基礎転写因子の一つである Sp1 が結合するとされる GC box が複数存在した．これら推定 Sp1 結合サイトにそれぞれ変異を導入することにより転写活性は低下し，また，Caco-2 細胞の核抽出液を用いた Gel Shift Assay より，推定 Sp1 結合サイトと Sp1 の結合が確認された．さらに，PEPT1 の転写活性は，Sp1 の過剰発現により上昇し，Sp1 と DNA との結合阻害剤である mithramycin A 処理により低下した．これらの結果から，ヒト PEPT1 プロモーターの basal activity には，Sp1 が複数の結合部位を通して寄与していることが示された[25]．

Sp1 の発現分布はユビキタスであり，PEPT1 の小腸特異的な発現を説明することはできない．そこで，PEPT1 の組織特異性を規定する因子の候補として，腸管特異的な転写因子であり，小腸上皮細胞の分化や機能維持に重要な役割を果している Cdx2 に着目し解析を行った[26]．Caco-2 細胞で Cdx2 を過剰発現させた場合，PEPT1 のプロモーター活性は顕著に上昇したが，プロモーター上における Cdx2 の反応領域は basal activity に重要であった領域と同一であり，Cdx2 の結合配列は存在しなかった．そこで，Cdx2 の作用機序について，共発現系，クロマチン免疫沈降法等の検討を加えた結果，Cdx2 は Sp1 と相互作用することにより PEPT1 プロモーター領域に作用し，転写を活性化させることが示された（図 4）．また，ヒトの胃組織（腸上皮化生の検体を含む）における PEPT1 と Cdx2 の mRNA 発現量は良好な相関を示し，PEPT1 発現調節における Cdx2 の重要性が *in vivo* の面からも示された．今後は，食餌，ホルモン，日周リズムなどの刺激による PEPT1 の発現調節が，どのような転写因子によって制御されているのかを解明していくことが課題である．

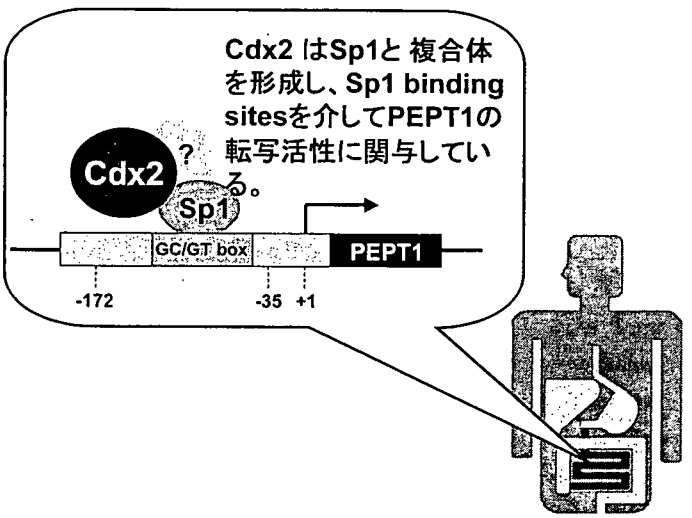

図 4. PEPT1 の転写制御機構

PEPT1 の基礎転写には Sp1 が，また小腸特異的な発現には Cdx2 が関与している[25-26]．Cdx2 は PEPT1 のプロモーター領域に直接結合せず，Sp1 を介して PEPT1 の転写を制御している．

II-C-1-4. おわりに

PEPT1 のクローニング以降，膨大な数のペプチドトランスポーターに関する情報が集積してきた．今後，これらの情報をどのように創薬や医薬品適正使用に応用していくかが，創薬・動態研究者の腕の見せ所だと思う．本稿では述べなかったが，潰瘍性大腸炎・クローン病などの炎症性腸疾患時には，元来発現の見られない大腸において PEPT1 が過剰発現し，大腸菌の産生する白血球走化性因子 N-formyl-methionyl-leucyl-phenylalanine（fMLP）を輸送することによって，慢性炎症に関与していることが示唆されている[27-28]．従って PEPT1 は薬効の標的分子として捉えることも可能であり，今後これら疾患時における，大腸 PEPT1 をターゲットとした，新たな薬物療法や栄養療法の開発も期待される．

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23

Impact of Drug Transport Proteins

Tomohiro Terada and Ken-ichi Inui

Abstract Drug transporters play critical roles in the absorption, distribution, and excretion of drugs and have been classified into five major families, peptide transporters (PEPT, SLC15), organic anion-transporting polypeptides (OATP, SLCO), organic ion transporters (OCT/OCTN/OAT/URAT, SLC22), H^+/organic cation antiporters (MATE, SLC47), and ABC drug transporters, such as P-glycoprotein (P-gp/MDR1, ABCB1). Their structures, tissue distribution, functions, and pharmacokinetic roles vary. The roles of drug transporters can be assessed in vitro and in vivo, using techniques spanning from cellular expression systems to gene knockout animals. Research outcomes from such studies have been applied to clinical science and drug development. In this chapter, the basic characteristics of drug transporters were reviewed with an emphasis on their impact on clinical/preclinical research.

Abbreviations

ABC	ATP-binding cassette
ACR	Acute cellular rejection
BCRP	Breast cancer resistance protein
BSEP	Bile salt export pump
CYP	Cytochrome P450
GFR	Glomerular filtration rate
HGNC	Human Gene Nomenclature Committee
HMG-CoA	3-Hydroxy-3-methylglutaryl coenzyme A
LDLT	Living-donor liver transplantation
MATE	Multidrug and toxin extrusion
MDR	Multidrug resistance protein
MRP	Multidrug resistance-associated protein
NBD	Nucleotide-binding domain
OAT	Organic anion transporter
OATP	Organic anion-transporting polypeptide
OCT	Organic cation transporter
OCTN	Novel organic cation transporter
PAH	P-aminohippurate
PCR	Polymerase chain reaction

PEPT	H^+/peptide cotransporter
P-gp	P-glycoprotein
SLC	Solute carrier
SNP	Single nucleotide polymorphism
SUR	Sulfonylurea receptor
TEA	Tetraethylammonium
TM	Transmembrane
URAT	Urate transporter

Keywords: Drug transporter; SLC-transporter; ABC transporter; Drug delivery; Intestinal absorption barrier1

23.1. Introduction

Drug efficacy and safety are determined by the interplay of multiple processes that regulate pharmacokinetics (e.g., absorption, distribution, metabolism, and excretion) and pharmacodynamics (e.g., drug action). For orally administered drugs, pharmacologic action is dependent on an adequate intestinal absorption and distribution before elimination via metabolic and excretory pathways. Drug-metabolizing enzymes have been believed to be the key determinants of pharmacokinetics. The membrane transport processes are also recognized as important to pharmacokinetic properties, but classical analyses were mainly performed in vivo or in excised tissues, mostly lacking in vitro methodologies to precisely evaluate the membrane transport characteristics of drugs.

In the early 1980s, studies of membrane vesicles and cultured epithelial cell lines were introduced into the research field of drug transport and the biochemical characterization of drug transport advanced remarkably. For example, the driving force and substrate specificity of a drug transporter were clearly demonstrated using membrane vesicles, and transepithelial transport and regulatory aspects were characterized by using cultured cell lines. At the end of 1980s, the molecular nature of drug transporters was unveiled by cDNA cloning and the first clinically important drug transporter, the P-glycoprotein (P-gp), was identified. Subsequently, various primary and secondary active drug transporters were isolated by expression cloning, polymerase chain reaction (PCR) cloning, and *in silico* homology screening strategies. The most recently identified drug transporters are the renal H^+/organic cation antiporters reported in 2005–2006. Although numerous drug transporters have been characterized so far, many others remain unidentified. For example, the molecular nature of the facilitative peptide transporters, which are located at the basolateral membranes of intestinal epithelial cells and are quite important for the transepithelial transport of peptide-like drugs, has not been elucidated.

Many drugs have been recognized to cross the intestinal epithelial cells via passive diffusion, thus their lipophilicity has been considered important. However, as described above, recent studies have demonstrated that a number of drug transporters including uptake and efflux systems determine the membrane transport process. In this chapter, we provide an overview of the basic characteristics of major drug transporters responsible not only for absorption but also for disposition and excretion in order to delineate the impact of drug transport proteins on pharmacokinetics.

23.2. Determination and Classification of Drug Transporters

Transporters have been functionally classified as primary and secondary active transporters. Primary active transporters include ATP-binding cassette (ABC) transporters that utilize the hydrolysis of ATP as a driving force. Secondary active transporters utilize various driving forces such as ion concentration gradients and electrical potential differences across cell membranes, according to the physicochemical properties of substrates and membrane localization of transporters. The Human Gene Nomenclature Committee (HGNC) has classified drug transporters based on sequence similarity as solute carriers (SLCs) and ABC transporters.

Although many members of the ABC and SLC families are categorized as drug transporters, because of their pharmacokinetic relevance and detailed characterization, only the following transporters are discussed in this chapter: peptide transporters (PEPT, SLC15), organic anion-transporting polypeptides (OATP, SLCO), organic ion transporters (OCT/OCTN/OAT/URAT, SLC22) and H^+/organic cation antiporters (MATE, SLC47), P-glycoprotein (P-gp/MDR1, ABCB1), multidrug resistance-associated proteins (MRP2 and MRP3, ABCC), and breast cancer-resistance protein (BCRP, ABCG2). Secondary structures of these transporters are shown in Figure 23.1.

23.3. Characteristics of Major Drug Transporters

23.3.1. PEPT (SLC15)

23.3.1.1. Structure and Tissue Distribution

A cDNA encoding the H^+/peptide cotransporter (PEPT1) was initially identified by expression cloning using a rabbit small intestinal cDNA library [1]. cDNA for the renal peptide transporter PEPT2 cDNA, an isoform of the intestinal PEPT1 has also been isolated [2]. PEPT1 and PEPT2 consist of 707–710 and 729 amino acid residues, respectively, and possess 12 transmembrane (TM) domains. The overall amino acid identity between them is ~50% [1–4]. PEPT1 is localized to brush-border membranes of intestinal and renal epithelial cells [5], whereas PEPT2 is preferentially expressed in the kidney and located at brush-border membranes of renal epithelial cells.

23.3.1.2. Function and Pharmacokinetic Roles

PEPT1 and PEPT2 can transport di- and tripeptides with different molecular sizes and charges, but not free amino acids and peptides composed of four or more peptide bonds [6]. Pharmacologically active peptide-like drugs such as β-lactam antibiotics, bestatin, and angiotensin-converting enzyme (ACE) inhibitors have been also reported to be transported by PEPT1 and PEPT2 [7]. It has been believed that the presence of peptide bonds is the most important factor in the recognition of substrates by peptide transporters. However, the structural requirements of PEPT1 and PEPT2 were reevaluated (most studies were performed with PEPT1), and it was demonstrated that even compounds without peptide bonds can be accepted as substrates (e.g., δ-amino levulinic acid [8], ω-amino fatty acid [9], and amino acid ester compounds [10–12]). Recently, a mathematical model of H^+-coupled transport phenomena via PEPT1 was proposed [13, 14].

Over the last decade, PEPT1 has been utilized as a target for improving the intestinal absorption of poorly absorbed drugs through amino acid-based modifications. For example, the enhanced oral bioavailability of valacyclovir and valganciclovir, L-valine ester prodrugs of acyclovir and ganciclovir,

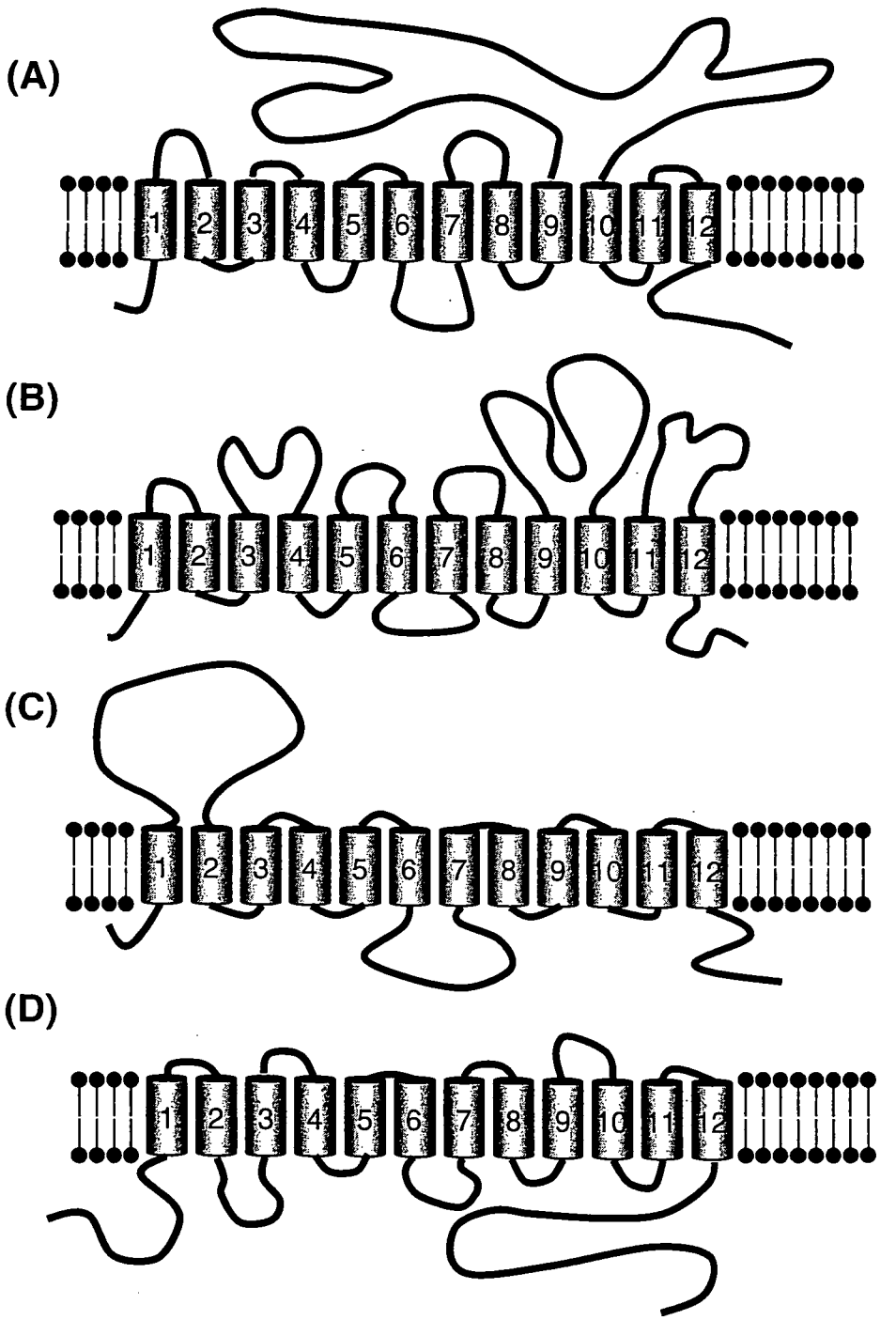

Figure 23.1 Putative secondary structures of various drug transporters. (A–D) Putative secondary structures of SLC drug transporters: (A) Peptide transporters (PEPT, SLC15), (B) Organic anion transporting polypeptides (OATP, SLCO), (C) Organic ion transporters (OCT/OCTN/OAT/URAT, SLC22), and (D) H^+/organic cation antiporter (MATE). (E–G) Putative secondary structures of ABC drug transporters: (E) P-glycoprotein (P-gp/MDR1, ABCB1), (F) Multidrug resistance-associated proteins (MRP, ABCC), and (G) Breast cancer-resistance protein (BCRP, ABCG2).

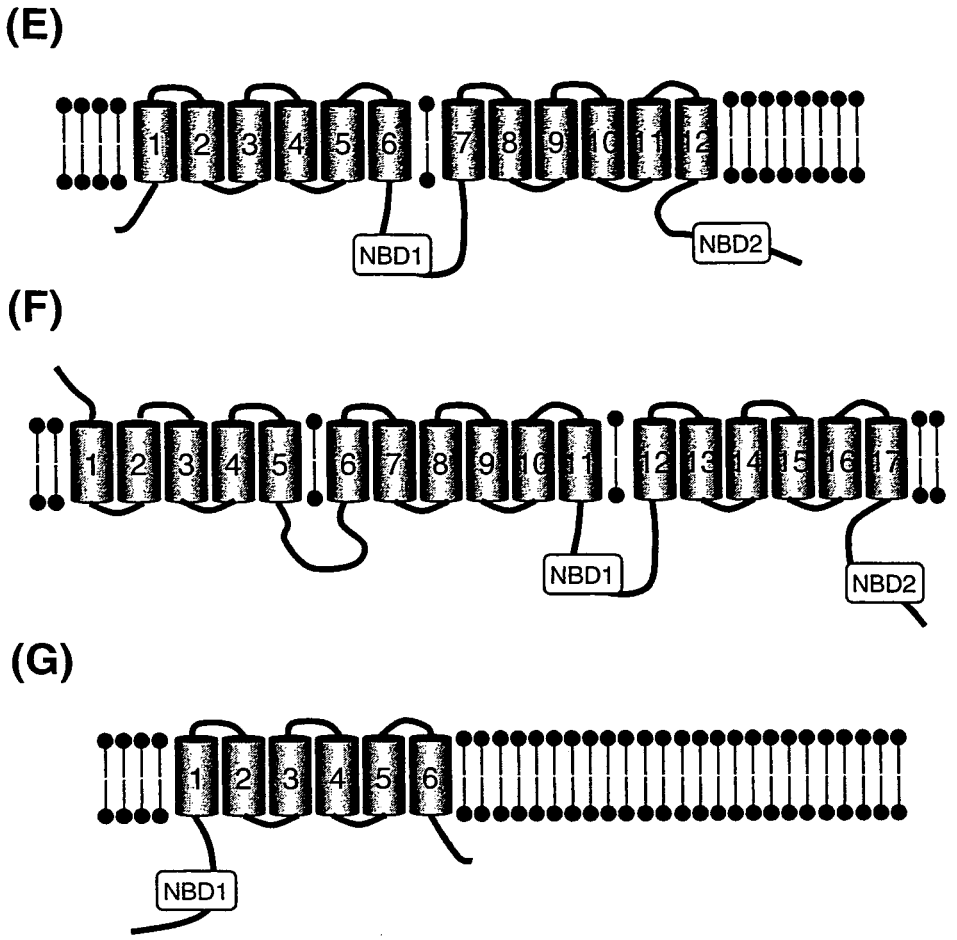

Figure 23.1 *(Continued.)*

respectively, has been attributed to their enhanced intestinal transport via PEPT1 [10, 12], and these drugs have been used in the clinical setting. The anticancer agents (e.g., gemcitabine [15] and floxuridine [16]) and antiviral drugs (e.g., azidothimidine [10] and levovirin [17]) were also converted to PEPT1 substrates by modifying the L-valine ester (Figure 23.2A). Another strategy for converting PEPT1 substrates is an amino acid peptide modification. For example, midodrine, an antihypotension prodrug for combining glycine via a peptide bond with an active drug, was recently demonstrated to be a substrate for PEPT1 [18] (Figure 23.2B). Thus, conversion of poorly absorbed drugs to PEPT1 substrates should be useful for improving oral bioavailability.

23.3.2. OATP (SLCO)

23.3.2.1. Structure and Tissue Distribution

In 1994, a Na^+-independent organic anion-transporting polypeptide (Oatp1) was originally cloned from a rat liver cDNA library [19]. Thereafter, many isoforms of Oatp (rodents)/OATP (human) were identified, but unlike other transporters, this family exhibits large interspecies differences [20]. HGNC designated the OATP family as the SLC21 family early on, but since the traditional *SLC21* gene classification does not permit an unequivocal and species-independent identification of genes and gene products, thereafter, all

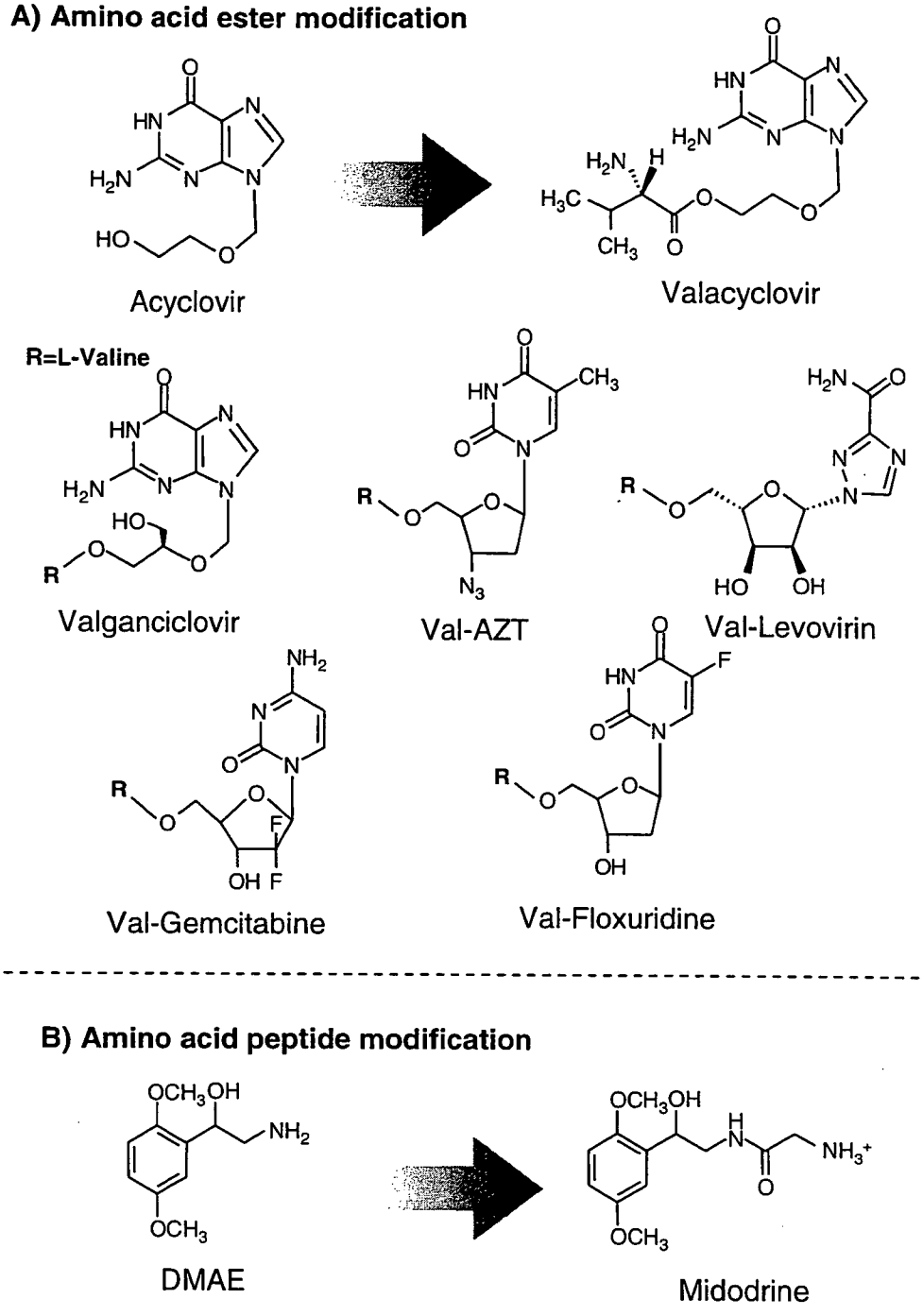

Figure 23.2 Improvement of poorly absorbed drugs using the broad substrate specificity of intestinal PEPT1. (A) Amino acid ester modification of various drugs (e.g., antiviral and anticancer drugs). Among amino acids, L-valine has been suggested to be suitable for this modification. (B) Amino acid peptide modification of the antihypotensive drug midodrine. Among amino acids, glycine has been suggested to be suitable for this modification.

Oatps/OATPs were newly classified within the OATP (protein)/*SLCO (gene)* (human) and Oatp (protein)/*Slco (gene)* (rodents) superfamily according to their phylogenetic relationships and chronology of identification. The methods of classification and the nomenclature were described in detail in a recent review [21].

All members of OATP/Oatp family contain 12 TM domains. Certain transporters show a more restricted tissue expression pattern (i.e., OATP1B1 [old name: OATP-C]/liver), while others such as OATP2B1 (old name: OATP-B) can be detected in almost every tissue that has been investigated [22]. This indicates that some OATPs/Oatps have organ-specific functions, while others might be involved in housekeeping functions.

23.3.2.2. Function and Pharmacokinetic Roles

OATP/Oatp families mediate the Na^+-independent transport of a wide range of amphipathic organic compounds, including bile salts, organic dyes, steroid conjugates, thyroid hormones, anionic oligopeptides, numerous drugs, and other xenobiotic substances [20]. Among the human OATP families, OATP1B1 (old name: OATP-C) has been well characterized. This transporter is exclusively expressed in the liver and located at sinusoidal membranes. Thus, the major pharmacokinetic role of OATP1B1 is hepatic uptake of various clinically important drugs such as pravastatin (3-Hydroxy-3-methylglutaryl coenzyme A [HMG-CoA] reductase inhibitor) [23], enalapril (ACE inhibitor) [24], and valsartan (an angiotensin II receptor antagonist) [25]. Recently, the clinical implications of single nucleotide polymorphisms (SNPs) for the *SLCO1B1* gene were reported first by Nishizato et al. [26], in that the 521T>C (Val174Ala) polymorphism in *SLCO1B1* is associated with increased systemic exposures to pravastatin in Japanese subjects. Thereafter, it was also reported that genetic polymorphisms in *SLCO1B1* are a major determinant of interindividual variability in the pharmacokinetics of pravastatin [27–29], the antidiabetic drug repaglinide [30], and atrasentan, a selective endothelin A receptor antagonist [31]. As described above, the OATP/Oatp family exhibits large interspecies differences and this feature may be responsible for the frequency with which SNPs in *SLCO* genes induce functional changes.

OATP2B1 (old name: OATP-B) is expressed at brush-border membranes of intestinal epithelial cells [32]. OATP2B1exhibited pH-sensitive transport activities for various organic anions such as estrone-3-sulfate, dehydroepiandrosterone sulfate, taurocholic acid, pravastatin, and fexofenadine [33]. However, further studies are needed to determine the specific physiological and pharmacokinetic contribution of OATP2B1 for intestinal absorption of these compounds.

23.3.3. OCT/OCTN/OAT/URAT (SLC22)

23.3.3.1. Structure and Tissue Distribution

The organic ion transporter superfamily is composed of various isoforms differing in the mode of transport (uniporters, symporters, and antiporters) and selectivity of substrate charges, although all isoforms have a similar secondary structure of 12 TM domains. In 1994, the first member of the SLC22 family, organic cation transporter 1 (OCT1), was identified from a rat kidney cDNA library by expression cloning [34]. Rat OCT2 was identified in 1996 [35], and the human zwitterion/cation transporter OCTN1 was discovered in 1997 [36]. In the same year, the first organic anion transporter (OAT1) was cloned from rats [37]. In 1998, OCT3 was identified in rats and humans [38, 39], and the human Na^+-carnitine cotransporter OCTN2 was cloned [40, 41]. Thereafter, various human OAT isoforms (OAT2–OAT4 and urate transporter [URAT1]) were identified [42–45].

In humans, the major expression sites of the SLC22 transporter family are as follows: OCT1/liver (sinusoidal membranes of hepatocytes), OCT2/kidney (basolateral membranes of renal proximal tubules), OCT3/skeletal muscle, placenta, heart, OCTN1/widely distributed, OCTN2/kidney and intestine (brush-border membranes), OAT1/kidney (basolateral membranes of renal proximal tubules), OAT2/liver (sinusoidal membranes of hepatocytes), OAT3/kidney (basolateral membranes of renal proximal tubules), OAT4/kidney (brush-border membranes of renal proximal tubules), and URAT1/kidney (brush-border membranes of renal proximal tubules) [46].

23.3.3.2. Function and Pharmacokinetic Roles

The SLC22 family plays important roles for renal secretion of various compounds (e.g., drugs, toxins, and endogenous metabolites via OCTs and OATs) [47–49], the reabsorption of urate (via URAT1) [50], and the intestinal and renal absorption of carnitine (via OCTN2) [51]. OCTs mediate the membrane potential-dependent uptake of organic cations such as tetraethylammonium (TEA, a typical substrate of OCTs), cimetidine (H_2 blocker), and metformin (antidiabetic agent). Previously, it was believed that the substrate recognition of OCT1, OCT2, and OCT3 is not very different, but a recent study revealed that creatinine is a specific substrate for OCT2 [52]. This finding is clinically relevant, because creatinine clearance is widely used to estimate the glomerular filtration rate (GFR). In other words, creatinine clearance may not reflect the true GFR. Moreover, if cationic drugs (OCT2 substrates) are coadministered, creatinine clearance may be decreased by inhibition of OCT2-mediated creatinine secretion, leading to an underestimation of the renal function. Cisplatin (anticancer agent) is a preferred substrate for OCT2 [53, 54], suggesting that the renal toxicity of cisplatin may be triggered by its uptake via OCT2 into renal proximal tubular cells.

OATs can transport various organic anions and the substrate specificity of each isoform has been characterized. P-aminohippurate (PAH) has been widely used as a typical substrate for renal organic anion transport systems. PAH uptake by OAT1 was stimulated by an outwardly directed gradient of α-ketoglutarate, which is consistent with experimental results from studies using renal basolateral membrane vesicles [55]. Antiviral drugs such as adefovir are preferably recognized by OAT1, suggesting that OAT1 may be responsible for the renal toxicity of antiviral agents [56]. Although OAT3 also recognizes PAH, its substrate specificity is different from that of OAT1. For example, estrone sulfate [43], cimetidine [43], and famotidine [57] are preferentially transported by OAT3, but not by OAT1. In addition, OAT3 exhibits a greater activity to transport cephalosporin antibiotics including cefazolin, as compared with OAT1 [58]. This is supported by clinical findings that the mRNA level of OAT3 is significantly correlated with the rate of elimination of cefazolin [59, 60].

OCTN2 is highly expressed in the human intestine from the jejunum to colon [61]. It was recently demonstrated that OCTN2 is predominantly responsible for the uptake of carnitine from the apical surface of mouse small intestinal epithelial cells, suggesting that OCTN2 could be a promising target for the oral delivery of therapeutic agents [62]. Mutations of transporters for the SLC22 family are responsible for specific diseases such as "primary systemic carnitine deficiency" (OCTN2) [63] or "idiopathic renal hypouricemia" (URAT1) [45],

and also thought to be linked with rheumatoid arthritis (OCTN1) [64] and Crohn's disease (OCTN2) [65].

23.3.4. MATE (SLC47)

23.3.4.1. Structure and Tissue Distribution

Organic cations are excreted by the H^+/organic cation antiporter in the brush-border membranes. As described above, the membrane potential-dependent organic cation transporters located to the basolateral membranes (OCT1–3, SLC22A1–3) have been identified and well characterized [47, 48], but the molecular nature of the H^+/organic cation antiporter has not been elucidated. Recently, based on *in silico* homology screening, human and mouse orthologs of the multidrug and toxin extrusion (MATE) family, which confers multidrug resistance to bacteria, have been identified [66, 67]. Rat MATE1 [68] and the kidney-specific human MATE2 (MATE2-K) [69] were identified next. This particular drug transporter family recently designated as SLC47 family.

MATE1 and MATE2-K consist of 566–570 amino acid residues with 12 TM domains and show about 50% amino acid identity. Human MATE1 is mostly expressed in luminal membranes of renal proximal tubules and liver canalicular membranes. Mouse MATE1 is also predominantly expressed in the kidney and liver, but it is also expressed in brain glia-like cells and capillaries, pancreatic duct cells, urinary bladder epithelium, and adrenal gland cortex [67]. Rat MATE1 mRNA is highly expressed in the kidney, especially in proximal tubules and placenta, but not in the liver [68]. These findings suggest a clear species difference in the distribution of MATE1 among human, mouse, and rat. Human MATE2-K as well as human MATE1 was located at brush-border membranes of renal proximal tubules [69].

23.3.4.2. Function and Pharmacokinetic Roles

MATE1 can transport not only organic cations such as cimetidine and metformin but also the zwitterionic compound cephalexin [68]. MATE2-K also transports various organic cations, but not cephalexin [69]. The substrate recognition characteristics of MATEs are quite similar, but not identical to those of OCTs. For example, cephalexin is a substrate for MATE1, but not for OCTs, while creatinine is a substrate for OCT2, but not for MATEs. MATE1 exhibits pH-dependent transport properties for cellular uptake and efflux studies using TEA as a substrate, while intracellular acidification by NH_4Cl pretreatment stimulates TEA transport [66–69]. Direct evidence that a proton gradient is the driving force for MATE1 activity was reported recently, utilizing membrane vesicles prepared from cells stably expressing MATE1. TEA transport exhibited the overshoot phenomenon only when there was an outwardly directed H^+ gradient across the vesicles [70], which has been also observed in rat renal brush-border membrane vesicles [71]. These findings indicate that an oppositely directed H^+ gradient serves as a driving force for MATE1.

23.3.5. ABC Transporters

23.3.5.1. Structure and Tissue Distribution

P-glycoprotein (P-gp, MDR1) was first isolated from cancer cells where it extrudes chemotherapeutic agents out of the cell thereby conferring multidrug resistance [72]. Subsequent analyses have demonstrated that P-gp is expressed

in various normal tissues and involved in the pharmacokinetics of a wide range of drugs, mediating the efflux of drugs from the intracellular to the extracellular space [73]. Many isoforms of ABC drug transporters have been isolated and characterized. Currently, the ABC superfamily is designated based on the sequence and organization of their ATP-binding domains, representing the largest family of TM proteins (e.g., transporters, ion channels, and receptors). This family is subdivided based on similarities in domain structure, nucleotide-binding folds, and TM domains [74]. Of the 48 members identified to date, several members are well characterized as drug transporters. P-gp/MDR1 is now designated as ABCB1. MDR3 (ABCB4) and BSEP (bile salt export pump or sister P-gp, ABCB11) are included in the ABCB subfamily. The MRP subfamily includes MRP1 (ABCC1), MRP2 (ABCC2), MRP3 (ABCC3), MRP4 (ABCC4), and other MRP isoforms [75]. Other members of the ABCC subfamily include CFTR (cystic fibrosis transmembrane conductance regulator, a Cl^- channel) and SUR1 and SUR2 (sulfonylurea receptors). BCRP (ABCG2) belongs to a different subfamily known as the White subfamily [76]. The clinical relevance of P-gp has been demonstrated in terms of drug interaction, gene polymorphisms, and expression levels.

The general structure of ABC transporters comprises 12 TM regions, split into two halves, each with a nucleotide-binding domain (NBD) [77]. However, there are a number of exceptions to this arrangement. For example, MRP1–3 have an additional five TM regions at the N terminus. BCRP has only six TM regions and one NBD and is known as a half transporter. In general, ABC transporters are expressed in blood–tissue barriers such as the blood–brain barrier and at the luminal surface of epithelial cells such as intestinal epithelial cells to protect the cells from toxic substances.

23.3.5.2. Function and Pharmacokinetic Roles

23.3.5.2.1. MDR1 (P-GP and ABCB1): P-gp has an extremely broad substrate specificity, with a tendency towards lipophilic, cationic compounds. The list of its substrates/inhibitors is continually growing and includes anticancer agents, antibiotics, antivirals, calcium channel blockers, and immunosuppressive agents. Its physiological function was clearly demonstrated by creating an *Mdr1a/1b*$^{-/-}$ mouse [78]. Studies in vivo using this mouse model revealed that MDR1 functions as a gatekeeper of the blood–brain barrier, blood–placental barrier, blood–testis barrier, and gut [79, 80]. P-gp is expressed at brush-border membranes of enterocytes, where it functions as the efflux pump for xenobiotics in the intestinal lumen before they can access the portal circulation.

The antituberculosis drug rifampicin (rifampin) is known to affect a number of drug-metabolizing enzymes such as cytochrome P450 (CYP) 3A4 in the liver and in the small intestine, causing a loss of efficacy of drugs metabolized by CYP3A4. In addition, rifampicin induces the intestinal expression of P-gp, decreasing the oral bioavailability of P-gp substrates such as digoxin [81] and talinorol [82]. Not only drugs but also herbal products, that is, St. John's wort, administered for a long term have been shown to induce the intestinal expression of CYP3A and P-gp, consistent with the loss of efficacy of various drug therapies in earlier case reports and specific clinical studies [83].

The expression level of intestinal MDR1 mRNA has been utilized to the personalized immunosuppressant therapy with tacrolimus in cases of living-donor liver transplantation (LDLT) [84]. Tacrolimus shows wide

intra- and interindividual pharmacokinetic variability, especially in bioavailability after oral administration. P-gp and CYP3A4 are suggested to cooperate in the intestinal absorption of tacrolimus. Our laboratories reported an inverse correlation between the tacrolimus concentration/dose (C/D) ratio and the intestinal mRNA level of MDR1 ($r = -0.776$), but not of CYP3A4 ($r = -0.096$), in 46 cases [85] which was confirmed in studies with a larger population ($r = -0.645$, $n = 104$) [86]. Furthermore, a higher level of intestinal MDR1 expression was strongly associated with the probability of acute cellular rejection (ACR), but there was no significant association between the intestinal CYP3A4 mRNA level and ACR. These results indicate that the expression level of intestinal MDR1 mRNA found with LDLT is not only a pharmacokinetic factor, but also a significant biomarker for ACR [87].

23.3.5.2.2. MRP2 (ABCC2): MRP2 was first functionally characterized as a canalicular multispecific organic anion transporter in canalicular membranes of hepatocytes [88]. This transporter can accept a diverse range of substrates, including glutathione, glucuronide, and sulfate conjugates of many endo- and xenobiotics and expressed at the apical domain of hepatocytes, enterocytes of the proximal small intestine and proximal renal tubular cells, as well as in the brain and placenta. Mutation of MRP2 causes the Dubin-Johnson syndrome [89].

23.3.5.2.3. MRP3 (ABCC3): In contrast to other ABC drug transporters, MRP3 is mainly expressed at basolateral membranes of epithelial cells in the liver and intestine [90]. Substrates for MRP3 include glucuronosyl and sulfated conjugates, whereas glutathione conjugates are relatively poor substrates for MRP3 compared with MRP1 and MRP2 [91]. As MRP3 also transports some bile salts [92], this transporter has been believed to play important roles in the enterohepatic circulation of bile salts by transporting them from enterocytes into the circulating blood to prevent the accumulation of intracellular bile acids. Mice lacking *Mrp3* were recently developed which are viable and fertile, exhibiting no apparent phenotype [93].

23.3.5.2.4. BCRP (ABCG2): Unlike P-gp and MRPs, BCRP has only one ABC and six putative TM domains, and therefore, is referred to as a half-ABC transporter, most likely functioning as a homodimer [76]. Among human tissues, the placenta showed the highest level of BCRP mRNA, followed by the liver and small intestine [94]. Unlike humans, mice exhibited high levels of mRNA in the kidney and only moderate levels in the placenta [95]. BCRP is capable of transporting a diverse array of substrates, which overlap those of P-gp and MRP1 to a certain extent [76]. Using mice lacking Bcrp, it was demonstrated that this transporter protects against the gastrointestinal absorption of a potent phototoxic agent, pheophorbide [96]. BCRP also mediates the intestinal efflux of an antibiotic, nitrofurantoin [97].

23.4. Conclusions and Perspectives

In this chapter, basic characteristics of major drug transporters and their preclinical/clinical implications are discussed. During the past 10 years, molecular information on each transporter has been organized. Novel technologies and various useful public databases such as SNP have improved our understanding

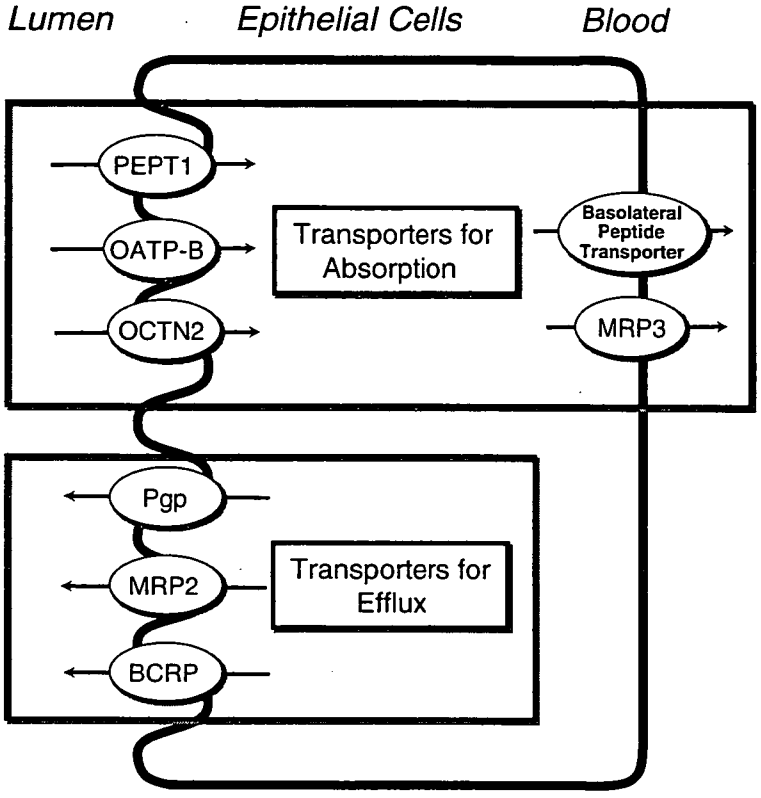

Figure 23.3 Drug transporters in the intestinal epithelial cells. PEPT1 is the most characterized transporter for intestinal drug absorption. The basolateral peptide transporter, which is not identified at the molecular level, also plays important roles. OATP-B, OCTN2 and MRP3 may be responsible for the intestinal absorption of some drugs. On the contrary, ABC transporters such as P-gp located at brush-border membranes mediated the efflux of drugs from intestinal epithelial cells, contributing to the low bioavailability of drugs such as the immunosuppressive agent, tacrolimus.

of the physiological, pharmacokinetic, and pharmacotherapeutic roles of these drug transporters. Drug transporter research can be actually applied to clinical science and drug development; that is, applications of drug delivery, the clarification of drug/drug interactions, application of personalized pharmacotherapy, and clarification of the relationship of each transporter to particular disease(s).

Various drug transporters are responsible for determining the oral bioavailability of drugs (Figure 23.3), although the extent of their contribution to the overall intestinal absorption process in vivo is not clear in some cases. Among them, peptide transporters, P-gp, MRP2, and BCRP unequivocally function as important factors regulating the oral bioavailability of drugs. Molecular determination of absorption by peptide transporters or secretion/excretion by ABC drug transporters will contribute to help the oral bioavailability of drugs under study.

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