknife analysis using TS or TP activity to predict 5-FU response demonstrated that these markers were less efficient at predicting response (r = 0.21 and P = 0.28 and r = 0.11 and P = 0.56 for TS and TP activity, respectively) than the gene expression profiling approach (r = 0.47, P = 0.008).

Likewise, no relationship between p53 and MMR status of the cell lines and response to 5-FU was observed. The lack of a significant difference in 5-FU response among p53 WT and mutant colon cancer cell lines is consistent with some previous reports in which a panel of cell lines has demonstrated that deletion of p53 from a p53 WT cell line did not result in a difference in 5-FU response among p53 WT and mutant colon cancer cell lines.

Table 4 Continued

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Gene expression ratios were correlated with 1 μM CPT-induced apoptosis across the panel of 30 colon carcinoma cell lines, and 308 significantly correlated genes were identified. *Gene also significantly correlated (in the same orientation) with 5 μM 5-FU-induced apoptosis.
(HCT116) results in marked resistance to 5-FU (79), whereas reintroduction of functional p53 into a p53 mutant colon cancer cell line significantly enhanced 5-FU-mediated cell killing (80). A similar disparity exists among in vivo studies in which some, but not others, have demonstrated improved 5-FU sensitivity in p53 WT tumors (13–15, 24). Use of an isogenic cell system has also demonstrated that MMR-deficient colon cancer cells are more resistant to 5-FU (73, 81). As for p53 status, however, studies in vivo have failed to consistently demonstrate a link between tumor MMR status and response to 5-FU (16–18, 74, 75). The present findings also reflect this lack of consistency for these markers in predicting sensitivity and support the concept that measurement of multiple, rather than single, markers may better predict 5-FU response.

A second advantage of gene expression profiling over single marker approaches is that predictors of response to each of multiple agents can potentially be determined from a single assay. In the present study, this was demonstrated for CPT, an alternative for treatment of tumors refractory to 5-FU (77, 78). Here, reanalysis of the same database used to predict response to 5-FU was able to identify a gene expression profile capable of predicting response to CPT.

For both 5-FU and CPT, a continuum of response in terms of induction of apoptosis was observed across the panel of 30 cell lines. This illustrates that simple classification of cell lines as sensitive or resistant to a given drug is a difficult process and that consideration of the relative magnitude of the response of a given cell line, or tumor, to multiple chemotherapeutic agents is likely to be a more practical approach. In this study, a jackknife cross-validation strategy demonstrated that selection of the 50 best-correlated genes with 5-FU response and the 149 best-correlated genes with CPT response maximally and significantly predicted response to each agent. Importantly, use of these gene expression profiles enables robust prediction of the magnitude of the apoptotic response to each of these agents, thereby adding an additional dimension to the predictive evaluation not afforded by dichotomous “yes” or “no” marker studies, such as p53 status.

Additionally, the ability to predict the likelihood of response to multiple agents could enhance the ability to determine whether single agents or combination therapies would be most appropriate for treatment of a specific tumor. The use of combination therapies is becoming increasingly common, and the ability to identify profiles of gene expression predictive of response to multiple agents in a given tumor could provide a basis for rational clinical decisions regarding the specific combination of therapies likely to result in maximal response and minimize avoidable toxicity.

Finally, the gene expression profiling approach identified a number of links between the mechanisms of action of chemotherapeutic agents and the likelihood of inducing a response. For example, a positive correlation between basal levels of Bak expression and sensitivity to 5-FU was identified. Furthermore, we demonstrated that 5-FU induced localization of Bak to the mitochondria, which was linked to release of cytochrome c. We also identified a significant negative correlation between the basal expression level of hypoxia inducible factor 1α (HIF1α) and sensitivity to 5-FU (Table 2). HIF1α is a transcription factor that is up-regulated under hypoxic conditions and plays a pivotal role in the adaptive response to hypoxia (82). There is evidence that hypoxia is associated with resistance to radiation therapy and chemotherapy (82), including 5-FU (83, 84). Although HIF1α is primarily regulated at the posttranslational level, transcription of HIF1α is also up-regulated under hypoxic conditions (85). It is possible that higher expression of HIF1α in 5-FU-resistant cell lines may serve as a surrogate marker of cellular redox status and, subsequently, sensitivity to 5-FU.

This study therefore demonstrates that the basal gene expression profile of a tumor can be used to predict probability of response to multiple chemotherapeutic options and can provide significant insight into underlying mechanisms. Our immediate challenge is to use similar analyses with resected tumor tissue or biopsy specimens. Such analyses will either confirm the predictive value of the gene sets for response to 5-FU and CPT or identify variations of the gene sets that may better predict clinical response. Collection of such gene expression/clinical data is ongoing at our institution. Because there are multiple strategies for analyzing the data, and there may be other investigators who have begun to accumulate gene expression data on colon cancer patient response and outcome to these as well as to other drugs, the entire gene expression data set for the 30 colon carcinoma cell lines is made available on our web site.5

Finally, in addition to gene expression profiling, considerable advances have now been made in other high-throughput profiling technologies, including mutation screening (Single Nucleotide Polymorphism analysis and complete genome hybridization), and proteomics. Combination of predictive gene sets identified by gene expression profiling with these methodologies may enhance the prediction of tumor response to chemotherapy and provide further insights into the molecular characterization of tumor cells.

ACKNOWLEDGMENTS

We thank Dr. Geoff Childs and Aldo Massimi for the printing and scanning of cDNA microarrays, Dr. Lauri A. Aaltonen and Päivi Laiho for assistance with determination of the MMR status of cell lines, Dr. Robert Whitehead for provision of LIM1215 and LIM2405 cell lines, Noa Cohen for technical assistance, and Drs. Anna Velcich and John Greally for valuable advice regarding the preparation of the manuscript.

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Gene profiling-based prediction of chemosensitivity


Gene Expression Profiling-Based Prediction of Response of Colon Carcinoma Cells to 5-Fluorouracil and Camptothecin

John M. Mariadason, Diego Arango, Qiuhu Shi, et al.


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