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# Gene expression profile of metastatic colon cancer cells resistant to cisplatin-induced apoptosis

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**Abstract.** The current chemotherapeutic modalities for advanced colorectal cancer are limited. DNA-platinating drugs such as cisplatin have poor efficacy against this malignancy. The aim of this study was to identify genes that render survival advantage after cisplatin treatment in metastatic colon cancer. Cell lines SW480 (primary colon cancer) and SW620 (metastatic lesion from the same patient) were obtained from ATCC. Apoptosis was measured by FACS analysis of cisplatin-treated (0.01-10 µg/ml) and untreated cells. Simultaneous analysis of ~1200 cDNAs was performed by microarray technique on untreated and treated cells from lines. Microarray results were confirmed by RT-PCR. The SW620 cell line was more resistant to apoptosis induced by cisplatin. Western blot analysis revealed equal expression of pro-caspases 3, 8, and 9 in both cell lines. Microarray analysis identified 15 genes and 9 expressed sequence tags (ESTs) significantly altered both by cell type (metastatic vs. non-metastatic) and treatment vs. non-treatment. Several of these transcripts are well-characterized genes including MCT, GAD67, P19, GSTM3, Cyclin D1, ATM, and CO-029 that have been implicated in various malignancies. In the present study, we have identified a set of genes responsible for apoptosis resistance following treatment with cisplatin in the late stages of carcinogenesis. Targeting these genes may increase chemotherapy effectiveness in advanced colon cancer and reduce toxicity in normal tissue.

## Introduction

Metastatic colon cancer is the result of accumulation of a series of genetic alterations in the colonocyte (1). Such alterations confer survival advantage to cells by making the colonocytes resistant to death in the presence of a genotoxic

challenge and chemotherapeutic agents. Defects in apoptosis can prolong the lifespan of cells, which in turn contribute to neoplastic cell expansion independent of cell division. An important mechanism of action of the main chemotherapeutic agent for the treatment of advanced colon cancer, 5-fluorouracil (5-FU), is by selective induction of apoptosis in colon cancer cells while sparing normal mucosa (2).

Other chemotherapeutic agents such as *cis*-diamminedichloroplatinum (II) (cisplatin or CDDP) have been used in colorectal cancer. However, their efficacy either alone or in combination for the treatment of this malignancy is disappointingly low (3-5). CDDP is a chemotherapeutic agent that induces apoptosis by DNA-platination, or formation of cisplatin-DNA adducts (6). This DNA damage induces a mitochondrial response and the release of pro-apoptotic elements that result in the activation of caspases, which are the central mediators of apoptosis (7). The observed resistance of colon cancer cells to CDDP is poorly understood.

With the advent of large-scale analysis of gene expression, many genes can be studied following pharmacological interventions. Microarray analysis is emerging as a powerful technique to study thousands of genes simultaneously in a single experiment. On GeneChip oligonucleotide arrays (Affymetix, Inc., Santa Clara, CA), a given gene is represented by 15-20 different 25-mer oligonucleotides that serve as unique, sequence-sequence detectors. An additional control element on these arrays is used as a mismatch sequence (MM). These are probes that are designed to be complementary to the reference sequence except for a homomeric base mismatch in the central position. The presence of the mismatched oligonucleotide allows cross-hybridization and local background to be estimated and subtracted from the perfect match (PM) signal. In the GeneChip expression assay, eukaryotic mRNA is converted to biotinylated cRNA from oligo-dT-primed cDNA (8). Each sample is hybridized to a separate array. Transcript levels are calculated by reference to cRNA spikes of known concentration added to the hybridization mixture. Differences in mRNA levels between samples are determined by comparison of any two hybridization patterns produced on separate arrays of the same array type.

An *in vitro* model for the study of the late events of colon carcinogenesis has been characterized and validated to represent a model for colon cancer progression (9). This

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model consists of two cell lines derived from the same patient at different stages of tumor progression. The SW480 cell line was derived from a primary lesion (Dukes stage B colon carcinoma) from a 50-year old Caucasian male patient. The SW620 cell line was cultured from a lymph node metastasis in the same patient at a later time (10,11). Since these two cell lines are from the same patient, it is likely that differences between the two represent changes that occur when cancer cells acquire metastatic potential. Thus, this system represents a unique model for the study of cell survival advantage in colon cancer progression.

Because loss of apoptotic capacity is associated with the development of malignant cancers, apoptosis induction and enhancement is a possible approach for cancer treatment. The aim of the present study was to investigate the genes that contribute to resistance to cisplatin-mediated apoptosis in an *in vitro* model of colon carcinogenesis by microarray analysis.

## Materials and methods

**Colon cancer cell lines.** The following two human colon cancer cell lines were obtained from the American Type Culture Collection (Rockville, MD): i) SW620 (colon adenocarcinoma, lymph node metastasis CCL-227 passage no.: 83); and ii) SW480 (colon adenocarcinoma CCL-228 passage no.: 96). These cell lines were grown according to ATCC protocol, including the recommended medium for propagation containing 10% fetal bovine serum (FBS) as previously described (12,13).

**Cell synchronization.** Cells were synchronized by FBS starvation for 24 h. Following a 24-h starvation period with no FBS present in the medium, cell growth was reactivated by incubation in medium containing 10% FBS. Cells grown to confluence were detached by mild trypsinization for RNA isolation.

**Propidium iodide-based flow cytometric DNA fragmentation assay.** Apoptosis was determined by DNA staining with propidium iodide (PI). Briefly,  $2 \times 10^6$  adherent cells were detached by trypsinization. Detached cells and the floating dead cells were centrifuged and washed twice with 1 ml cold 1X PBS (Life Technologies). Supernatant was aspirated and 1 ml cold 75% ethanol was added and cells were incubated at  $-20^\circ\text{C}$  for 1 h. Thereafter, the cells were washed with 1 ml of 1X PBS twice. After the last wash, 100  $\mu\text{l}$  of PI solution (50  $\mu\text{g}/\text{ml}$  PI + 0.05 mg/ml RNase A) (Sigma, St. Louis, MO) was added and the cells were incubated protected from light at room temperature for at least 2 h prior to analysis. DNA analysis was performed using fluorescence channel 3 in an Epic XL flow cytometer (Coulter Electronics, Inc., Miami, FL).

For analysis of apoptosis, cells (SW480 and SW620) were treated with cis-platinum (II) diamine dichloride (CDDP) (Sigma, St. Louis, MO) at the following concentrations: 0.01  $\mu\text{g}/\text{ml}$ , 0.1  $\mu\text{g}/\text{ml}$ , 1  $\mu\text{g}/\text{ml}$ , 5  $\mu\text{g}/\text{ml}$ , and 10  $\mu\text{g}/\text{ml}$  for 24 h.

**Western blot analysis.** Colon carcinoma cell lines were incubated for 48 h in the presence or absence of 10  $\mu\text{g}$  of

CDDP. The cells were then detached by trypsin treatment and lysed at  $4^\circ\text{C}$  in RIPA buffer [50 mM Tris-HCl (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, supplemented with one tablet of protease inhibitor cocktail, Complete Mini (Roche, Indianapolis, IN)]. The cell lysates (40  $\mu\text{g}$ ) were then electrophoresed on 15% SDS-PAGE (Bio-Rad, Hercules, CA) and were subjected to Western blot analysis. The proteins were transferred from the gels onto Hybond nitrocellulose membranes (Amersham, Arlington Heights, IL) in a semi-dry transblotting cell (Bio-Rad, Hercules, CA). The membranes were blocked for 1 h at room temperature with 5% non-fat skim milk/PBS and then incubated with the respective antibody for 1 h at room temperature. Rabbit anti-caspase-3 polyclonal antibody was purchased from PharMingen (San Diego, CA). Mouse anti-caspases-8 and -9 polyclonal antibodies were purchased from Cell Signalling (New England Biolabs, Beverly, MA). After washing with PBS/0.1% Tween-20 twice, the membranes were incubated for 30 min with horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG Ab (New England Biolabs, Beverly, MA). After washing with TBS/0.1% Tween-20 three times, the membranes were developed with a Lumiglo Western blot detection kit (New England Biolabs, Beverly, MA).

## Microarray analysis

**Target preparation.** SW480 and SW620 cell lines were left untreated or treated with 0.1  $\mu\text{g}/\text{ml}$  cisplatin for four hours and total RNA was extracted from frozen cell pellets using a Qiagen RNeasy Isolation Kit (Qiagen Inc., Valencia, CA). Five micrograms of total RNA were transcribed to cDNA using a T7-(dT)<sub>7</sub> oligomer (Genset Inc., La Jolla, CA) and a Superscript Choice System (Gibco-BRL, Rockville, MD). Double-stranded cDNA was purified by phenol-chloroform extraction with Phase-Lock Gel (Fisher Scientific, Pittsburgh, PA). Biotin-labeled cRNA was synthesized *in vitro* with an Enzo BioArray RNA Transcription Labeling Kit (Affymetrix Inc., San Jose, CA). Synthesized cRNA was purified with an affinity resin column (Qiagen Inc., Valencia, CA). Ten to fifteen micrograms of cRNA was fragmented by incubating at  $86^\circ\text{C}$  for 30 min in 40 mM Tris-acetate (pH 8.1), 100 mM potassium acetate, and 30 mM magnesium acetate.

**Microarray.** Microarray analysis was performed using human microarray chips HG-U95A (Affymetrix Inc., San Jose, CA). The Human Genome U95 Set contains the most comprehensive transcript coverage of the human genome. This set evaluates the expression level of >60,000 human genes and ESTs. The U95A Array contains all full-length genes. This single array represents ~12,000 sequences that have been previously characterized in terms of function or disease association (8).

Five micrograms of cRNA, 1 microgram of cRNA control cocktail, and 0.1 mg/ml of sonicated herring sperm DNA were added to the hybridization buffer containing 100 mM MES, 1 M [Na], 20 mM EDTA, 0.01% Tween-20, and 0.5 mg/ml acetylated BSA. The hybridization mixture was heated to  $99^\circ\text{C}$  for 5 min, followed by incubation at  $45^\circ\text{C}$  for 16 h with mixing on a rotisserie at 60 rev/min. Following hybridization, the solution was removed and automated washing and staining were performed with a stringent buffer [100 mM

MES, 0.1 M (Na), 0.01% Tween-20], a non-stringent buffer (6 SSPE, 0.01% Tween-20, 0.005% Antifoam), streptavidin-phycoerythrin (SAPE) stain solution [10 µg/ml SAPE, 2 µg/µl acetylated BSA, 100 mM MES, 1 M (Na), 0.05% Tween-20, 0.005% Antifoam], and antibody solution [3 µg/ml acetylated BSA, 100 mM MES, 1 M (Na), 0.05% Tween-20, 0.005% Antifoam]. Following washing and staining cycles, the probe array was scanned twice at 3 µm resolution using the HP GeneArray scanner (Hewlett-Packard, Palo Alto, CA). The Gene Chip Eukaryotic Hybridization Control Kit was used as a standard to estimate the abundance of RNA in the samples.

**Reverse transcriptase polymerase chain reaction (RT-PCR).** The genes extracted from microarray analysis were searched by accession number in the GenBank Entry data base and primers were designed from the cDNA sequence for each specific gene (as shown in Table I). RT-PCR was performed as previously described (12,13). Briefly, SW480 and SW620 cell lines were left untreated or treated with 0.1 µg/ml cisplatin for four hours and total RNA was extracted from frozen cell pellets using a Qiagen RNeasy Isolation Kit. Random hexamers (Gibco-BRL, Rockville, MD) were used for priming first-strand cDNA. Synthesis of cDNA was performed using a Superscript Choice System (Gibco-BRL, Rockville, MD). To amplify the various PCR products, we used the primers shown in Table I. The positive control was amplified by PCR using primers specific for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward: TTT GGT ATT GAA GAG GGC CT; reverse: ATT AAA GCC AAA GTA AAA GC). A negative control for the RT-PCR was included by running parallel reactions for each cDNA with GAPDH control primers and exclusion of the reverse transcriptase enzyme. PCR conditions were: 94°C for 30 sec, 52-55°C for 30 sec, and 72°C for 30 sec for 30 cycles. PCR products were separated on a 2% agarose gel containing ethidium bromide and visualized under UV light. The intensity of each band was evaluated by an image analyzer (Bio-Rad, Burlington, MA) and the intensity of each experimental primer for the cisplatin-treated cell line (SW620) was compared to that of the untreated parent cell line (SW480).

#### Statistical methods

**Effects of CDDP treatment on apoptosis induction.** Treatment effects on apoptosis in cell line SW480 vs. SW620 at the various concentrations of CDDP were evaluated by Student's t-test. Data are expressed as means ± SEM. All values are considered statistically significant at  $P \leq 0.05$ .

**Microarray data acquisition and analysis.** The microarray was scanned and the image files loaded into the Dchip software package (14). This package computes two summary measures for each gene. The first measure is the model-based expression value that summarizes the approximate expression level over the 20 individual probes. The second measure is the present/absent call. This call is a decision rule utilizing a number of details of the probe pairs to determine if there was or was not any mRNA corresponding to that particular gene in the sample. Genes were considered to have significant differential expression if they met the following criteria: i) a

significant main effect in the ANOVA model; ii) a fold change greater than 2 in one of the comparisons. P-values for the ANOVA coefficients are based on two-sided tests and have not been adjusted for multiple comparisons. A threshold was considered significant at  $P \leq 0.05$  for each of the effects (i.e. cell type and treatment).

## Results

**Apoptosis induction with cisplatin.** To evaluate the effects of cisplatin on colon carcinoma cell lines, cells were either left untreated or treated with various concentrations of cisplatin (0.01-10 µg/ml) for 24 h. The SW620 cell line exhibited a smaller proportion of cells undergoing apoptosis than the SW480 cell line at baseline (35±1.1% fewer apoptotic cells;  $P \leq 0.05$ ). Cisplatin treatment resulted in a concentration-dependent induction of apoptosis in both cell lines. However, the SW620 cell line was consistently more resistant to apoptosis induced by cisplatin than the SW480. The maximum effect of 35±3% increase in apoptotic cells relative to untreated cells was observed at 10 µg/ml of cisplatin compared to 22±0.1% in the SW620 cell line ( $P \leq 0.05$ ). The greatest difference was observed at 1 µg/ml with a differential degree of apoptosis relative to untreated cells of 65% ( $P \leq 0.05$ ). Cisplatin treatment resulted in a 34±3.0% ( $P \leq 0.05$ ) induction of apoptosis in the SW480 cell line at 10 µg/ml and 21±0.1% ( $P \leq 0.05$ ) in the SW620 cell line at the same concentration (Fig. 1). These results demonstrate that the SW620 cells are more resistant to apoptosis at baseline and with cisplatin treatment than their SW480 parental non-metastatic cell line SW480.

**Activation of caspase cascade by cisplatin.** Once we demonstrated that cisplatin was capable of apoptosis induction in our cells, we evaluated the effects of treatment on the caspase cascade in the SW480 and SW620 cell lines. Cells were either left untreated or treated with 10 µg/ml cisplatin for 24 h. Western blot analysis showed that both cell lines express equal amounts of pro-caspases 3, 8, and 9. Cisplatin treatment initiated equal caspase activation in both cell lines shown by the presence of the active form of these proteins (Fig. 2). These data demonstrated that the differential sensitivity of colon carcinoma cell lines to cisplatin is not at the level of the caspases and might be due to other apoptotic regulatory gene products.

**Microarray analysis.** The data set had 12,625 genes. The first step of the analysis removed genes that were absent in all four samples (2 cell lines x 2 treatments). This step eliminated 8564 genes from the analysis. Step two involved the construction of an analysis of variance (ANOVA) model. A separate ANOVA model was fit for each of the remaining 4061 genes that meet the present/absent criteria for the analysis. The ANOVA model used the expression value as the outcome measure and estimated the two main effects of treatment by cell line. There were 199 genes with significant effects in the ANOVA model. The next step involved computing the fold-change between each of the experimental conditions. A fold-change of greater than 2 or less than negative 2 was considered significant. Of the 199 genes with

Table I. Sequences for each primer obtained from GenBank.

Gene	Primer set and product size
Monocarboxylate transporter (MCT)	Forward: 5'-TTC ACT ATC GGC TTC AGC AA-3' Reverse: 5'-CCG ATG CCA CTC ATG GAC AC-3' Product: 205 bp
Human glutamate decarboxylase (GAD67)	Forward: 5'-CTA GGG GCC AAG GGA AAT GC-3' Reverse: 5'-CCT CCC ACC ACC AAG GTC CAG-3' Product: 258 bp
Human serine proteinase inhibitor (P19)	Forward: 5'-TGT GTC CCC AGA TCC CCA CT-3' Reverse: 5'-AAA GCA CAG GGT CGC CAG AG-3' Product: 275 bp
Human glutathione transferase M3 (GSTM3)	Forward: 5'-ACA TCG CTC GCA AGC ACA AC-3' Reverse: 5'-ATC CAG GCA CTT GGG GTC AA-3' Product: 292 bp
Human subunit C of V-ATPase (vat C)	Forward: 5'-TGG CTT GCC AGT GAA CTT CC-3' Reverse: 5'-ACA GGA TTG TCG GGG AAG GAG-3' Product: 247 bp
<i>Homo sapiens</i> mRNA for translocation protein-1	Forward: 5'-ACA GGA TTG TCG GGG AAG GAG-3' Reverse: 5'-TGA AGC CCA CAT CAG CAG TCA-3' Product: 202 bp
Human cyclin D (cyclin D1)	Forward: 5'-GCA GTA GCA GCG AGC AGC AG-3' Reverse: 3'-CTA CAC CGA CGG CTC CAT CC-3' Product: 541 bp
Human ataxia-telangiectasia locus protein (ATM) gene	Forward: 5'-TGC AGG CAC ATG CTA CCA CA-3' Reverse: 5'-TGC CCC GAT TCA ATC TCA CA-3' Product: 301 bp
<i>Homo sapiens</i> apoptosis-related protein TFAR15 (TFAR15)	Forward: 5'-CGA CCA GAG CCA GAA TTC CAA-3' Reverse: 5'-TGC CCT GCG GTT CTG GTA TT-3' Product: 194 bp
Human CO-029	Forward: 5'-GCA GGT GGC GAC AGG TAT CC-3' Reverse: 5'-TCC CCA ATC AGC AGC TCC AT-3' Product: 192 bp
Human surface antigen (ESA)	Forward: 5'-TGG TTT CAG GGG GCT GTT GT-3' Reverse: 5'-TCG GCC GTC TCT ACG TCC TC-3' Product: 147 bp
<i>Homo sapiens</i> motilin gene	Forward: 5'-CTG CTC TGC TGG TGG TGC AT-3' Reverse: 5'-GGT GAA GAT GGG GAC GAA GG-3' Product: 71 bp
<i>Homo sapiens</i> mRNA for ASM-like phosphodiesterase 3b	Forward: 5'-CTG CTC GCC TGG CTG ATT TT-3' Reverse: 5'-AGC TTG GTC AGG CGT TCC AC-3' Product: 346 bp
Human interferon regulatory factor 5 (Humirf5)	Forward: 5'-AAG AAT GGC CTG ACC GCA AA-3' Reverse: 5'-CAT GCC AGC TGG GTG CAT AG-3' Product: 289 bp
<i>Homo sapiens</i> mRNA for IFN-inducible $\gamma$ 2 protein	Forward: 5'-TCA AAG CAC CGC CAC CTG TA-3' Reverse: 5'-CCA GCC AAA GGC TCC ACT GT-3' Product: 228 bp

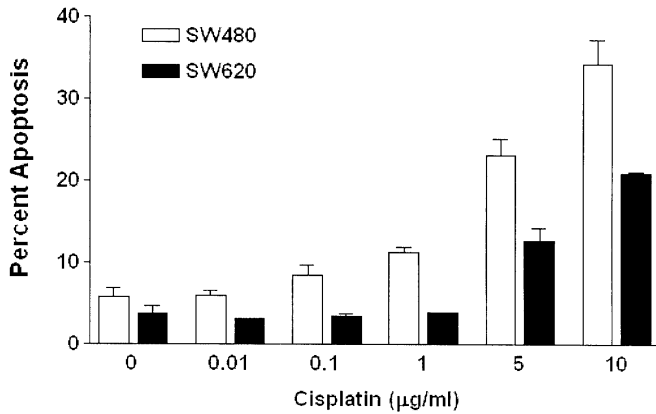


Figure 1. Effects of cisplatin treatment on induction of apoptosis in two colon cancer cell lines. Apoptosis was measured by PI staining in triplicate samples. The SW620 cell line is more resistant to apoptosis than the SW480 cell line at baseline. Cisplatin treatment resulted in a concentration-dependent induction of apoptosis. However, the SW620 cell line is more resistant at all concentrations of cisplatin. Data are expressed as means  $\pm$  SEM. Difference in treatment effects was considered significant at  $P \leq 0.05$ .

Table II. ANOVA analysis of differential expression of genes identified by microarray analysis that were significant in a 2x2 filtered design with treatment and cell type as main effects.

	Treatment		
	Significant	Non-significant	
Cell type			
Significant	24	45	70
Non-significant	8	0	7
Total	32	45	77

Thirty-two genes are significantly affected by treatment, some of which may also be significantly altered by cell type effect. Similarly, 70 genes are significantly altered by cell type effect, some of which may also be altered by treatment effect. Only 24 genes are significantly affected with respect to both parameters. Effects were considered statistically significant at  $P \leq 0.05$ .

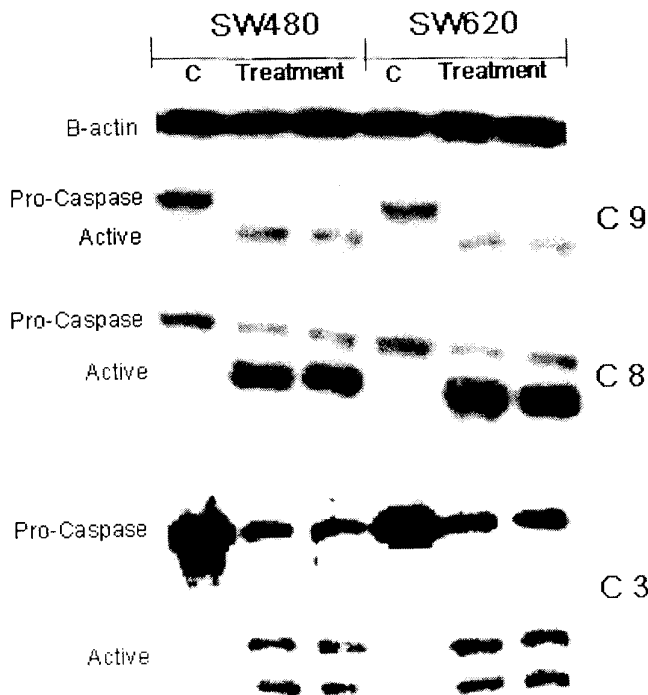


Figure 2. Effect of cisplatin treatment (10 µg/ml) on expression of caspase proteins shown by Western blot analysis. Pro-caspases 3, 8, and 9 are equally present in both cell lines relative to  $\beta$ -actin. Similarly, activated caspases are equally present in both cell lines relative to control following cisplatin treatment.

genes, most of which clustered to genes involved in cellular metabolism (Table III).

**RT-PCR.** The microarray results for all 15 genes were confirmed by RT-PCR. Consistent with microarray data, 12 out of 15 genes demonstrated differential gene expression by RT-PCR. Three genes, however, did not show difference in gene expression (Table IV).

## Discussion

A balance between cell renewal and apoptosis is crucial in regulating the size and structure of tissues (15). Tumor growth depends not only on the rate of proliferation, but also on the rate of apoptosis. Loss of the ability of colonocytes to die in response to a genotoxic challenge may be an important indicator of propensity for malignant transformation. In the present study, we demonstrated that a metastatic colon cancer cell line (SW620) was more resistant to apoptosis than its non-malignant parent cell (SW480) after treatment with cisplatin. This finding is similar to other observations where resistance to apoptosis in the SW620 cell line relative to the SW480 cell line via the death receptor pathway has also been reported (9). Additionally, we have also demonstrated that the SW620 cell line is more resistant to apoptosis following treatment with the anti-apoptotic herbal mixture PC-SPES as determined by the TUNEL method (16). Given the previous observations, we found that for this study, FACS analysis of apoptosis by DNA content was sufficient to establish a dose-response effect following cisplatin treatment, which was observed in this experiment.

Since the caspases are the central mediators of apoptosis, we investigated the effect of cisplatin in both of the cell lines following cisplatin treatment. In the present study, we found that pro-caspases 3, 8 and 9 were equal in both cell lines. Activation of the caspase cascade was observed with high concentrations of cisplatin (10 µg/ml). The results presented

significant ANOVA effects, 77 had significant fold-changes (Table II).

Of the 77 genes, there were 24 genes whose expression changes were statistically significant both relative to cisplatin treatment and cell type (metastatic vs. non-metastatic). Nine of these genes were ESTs and 15 were well-characterized

Table III. Genes demonstrating a significant change between cell type and treatment by microarray analysis.

Accession no.	Gene	Function
U59299	<i>Homo sapiens</i> putative monocarboxylate transporter MCT mRNA	
M81883	Human glutamate decarboxylase (GAD67) mRNA	
U71364	Human serine proteinase inhibitor (P19) mRNA	
J05459	Human glutathione transferase M3 (GSTM3) mRNA, complete cds	Cellular metabolism
J05682	Human subunit C of V-ATPase (vat C) mRNA	
D87127	<i>Homo sapiens</i> mRNA for translocation protein-1 Guanine nucleotide-binding protein Hsr1	
M64349	Human cyclin D (cyclin D1) mRNA, complete cds	Cell cycle
U67092	Human ataxia-telangiectasia locus protein (ATM) gene	
AF022385	<i>Homo sapiens</i> apoptosis-related protein TFAR15 (TFAR15)	Apoptosis
M35252	Human CO-029	Cell adhesion
M60922	Human surface antigen (ESA) mRNA	
X15393	<i>Homo sapiens</i> motilin gene exon 2 (and joined CDS)	Cell motility
Y08134	<i>Homo sapiens</i> mRNA for ASM-like phosphodiesterase 3b	Syringomyelin synthesis
U51127	Human interferon regulatory factor 5 (Humirf5) mRNA	Immunology
X59892	<i>Homo sapiens</i> mRNA for IFN-inducible $\gamma$ 2 protein	Translation
D50917	Human mRNA for KIAA0127 gene	
AF091080	<i>Homo sapiens</i> clone 614 unknown mRNA	
W32483	<i>Homo sapiens</i> cDNA, 5' end/clone	
D14661	Human mRNA for KIAA0105 gene, complete cds	
AB018264	<i>Homo sapiens</i> mRNA for KIAA0721 protein	EST
AB028949	<i>Homo sapiens</i> mRNA for KIAA1026 protein	
AL050172	<i>Homo sapiens</i> mRNA; cDNA DKFZp586F1322 (from clone DKFZp586F1322)	
AI597616	<i>Homo sapiens</i> cDNA, 3' end/clone	
AL022318	Putative novel protein similar to APOBEC1	

Table IV. Differential gene expression of the cisplatin treated metastatic cell line (SW620) compared to its untreated parent cell line (SW480).

Gene	Cisplatin-treated SW620
<i>Homo sapiens</i> putative monocarboxylate transporter MCT mRNA	↑
Human glutamate decarboxylase (GAD67) mRNA	↑
Human serine proteinase inhibitor (P19) mRNA	↑
Human glutathione transferase M3 (GSTM3) mRNA, complete cds	↑
Human subunit C of V-ATPase (vat C) mRNA	↑
<i>Homo sapiens</i> mRNA for translocation protein-1	↑
Human cyclin D (cyclin D1) mRNA, complete cds	↑
Human ataxia-telangiectasia locus protein (ATM) gene	↑
<i>Homo sapiens</i> apoptosis-related protein TFAR15 (TFAR15)	↓
Human CO-029	↑
Human surface antigen (ESA) mRNA	↑
<i>Homo sapiens</i> motilin gene exon 2 (and joined CDS)	↑
<i>Homo sapiens</i> mRNA for ASM-like phosphodiesterase 3b	↓
Human interferon regulatory factor 5 (Humirf5) mRNA	↑
<i>Homo sapiens</i> mRNA for IFN-inducible $\gamma$ 2 protein	↑

Increased expression relative to expression in untreated SW 480 is depicted by an upward arrow and decreased expression by a downward arrow.

in Fig. 2 demonstrate that the differential sensitivity to cisplatin-induced apoptosis exhibited by SW480 and SW620 cells is not due to differential levels of caspases.

Other investigators have demonstrated that the resistance to apoptosis in colon cancer cell lines SW480 versus SW620 following cisplatin treatment is not related to DNA mismatch repair enzymes (5). Therefore, having excluded the involvement of the major caspases and DNA mismatch repair in the mechanism of resistance to apoptosis in these cell lines, we elected to undertake simultaneous analysis of several thousand genes following induction of apoptosis with cisplatin, which is a well-characterized anticancer agent known to induce apoptosis. It is known that the efficacy of cisplatin against colon cancer is low although another chemotherapeutic agent, 5-FU, is capable of inducing apoptosis in colon cancer cells (2). Therefore, we anticipate that identification of the genes contributing to cellular resistance to apoptosis by cisplatin may lead to a better chemotherapeutic response.

In this study, we used a well-characterized *in vitro* system for colon cancer progression (9). In order to identify multiple genes participating in apoptosis, we performed microarray analysis in cisplatin-treated and untreated cell lines SW480 and SW620. An important advantage of this cell line system is that the background genetic variation is minimal because these two cell lines were derived from the same patient.

Our study analyzed differential gene expression that resulted from a system that met two criteria: genes differentially expressed in a metastatic versus a non-metastatic cell line and genes which were altered as a result of cisplatin treatment. Of 12,625 genes present in the oligonucleotide array, 24 genes met both criteria. Thus, these are the genes most likely responsible for cisplatin resistance as the cell acquires metastatic potential.

Of the 24 genes found to be differentially regulated, 15 had cDNA sequences available on GenBank. This allowed the design of primers for RT-PCR which confirmed the differential expression observed by microarray analysis for all but three genes. More sensitive methods to assess differential expression with these genes such as real-time PCR are continuing.

Our analysis demonstrated that a significant number of transcripts clustered to genes involved in cellular metabolism. Several of these genes have been shown to be important in oncogenesis. For instance, overexpression of monocarboxylate transporter (MTC1) has been shown to occur in malignancies of the central nervous system (17) and P19, a novel serine proteinase inhibitor, in lung squamous cell carcinoma (18). Similarly, glutamate decarboxylase (GAD67) activity and GABA content have been demonstrated to be increased in breast and colon neoplastic tissues (19). Glutathione transferase (GST) activity has been shown to be lower in colonocytes of control patients versus those at risk of developing colon cancer (20). In addition, overexpressed GST leads to chemotherapeutic resistance in ovarian cancer cell lines (21).

Our analysis identified two genes involved in cell cycle regulation. Both cyclin D1 and ataxia-telangiectasia (ATM) proteins have been implicated in carcinogenesis. Cyclin D1 has been shown to be overexpressed in acute leukemia in children (22) and glioblastoma cell lines (23). ATM mutations

are involved in the development of sporadic human cancers such as T-cell prolymphocytic leukemia and B-cell chronic lymphocytic leukemia (22). In addition, mutations of the ATM gene resulting from aberrant alternative splicing have been identified in colon cancer (24).

Other genes found in our analysis include genes participating in cell adhesion. Cell-cell adhesion is an important process in metastasis. We have previously characterized certain adhesion molecules in this cell line system that are expressed and others that are lost. *In vivo*, loss of cell adhesion would enable the aberrant cell to migrate in the process of metastasis (12). In the present study, we found that tetraspanning CO-029 was significantly altered. CO-029 has been shown to be overexpressed in hepatocellular carcinoma and may be an important gene product promoting tumor metastasis (25). A novel extracellular epidermal molecule, epidermal surface antigen (ESA), which is thought to play a role in intercellular epidermal adhesion, was also one of the genes differentially expressed in our analysis.

Our analysis also resulted in the identification of 9 ESTs significantly changed in our experiment. Until the genes corresponding to these ESTs are fully characterized, the significance of the EST results in cisplatin-induced apoptosis remain unclear.

In the present study, we have identified 15 genes and 9 ESTs that are significantly altered both due to cell type (metastatic vs. non-metastatic) in colon cancer progression and cisplatin treatment vs. no treatment. Because cisplatin is a potent inducer of apoptosis, these transcripts are likely to represent genes responsible for resistance to apoptosis in malignant colon cancer. Identification of these genes may allow further diagnosis and therapeutic treatment approaches for metastatic colon cancer. Thus, interventions with other chemotherapeutic DNA-platinating agents such as cisplatin or oxaliplatin either alone or in combination may prove to be efficient against advanced colon cancer. These genes may also provide insight into the biology of colon cancer and the process of metastasis.

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## References

1. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM and Bos JL: Genetic alterations during colorectal-tumor development. *N Engl J Med* 319: 525-532, 1988.
2. Rigas A, Dervenis C, Giannakou N, Kozoni V, Shiff SJ and Rigas B: Selective induction of colon cancer cell apoptosis by 5-fluorouracil in humans. *Cancer Invest* 20: 657-665, 2002.
3. Grem JL, McAtee N, Balis F, Murphy R, Venzon D, Kramer B, Goldspiel B, Begley M and Allegra CJ: A phase II study of continuous infusion 5-fluorouracil and leucovorin with weekly cisplatin in metastatic colorectal carcinoma. *Cancer* 72: 663-668, 1993.
4. Planting AS, van der Burg ME, van den Bent MJ, Boer-Dennert M, Stoter G and Verweij J: Phase II study of a short course of weekly high-dose cisplatin combined with long-term oral etoposide in metastatic colorectal cancer. *Br J Cancer* 73: 1265-1267, 1996.



5. Sergent C, Franco N, Chapusot C, Lizard-Nacol S, Isambert N, Correia M and Chauffert B: Human colon cancer cells surviving high doses of cisplatin or oxaliplatin *in vitro* are not defective in DNA mismatch repair proteins. *Cancer Chemother Pharmacol* 49: 445-452, 2002.
6. Kartalou M and Essigmann JM: Mechanisms of resistance to cisplatin. *Mutat Res* 478: 23-43, 2001.
7. Thornberry NA and Lazebnik Y: Caspases: enemies within. *Science* 281: 1312-1316, 1998.
8. Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV, Chee MS, Mittmann M, Wang C, Kobayashi M, Horton H and Brown EL: Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol* 14: 1675-1680, 1996.
9. Hewitt RE, McMarlin A, Kleiner D, Wersto R, Martin P, Tsoskas M, Stamp GW and Stetler-Stevenson WG: Validation of a model of colon cancer progression. *J Pathol* 192: 446-454, 2000.
10. Fogh J, Fogh JM and Orfeo T: One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. *J Natl Cancer Inst* 59: 221-226, 1977.
11. Leibovitz A, Stinson JC, McCombs WB III, McCoy CE, Mazur KC and Mabry ND: Classification of human colorectal adenocarcinoma cell lines. *Cancer Res* 36: 4562-4569, 1976.
12. Huerta S, Srivatsan ES, Venkatesan N, Peters J, Moatamed F, Renner S and Livingston EH: Alternative mRNA splicing in colon cancer causes loss of expression of neural cell adhesion molecule. *Surgery* 130: 834-843, 2001.
13. Huerta S, Srivatsan ES, Venkatesan N and Livingston EH: Human colon cancer cells deficient in DCC produce abnormal transcripts in progression of carcinogenesis. *Dig Dis Sci* 46: 1884-1891, 2001.
14. Li C and Wong WH: Model-based analysis of oligonucleotide arrays: expression index computation and outlier detection. *Proc Natl Acad Sci USA* 98: 31-36, 2001.
15. McDonnell TJ: Cell division versus cell death: a functional model of multistep neoplasia. *Mol Carcinog* 8: 209-213, 1993.
16. Huerta S, Arteaga RJ, Irwin RW, Takayuki I, Heber D and Koeffler HP: PC-SPES inhibits colon cancer growth *in vitro* and *in vivo*. *Cancer Res* 62: 5204-5209, 2002.
17. Froberg MK, Gerhart DZ, Enerson BE, Manivel C, Guzman-Paz M, Seacotte N and Drewes LR: Expression of monocarboxylate transporter MCT1 in normal and neoplastic human CNS tissues. *Neuroreport* 12: 761-765, 2001.
18. Wang T, Hopkins DA, Fan L, Fanger GR, Houghton R, Vedvick TS, Repasky E and Reed SG: A p53 homologue and a novel serine proteinase inhibitor are over-expressed in lung squamous cell carcinoma. *Lung Cancer* 34: 363-374, 2001.
19. Matuszek M, Jesipowicz M and Kleinrok Z: GABA content and GAD activity in gastric cancer. *Med Sci Monit* 7: 377-381, 2001.
20. Grubben MJ, Nagengast FM, Katan MB and Peters WH: The glutathione biotransformation system and colorectal cancer risk in humans. *Scand J Gastroenterol (Suppl.)*: 68-76, 2001.
21. Zhang F, Qi L and Chen H: Value of P-glycoprotein and glutathione S-transferase-pi as chemo-resistant indicators in ovarian cancers. *Zhonghua Zhong Liu Za Zhi* 23: 313-316, 2001.
22. Zhang L and Li J: Expression and role of cyclin D3 in childhood acute leukemia. *Zhonghua Xue Ye Xue Za Zhi* 20: 347-349, 1999.
23. Xiong Y, Connolly T, Futcher B and Beach D: Human D-type cyclin. *Cell* 65: 691-699, 1991.
24. Ejima Y, Yang L and Sasaki MS: Aberrant splicing of the ATM gene associated with shortening of the intronic mononucleotide tract in human colon tumor cell lines: a novel mutation target of microsatellite instability. *Int J Cancer* 86: 262-268, 2000.
25. Kanetaka K, Sakamoto M, Yamamoto Y, Yamasaki S, Lanza F, Kanematsu T and Hirohashi S: Overexpression of tetraspanin CO-029 in hepatocellular carcinoma. *J Hepatol* 35: 637-642, 2001.