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Comparison of Drug Transporter Levels in Normal Colon, Colon Cancer, and Caco-2 Cells: Impact on Drug Disposition and Discovery

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Abstract: A critical step in early phase drug development is the determination of oral bioavailability. In part, the ability to predict whether a drug will be effectively transported across the gastrointestinal mucosa can be estimated from the physicochemical properties of the compound. Although advancements through rational drug design have more correctly predicted bioavailability, considerable variability remains to be explained. Transporter expression throughout the gastrointestinal tract may explain much of this variation. ATP-binding cassette (ABC) transporters were the first family of transporters identified to modify bioavailability. More recently, the solute carrier family has also been shown to alter the pharmacokinetic profile of drugs. Currently, the Caco-2 human colon carcinoma cell line is often used by the pharmaceutical industry to evaluate intestinal absorption of drugs; however, in vivo/in vitro permeabilities with carrier mediated drugs do not correlate well, suggesting that Caco-2 transporter expression varies from that of the small intestine. With this is mind, we integrated U133A GeneChip expression data from the NCBIs Gene Expression Omnibus (GEO) collection and then compared the expression pattern of Caco-2 cells to normal colon to determine if the Caco-2 cell line is a reliable model for colonic delivery. Furthermore, transporter expression of Caco-2 cells was compared to that of human colon tumors to assess whether this cell line could be useful to predict drug absorption for colon cancer. Our analysis shows that the expression pattern for Caco-2 cells closely resembles the gene expression profile of transporters within the normal colon, suggesting that this cell line may serve as an in vitro model of colonic drug adsorption. However, the molecular "fingerprint" of Caco-2 was distinctly different from tumor samples, indicating that the Caco-2 model would unlikely predict accurate drug absorption for colon cancer sites.

Keywords: Gene expression; ATP-binding cassette (ABC) transporters; solute carriers; colon; Caco-2 cells; Affymetrix HG-U133A GeneChip

Introduction

Poor drug absorption is one of the major obstacles to effective oral drug delivery. To circumvent this barrier, rational drug design is commonly employed to optimize various physicochemical attributes for sufficient drug absorption. However, for drug absorption, this tells only part of the story. The phenomenon of oral bioavailability can be better understood when the gastrointestinal expression of ATP-binding cassette (ABC) transporters and solute carriers (SLC) is taken into account.

Transporters can alter drug bioavailability through numerous mechanisms. First, uptake of drugs can be enhanced by many members of the SLC family,[1] and the pharmaceutical industry should consider these SLCs to provide improved oral drug delivery. Second, drug−drug interactions can also occur when the administration of one drug either induces transporter expression or blocks transporter function.[2,3] These secondary changes in transporter levels and/or function will

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consequently influence the pharmacokinetics of a second drug. Third, food−drug interactions are also possible. For example, the induction of both efflux and uptake transporter levels in Caco-2 cells has been reported following changes in nutrient exposure and drug treatment.[4,5] When considering drug absorption, the function most attributed to ABC transporters, in particular for those transporters situated on the apical membrane of the gastrointestinal tract, is decreased drug bioavailability through efflux from the intestinal mucosa into the gut lumen. ABC transporters are also responsible for the development of multidrug resistance in cancer therapy where these transporters effectively reduce the intracellular accumulation of chemotherapies to levels insufficient for therapeutic efficacy.[6] Last, polymorphic changes within transporters can also change absorption profiles as seen for diflomotecan[7] and (reviewed in ref 8).

Members of the ABC superfamily are identified by a consensus ATP-binding site and classified into 7 subfamilies (A−G) based on gene structure similarity, order of the domains, and sequence homology in nucleotide binding folds and transmembrane domains.[9,10] The first known ABC transporter, P-glycoprotein, coded by the ABCB1/MDR1 gene, was discovered by Dano in 1973 in multidrug-resistant cells which effluxed daunomycin.[11] During the last three decades, other members of the ABC superfamily, namely, members of the ABCC family and ABCG2, have been reported to efflux drugs and thus be involved in multidrug resistance.[12] Many of these transporters have promiscuous substrate binding and share great overlap in substrate specificity, causing major barriers for effective drug delivery.

The SLC superfamily consists of nearly 300 genes which have been divided into 43 families based upon amino acid homology of at least 25% between family members.[13] Drug transport has been linked to the following SLC families: SLC15, SLC21A, and SLC22A. For example, the absorption of a drug can be increased when it is a substrate for PepT1 (SLC15A1), an uptake transporter on the apical side of intestinal cells. Behrens et al. have shown that decreased PepT1 mRNA expression correlates with decreased cephradine apparent permeability coefficients.[14] The SLC21A family, now referred to as the organic anion transporting polypeptides (OATP/SLCO) superfamily, consists of 12 transmembrane domain proteins whose substrates are anionic amphipathic high molecular weight (>450) molecules with a great degree of binding to albumin.[15] The mechanism of transport is based upon an anion exchange which couples cellular uptake of organic compounds with the efflux of bicarbonate, glutathione, and/or glutathione-S-conjugates. The final SLC family linked to drug absorption, SLC22, includes organic cation transporters (OCTs), zwitterions/cation transporters (OCTNs), and organic anion transporters (OATs). These transporters also contain 12 membrane spanning domains; however, transport function varies among the members of this SLC family. OCTs act as uniporters whereas the OAT members use anion exchange to facilitate transport.[16] Na^+/L-carnitine cotransport occurs with OCTN2. The organic cation uptake transporters such as other members of the SLC22 family show broad substrate specificity which makes this family important in drug absorption.[17]

Caco-2 cells,[18] a human colon carcinoma cell line, are commonly used as an in vitro model for intestinal absorption and, in that context, have been compared to the duodenum and jejunum and other colon carcinoma cell lines.[19−21]

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However, this cell line often poorly predicts absorption of carrier-mediated drugs,[20] suggesting that the small intestine and Caco-2 cells vary considerably in their transporter expression. Although the colon is not normally identified as the major tissue of drug absorption, it is especially important when considering specialized delivery systems (e.g., enteric coated tablets and sustained or controlled release formulations). Such delivery systems are often utilized to ensure more constant absorption and, occasionally, are important when targeting drug delivery to a specific gastrointestinal disease site, as is the case when treating ulcerative colitis.[22] Therefore, alterations in drug absorption in this region can often lead to unexpected or deleterious clinical effects. In an attempt to better explain the poor correlation between absorption of carrier-mediated drugs and predictions based on Caco-2 permeabilities which have been previously reported,[20] we compared expression patterns of select ABC transporters and SLCs in Caco-2 cells with gene expression in normal colon samples. We hypothesized that the transporter expression of normal colon would be similar to that of Caco-2, thereby suggesting that Caco-2 cells are of utility for modeling drug delivery across the colon. The transporter expression in Caco-2 cells was also compared to human tumor samples to determine if this cell line might also have applicability for predicting drug bioavailability to colonic tumors. U133A GeneChip data was obtained from the NCBIs Gene Expression Omnibus (GEO) database for each of the sample types examined.[23]

Methods

Collection of GeneChip Data from GEO. The data discussed in this publication have been deposited in NCBIs GEO (http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession numbers. Data sets were examined for four samples types: normal colon, colon cancer, small intestine, and Caco-2 cells. The GEO Series accession numbers utilized in this work are the following: GDS690, GSE2232, GDS756, GDS1096, GSE1141, GSE2719, GSE1323, GSE2138, and GSE2361.

Selection of Transporters To Examine. The following ABC transporters and SLC family members were examined in our analysis (Table 1). ABC transporters include ABCA2, ABCA3, ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCC4, ABCC5, and ABCG2. SLC members include SLC10A1, SLC10A2, SLC15A1, SLC15A2, SLC21A3, SLC21A6, SLC21A8, SLC21A9, SLC21A11, SLC21A12, SLC21A14, SLC22A1, SLC22A2, SLC22A3, SLC22A4, SLC22A5, SLC22A6, SLC22A7, SLC22A8, and SLC22A9.

Table 1. Summary of New and Old Nomenclature for ABC Transporters and Solute Carriers

gene symbol	alternative names
ABC Transporters	
ABCA2	
ABCA3	ABC-C
ABCB1	MDR1, P-glycoprotein
ABCB11	BSEP, SPGP
ABCC1	MRP1
ABCC2	MRP2, Cmoat
ABCC3	MRP3
ABCC4	MRP4
ABCC5	MRP5
ABCG2	BCRP, MXR, ABCP
Solute Carriers	
SLC10A1	NTCP
SLC10A2	ASBT
SLC15A1	PEPT1
SLC15A2	PEPT2
SLC21A3/SLCO1A2	OATP-A, OATP, OATP1A2
SLC21A6/SLCO1B1	OATP-C, LST-1, OATP2, OATP1B1
SLC21A8/SLCO1B3	OATP-8, OATP1B3
SLC21A9/SLCO2B1	OATP-B, OATP-RP2, OATP2B1
SLC21A11/SLCO3A1	OATP-D, OATP3A1
SLC21A12/SLCO4A1	OATP-E, OATP-RP1, OATP4A1
SLC21A14/SLCO1C1	OATP-F, OATP-RP5, OATP1C1
SLC22A1	OCT1
SLC22A2	OCT2
SLC22A3	OCT3, EMT
SLC22A4	OCTN1
SLC22A5	OCTN2, CT1
SLC22A6	OAT1
SLC22A7	OAT2
SLC22A8	OAT3
SLC22A9	UST3

Analysis of GeneChip Data. Mas5 data from the Affymetrix Microarray Suite 5.0 software was obtained for each sample type from the GEO database. All samples were analyzed in unison after normalizing for variances in chip intensity. The Mas5 gene signal was centered first by median chip intensity, then by mean gene expression across samples. Gene expression data was transformed and analyzed after log 2 transformation. Spotfire Functional Genomics software (Somerville, MA) was used to generate clustered image maps (CIMs) and gene-sample dendrograms. The Euclidean distance measure (average linkage) was used with the unweighted pair-group method/arithmetic mean (UPGMA) for double-hierarchical clustering. Principal component analysis (PCA) was used to determine pattern recognition within the various datasets. Statisical analysis of microarrys (SAM),[24] available for public use by Stanford University Labs, was used to identify genes that were differentially expressed in

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Figure 1

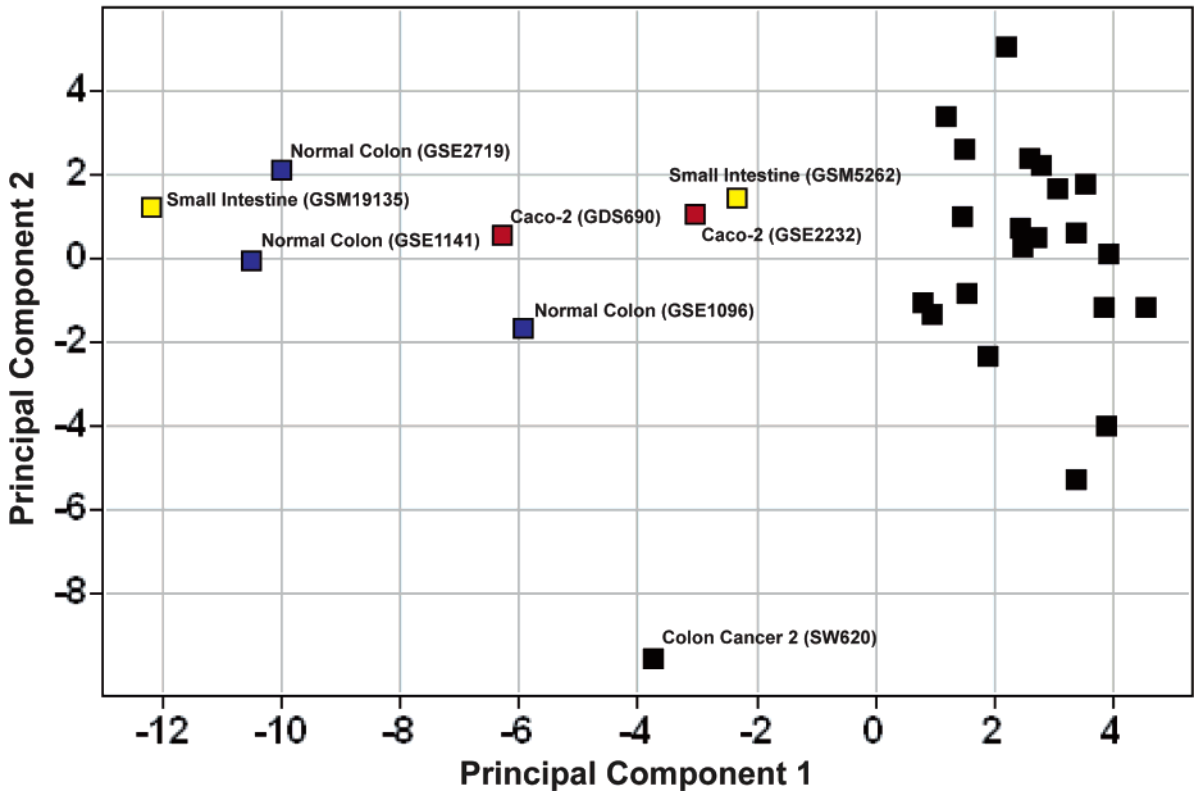

Figure 1. Principal component analysis of U133A GeneChip Data. The Caco-2 samples (red) have a pattern most closely resembling that of both normal colon (blue) and small intestine samples (yellow). The samples grouped on the right include most of the colon cancer samples (black); however, the SW620 cell line, the metastatic cell line, is separate from all other samples.

Caco-2/normal colon samples, as compared to tumor samples. The false discovery rate (FDR) was set to <10%.

Results and Discussion

Transporter Expression among Caco-2 Cells Varies Minimally Compared to Normal Colon. To evaluate if gene expression patterns for transporters of Caco-2 cells and normal colon were similar, principal component analysis (PCA) was performed using U133A GeneChip data from the GEO database (Figure 1). Although oversimplified, PCA has the effect of reducing the gene expression data across the 30 selected transporters into linear expression patterns that are best able to distinguish sample differences. We compared transporter data from Caco-2 cells to small intestine, normal colon, and colon cancer. Although others have compared Caco-2 cells to the distinct regions of the small intestine,[19,20] a recent study in mice shows that most differentially regulated transporters are similarly expressed along the entire small intestine.[25] PCA revealed two distinct regions which were well separated by the first principal component. The first region, located in the top left quadrant of the figure, contains the Caco-2 cell line as well as the normal colon and small intestine (colored squares). The second region contains a tight grouping of colon tumor samples (black squares), with the exception of the one outlier.

Colon Tumor Drug Bioavailability Is Unlikely To Be Accurately Modeled by Caco-2 Cells. Since Caco-2 cells were originally derived from a human colon tumor, one might expect that this cell line would also be useful for predicting sensitivity or resistance to antineoplastic chemotherapeutics. However, as shown in Figure 2, double-hierarchical clustering displayed a much tighter association between transporter expression in normal colon and Caco-2 cells than with tumor tissue. Although several of the ABC transporter/SLCs varied little across all the samples, two gene subsets (bottom corners of Figure 2) appeared to distinguish the Caco-2/normal colon cluster from colon cancer samples. Within the lower right region of this heat map, several differences between Caco-2 cells and normal colon can be seen. As reported previously, PepT1 (SLC15A1) expression in Caco-2 cells is lower, which would predict lower bioavailability for drugs that are substrates for PepT1.[20] The

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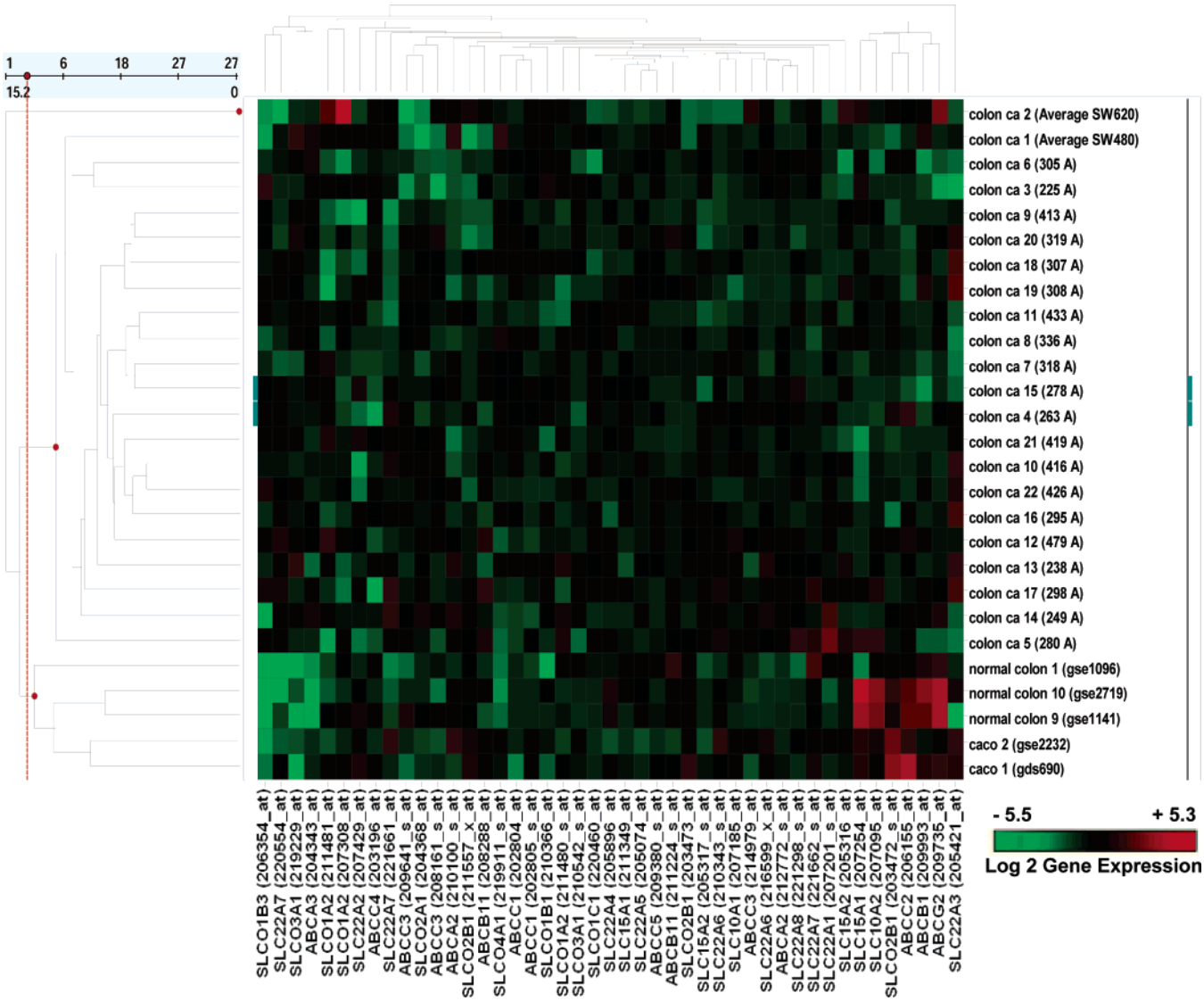

Figure 2. Hierarchical clustering analysis of U133A GeneChip Data for Caco-2, normal colon, and colon cancer samples. Three main clusters were seen on this dendrogram. The first cluster consists of the Caco-2 cells and the three normal colon samples, which display a surprisingly similar transporter expression profile. The second cluster contains most of the colon cancer samples. Finally, the third cluster contains the SW620 cell line, which shows a distinct gene expression profile compared to the other samples. Red represents highest expression, and green represents lowest expression.

expression of ABCB1, ABCG2, and SLC10A2 are lower in Caco-2 cells; these differences again would cause variations in predicted bioavailability compared to what was actually seen in vivo.

The last branch of the dendrogram contained the SW620 cancer cell line from a metastatic colon cancer. This was also the outlier found in Figure 1. Several interesting observations can be made from the SW620 gene profile compared to those of the other colon cancer samples. First of all, there is great overexpression of the SLCO1A2 gene. A recent study about SLCO1A2 reported its expression in brain capillary, renal distal nephron, and liver.[26] As mentioned previously, this cell line was derived from an isogenic metastatic colon cancer as opposed to a primary colon tumor, which may account for this higher level of SLCO1A2, which is not seen in any of the other samples. Second, SLC22A7 gene expression is the lowest for the SW620 cell line compared to all other tumor samples and resembles those seen in normal colon. Last, there is an increase in ABCG2 compared to the other colon cancers, suggesting that this tumor may be resistant to treatment with anthracyclines or mitoxantrone.

Statistical analysis of microarrays (SAM) was used to clearly identify those genes which accounted for the difference between Caco-2/normal colon cluster and colon tumors. Four ABC transporters and six SLCs were differentially

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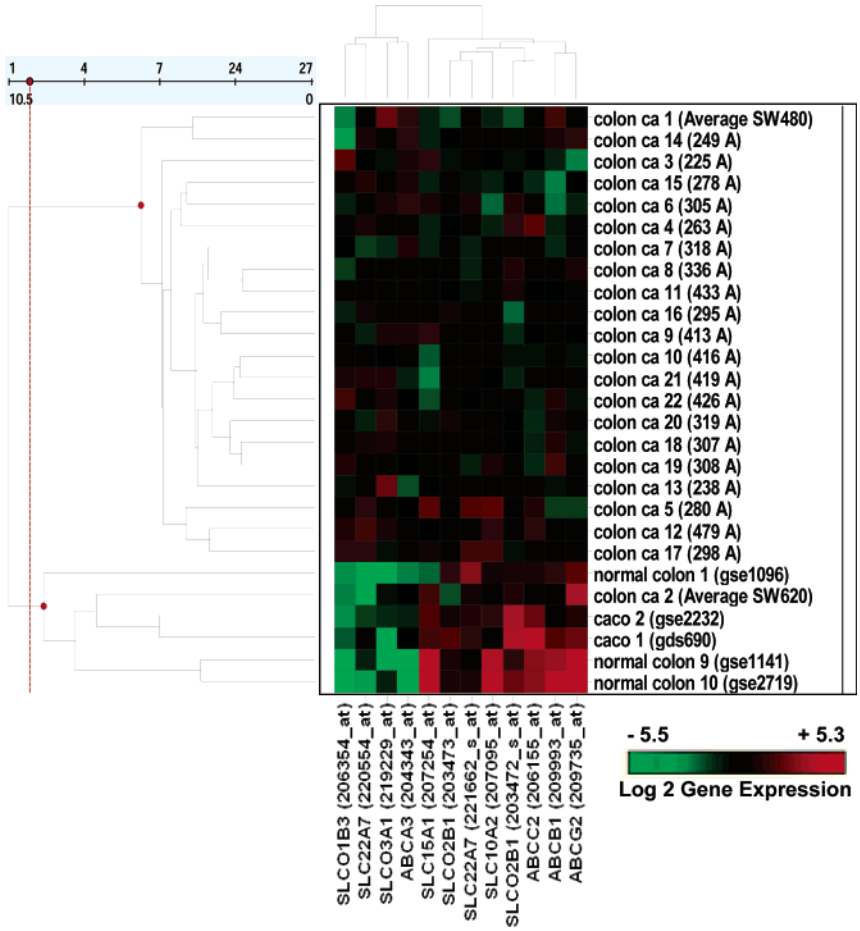

Figure 3. Hierarchical clustering analysis of select transporters that distinguish Caco-2/normal colon from colon tumor. Four ABC transporters and six SLCs were differentially expressed among the two classes, Caco-2/normal colon and colon tumor. Red represents highest expression, and green represents lowest expression.

expressed among those two classes (Figure 3). Of probable clinical significance, ABCB1 and ABCG2 were markedly overexpressed in Caco-2 and normal colon compared to colon cancer. This would suggest that Caco-2 would overestimate transporter-mediated drug resistance in tumors. These results, indicating relatively high expression of ABCB1, ABCC2, and ABCG2 within Caco-2 cells, are consistent with those previously reported for Caco-2 ABC transporter expression performed by RT-PCR.[19] Colon tumor samples appear to express less PepT1 (SLC15A1), SLCO2B1, and SLC10A2 (ASBT). Lower expression of these SLCs can potentially lead to a lower bioavailability for cancer drugs such as bestatin.[27] Interestingly, more ABCA3 is expressed which is normally found in lung tissue, and again a list of SLCs is slightly higher expressed in these colon tumor samples including SLCO3A1, SLCO1B3, and SLC22A7. Both SLCO3A1 and SLCO1B3 have been found in different tumor cells, but their significance in cancer is yet to be determined.[28,29] Alternatively, SLC22A7 is normally found in the liver and kidneys.[16]

There are a number of challenges to using data from the GEO database: each of the nine laboratories of which data was collected almost certainly had their own methods for sample acquisition and storage, mRNA isolation, chip hybridization, and image capture. Alternatively, the expression of transporters in normal colon is not uniform due to various environmental conditions and genetic factors which are difficult to account for in this work. Our dendrogram displays that for all experimental conditions the samples of

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similar tissue type but from different GEO data sets cluster together, indicating that the results from the U133A microarray were reproducible despite the challenges mentioned above. Often, duplicate probe sets of the same transporter also clustered together. However, several probe sets failed to cluster together such as those for SLC22A7. This factor indicates that the detection of a particular gene depends highly on the design of the probe, and this is a limitation of this technology. In addition, the transporter genes that were chosen for our analysis were not reported to increase in expression with increasing passage number of the cells;[30] therefore, this was not a concern for selecting this data from GEO. Ideally, tissue selection for analysis would be performed using laser capture microdissection to ensure a homeogeneous population of cells, and a follow-up using RT-PCR would be performed to verify results obtained by the microarray. One final caveat concerning the use of microarray data is that further evaluation at the protein level is often necessary to verify the changes seen at the mRNA transcript level since a positive correlation between gene expression and protein expression is not always detected due to posttranscriptional processes.

In conclusion, transporter expression among Caco-2 cells varies minimally compared to normal colon. The Caco-2 system may serve as an effective model for estimating drug absorption through normal colon; however, one must do so with the understanding that there are still differences seen between normal colon and Caco-2 cells. It appears that many scientists realize the advantages and the shortcomings of this system and have begun to engineer polarized transepithelial cell model systems to represent transport with their transporters of interest as has been done for hepatic efflux models.[31,32] Colon tumor drug bioavailability is unlikely to be accurately modeled by Caco-2 cells, and identification of a model cell line for colon tumor delivery is currently ongoing. Our analysis has also shown the utility of the GEO database for analyzing diverse datasets using a commercially available microarray platform to answer questions about transporter gene expression patterns.

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