Membrane Transporters and Channels: Role of the Transportome in Cancer Chemosensitivity and Chemoresistance

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ABSTRACT

Membrane transporters and channels (collectively the transportome) govern cellular influx and efflux of ions, nutrients, and drugs. We used oligonucleotide arrays to analyze gene expression of the transportome in 60 human cancer cell lines used by the National Cancer Institute for drug screening. Correlating gene expression with the potencies of 119 standard anticancer drugs identified known drug-transporter interactions and suggested novel ones. Folate, nucleoside, and amino acid transporters positively correlated with chemosensitivity to their respective drug substrates. We validated the positive correlation between SLC29A1 (nucleoside transporter ENTI) expression and potency of nucleoside analogues, azacytidine and inosine-glycocidaldehyde. Application of an inhibitor of SLC29A1, nitrobenzylmercaptopurine ribonucleoside, significantly reduced the potency of these two drugs, indicating that SLC29A1 plays a role in cellular uptake. Three ABC efflux transporters (ABCB1, ABCC3, and ABCB5) showed significant negative correlations with multiple drugs, suggesting a mechanism of drug resistance. ABCB1 expression correlated negatively with potencies of 19 known ABCB1 substrates and with Baker’s antifolin aminopterin. Use of RNA interference reduced ABCB1 mRNA levels and concomitantly increased sensitivity to these two drugs, as expected for ABCB1 substrates. Similarly, specific silencing of ABCB5 by small interfering RNA increased sensitivity to several drugs in melanoma cells, implicating ABCB5 as a novel chemoresistance factor. Ion exchangers, ion channels, and subunits of proton and sodium pumps variably correlated with drug potency. This study identifies numerous potential drug-transporter relationships and supports a prominent role for membrane transport in determining chemosensitivity. Measurement of transporter gene expression may prove useful in predicting anticancer drug response.

INTRODUCTION

Membrane transporters, ion exchangers, and ion channels are encoded by numerous gene families, comprising ~4% of genes in the human genome, with 406 genes encoding ion channels and 883 encoding a broad variety of transporters, of which, 350 are intracellular transporters (1). These genes and their encoded proteins—the transportome—perform important functions for the cell: they provide nutrients, remove unwanted materials, and establish electrochemical gradients across membranes (2). Numerous Mendelian disorders caused by mutations in transporter and channel genes underscore their physiological relevance (3). Membrane transporters also play key roles in pharmacology, affecting the entry of drugs into cells and extrusion of drugs from them. In particular, some ABC (ATP-binding cassette) transporters such as the multiple drug resistance transporter ABCB1 (multidrug resistance 1, P-glycoprotein) mediate energy-dependent efflux of drugs and thereby play major roles in the development of drug resistance (4, 5). Electrochemical gradients established across membranes by transporters and channels influence drug partitioning into and out of cells and cell organelles, e.g., the mitochondria (6).

Given the large number of transport molecules and potential drug substrates for them, only a very small percentage of the possible pharmacological interactions among them have been studied. Those interactions may be particularly important in the chemotherapy of cancer because the balance between beneficial and toxic effects is so finely tuned. The effectiveness of cancer chemotherapy may often depend on the relative transport capacities of normal and cancer cells. Nonetheless, a systematic study of the transportome’s roles in chemosensitivity and chemoresistance is lacking.

Robustly positive or negative gene-drug correlations can reflect a role in sensitivity or resistance. Gene-drug relationships have been studied productively in the panel of 60 human cancer cell lines (the NCI-60) used since 1990 by the United States National Cancer Institute (NCI) to screen for anticancer agents (7). For example, in a study using cDNA microarrays, (8, 9), the potency of L-asparaginase, used in the treatment of acute lymphoblastic leukemia, was shown to correlate negatively with asparagine synthetase expression in the leukemia cell subpanel. That relationship provided support for the mechanism by which L-asparaginase was thought to work. Because ovarian cell lines in the panel showed a similar negative correlation, it was hypothesized that a subset of ovarian cancer patients might benefit from L-asparaginase treatment. Analogously, a negative correlation between dihydropyrimidine dehydrogenase expression and 5-fluorouracil (5FU) activity was consistent with the mechanism of 5FU inactivation by the enzyme (9). Similar studies have been done using Affymetrix oligonucleotide arrays (10) to establish gene-drug relationships across the NCI-60. However, none of these microarray studies included more than a modest fraction of the transporter genes, and their results were not always concordant (11).

In light of the importance of the transportome, we designed and produced microarrays (70-mer oligonucleotides) that detect mRNA expression of genes encoding the transporters and channels. Here, we focus on solute carriers (SLCs), ABC transporters acting as extrusion pumps, ion transport ATPases, and select ion channel and pore families. In combination, these genes encode a majority of proteins involved in membrane transport, specifically of drugs. We then applied those arrays to a study of the NCI-60 cells and correlated the resulting expression patterns with potency data for a set of 119 anticancer agents with putatively known mechanisms of action (7). This analysis established numerous significant drug-transporter relationships. We then exploited RNA interference (12, 13) and transport inhibitors to validate three of those relationships. This study provides a basis for additional exploration of drug transport pathways and for optimizing cancer chemotherapy.
Correlation Analysis between Gene Expression and Drug Activity. Growth inhibition data (Ch_{50}, values for 60 human tumor cell lines) were those obtained by the Developmental Therapeutics Program. 7 Values were expressed as potencies by using the negative log of the molconcentration calculated in the NCI screen. We focused on 119 drugs for which the mechanism of action is largely understood. 7 The drug data can be found online. 7 Pearson correlation coefficients were calculated for assessment of gene-drug relationships. Confidence intervals and unadjusted P values were obtained using Efron’s bootstrap resampling method (18), with 10,000 bootstrap samples for each gene-drug comparison. To reduce the number of false-positive correlations among 87,000 comparisons, we controlled for false discovery rate as described previously (19). However, because of computational limitations introduced by the bootstrapping technique, using 10,000 samplings yielded only bootstrap estimators with a resolution of 0.0001. To control false discovery rate at the level 0.05, criteria would have to be too stringent, i.e., if only P = 0 was regarded as significant. Therefore, an arbitrary cutoff of 0.001 was used for the unadjusted bootstrap P values. This cutoff is expected to detect truly more gene-drug correlations at the expense of increasing the number of false-positive ones to be validated by other means.

Small Interfering RNA (siRNA)-Mediated Down-Regulation of Gene Expression. siRNA duplexes for ABCB1 were chemically synthesized by Qiagen, Inc. (Valencia, CA). The target sequence was 5’-AACGCGAG- CAGTGTTTCAGTGTT-3’, beginning from nt 2113 of the ABCB1 mRNA sequence NM_000927, as recommended. 6 Chemically synthesized mock siRNA (fluorescein-labeled, nonsilencing) was also purchased from Qiagen, Inc. siRNA duplexes for ABCB5 were synthesized using the Silencer siRNA construction kit (Ambion, Austin, TX). The three target sequences were as follows: 5’-AAAGAGGCACCTTAAATGGAAG-3’ (ABCB5_754); 5’-AAGTT- GGAGAATCGCTGACCTT-3’ (ABCB5_930); and 5’-AACAGTTCTC- GATGGCCCTG-3’ (ABCB5_1114), which are located at nt 745, 930, and 1114 of the ABCB5 mRNA sequence NM_005424, respectively.

Cell lines, obtained from Division of Cancer Treatment and Diagnosis at NCI were cultured in RPMI 1640 containing 10% heat-inactivated FCS in a 5% CO2 incubator at 37°C. Transfection was performed with TransMessenger Transfection Reagent (Qiagen, Inc.). To down-regulate ABCB1 or ABCB5, cancer cells were transfected with 0.3 or 0.6 μm siRNA. For RNA extraction, cells were harvested 48 h after transfection. To measure cytotoxic drug potency, cells grown in 6-well plates were subcultured into 96-well plates 24 h after transfection.

Cytotoxicity Assay. 5FU, azacytidine, camptothecin, and mitoxantrone were obtained from Sigma. The other compounds were from the Developmental Therapeutics Program at NCI. Drug potency was tested using a proliferation assay with sulforhodamine B, a protein-binding reagent (20), except for cell lines HL-60 and K-562. In each experiment, 3000 cells/well were seeded in 96-well plates and incubated for 24 h. Anticancer drugs were added in a dilution series in 6 replicated wells. After 4 days, incubation was terminated by replacing the medium with 100 μl of 10% trichloroacetic acid (Sigma, St. Louis, MO) in 1 × PBS, followed by incubation at 4°C for at least 1 h. Subsequently, the plates were washed with water and air-dried. The plates were stained with 100 μl of 0.4% sulforhodamine B (Sigma) in 1% acetic acid for 30 min at room temperature. Unbound dye was washed off with 1% acetic acid. After air-drying and resolubilization of the protein-bound dye in 10 mM Tris-HCl (pH 8.0), absorbance was read in a microplate reader at 570 nm. To determine IC_{50} values, the absorbance of control cells without drug was set at 1. Dose-response curves were plotted using SigmaPlot software (RockWare, Golden, CO). Each experiment was performed independently at least three times. Student’s t test was used to determine the degree of significance. To study the effect of an ABCB1 inhibitor, 10 μM verapamil (Sigma) was added to the cells 20 min before and during exposure to drugs, and the plates were incubated for 3 days.

The chemosensitivity of HL-60 and K-562 cells to inosine-glycodialdehyde and azacytidine was assessed with the 2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt assay (Sigma). Cells were seeded (5,000 cells/well for K-562 and 10,000 cells/well for HL-60) in 96-well plates and incubated for 24 h before exposure to graded concentration of each
drug for 72–96 h. The effect of a SLC29A1 transport inhibitor was assessed by adding 100 nM nitrobenzylmercaptopurine ribonucleoside (Sigma) for 20 min before and during drug exposure. Each experiment was performed independently at least twice.

**Real-Time Quantitative Reverse Transcriptase-PCR (RT-PCR).** Total RNA was prepared by using the RNeasy Mini Kit (Qiagen), following the manufacturer’s protocol. The integrity of the RNA was assessed by denaturing agarose gel electrophoresis (visual presence of sharp 28S and 18S bands). The RNA was quantitated by spectrophotometry. One µg of total RNA was incubated with DNase I and reverse transcribed with oligo(dT) with Superscript II RT-PCR (Life Technologies, Inc.). One µl of reverse transcriptase product was amplified by primer pairs specific for selected genes. Primers were designed with Primer Express software (Applied Biosystems, Foster City, CA), and ACTB (β-actin) was used as a normalizing control. Relative gene expression was measured with the GeneAmp 7000 Sequence Detection system (Applied Biosystems). Conditions and primer sequences are available on request.

**RESULTS**

**Correlating Transportome Gene Expression with Drug Activity.** The 70-mer microarray comprises 632 probes targeting 461 transporter genes and 151 channel genes, as well as 100 probes for unrelated genes (14, 15). The array was designed before the human genome sequence became available. It includes probes for 215 SLC genes of ~300 SLC genes that have been cloned to date (SLC, transporter of SLC families, including the structurally similar SLC ion exchangers), 40 of the 48 ABC transporters (ATP-driven extrusion pumps), plus a few contigs assembled from expressed sequence tags of putative SLC transporters not represented in the current annotation of the human genome. Furthermore, we include probes for a majority of ATPases (active ATPase ion transporters other than the ABC transporters) and channel genes encoding Na⁺, K⁺, Ca²⁺, and Cl⁻ channels. Therefore, our array covers a majority of genes relevant to drug transport. Twenty genes were represented by probes for two different domains of the target molecule to permit comparison of the results from different sites of hybridization.

After the experimental data on transporter expression patterns had been analyzed, previously published cDNA and Affymetrix expression data for limited numbers of the genes (9, 10) were used as controls against which to compare our results. Hierarchical clustering of cell lines based on gene expression and corroboration of gene expression data by comparing multiple expression data sets are reported as supplementary information online.

To determine the relationship between gene expression for the 732 probes and growth inhibitory potencies of the 119 drugs, we calculated the Pearson correlation coefficient for each gene-drug pair. Of the resulting 87,108 gene-drug correlations, 2.5% were distributed uncorrelated, we used r = 0.3 as a heuristic criterion for potentially significant correlations to cut down on the number of gene-drug pairs, the statistical significance of which we then assessed by computing the statistical significance of which we then assessed by computing -fold in both cell types (Table 2), indicating that SLC29A1 (equilibrative nucleoside transporter 1) plays an important role in cellular uptake of these compounds.

Impaired transport of folate drugs is a potential mechanism of drug resistance, whereas high transport capacity would be expected to increase chemosensitivity. As shown in Fig. 1B, SLC19A1, a member of the reduced folate carrier protein family, correlated positively with folate analogues such as aminopterin derivative and methotrexate. These results are consistent with previous findings (24, 25) and extend the spectrum of putative substrates, although the known SLC19A1 substrate aminopterin did not reach statistical significance (r = 0.20; P = 0.07), possibly because other sensitivity/resistance mechanisms may predominate. Although not known to transport folates, SLC19A2 and A3 also showed positive correlations with antifolate drugs, a finding that requires additional verification.

Amino acid transporters have received less attention as drug carriers, although the activity of polar amino acid analogues is likely to depend on transporter function. Fig. 1C shows several amino acid transporters that correlated with the potency of amino acid analogues, a finding not previously noted. For example, SLC38A2 (or ATA2), a member of the amino acid transport system A, correlated positively with acivicin and L-alanosine, the amino acid analogue drugs. SLC25A12, which encodes a calcium-stimulated aspartate/glutamate...
carrier protein (Aralar1) located in the mitochondrial inner membrane, showed positive correlation with N-phosphonoacetyl-L-aspartic-acid. In contrast, SLC25A13, which encodes Citrin, another calcium-stimulated aspartate/glutamate transporter in mitochondria homologous to Aralar 1, showed negative correlation with l-asparaginase (−0.55), possibly by providing aspartate precursor to the cells. Moreover, the correlation coefficient was −0.96 (confidence interval −1.00 to −0.87) for the six leukemic lines and −0.98 (confidence interval −1.00 to −0.92) for the six ovarian lines. This result parallels previous studies with the NCI-60 that implicated asparagine synthetase in drug resistance, particularly in leukemic cells (9). SLC25A13 and asparagine synthetase play roles in urea and arginine synthesis (26), and both are located in chromosome 7q21.3, separated by <100 kb (SLC25A13 is centromeric of asparagine synthetase). Possible coordinate expression or chromosomal amplification involving these two genes should be considered for future study.

Several SLC genes correlated with multiple drugs of different structures (Supplementary Table 1). This correlation pattern may reflect functions of the transporter other than a transporter-substrate relationship. For example, some nutrient transporters (glucose, amino acids, organic anions, and peptides) may be up-regulated because of the increased energy needs of cancer cells. Although amino acid transporter SLC7A11 positively correlated with l-alanosine (Fig. 1), it also showed significant negative correlations (Table 1). SLC7A11 forms a heteromultimeric complex with SLC3A2 (the amino acid transport system XC\(^{-}\)), and both showed negative correlation with multiple drugs, including cisplatin (Supplementary Table 1). The amino acid transport system X\(^{-}\) mediates cystine entry coupled with the exodus of glutamate and thereby regulates intracellular glutathione levels. This has been shown to contribute to cisplatin resistance in cancer cell lines (27). Similarly, negative correlations between drugs and glucose transporters may be related to the impact of glucose metabolism and apoptosis (28, 29). Thus, glucose transporters could affect drug potency either by serving as drug carriers or by modulating

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**Table 1** Select transporter and channel genes showing significant correlations with chemosensitivity

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\(^{a}\) Underlined drugs negatively correlate with expression of the corresponding genes while all other drugs have positive correlations. For each gene, the number of drugs with positive or negative correlation (r) is shown with two cutoff points, \( P < 0.05 \) and \( P < 0.001 \). \( r > 0 \), positive correlation; \( r < 0 \), negative correlation. For a complete list, see Supplementary Tables 1–3.

\(^{b}\) SLC, solute carrier.
ABCC2A5 are positively or negatively correlated with numerous drugs. These results provide the rationale for additional analysis of underlying mechanisms, including drug transport and other biological functions of the SLC transporters.

Intracellular pH has been shown to affect cellular response to anticancer drugs. Two SLC ion exchangers, a bicarbonate transporter and a sodium-proton exchanger (30) function as pH regulators in tumor cells (30). Among members of the Na\(^+\)H\(^+\) exchanger family, SLC9A3R2 showed positive association with multiple drugs (Table 1). Moreover, an expressed sequence tag encoding a hypothetical protein, LOC133308, that contains a Na\(^+\)H\(^+\) exchanger motif positively correlated with several drugs. Among the bicarbonate transporters, SLC4A7 correlated positively with 56 drugs. Therefore, genes that affect pH may influence many different ionizable drugs by changing pH gradients that determine drug partitioning into cells and cell organelles.

ABC Transporters and Chemoresistance. Negative correlations indicative of a possible role in chemoresistance occurred with 11 ABC genes (Supplementary Table 2), of which 9 had previously been implicated in drug resistance (4, 31). However, only 4 ABC transporter genes showed highly significant negative correlations with drugs (P < 0.001). Expression data obtained by other methods validated results for three of these genes, ABCB1, ABCB3, and ABCB5. (Table 1). The fourth gene, a known chemoresistance transporter, ABCC1 (multidrug-resistant protein 1), was not additionally considered because various array types gave discrepant results.

Expression levels of ABCB1 (or multidrug resistance 1, P-glycoprotein) significantly correlated with potency of many drugs (Table 1), consistent with numerous previous studies (8, 32, 33). Previously ABCB1 mRNA and protein levels (32, 34) and function in terms of rhodamine efflux (33) have been correlated with drug activity data against NCI-60. These studies produced a list of drugs that have been predicted as P-glycoprotein substrates and experimentally verified. We plotted the ordered ABCB1 correlation coefficients for all 119 drugs and obtained a clear separation between known ABCB1 substrates and nonsubstrates (Fig. 2B). Using the dual criteria of P < 0.05 and r < –0.3, we identified all known substrates of ABCB1 plus a geldanamycin analogue (GA) (NSC 330500) and Baker’s antifol (BAF) (NSC 139105) (Table 3). Negative ABCB1-GA and ABCB1-BAF correlations suggested that these drugs are substrates of the transporter. To validate this new finding, we used a chemically synthesized siRNA duplex to target ABCB1 in NCI/ADR-RES and HCT-15 cells, which express high levels of ABCB1. Real-time RT-PCR demonstrated that 40 h after treatment, siRNA substantially reduced ABCB1 mRNA levels by 74 and 68% in NCI/ADR-RES and HCT-15, respectively. When we compared growth inhibitory IC\(_{50}\) values for siRNA-treated and mock-treated control cells using a sulforhodamine B cell proliferation assay, the sensitivity of NCI/ADR-RES to paclitaxel, bisantrene, GA, and BAF was 2.0–7.6-fold greater in the siRNA-treated cells (Table 2 and Supplementary Fig. 3A). The sensitivity to 5FU, a non-P-glycoprotein substrate, was unaffected by siRNA silencing. The sensitivity of HCT-15 to GA and BAF was 1.3- and 1.5-fold greater in the siRNA-treated cells (Table 2). Because these changes were relatively small, we used chemical inhibitors to determine whether GA and BAF are indeed substrates of ABCB1 in HCT-15. We treated the HCT-15 cells with increasing concentrations of GA or BAF, with and without the presence of ABCB1 antagonist verapamil. For GA and BAF, respectively, increase in potency was 3.5- and 43.8-fold after verapamil treatment. Therefore, application of RNA interference gene silencing and ABCB1 antagonist supports the hypothesis that GA and BAF are ABCB1 substrates.

ABCC3, which encodes multidrug resistance-associated protein 3, showed significant negative correlation with a methotrexate derivative. That finding is consistent with a study that overexpression of ABCC3 results in high-level resistance to methotrexate (35). ABCB5, a putative ABC transporter of unknown physiological function, showed significant negative correlation with a methotrexate derivative and a folate analogue: aminopterin (NSC 132483); aminopterin-d, aminopterin derivative (NSC 134033); an-antifol (NSC 623017 and NSC 633713); BAF, Baker’s-antifolate (NSC 139105); methotrexate (NSC 740); methotrexate-d, methotrexate derivative (NSC 174121); trimetrexate (NSC 352122). C, amino acid transporters and amino acid analogues: l-asparaginase (NSC 109299); acivicin (NSC 163501); t-alanosine (NSC 153353); PALA, N-phosphonoacetly-l-aspartic acid (NSC 224131).
function, showed strong negative correlation with campothecin, 7-Chl (P < 0.001). Our array data indicated that ABCB5 is selectively expressed in melanoma cells, suggesting a tissue-specific role in chemoresistance. To identify suitable ABCB5 domains for siRNA-mediated gene silencing, we synthesized siRNA duplexes against chemoresistance. To identify suitable expressed in melanoma cells, suggesting a tissue-specific role in expression of ABCB5. A correlation coefficient of 0.3 is the approximate cutoff for statistical significance (see also Table 3).

Role of Ion Pumps (ATPases) and Channels in Chemosensitivity/Resistance. To identify ion pumps associated with drug activity, we investigated ATPases that maintain cellular electrical gradients (Table 1 and Supplementary Table 3). Genes that encode ATPases involved in cholesterol and phospholipid transport (37), correlated positively with doxorubicin and other ABCB1 substrates. The mechanism underlying these observations remains to be determined.

Several ABC transporters (e.g., ABCA1, ABCB3, and ABCC1) showed positive drug correlations (Table 1). ABCC1, a half-transporter involved in cholesterol and phospholipid transport (37), correlated positively with doxorubicin and other ABCB1 substrates. The mechanism underlying these observations remains to be determined.

Ion channels modulate electrochemical gradients generated by ion pumps and ion exchangers. Maintenance of a strong electrochemical gradient is vital to the cell and a potentially strong influence on drug activity. K+ and Cl- leakage currents tend to polarize cells, whereas derived from overlapping human expressed sequence tags assembled into a contig (14, 15), which is partially homologous with the cloned cDNA (36). Subsequent analysis of the mRNA expression of the ABCB5 expressed sequence tag and the cloned ABCB5 gene by real-time RT-PCR in 20 of the NCI-60 cell lines revealed that both mRNA isoforms are expressed at a constant ratio. Moreover, correlating the RT-PCR to drug activity data confirmed the notion that ABCB5 may serve as a broadly selective drug resistance transporter.10

The known chemoresistance genes ABCA2, ABCB2, ABCB11, ABCC1, ABCC2, ABCC4, and ABCC5 were negatively associated with several drugs (P < 0.05; Supplementary Table 2). However, the suggested drug substrates differed from those previously reported and measured expression of these genes did not correlate well with results obtained from other array studies (Supplementary information online). This discrepancy may have been related to insufficient sensitivity of the 70-mer arrays, to cross-hybridization, or to other technical problems involving hybridization for these genes. As with all microarray gene expression studies, not all probes function optimally. Additional validation studies would be needed before chemoresistance could be inferred or excluded.

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Ca$^{2+}$ and Na$^+$ channels depolarize them. These two types of flux would be expected to have opposite effects on drug equilibration across cell membranes. However, Ca$^{2+}$ flux is also important in apoptotic signaling, as noted above, so the net effect on drug potency is difficult to predict. In this context, CACNAID, which encodes the α1D subunit of the l-type calcium channel, showed negative correlation with several drugs, including deoxydoxorubicin (Table 1). Interestingly, l-type calcium channel antagonists block ABCB1 and thereby are thought to overcome drug resistance (43). It remains to be seen whether blocking CACNAID could have contributed to this effect. Several genes that encode subunits of sodium, chloride, potassium, and other cation channels correlated with drug activity, confirming that ion channels can modulate drug response—possibly by affecting the cell’s resting potential or by providing key metal ion cofactors. It will be important to understand the role of ion channels in the cell’s response to toxic stimuli because perturbation of the ADP-ATP ratio during the course of a cytotoxic reaction directly alters electrochemical gradients.

**DISCUSSION**

This study is the first to assess drug-transporter relationships comprehensively at the genomic level. Correlations between expression of the transportome and chemosensitivity in the NCI-60 panel reveal numerous significant gene-drug correlations. A number of those correlations correspond to known transporter-drug substrate relationships, thereby validating the approach. Moreover, the use of siRNA to down-regulate transporters provides additional validation for ABCB1 and ABCB5 as resistance genes and for SLC29A1 as a sensitivity gene. In aggregate, the results here emphasize the important role that multiple types of membrane transport molecules can play in sensitivity and resistance to cytotoxic drugs. In addition to the direct effects on drug transport that have already been discussed, indirect mechanisms may also modulate sensitivity. Transporters and channels may, for example, affect chemosensitivity indirectly by providing nutrients to cancer cells, modifying the propensity to apoptosis or modulating the electrochemical gradient. For validated transporter-drug pairs, the correlations calculated here enable one to search for additional genes that we could mine all of the useful information in them. Others who have domain expertise with respect to particular transport molecules or drugs will see and focus on relationships that are not apparent—or not apparently important—to us.
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