*Review*

# Drug Transporters as Targets for Cancer Chemotherapy

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**Abstract.** *Transporter proteins play an important role in taking up nutrients into and effluxing xenobiotics out of cells to sustain cell survival. Transporters that affect drug absorption, distribution and excretion are the so-called drug transporters. In the last decade, a number of studies revealed interactions between drug transporters and clinically important anticancer agents. Utilizing the knowledge of transporter functions offers us the possibility of delivering a drug to the target tissues, avoiding distribution to other tissues and improving oral bioavailability. Many transporters have been reported to be differentially up-regulated in cancer cells compared to normal tissues, suggesting that the differential expression of transporters in cancer cells may provide good targets for enhancing drug delivery as well as diagnostic markers for cancer therapy. This review will focus on the role of drug transporters in the adaptation and growth of tumors and in their potential usefulness as therapeutic targets in cancer.*

Approximately 4% of the genes in the human genome encode transporter proteins, including ion channels (1). Plasma membrane transporter proteins are encoded by numerous gene families. They are key players in the uptake of nutrients, such as sugars, amino acids, nucleosides and inorganic ions, into and the efflux of xenobiotic toxins, including many anticancer drugs out of cells to sustain cell survival. Transporter proteins can be classified into two major families, the solute carrier (SLC) and the ATP-binding cassette (ABC) transporters. Most ABC proteins transport a substrate molecule by utilizing the energy generated from ATP hydrolysis, while some form specific membrane channels. SLC transporters comprise both facilitated and secondary active transporters. Facilitated transporters move a molecule across plasma membranes following its concentration gradient, whereas secondary active transporters utilize the downhill flow of an ion to transport another molecule against its concentration gradient across the membranes in either the same (symport or cotransport) or opposite (antiport) direction. Currently the SLC and ABC family include 360 transporter genes in 46 subfamilies (2) and 48 transporter genes in 7 subfamilies (3, 4), respectively.

Transporters that affect drug absorption, distribution and excretion are often called drug transporters, these confer sensitivity or resistance to anticancer drugs. The functional characterization of drug transporters provides important information crucial to drug design by targeting specific transporter proteins (5). The use of specific transporters as molecular targets in cancer therapy has been proposed as a promising strategy for tissue-selective drug delivery and for reducing systemic toxicity (6). More recent, transporter-targeted drug delivery approaches have been tested for their usefulness in the clinic. A number of studies revealed that many transporters are up-regulated in cancer cell lines and malignant tumors compared to normal tissues, suggesting that a differential expression of transporters in cancer exists and can be exploited for drug delivery or as diagnostic markers. This review will focus on the role of drug transporters for tumor growth and the usefulness of drug transporters as targets for specific drug delivery into cancer cells.

*Abbreviations:* SLC: solute carrier; ABC: ATP-binding cassette; PEPT: the $H^+$/peptide co-transporter; PDH: photodynamic therapy; NOS: nitric oxide synthase; CNS: central nervous system; OATP: organic anion transporting polypeptide; CNT: concentrative nucleoside transporter; ENT: equilibrative nucleoside transporter; NBMPR: nitrobenzylthioinosine; 5FU: fluorouracil; BBB: blood-brain barrier; MDR: multidrug resistance.

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*Key Words:* SLC transporters, ABC transporters, cancer therapy, multidrug resistance, review.

Table I. *SLC transporters, their substrate and their anticancer drugs/agents.*

| Transport system | Protein name | Gene symbol | Predominant substrate | Transport type*/Coupling ion | Anticancer drug/agent |
|---|---|---|---|---|---|
| Peptide | PEPT1 | SLC15A1 | Oligopeptide | $C/H^+$ | Bestatin, δ-ALA |
| | PEPT2 | SLC15A2 | Amino acid ester of floxuridine | | |
| Amino Acid | ATB⁰/ASCT2 | SLC1A5 | A,S,C,T,Q** | $C/Na^+$ | - |
| | LAT1 | SLC7A5 | H,M,L,I,V,F,Y,W,Q** | E/amino acid | Melphalan, BCH (inhibitor) |
| | ATB⁰,⁺ | SLC6A14 | K,R,A,S,C,T,N,Q,H, M,I,L,V,F,Y,W** | $C/Na^+\&Cl^-$ | NOS inhibitors |
| | xCT | SLC7A11 | E, Ci** | E/amino acid | L-Alanosine*** |
| Organic Anion | NTCP | SLC10A1 | Bile salts | $C/Na^+$ | Chlorambucil-bile acid conjugates Ursodeoxycholic acid derivatives Bamet-R2, Bamet-UD2 |
| | ASBT | SLC10A2 | Bile salts | $C/Na^+$ | Bamet-UD2 |
| | OATP1A2 | SLCO1A2 | Bile salts, Organic anion, Organic cation | F | Methotrexate Bamet-R2, Bamet-UD2 |
| | OATP1B1 | SLCO1B1 | Bile salts, Organic anion | F | Methotrexate Irinotecan (SN38) Bamet-R2, Bamet-UD2 |
| | OATP1B3 | SLCO1B3 | Bile salts | F | Methotrexate |
| Organic Cation | OCT1 | SLC22A1 | Organic cation, polyspecific | F | Oxaliplatin, Imatinib Bamet-R2, Bamet-UD2 |
| | OCT2 | SLC22A2 | Organic cation, polyspecific | F | Bamet-R2, Bamet-UD2 |
| | CT2 | SLC22A16 | Carnitine, Betain | F | Doxorubicin |
| Nucleoside | CNT1 | SLC28A1 | Nucleoside | $C/Na^+$ | Cytarabine (ara-C), Gemcitabine Fluorouridine 5dFU |
| | ENT1 | SLC29A1 | Nucleoside Inosine | F | Cytarabine (ara-C), Gemcitabine Cladribine, Fluorouridine, 5dFU, Fludarabine |
| Folate | RFC | SLC19A1 | Folate | $E/OH^-$ | Methotrexate, Tomudex, Edatrexate, Alimta (LF23514) |
| Monocarboxylate | MCT1 | SLC19A1 | Lactate, Pyruvate | $C/H^+$ or E | Lonidamine (inhibitor) alpha-Cyano-4-OH-cinnamate (inhibitor) |
| | SMCT | SLC5A8 | Lactate, Pyruvate, Butyrate | $C/Na^+$ | - |
| Glucose | GLUT1 | SLC2A1 | Glucose | F | 18-FDG |

*C; cotransporter, E; exchanger, F; facilitated transporter; **Amino acid substrates are shown in one-letter code; ***Proposed substrates (or not confirmed).
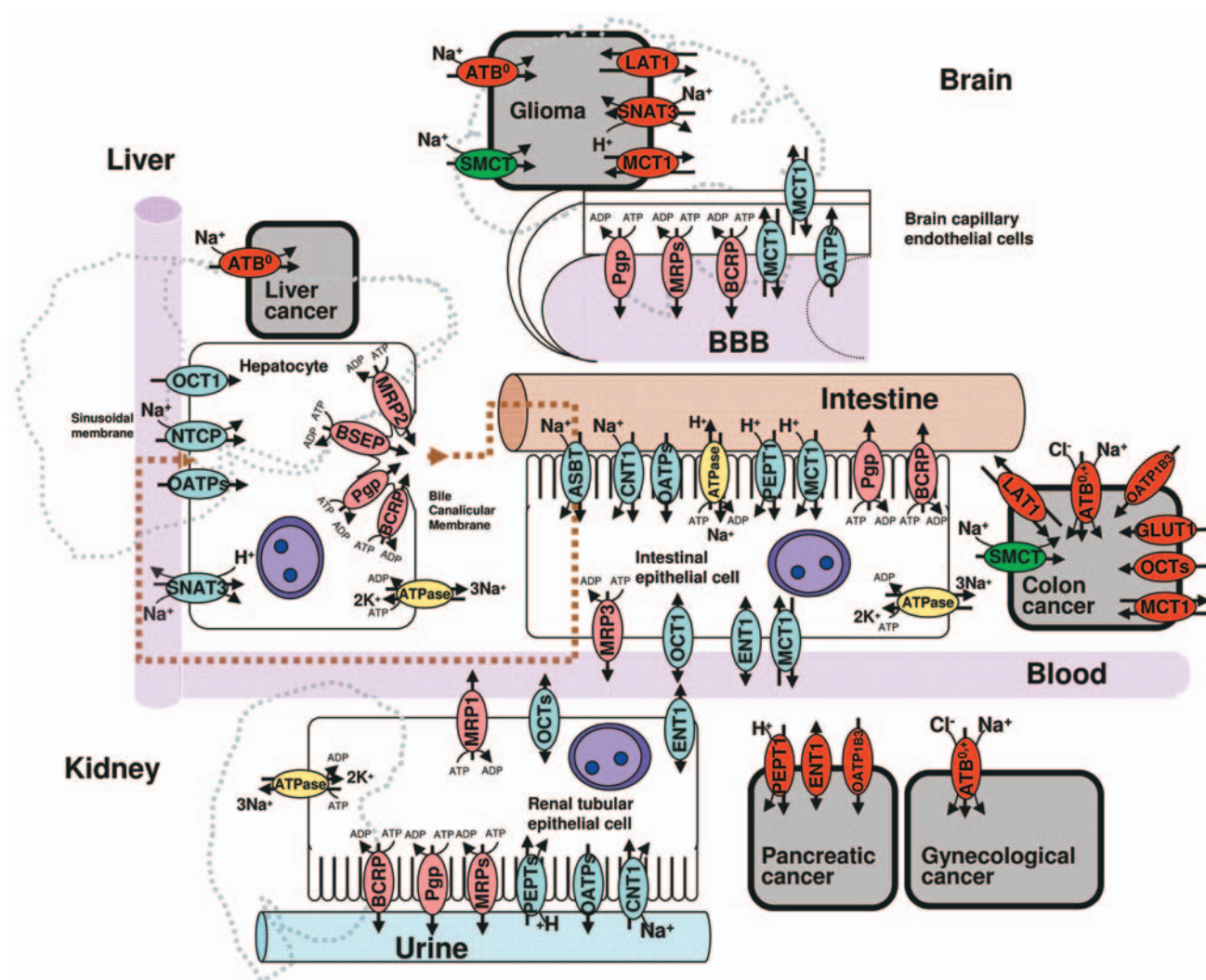
Figure 1. *Human transporters expressed in major tissues affecting absorption, metabolism and disposition of drugs and at the blood-brain barrier (BBB). Dotted line indicates the enterohepatic circulation of bile salts. Transporters in blue, pink and yellow indicate SLC transporters, ABC transporters and ATPases, respectively. Transporters in red and green indicate transporters that are reported to be up-regulated and down-regulated, respectively, in cancer as compared to their normal counterparts.*

## 1. SLC Transporters as Targets for Cancer Chemotherapy

Most SLC transporters function as influx transporters and could be useful as tools for delivery of anticancer drugs to tumor tissues. The interaction of anticancer agents with various types of SLC transporters is reviewed (as summarized in Figure 1 and Table I) and the possible role of transporters in cancer chemotherapy is discussed.

### 1.1 Oligopeptide Transporters

The $H^+$/peptide co-transporters PEPT1 (*SLC15A1*) and PEPT2 (*SLC15A2*) have been extensively characterized as

drug transporters in the intestine. PEPT1 and PEPT2 mediate transport of di- and tri-peptides across plasma membranes utilizing an inwardly directed $H^+$ gradient into cells. PEPT1 is functionally expressed in epithelial cells of the small intestine, kidney and bile duct, whereas PEPT2 is predominantly expressed in the epithelial cells of the kidney and the choroid plexus (7). The expression of these transporters in epithelial cells in the intestine and kidney is important for (re)absorption of nutrients and peptide-mimetic drugs including β-lactam antibiotics, angiotensin-converting enzyme inhibitors and even non-peptide antiviral drugs as valacyclovir (Figure 1 and Table I, (8)). The limited expression pattern (intestine and kidneys), but broad substrate specificity of PEPTs could be utilized to deliver

peptidomimetics to the target tissues differentially expressing peptide transporters. Cancer cells appear to up-regulate peptide transport. Since the finding that a pH-dependent carrier-mediated uptake of dipeptides was enhanced in human fibrosarcoma HT1080 cells compared to that of normal fibroblast IMR90 cells (9), functional expression of PEPT1 has been found in variety of human cancer cell lines (10-12). However little is known about the expression of PEPT2 in these cancer cells. The hypothesis whether the differential expression of PEPT1 is a valid target for cancer therapy was tested in animal models implanted with HeLa cells stably transfected with *PEPT1*. Bestatin is a dipeptide mimetic that competitively inhibits amino peptidase B and leucine amino peptidase. Bestatin is a good substrate for PEPT1 and PEPT2. Four-week consecutive oral administration of bestatin suppressed the growth of tumors expressing PEPT1, but not mock-transfected control tumors (13). Future studies are needed to clarify expression of peptide transporters in primary tumors and to determine if the basal expression is sufficient to deliver drugs. For this approach to succeed, it will be important to develop anticancer agents that specifically target PEPT1. Recently, amino acid ester pro-drugs of the nucleoside analog floxuridine were reported to have promising anticancer activity in cancer cells expressing PEPT1 (14).

Other applications for using PEPT1 expressed in cancer chemotherapy lie in fluorescence imaging and photodynamic therapy (PDH). δ-Aminolevulinic acid (ALA) has gained interest in PDH of tumors including gastrointestinal adenocarcinoma and dysplastic mucosa (15, 16), because it is metabolized to produce the photosensitizer protoprophyrin IX. Since this compound is transported by PEPT1, the expression of PEPT1 on epithelial cells of the bile duct has been proposed as a useful target for delivery of δ-ALA to extrahepatic biliary duct cancers (17).

## 1.2 Amino Acid Transporters

Amino acid transporters have been classified into a number of distinct 'systems' dependent on their substrate specificity, transport mechanisms and regulatory properties in humans and rodents. Neoplastic transformation is accompanied by adaptive increases in nucleotide and protein biosynthesis. Enzymatic conversion of glutamine to glutamate and ammonia is the first step in a series of reactions that generate the metabolic intermediates required for cell growth. Therefore glutamine is essential for tumor growth; indeed tumor cells are known to consume large amounts of glutamine (18). In humans, glutamine is principally taken up into cells by the $Na^+$-dependent systems A and ASC (or $B^0$), with the exception of the liver, where the pH-dependent system N predominantly mediates glutamine

influx from the sinusoidal blood. Recently a distinct carrier-mediated glutamine transport rather than system N has been suggested in transformed human hepatocytes (Figure 1, (19)). This particular transport event to regulate glutamine uptake was identified as the amino acid transporter $ATB^0$/ASCT2 (*SLC1A5*) that is an isotype of system ASC for neutral amino acids. The differential expression of $ATB^0$/ASCT2 in human hepatocellular carcinoma and hepatoblastoma is of pharmacological interest (20), although it is not known yet why a major transport system is altered during hepatocellular malignant transformation. Similar to liver, such up-regulation was found in human colorectal cancer (21), glioma and glioma metastases (22, 23). In addition to $ATB^0$/ASCT2, SNAT3 (*SLC38A3*, an isotype of system N) was found to be overexpressed in malignant glioma, suggesting an immunohistological application as a potential diagnostic maker for these brain cancers (23).

Tumor cells have an increased demand for essential amino acids as well as glutamine. Increased expression of the system L transporter LAT1 (*SLC7A5*, also termed TA1/E16) is well documented in various types of human cancer cell lines and primary malignant tumors. The LAT1 protein mediates an $Na^+$-independent exchange of neutral amino acids including several essential amino acids (*e.g.* L, I and V) (24), but requires a single-membrane spanning protein, the heavy chain of 4F2 (CD98) antigen (4F2hc also termed CD98hc, *SLC3A2*) to function. In contrast to the restricted normal tissue distribution of LAT1, its broad and high expression in a variety of cancer cell lines has drawn attention to this transporter although the expression of 4F2hc was variable among the cell lines, particularly in acute leukemia (25). Current evidence also suggests a differential expression of LAT1 in human colorectal tumor tissues (26), glioma (27) and esophageal carcinoma (28). It is important to note that the expression of LAT1 has been associated with clinicopathological variables. Kaplan-Meier analyses demonstrated that high LAT1 expression correlated with poor survival for sixty patients with astrocytoma, indicating that LAT1 is an independent predictive factor for this tumor type (27). Of therapeutic importance, the differential expression of LAT1 could be exploited in the treatment of tumor with melphalan, a substrate for LAT1. Thus LAT1 levels should be determined prior to therapy; only those who express LAT1 should receive melphalan.

$ATB^0$/ASCT2 and LAT1 themselves might be good molecular targets for therapeutic intervention in certain cancers. These two amino acid transporters were previously validated as molecular targets for cancer therapy. Fuchs *et al.* revealed that disruption of $ATB^0$/ASCT2 mRNA by transfecting human hepatoma SK-Hep cells with antisense RNA to $ATB^0$/ASCT2 elicited apoptosis in human hepatocellular carcinoma cells, resulting in a 98% decrease

in their proliferative activity (29), while competitive inhibition of $ATB^0$-mediated glutamine uptake only blocked the growth of hepatocellular carcinoma cell lines which lacked expression of system N transporters (SNAT3 and SNAT5; *SLC38A5*) and exhibited low activity of glutamine synthase (20). Specific inhibition of LAT1 was also tested by utilizing 2-aminobicyclo-2(2, 2, 1)-heptane-2-carboxylic acid (BCH), a known specific inhibitor for LAT1. In rat models, BCH exerted an antiproliferative activity in rat ortholog Lat1-expressing C6 glioma cells by showing prolonged overall survival of animals treated with BCH. Studies are currently underway to develop compounds that efficiently and specifically inhibit these amino acid transporters.

The amino acid transporter, $ATB^{0,+}$ (*SLC6A14*) may provide a possibility for targeted drug delivery. The $ATB^{0,+}$ transporter might target antiangiogenic nitric oxide synthase (NOS) inhibitors to colorectal or gynecological cancers. $ATB^{0,+}$ accepts zwitterionic as well as cationic amino acids, hence this differentiates it from $ATB^0$. $ATB^{0,+}$, unlike $ATB^0$ is able to transport a series of NOS inhibitors (30), most of which are structurally related to L-arginine, because L-arginine is converted to citrulline and nitric oxide by NOS (31). Expression of inducible NOS (iNOS) is known to be increased in several types of cancers, including colorectal (32, 33) and gynecological (34), and appears to be correlated with proliferation and vascular invasion of these cancers (35). Recently, quantitative RT-PCR studies showed that $ATB^{0,+}$ mRNA increased 23-fold in human colorectal and 5.6-fold in cervical tumors, respectively, compared to their normal surrounding tissues (33, 36). NOS inhibitors can be transported by three amino acid transporter systems: system $y^+$, system $b^{0,+}$ and system $B^{0,+}$. Of these, the ability of $ATB^{0,+}$ (an isotype of system $B^{0,+}$) to concentrate substrates inside the cell is the greatest because it utilizes multiple driving forces ($Na^+$, $Cl^-$ and membrane potential) to transport substrates (37). Taking account of the capability and expression pattern of $ATB^{0,+}$, it would be a promising target for NOS inhibitors in these cancers.

The amino acid transporter xCT (*SLC7A11*), together with 4F2hc, encodes the heterodimeric amino acid transport system $Xc^-$, which mediates cystine entry into cells in exchange for glutamate. Recent microarray studies profiling gene expression of transporters in the NCI-60 human cancer cell line panel showed that xCT and 4F2hc were expressed at relatively high levels in lung, colon and CNS cancer cells (38). The linking of the expression of xCT in the 60 cancer cell line panel to the antiproliferative potencies of 1,400 candidate anticancer drugs identified an amino acid analog L-alanosine as the drug with a positive correlation, which could be a substrate for xCT (38). Microarray profiling analysis of drug transporter genes with the cytotoxicity of anticancer drugs amongst multiple cancer cell lines is likely

to be an efficient methodology to reveal novel interactions of drugs with the transporters, hence providing important information on chemosensitivity, chemoresistance and effective drug delivery.

## 1.3 Bile Acid Transporters and Organic Anion Transporting Polypeptides (OATPs) Responsible for Enterohepatic Circulation

Of more than 50 $Na^+$/bile salt cotransporters in the SLC10 family, the $Na^+$/taurocholate cotransporting polypeptide (NTCP; *SLC10A1*) and the apical $Na^+$-dependent bile salt transporter (ASBT; *SLC10A2*, also termed IBAT) are best known members for the enterohepatic circulation of bile salts in humans. OATPs are membrane transporters that are currently classified within the new gene symbol "OATP/SLCO" superfamily (39). Members of this family mediate the $Na^+$-independent transport of a wide range of amphipathic organic compounds including bile salts, steroid conjugates, thyroid hormones, anionic oligopeptides, numerous drugs and xenobiotics (40). In humans, members of OATP1A2 (*SLCO1A2*, also termed OATP-A, OATP), OATP1B1 (*SLCO1B1*, also termed OATP-C, LST1, OATP2) and OATP1B3 (*SLCO1B3*, also termed OATP8, LST2) play an important role in drug absorption and disposition, particularly in the enterohepatic circulation. Every step of the enterohepatic circulation of bile salts is maintained by transporter proteins (Figure 1). Bile salts are taken up into the liver across sinusoids by NCTP and OATPs including OATP1A2, OATP1B1 and OATP1B3, while the exit step from the liver across the canalicular membranes is mediated mainly by the ATP-dependent bile salt export pump (BSEP; *ABCB11*) or the multidrug resitance associated protein 2 (MRP2; *ABCC2*). About 90% of bile salts excreted into the gastrointestinal tract are actively reabsorbed by the ABST and then recycled. The recycling performed by several transporters in a concerted manner is a pharmacologically attractive drug delivery system for bile acid derivatives. Such a strategy could target organs like the liver, leading to both a prolonged exposure of target organ to and limited systemic toxicity of the drug. With respect to chemotherapy, bile acids conjugated with the alkylating drug chlorambucil (41, 42) or cisplatin (43, 44), and ursodeoxycholic acid derivatives (45) have been studied for efficacy, as well as their interaction with these liver-specific transporters (42, 46). Liver perfusion experiments revealed a secretion profile of the chlorambucil-bile acid conjugates into bile very similar to taurocholate, because of NTCP-mediated high-affinity uptake of this compound into the liver (45). In contrast, unconjugated chlorambucil was predominantly excreted by the kidney (41). The anticancer activity of bile acid conjugated agents for hepatocellular carcinoma was tested with a series of bile acid cisplatin derivatives designated as "Bamet" (43, 44). Two

Bamets seem to have potential in treating hepatocarcinomas: Bamet-R2 [cis-diammine-chloro-cholylglycinate-platinum (II)] and Bamet-UD2 [cis-diammine-bisursodeoxycholate-platinum (II)]. The anticancer activity of these compounds against liver tumor xenografts was almost comparable to that of cisplatin, however, the Pt intracellular accumulation in other organs, particularly the kidneys was much lower, which may translate into reduced systemic toxicity of cisplatin (47). Later *in vitro* transport studies of Bamets, clearly showed that they act *via* a liver-specific distribution owing to the interaction with transporters involved in enterohepatic circulation of bile salts (46). Drug design targeting this circuit may have potential in increasing the clinical efficiency of standard chemotherapeutic agents for the treatment of hepatocellular carcinoma and liver-related malignant diseases.

Some members of the SLCO family are reported to mediate transport of anticancer drug into cells. For example, methotrexate is a folate analog with anti-neoplastic effect and can be transported by OATP1A2 (48), OATP1B1 (40) and OATP1B3 (39). Recently, irinotecan and its major metabolite SN38 were reported to be efficiently transported by OATP1B1 (49). It is important to note that OATP1B3 is up-regulated in gastric, colorectal and pancreatic cancer, but not in hepatocellular carcinomas, whereas it is almost exclusively expressed in liver among normal tissues (50).

## 1.4 Organic Cation/Anion/Zwitterion Transporters

The SLC22 family consists of bidirectional facilitated organic cation (OCTs) and anion (OATs) transporters (Figure 1) and Na$^+$-dependent zwitterions/cation transporters (OCTNs) (51). Amongst these transporters, recent studies highlight the role of OCT1 in cancer chemotherapy. OCT1 (*SLC22A1*)-mediated influx may be a key determinant of molecular response to the clinically used BCR-ABL tyrosine kinase inhibitor imatinib, but not nilotinib, in untreated chronic myeloid leukemia patients (52). In addition to imatinib, oxaliplatin, which is a potent cisplatin-derivative against colorectal cancer, was found to be transported by OCT1 and 2 (*SLC22A2*) (53). Because OCT1 and 2 markedly increased oxaliplatin accumulation and cytotoxicity in transfected cells, they could be major determinants of the anticancer activity of oxaliplatin. In fact, RT-PCR showed that expression of OCT1 and OCT2 mRNA was detected at different levels among colorectal tumors (53), therefore OCTs in tumors should be investigated as markers for selecting specific platinum-based therapies in individual patients. Since imatinib and oxaliplatin are structurally unrelated, substrate specificity of OCTs may provide a benefit for the development of new anticancer drugs specifically targeted to OCTs, providing a novel strategy for targeted drug therapy.

CT2 (*SLC22A16*, also termed FLIPT2 or OCT6) is another transporter with potential for the targeted drug delivery of anticancer agents to tumors. CT2 transports carnitine and betain bidirectionally across plasma membranes (51). A recent study demonstrated CT2 as a mediator of doxorubicin uptake into cancer cells. Quantitative real-time RT-PCR analyses detected that mRNA of this gene is expressed in primary specimens from patients with acute leukemia. Characterization of protein function and gene expression in leukemia and solid tumors may prove valuable for improving existing treatments and designing novel cancer therapeutic regimens (54).

## 1.5 Nucleoside Transporters

A number of synthetic nucleoside analogs are known to be therapeutically useful agents in the treatment of AIDS and cancer. Potent antineoplastic nucleoside analogs in clinical use include the purines cladribine and fludarabine, and the pyrimidines cytarabine (ara-C) and gemcitabine. These agents are so lipophobic that they are unable to enter cells without nucleoside transporters (NTs, Table I). Therefore, the interaction of nucleoside analogs with NTs is one of the important determinants of their clinical efficacy in cancer therapy (55). Physiologically, NTs play an important role in the transport of purine and pyrimidine nucleosides and bases during nucleic acid synthesis. There are two families of NTs in human and rodents: concentrative (CNTs, SLC28) and equilibrative (ENTs, SLC29) nucleoside transporters. The SLC28 family consists of the three Na$^+$-coupled concentrative nucleoside transporters designated as CNT1 to 3. The three isotypes differ in their substrate specificities: CNT1 (*SLC28A1*, also known as "cit") prefers pyrimidine-nucleosides, CNT2 (*SLC28A2*, "cif") prefers purine-nucleosides, and CNT3 (*SLC28A3*, "cib") transports both pyrimidine and purine nucleosides (56, 57). The SLC29 family comprises four members designated as bidirectional ENT1 to 4 (*SLC29A1-4*). Two of these are the best characterized members of this family: ENT1 (NBMPR-sensitive, also known as "es") and ENT2 (NBMPR-insensitive, "ei"). Both are expressed on plasma membranes with similar broad substrate specificities for purine and pyrimidine nucleosides, but ENT2 efficiently transports nucleobases in addition to nucleosides (57, 58).

Gemcitabine was the first agent that showed antitumor activity in patients with pancreatic cancer and is currently also used for advanced breast, non-small cell lung, and ovarian cancer (59). Gemcitabine is efficiently transported by CNT1 and ENT1, and to a lesser extent by CNT3 and ENT2 (60). Recently, ENT1 was reported to be overexpressed in human pancreatic cancer cell lines, demonstrating that ENT1, rather than CNT1, is an NT

isotype that confers sensitivity to gemcitabine (61). This observation is in agreement with the outcome of clinical studies which documented the prognostic significance of ENT1 for efficacy of gemcitabine in pancreatic cancer (62, 63). Although ENT1 is almost ubiquitously expressed in human tissues, significant overexpression of this transporter in cancer may provide a therapeutic window for gemcitabine and other nucleoside analogs. Therefore, an increase in ENT1 expression can potentially augment the effect of gemcitabine. 5Fluorouracil inhibits *de novo* DNA synthesis by blocking thymidylate synthase, which in turn allows cells to alternatively enhance the salvage pathway of DNA synthesis by up-regulating NBMPR-sensitive nucleoside transport (64). In mice implanted with human pancreatic cancer MiaPaCa-2 cells, Tsujie *et al.* showed that animals treated with uracil-tegafur (UFT) exhibited significantly increased ENT1 mRNA in xenografts. As a consequence of this, treatment with UFT followed by gemcitabine led to significant growth inhibition of xenografts as compared to either untreated mice or mice treated with UFT alone (65), suggesting that optimization of an administration schedule of gemcitabine in combination with thymidylate synthase inhibitors might be important for clinical efficacy of antineoplastic nucleoside analogs that are transported by ENT1 in chemotherapy.

More interestingly, recent studies have shown that, in addition to NBMPR, coronary vasodilators such as dipyridamole and lidoflazine analogs, protein kinase inhibitors such as imatinib, and immunosuppressive agents such as rapamycin are potent modulators of ENT1 and ENT2 (66). Thus, modifying the activity of nucleoside transporters by protein kinase inhibitors may offer an attractive rationale for targeting both protein kinases and nucleoside transporters as a salvage pathway of DNA synthesis, resulting in enhancement of anticancer activity of inhibitors of *de novo* nucleic acid synthesis such as methotrexate and leflunomide.

## 1.6 The Reduced Folate Carrier

The human reduced folate carrier (RFC; *SLC19A1*, also termed RFT or FOLT) mediates the transport of reduced folates and antifolates as divalent anions, and to a lesser extent the phosphate esters of thiamine (67). Disruption of folate metabolism has long been known to inhibit cell growth and is an established target in cancer chemotherapy. Antifolates in clinical use include the dihydrofolate reductase inhibitor methotrexate (MTX) and the thymidylate synthase inhibitor Tomudex (ZD1694). Since folate and its derivatives are hydrophilic divalent anions, they must be transported by carriers into cells to elicit their action. Although the role of RFC in antifolate transport is undisputed (Table I), the folate receptor α (FRα or FOLRs,

also termed FBP) also plays a role in uptake of antifolates to a lesser extent (68). Differential expression levels of RFC and FRα are recognized in normal and cancer cells, hence folate antagonist structure-activity relationships could be of value for predicting drug sensitivity and resistance of tumor cells, or drug related toxicity to normal cells, and for the rational design and development of novel antifolates. In terms of chemoresistance to antifolates in cancer, the level of expression of the RFC might be an important factor in determining the sensitivity of breast cancer cells as well as leukemia cells to antifolate compounds. Loss of function associated with inactivating mutations and reduced gene expression of RFC are well-documented mechanisms of MTX resistance in leukemia and breast cancer. Decreased expression of RFC in primary tumor cells was described with poor response to MTX in patients with osteosarcoma (69) and acute leukemia (70).

## 1.7 Monocarboxylate Transporters

Monocarboxylate transporters (MCT1-4) mediate transport of lactate, pyruvate and ketone bodies across the plasma membranes of mammalian cells in a proton-dependent manner (71). MCT1 (*SLC16A1*) is ubiquitously expressed in tissues in mammals and functions as an $H^+$/monocarboxylate cotransporter or bidirectional monocarboxylate exchanger. Expression of MCT1 in epithelium of the small intestine and the blood-brain barrier (BBB) is important for intestinal absorption and distribution to the CNS of nutrients and exogenous compounds (Figure 1). Monocarboxylic acids, such as lactate and pyruvate, play a major role in glycolysis that is a key mechanism in generating ATP by converting glucose to pyruvate in all mammalian cells. In normal aerobic cells, pyruvate is transformed into acetyl-CoA in the mitochondrion and then utilized to produce ATPs through the Krebs cycle. However, in cancer cells, where the demand for oxygen is always high, the hypoxic tumor environment favors the intensification of anaerobic metabolic pathways, resulting in the high intracellular accumulation of lactate converted from pyruvate for acquisition of ATP by lactate dehydrogenase (LDH) 5, which is up-regulated by hypoxia inducible factor (HIF) 1α. MCT1 appears to be up-regulated in a variety of human malignancies (72), although studies regarding MCT in cancer are rare. Koukourakis *et al.* hypothesized that MCT1 plays a vital role in transporting lactate out of tumor cells in exchange for pyruvate that is regenerated from lactate by LDH1 in stroma cells surrounding tumor cells, where LDH5 is turned off because of the aerobic condition. The differential expression of MCT1 could be a new therapeutic target for cancer chemotherapy. Experimental data from primary neuroblastomas indicate

that inhibition of MCT1 by an indazole-carboxylic acid, lonidamine, caused cell death to a similar extent as did α-cyano-4-OH-cinnamate, a well established MCT1 inhibitor, because of intracellular acidification (73). Such treatment would be selective by virtue of the acidic milieu surrounding tumors, because MCT1 is increasingly active as extracellular pH decreases below 7.0 and lactic acid production increases.

On the other hand, short-chain fatty acids such as pyruvate and butyrate are known inhibitors of histone deacetylase (HDAC). SMCT *(SLC5A8)* is a $Na^+$-dependent monocarbo-xylate transporter for these short-chain fatty acids. This protein is the first membrane transporter postulated to function as a tumor suppressor (74) because the silencing of its expression represents an early event in the progression of cancer cells, so that it would prevent the entry of circulating pyruvate into tumor cells and thus avoid pyruvate-induced cell-death (75). Therefore, pharmacological means to increase the intracellular concentrations of pyruvate in tumor cells *via* induction of SMCT and/or inhibition of LDH5 may have potential in the treatment of cancer.

### 1.8 Glucose Transporter as a Diagnostic Marker

Increased rates of glucose uptake and glucose metabolism in malignant tumors have been documeted and are mediated by the overexpression of the gluose transporter (GLUT1; *SLC2A1*) and key glycolytic enzymes (76, 77). GLUT1 expression in tumor tissues is one of the most characteristic biochemical markers for the transformed phenotype. Positron emission tomography (PET) is an advanced imaging tool for the diagnosis and staging of cancers that was developed based on identifying enhanced glucose uptake in malignant tumor cells. 2-[Fluorine-18]-fluoro-2-deoxy-*D*-glucose (FDG) is taken up by GLUT1 in tumor cells and phosphorylated by hexokinase to FDG-6-phosphate. FDG-6, however is not a good substrate for glycolytic enzymes in subsequent reactions and hence accumulates within cells in a tumor-specific manner. With the availability of senstive PET scanners and successful application of 18-FDG for measuring regional tissue glucose metabolism, enhanced tumoral glucose consumption has gained considerable clinical interest, representing one of the most important pathophysiological mechanisms for detecting human malignant tumors. Numerous studies have revealed correlation of 18-FDG uptake with the expression of GLUT1 in various tumor types inlcuding lung, colorectal, lymphoma, esohageal, melanoma, head and neck, and breast cancer. This suggests that cellular uptake of FDG *via* GLUT-1 is a useful diagnostic tool for carcinoma and should be compared to standard pathological characteristics (78).

## 2. ABC Transporters as Target for Cancer Chemotherapy

### 2.1 ABC Transporters and Multidrug Resistance

The ABC transporters are primary active transport systems of the cell which are widespread from bacteria to humans. ABC transporter proteins are located in the plasma membranes of cells, or in the membranes of different cellular organelles and mediate the translocation of various molecules across these barriers. Most ABC proteins transport a substrate molecule by utilizing the energy generated from ATP hydrolysis, while some form specific membrane channels. Several ABC transporters function as efflux pumps of xenobiotics or toxins and their transport activity has a significant role in modulating the absorption, distribution and excretion of numerous clinically important drugs, particularly anticancer drugs. In terms of self-defense, cells can develop resistance to these cytotoxic compounds by a number of mechanisms including decreased uptake carriers, increased detoxification, alteration in target proteins, or increased excretion. Multidrug resistance (MDR) is a phenomenon where cells become simultaneously resistant to structurally unrelated compounds when exposed to a single cytotoxic agent. MDR was first observed three decades ago but is still a severe obstacle in cancer chemotherapy because it limits clinical efficiency of major anticancer drugs on repeated use. Numerous clinical studies have revealed that the MDR phenotype in tumors is associated with the overexpression of certain ABC proteins, termed MDR transporters, including Pgp, MRP1, MRP2, MRP3, MRP4, MRP5 and BCRP, which mediate efflux transport of xenobiotics and drugs. The MDR mediating Pgp (product of *MDR1*; *ABCB1*) was the first ABC transporter-based resistance mechanism discovered (79) and is probably still the most widely observed mechanism in MDR in human cancer. There are two other ABC transporters which have been demonstrated to participate in the MDR of tumors: the MDR-associated protein 1 (MRP1; *ABCC1*) (80) and the breast cancer resistance protein (BCRP; *ABCG2*, also termed MXR or ABCP1) (81). Since no genes other than *Abcg2* have been found overexpressed in cells from mice with disrupted *Abcb1a, Abcb1b* and *Abcc1* genes in response to topotecan, mitoxantrone, or doxorubicin (82), these three transporters appear to account, at least in part, for MDR observed in tumor cells of both humans and rodents.

### 2.2 MDR transporters in Targeted Cancer Therapy

Inhibitors of the major MDR transporter genes have been developed and extensive experimentation and clinical research have been performed in attempts to block the development of drug resistance. MDR modifying agents, which inhibit the

Table II. *Major substrate drugs and inhibitors/modulators of Pgp, MRP1 and BCRP.*

| Transporter (protein name) | Gene symbol | Substrate anticancer drug | Inhibitor/modulator |
|---|---|---|---|
| P-glycoprotein Pgp | MDR1 ABCB1 | Alkylating agents   Mitomycin Plant alkaloids   Vinblastine, Vincristine, Etoposide   Paclitaxel Antibiotics   Anthracyclines   (Doxorubicin, Daunorubicin, Epirubicin)   Actinomycin D, Dactinomycin, Mitoxantrone TKI-inhibitors   Imatinib Other cytotoxic agents   Topotecan | Cyclosporin A Valspodar (PSC-833) LY335979 (Zosuquidar trihydrochloride) Verapamil Staurosporine Econazole Prazosine GF120918 Reserpine |
| MRP1 | ABCC1 | Alkylating agents   Cisplatin,   Cyclophosphamide, Chlorambucil,   Carmustine Plant alkaloids   Vincristine, Vinblastine, Etoposide   Paclitaxel, Irinotecan (SN-38) Antibiotics   Anthracyclines   (Doxorubicin, Daunorubicin,   Epirubicin, Idarubicin) Other cytotoxic agents   Anti-folates (*e.g.* Methotrexate)   Hydroxyurea, Tamoxifen | MK571 Staurosporine Probenecide Benzbromarone Sulfinpyrazone Indomethacin Genistein CCP (Chlorocarbonyl cyanide phenylhydrazone) |
| BCRP/MXR/ABCP1 | ABCG2 | Antibiotics   Anthracyclines   (Doxorubicin\*, Daunorubicin\*,   Epirubicin\*, Idarubicinol\*)   Mitoxantrone Plant alkaloids   Irinotecan (SN-38) TKI-inhibitors   Imatinib Topoisomerase I inhibitors   Topotecan, 9-Aminocamptothecin   NB-506, J-10788 Other cytotoxic agents   Anti-folates (*e.g.* Methotrexate\*\*)   Flavopiridol | Fumitremorgin C (FTC) FTC analog (*e.g.* Ko-143) GF129018 Iressa (Geftinib) Reserpine Tamoxifen Estrone Anti-estrogen derivatives (TAG-11, TAG-139) |

\*Transport substrate for BCRP mutant (R482T); \*\*Transport substrate for BCRP wild-type.

function of the MDR transporters either competitively or non-competitively, are good candidates for pharmacological modulation. Such compounds are expected to increase the cytotoxic action of drugs that are substrates of MDR transporters by preventing their extrusion by the target cells. Substrates and inhibitors/modulators for the three major ABC transporters are listed in Table II. Since both Pgp and BCRP are located in the apical membranes of epithelial cells of the intestines and BBB, oral bioavailability and CNS penetration of substrate drugs can be low and variable. The inhibition of Pgp and/or BCRP is therefore a logical strategy to improve oral absorption and delivery of anticancer drugs to brain tumors or CNS metastases across BBB (83). For example, coadministration of the Pgp and BCRP inhibitor GF120918 significantly increased the systemic exposure of oral topotecan, a substrate for BCRP and to lesser extent for Pgp,

resulting in an apparent oral bioavailability increase from 40% to 97% (84). In addition, a high throughput cell-based screen for *BCRP* has been established and is being used to identify new inhibitors (85). Inhibition of these transporters is likely to cause toxicity in a patient's normal stem cells, particularly those of the bone marrow, or might enhance drug distribution to the CNS across the BBB. Therefore, Pgp and BCRP inhibitors would have to be carefully titrated in order to avoid excessive side-effects.

The use of monoclonal antibodies to block MDR transporters may be another approach to reverse MDR. Several antibodies have been reported to inhibit *in vitro* Pgp-mediated drug efflux (86). Antibodies that bind to extracellular epitopes of transporters may provide a supplement to chemical agents for the reversal of MDR in cancer. In addition, a method to eliminate MDR is the use of macromolecular carriers. Conjugation of anti-tumor agents to various drug carriers, including antibodies specific for MDR transporters may be a clinically useful application. Methods involving inhibition of expression or functional removal of mRNA using antisense oligonucleotides or ribozymes targeting MDR proteins were recently reviewed (87, 88).

## 2.3 Stem cells/Cancer Stem Cells and MDR Transporters

Over the past three decades, the cancer stem cell (CSC) hypothesis has postulated that only a small population of cancer cells is capable of giving rise to new tumors. Since tumorigenic CSCs were first identified in acute myeloid leukemia (89), the concept that cancer develops from CSCs is gaining wider acceptance. Almost all current therapies against cancer target differentiated cancer cells, but not CSCs, hence CSCs that are more resistant to therapy and relatively quiescent can escape the treatment, resulting in recurrence of the tumor. Although CSCs and normal stem cells are expected to have many similar properties, the challenge in eradicating CSCs from tumor cells provides a new rationale for CSC-targeted cancer chemotherapy without affecting normal tissue stem cells. Hence, finding unique pathways in CSCs is of the utmost importance.

Pgp and BCRP are the most extensively studied MDR transporters in stem cells (90) and are found to be expressed in normal stem cells and CSCs (91-93). Recently, flow cytomet-ric methods demonstrated that a small population of cells ($\sim 0.1\%$) called the side population (SP), with low Hoechst dye accumulation, was able to reconstitute tumors similar to the tumor of origin in NOD-SCID mice (94). The SP is a CSC/progenitor-enriched population and has been identified in many normal tissues including mammary glands, lung, muscle, heart, liver, brain and skin in both human and animal models (90), and in most human tumor stem cells (92, 93). Current evidence suggests that BCRP contributes

significantly to the generation of the SP phenotype, which in turn protects stem cells from xenobiotic toxins. With respect to MDR in cancer, it may be important for efficient clinical outcome to modulate or block the function of BCRP in CSCs. Therefore the combination of anticancer drugs with inhibitors targeting ABC transporters, in particular BCRP, may be an efficient way to combat malignant tumors (95). Moreover, recent reports suggest that transcription of BCRP and mouse ortholog is likely regulated by multiple promoters in response to drug exposure (96), or in a hematopoietic stem cell-specific manner (97). Previously a sharp down-regulation of BCRP expression at the stage of lineage commitment was observed, suggesting that this gene may play an important role in the unique physiology of pluripotent stem cells. In this respect, determination of precise transcriptional control of this gene in human hematopoietic stem or SP cells may provide a new drug target for modulating BCRP expression in a stem cell-specific manner, although differential promoter usage by CSCs remains to be proven. Malignancies appear to be heterogeneous with respect to drug responsiveness. Cancer that responds to therapy initially appears to acquire drug resistance during the course of treatment, the mechanism of which may be different from the mechanism that intrinsically occurs. If normal tissue stem cells are subject to transforming events leading to the generation of CSCs, CSCs and normal stem cells are expected to have many similar properties. However, cancer stem cells can also arise from a mature (*i.e.* differentiated or committed) cell (98). Whether these two types of CSCs have similar characteristics or not remains unclear.

Finally, in addition to ABC transporters, little is known about expression of SLC transporters in cancer stem cells. Differential expression of influx transporters in CSCs could be targets for delivery of anticancer agents to CSCs. In this respect, expression profiles of SLC transporters should be investigated in normal tissue stem cells and CSCs.

## Conclusion

It is essential to specifically target anticancer drugs to the tumor cells. This will increase clinical efficiency and reduce side-effects of anticancer agents. The effectiveness of cancer chemotherapy will often depend on the relative transport capacities of normal and cancer cells. In as much as transporters are gatekeepers that regulate entry of anticancer drugs into cells, transporters that are differentially expressed in cancer and tumor cells are also targets for cancer chemotherapy. Therefore drug design or pro-drug derivatization of anticancer agents that consider substrate specificities and tissue distribution of transporters could create efficient anticancer agents and improve currently available therapies. In addition, the development of inhibitors specific to transporters that play a role in tumor cell survival may have

pharmacological potential as anticancer agents. Moreover investigations into transporters overexpressed by cancer stem cells may provide new targets for cancer chemotherapy. Finally, this review of drug transporters and their importance in cancer biology and chemotherapy clearly highlights the pivotal role of drug transporters in translational research. The large variety of human transporter proteins underscores the inclusion of the study of drug transporter expression patterns for optimization and individualized use of cancer medicines.

## Acknowledgements

## References

1   Venter JC, Adams MD, Myers EW *et al*: The sequence of the human genome. Science *291*: 1304-1351, 2001.

2   Hediger MA, Romero MF, Peng J-B, Rolfs A, Takanaga H and Bruford EA: The ABCs of solute carriers: Physiological, pathological and therapeutic implications of human membrane transport proteins. Pflugers Arch *447*: 465-468, 2004.

3   Dean M and Allikmets R: Complete characterization of the human ABC gene family. J Bioenergetics and Biomembranes *33*: 475-479, 2001.

4   Dean M: The genetics of ATP-binding cassette transporters. Methods Enzymol *400*: 409-429, 2005.

5   Mizuno N, Niwa T, Yotsumoto Y and Sugiyama Y: Impact of drug transporter studies on drug discovery and development. Pharmacol Rev *55*: 425-461, 2003.

6   Tsuji A: Tissue selective drug delivery utilizing carrier-mediated transport systems. J Control Rel *62*: 239-244, 1999.

7   Shu C, Shen H, Teuscher NS, Lorenzi PJ, Keep RF and Smith DE: Role of PEPT2 in peptide/mimetic trafficking at the blood-cerebrospinal fluid barrier: Studies in rat choroid plexus epithelial cells in primary culture. J Pharmacol Exp Ther *301*: 820-829, 2002.

8   Tsuji A: Transporter-mediated drug interactions. Drug Metab Pharmacokinet *17*: 253-274, 2002.

9   Nakanishi T, Tamai I, Sai Y, Sasaki T and Tsuji A: Carrier-mediated transport of oligopeptides in the human fibrosarcoma cell line HT1080. Cancer Res *57*: 4118-4122, 1997.

10  Gonzalez DE, Covitz K-MY, Sadee W and Mrsny RJ: An oligopeptide transporter is expressed at high levels in the pancreatic carcinoma cell lines AsPc-1 and Capan-2. Cancer Res *58*: 519-525, 1998.

11  Knutter I, Rubio-Aliaga I, Boll M, Hause G, Daniel H, Neubert K and Brandsch M: H$^+$-peptide cotransport in the human bile duct epithelium cell line SK-ChA-1. Am J Physiol Gastrointest Liver Physiol *283*: G222-229, 2002.

12  Inoue M, Terada T, Okuda M and Inui K: Regulation of human peptide transporter 1 (PEPT1) in gastric cancer cells by anticancer drugs. Cancer Letters *230*: 72-80, 2005.

13  Nakanishi T, Tamai I, Takaki A and Tsuji A: Cancer cell-targeted drug delivery utilizing oligopeptide transport activity. Int J Cancer *88*: 274-280, 2000.

14  Landowski CP, Vig BS, Song X and Amidon GL: Targeted delivery to PEPT1-overexpressing cells: Acidic, basic and secondary floxuridine amino acid ester prodrugs. Mol Cancer Ther *4*: 659-667, 2005.

15  Peng Q, Warloe T, Berg K, Moan J, Kongshaug M, Giercksky K and Nesland J: 5-Aminolevulinic acid-based photodynamic therapy. Cancer *79*: 2282-2308, 1997.

16  Zopf T and Riemann JF: The change in laser usage in gastroenterology-the status in 1997. Z Gastroenterol *35*: 987-997, 1997.

17  Neumann J and Brandsch M: delta -Aminolevulinic acid transport in cancer cells of the human extrahepatic biliary duct. J Pharmacol Exp Ther *305*: 219-224, 2003.

18  Souba WW: Glutamine and cancer. Ann Surg *218*: 715-728, 1993.

19  Bode BP, Kaminski DL, Souba WW and Li AP: Glutamine transport in isolated human hepatocytes and transformed liver cells. Hepatology *21*: 511-520, 1995.

20  Bode BP, Fuchs BC, Hurley BP, Conroy JL, Suetterlin JE, Tanabe KK, Rhoads DB, Abcouwer SF and Souba WW: Molecular and functional analysis of glutamine uptake in human hepatoma and liver-derived cells. Am J Physiol Gastrointest Liver Physiol *283*: G1062-1073, 2002.

21  Witte D, Ali N, Carlson N and Younes M: Pharmacology and therapeutics. Anticancer Res *22*: 2555-2557, 2002.

22  Dolinska M, Dybel A, Zablocka B and Albrecht J: Glutamine transport in C6 glioma cells shows ASCT2 system characteristics. Neurochem Int *43*: 501-507, 2003.

23  Sidoryk M, Matyja E, Dybel A, Zielinska M, Bogucki J, Jaskolski DJ, Liverski PP, Kowalczyk P and Albrecht J: Increased expression of a glutamine transporter SNAT3 is a marker of malignant gliomas. Neuroreport *15*: 575-578, 2004.

24  Hyde R, Taylor PM and Hundal HS: Amino acid transporters: Roles in amino acid sensing and signalling in animal cells. Biochem J *373*: 1-18, 2003.

25  Yanagida O, Kanai Y, Chairoungdua A, Kim DK, Segawa H, Nii T, Cha SH, Matsuo H, Fukushima J-I, Fukasawa Y, Tani Y, Taketani Y, Uchino H, Kim JY, Inatomi J, Okayasu I, Miyamoto K-I, Takeda E, Goya T and Endou H: Human L-type amino acid transporter 1 (LAT1): Characterization of function and expression in tumor cell lines. Biochim Biophys Acta *1514*: 291-302, 2001.

26  Wolf DA, Wang S, Panzica MA, Bassily NH and Thompson NL: Expression of a highly conserved oncofetal gene, TA1/E16, in human colon carcinoma and other primary cancers: Homology to Schistosoma mansoni amino acid permease and Caenorhabditis elegans gene products. Cancer Res *56*: 5012-5022, 1996.

27  Nawashiro H, Otani N, Shinomiya N, Fukui S, Ooigawa H, Shima K, Matsuo H, Kanai Y and Endou H: L-type amino acid transporter 1 as a potential molecular target in human astrocytic tumors. Int J Cancer *119*: 484-492, 2006.

28  Kobayashi H, Ishii Y and Takayama T: Expression of L -type amino acid transporter 1 (LAT1) in esophageal carcinoma. J Surg Oncol *90*: 233-238, 2005.

29  Fuchs BC, Perez JC, Suetterlin JE, Chaudhry SB and Bode BP: Inducible antisense RNA targeting amino acid transporter ATB$^0$/ASCT2 elicits apoptosis in human hepatoma cells. Am J Physiol Gastrointest Liver Physiol *286*: G467-478, 2004.

30 Hatanaka T, Nakanishi T, Huang W, Leibach FH, Prasad PD, Ganapathy V and Ganapathy ME: Na$^+$- and Cl$^-$-coupled active transport of nitric oxide synthase inhibitors *via* amino acid transport system B$^{0,+}$. J Clin Invest *107*: 1035-1043, 2001.

31 Jenner P and Olanow C: Oxidative stress and the pathogenesis of Parkinson's disease. Neurology *47*: 161S-170, 1996.

32 Bing RJ, Miyataka M, Rich KA, Hanson N, Wang X, Slosser HD and Shi S-R: Nitric oxide, prostanoids, cyclooxygenase and angiogenesis in colon and breast cancer. Clin Cancer Res *7*: 3385-3392, 2001.

33 Gupta N, Miyauchi S, Martindale RG, Herdman AV, Podolsky R, Miyake K, Mager S, Prasad PD, Ganapathy ME and Ganapathy V: Upregulation of the amino acid transporter ATB$^{0,+}$ (SLC6A14) in colorectal cancer and metastasis in humans. Biochim Biophys Acta *1741*: 215-223, 2005.

34 Thomsen LL, Lawton FG, Knowles RG, Beesley JE, Riveros-Moreno V and Moncada S: Nitric oxide synthase activity in human gynecological cancer. Cancer Res *54*: 1352-1354, 1994.

35 Yagihashi N, Kasajima H, Sugai S, Matsumoto K, Ebina Y, Morita T, Murakami T and Yagihashi S: Increased *in situ* expression of nitric oxide synthase in human colorectal cancer. Virchows Archiv *V436*: 109-114, 2000.

36 Gupta N, Prasad PD, Ghamande S, Moore-Martin P, Herdman AV, Martindale RG, Podolsky R, Mager S, Ganapathy ME and Ganapathy V: Up-regulation of the amino acid transporter ATB$^{0,+}$ (SLC6A14) in carcinoma of the cervix. Gynec Oncol *100*: 8-13, 2006.

37 Nakanishi T, Hatanaka T, Huang W, Prasad PD, Leibach FH, Ganapathy ME and Ganapathy V: Na$^+$- and Cl$^-$-coupled active transport of carnitine by the amino acid transporter ATB$^{0,+}$ from mouse colon expressed in HRPE cells and Xenopus oocytes. J Physiol (Lond) *532*: 297-304, 2001.

38 Huang Y, Dai Z, Barbacioru C and Sadee W: Cystine-glutamate transporter SLC7A11 in cancer chemosensitivity and chemoresistance. Cancer Res *65*: 7446-7454, 2005.

39 Hagenbuch B and Meier P: Organic anion transporting polypeptides of the OATP/SLC21 family: Phylogenetic classification as OATP/SLCO superfamily, new nomenclature and molecular/functional properties. Pflugers Archiv *447*: 653-65, 2004.

40 Hagenbuch B and Meier PJ: The superfamily of organic anion transporting polypeptides. Biochim Biophys Acta *1609*: 1-18, 2003.

41 Kramer W, Wess G, Schubert G, Bickel M, Girbig F, Gutjahr U, Kowalewski S, Baringhaus K, Enhsen A and Glombik H: Liver-specific drug targeting by coupling to bile acids. J Biol Chem *267*: 18598-18604, 1992.

42 Kullak-Ublick GA, Glasa J, Boker C, Oswald M, Grutzner U, Hagenbuch B, Stieger B, Meier PJ, Beuers U, Kramer W, Wess G and Paumgartner G: Chlorambucil-taurocholate is transported by bile acid carriers expressed in human hepatocellular carcinomas. Gastroenterology *113*: 1295-1305, 1997.

43 Criado J, Herrera M, Palomero M, Medarde M, Rodriguez E and Marin J: Synthesis and characterization of a new bile acid and platinum(II) complex with cytostatic activity. J Lipid Res *38*: 1022-1032, 1997.

44 Criado JJ, Dominguez MF, Medarde M, Fernandez ER, Macias RIR and Marin JJG: Structural characterization, kinetic studies and *in vitro* biological activity of new cis-diamminebis-cholylglycinate(O,O') Pt(II) and cis-diamminebis-ursodeoxycholate (O,O') Pt(II) complexes. Bioconjugate Chem. *11*: 167-174, 2000.

45 Eun-ok I, Choi YH, Paik K-J, Suh H, Jin Y, Kim K-W, Yoo YH and Kim ND: Novel bile acid derivatives induce apoptosis *via* a p53-independent pathway in human breast carcinoma cells. Cancer Letters *163*: 83-93, 2001.

46 Briz O, Serrano MA, Rebollo N, Hagenbuch B, Meier PJ, Koepsell H and Marin JJG: Carriers involved in targeting the cytostatic bile acid-cisplatin derivatives cis-diammine-chloro-cholylglycinate-platinum(II) and cis-diammine-bisursodeoxy-cholate-platinum(II) toward liver cells. Mol Pharmacol *61*: 853-860, 2002.

47 Dominguez MF, Macias RIR, Izco-Basurko I, de la Fuente A, Pascual MJ, Criado JM, Monte MJ, Yajeya J and Marin JJG: Low *in vivo* toxicity of a novel cisplatin-ursodeoxycholic derivative (Bamet-UD2) with enhanced cytostatic activity *versus* liver tumors. J Pharmacol Exp Ther *297*: 1106-1112, 2001.

48 Badagnani I, Castro RA, Taylor TR, Brett CM, Huang CC, Stryke D, Kawamoto M, Johns SJ, Ferrin TE, Carlson EJ, Burchard EG and Giacomini KM: Interaction of methotrexate with organic-anion transporting polypeptide 1A2 and its genetic variants. J Pharmacol Exp Ther *318*: 521-529, 2006.

49 Nozawa T, Minami H, Sugiura S, Tsuji A and Tamai I: Role of organic anion transporter OATP1B1 (OATP-C) in hepatic upatke of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: *In vitro* evidence and effect of single nucleotide polymorphisms. Drug Metab Dispos *33*: 434-439, 2005.

50 Abe T, Unno M, Onogawa T, Tokui T, Kondo TN, Nakagomi R, Adachi H, Fujiwara K, Okabe M, Suzuki T, Nunoki K, Sato E, Kakyo M, Nishio T, Sugita J, Asano N, Tanemoto M, Seki M, Date F, Ono K, Kondo Y, Shiiba K, Suzuki M, Ohtani H, Shimosegawa T, Iinuma K, Nagura H, Ito S and Matsuno S: LST-2, a human liver-specific organic anion transporter, determines methotrexate sensitivity in gastrointestinal cancers. Gastroenterology *120*: 1689-1699, 2001.

51 Koepsell H and Endou H: The SLC22 drug transporter family. Pflugers Archiv *V447*: 666-676, 2004.

52 White DL, Saunders VA, Dang P, Engler J, Zannettino ACW, Cambareri AC, Quinn SR, Manley PW and Hughes TP: OCT-1-mediated influx is a key determinant of the intracellular uptake of imatinib but not nilotinib (AMN107): Reduced OCT-1 activity is the cause of low *in vitro* sensitivity to imatinib. Blood *108*: 697-704, 2006.

53 Zhang S, Lovejoy KS, Shima JE, Lagpacan LL, Shu Y, Lapuk A, Chen Y, Komori T, Gray JW, Chen X, Lippard SJ and Giacomini KM: Organic cation transporters are determinants of oxaliplatin cytotoxicity. Cancer Res *66*: 8847-8857, 2006.

54 Okabe M, Unno M, Harigae H, Kaku M, Okitsu Y, Sasaki T, Mizoi T, Shiiba K, Takanaga H, Terasaki T, Matsuno S, Sasaki I, Ito S and Abe T: Characterization of the organic cation transporter SLC22A16: A doxorubicin importer. Biochem Biophys Research Comm *333*: 754-762, 2005.

55 Damaraju V, Damaraju S, Young J, Baldwin S, Mackey J, Sawyer M and Cass E: Nucleoside anticancer drugs: The role of nucleoside transporters in resistance to cancer chemotherapy. Oncogene *22*: 7524-7536, 2003.

56 Gray J, Owen R and Giacomini K: The concentrative nucleoside transporter family, SLC28. Pflugers Archiv *447*: 728-734, 2004.

57 Baldwin S, Mackey J, Cass C and Young JD: Nucleoside transporters: Molecular biology and implications for therapeutic development. Mol Med Today *5*: 216-224, 1999.

58 Baldwin SA, Beal P, Yao SM, King A, Cass C and Young J: The equilibrative nucleoside transporter family, SLC29. Pflugers Archiv 447: 735-743, 2004.

59 Barton-Burke M: Gemcitabine: A pharmacologic and clinical overview. Cancer Nurs 22: 176-183, 1999.

60 Mackey JR, Mani RS, Selner M, Mowles D, Young JD, Belt JA, Crawford CR and Cass CE: Functional nucleoside transporters are required for gemcitabine influx and manifestation of toxicity in cancer cell lines. Cancer Res 58: 4349-4357, 1998.

61 Garcia-Manteiga J, Molina-Arcas M, Casado FJ, Mazo A and Pastor-Anglada M: Nucleoside transporter profiles in human pancreatic cancer cells: Role of hCNT1 in 2',2'-difluorodeo-xycytidine-induced cytotoxicity. Clin Cancer Res 9: 5000-5008, 2003.

62 Spratlin J, Sangha R, Glubrecht D, Dabbagh L, Young JD, Dumontet C, Cass C, Lai R and Mackey JR: The absence of human equilibrative nucleoside transporter 1 is associated with reduced survival in patients with gemcitabine-treated pancreas adenocarcinoma. Clin Cancer Res 10: 6956-6961, 2004.

63 Giovannetti E, Del Tacca M, Mey V, Funel N, Nannizzi S, Ricci S, Orlandini C, Boggi U, Campani D, Del Chiaro M, Iannopollo M, Bevilacqua G, Mosca F and Danesi R: Transcription analysis of human equilibrative nucleoside transporter-1 predicts survival in pancreas cancer patients treated with gemcitabine. Cancer Res 66: 3928-3935, 2006.

64 Pressacco J, Mitrovski B, Erlichman C and Hedley DW: Effects of thymidylate synthase inhibition on thymidine kinase activity and nucleoside transporter expression. Cancer Res 55: 1505-1508, 1995.

65 Tsujie M, Nakamori S, Nakahara S, Takeda S, Takahashi Y, Hayashi N, Okami J, Nagano H, Dono K, Umeshita K, Sakon M and Monden M: Schedule-dependent therapeutic effects of gemcitabine combined with uracil-tegafur in a human pancreatic cancer xenograft model. Pancreas 33: 142-147, 2006.

66 Huang M, Wang Y, Cogut SB, Mitchell BS and Graves LM: Inhibition of nucleoside transport by protein kinase inhibitors. J Pharmacol Exp Ther 304: 753-760, 2003.

67 Ganapathy V, Smith S and Prasad P: SLC19: The folate/ thiamine transporter family. Pflugers Archiv V447: 641-646, 2004.

68 Westerhof G, Schornagel J, Kathmann I, Jackman A, Rosowsky A, Forsch R, Hynes J, Boyle F, Peters G and Pinedo H: Carrier- and receptor-mediated transport of folate antagonists targeting folate-dependent enzymes: Correlates of molecular-structure and biological activity. Mol Pharmacol 48: 459-471, 1995.

69 Guo W, Healey JH, Meyers PA, Ladanyi M, Huvos AG, Bertino JR and Gorlick R: Mechanisms of methotrexate resistance in osteosarcoma. Clin Cancer Res 5: 621-627, 1999.

70 Gorlick R, Cole P, Banerjee D, Longo G, Li WW and Bertino JR: Mechanisms of methotrexate resistance in acute leukemia. Decreased transport and polyglutamylation. Adv Exp Med Biol 457: 543-550, 1999.

71 Halestrap AP and Meredith D: The SLC16 gene family from monocarboxylate transporters (MCTs) to aromatic amino acid transporters and beyond. Pflugers Archiv V447: 619-628, 2004.

72 Koukourakis MI, Giatromanolaki A, Harris AL and Sivridis E: Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: A metabolic survival role for tumor-associated stroma. Cancer Res 66: 632-637, 2006.

73 Fang J, Quinones QJ, Holman TL, Morowitz MJ, Wang Q, Zhao H, Sivo F, Maris JM and Wahl ML: The $H^+$-linked monocarboxylate transporter (MCT1/SLC16A1): A potential therapeutic target for high-risk neuroblastoma. Mol Pharmacol 70: 2108-2115, 2006.

74 Li H, Myeroff L, Smiraglia D, Romero MF, Pretlow TP, Kasturi L, Lutterbaugh J, Rerko RM, Casey G, Issa J-P, Willis J, Willson JKV, Plass C and Markowitz SD: SLC5A8, a sodium transporter, is a tumor suppressor gene silenced by methylation in human colon aberrant crypt foci and cancers. PNAS 100: 8412-8417, 2003.

75 Thangaraju M, Gopal E, Martin PM, Ananth S, Smith SB, Prasad PD, Sterneck E and Ganapathy V: SLC5A8 triggers tumor cell apoptosis through pyruvate-dependent inhibition of histone deacetylases. Cancer Res 66: 11560-4, 2006.

76 McGowan KM, Long SD and Pekala PH: Glucose transporter gene expression: Regulation of transcription and mRNA stability. Pharmacol & Therap 66: 465-505, 1995.

77 Wahl RL: Targeting glucose transporters for tumor imaging: "Sweet" idea, "Sour" result. J Nucl Med 37: 1038-1041, 1996.

78 Kostakoglu L, Agress H Jr and Goldsmith SJ: Clinical role of FDG PET in evaluation of cancer patients. Radiographics 23: 315-340, 2003.

79 Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE and Gottesman MM: P-glycoprotein: From genomics to mechanism. Oncogene 22: 7468-7485, 2003.

80 Leslie EM, Deeley RG and Cole SPC: Toxicological relevance of the multidrug resistance protein 1, MRP1 (ABCC1) and related transporters. Toxicology 167: 3-23, 2001.

81 Doyle LA and Ross DD: Multidrug resistance mediated by the breast cancer resistance protein BCRP (ABCG2). Oncogene 22: 7340-7358, 2003.

82 Allen JD, Brinkhuis RF, Wijnholds J and Schinkel AH: The mouse BCRP1/MXR/ABCP gene: Amplification and overexpression in cell lines selected for resistance to topotecan, mitoxantrone, or doxorubicin. Cancer Res 59: 4237-4241, 1999.

83 Breedveld P, Beijnen JH and Schellens JHM: Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and cns penetration of anticancer drugs. Trends in Pharmacol Sci 27: 17-24, 2006.

84 Kruijtzer CM, Beijnen JH, Rosing H, ten Bokkel Huinink WW, Schot M, Jewell RC, Paul EM and Schellens JH: Increased oral bioavailability of topotecan in combination with the breast cancer resistance protein and p-glycoprotein inhibitor GF120918. J Clin Oncol 20: 2943-2950, 2002.

85 Henrich CJ, Bokesch HR, Dean M, Bates SE, Robey RW, Goncharova EI, Wilson JA and McMahon JB: A high-throughput cell-based assay for inhibitors of ABCG2 activity. J Biomol Screen 11: 176-183, 2006.

86 Mechetner E and Roninson I: Efficient inhibition of p-glycoprotein-mediated multidrug resistance with a monoclonal antibody. PNAS 89: 5824-5828, 1992.

87 Pawlak W, Zolnierek J, Sarosiek T and Szczylik C: Antisense therapy in cancer. Cancer Treatment Reviews 26: 333-350, 2000.

88 Gottesman MM, Fojo T and Bates SE: Multidrug resistance in cancer: Role of ATP-dependent transporters. Nat Rev Cancer 2: 48-58, 2002.

89 Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA and Dick JE: A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. 367: 645-648, 1994.

90 Hadnagy A, Gaboury L, Beaulieu R and Balicki D: Sp analysis may be used to identify cancer stem cell populations. Exp Cell Res *312*: 3701-3710, 2006.

91 Kim M, Turnquist H, Jackson J, Sgagias M, Yan Y, Gong M, Dean M, Sharp JG and Cowan K: The multidrug resistance transporter ABCG2 (breast cancer resistance protein 1) effluxes Hoechst 33342 and is overexpressed in hematopoietic stem cells. Clin Cancer Res *8*: 22-28, 2002.

92 Scharenberg CW, Harkey MA and Torok-Storb B: The ABCG2 transporter is an efficient Hoechst 33342 efflux pump and is preferentially expressed by immature human hematopoietic progenitors. Blood *99*: 507-512, 2002.

93 Hirschmann-Jax C, Foster AE, Wulf GG, Nuchtern JG, Jax TW, Gobel U, Goodell MA and Brenner MK: A distinct "side population" of cells with high drug efflux capacity in human tumor cells. PNAS *101*: 14228-14233, 2004.

94 Goodell M, Brose K, Paradis G, Conner A and Mulligan R: Isolation and functional properties of murine hematopoietic stem cells that are replicating *in vivo*. J Exp Med *183*: 1797-1806, 1996.

95 Dean M, Fojo T and Bates S: Tumour stem cells and drug resistance. Nat Rev Cancer *5*: 275-284, 2005.

96 Nakanishi T, Bailey-Dell KJ, Hassel BA, Shiozawa K, Sullivan DM, Turner J and Ross DD: Novel 5' untranslated region variants of BCRP mRNA are differentially expressed in drug-selected cancer cells and in normal human tissues: Implications for drug resistance, tissue-specific expression and alternative promoter usage. Cancer Res *66*: 5007-5011, 2006.

97 Zong Y, Zhou S, Fatima S and Sorrentino BP: Expression of mouse Abcg2 mRNA during hematopoiesis is regulated by alternative use of multiple leader exons and promoters. J Biol Chem *281*: 29625-29632, 2006.

98 Dean M: Cancer stem cells. Mol Interventions *6*: 140-148, 2006.