

Intestinal transporters and oral absorption enhancing strategies based on these transporters

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ABSTRACT

Utilizing transporter-mediated drug delivery to achieve effective oral absorption emerges as a promising strategy. Researchers have been concentrated on discovering solutions to the issues of low solubility and poor permeability of insoluble drugs, whereas, current reports have revealed that drug transporter proteins are abundantly expressed in the mucosa of intestinal epithelial cells, and that their mediated drug absorption effectively improved the bioavailability of orally administered drugs. There are two main categories based on the transporter mechanism, which include the family of ATP-binding cassette (ABC) transporters with efflux effects that reduce drug bioavailability and the family of solute carriers (SLC) transporters with uptake effects that promote drug absorption, respectively. Thus, we review studies of intestinal transporter-mediated delivery of drugs to enhance oral absorption, including the types of intestinal transporters, distribution characteristics, and strategies for enhancing oral absorption using transporter-mediated drug delivery systems are summarized, with the aim of providing important theoretical references for the development of intestinal-targeted delivery system.

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1. Introduction

Drug transporters are functional proteins located on the cell membrane, which are specifically expressed in the cells of various tissues and organs, affecting the absorption, distribution, metabolism and excretion (ADME) processes of drugs in the body [1], which are responsible for transporting endogenous substances (amino acids, glucose, vitamins, *etc.*) in the body, a majority of drugs with structural class similarities to physiological substrates may also recognized and transported by these transporters, hence, it has a broad range of substrate properties and multi-specificity [2]. They can be classified into two forms, the easy diffusion type, which does not require energy participation, and the active transport type, which need energy participation, and their transport processes are in accordance with the Michaelis-Menten equation [3]. For instance, the glucose transporter 2 (GLUT2) in the lateral basement membrane of small intestinal epithelial cells, which transports glucose from the epithelial cells down the concentration difference towards the capillaries is classified as easy diffusion,

whereas drugs, amino acids, oligopeptides, *etc.*, pass through cellular membranes mainly in an active transport mode [4]. Additionally, drug transporters have a deeper binding site to the substrate, which is typically found within the transport channel on the plasma membrane, and can accept more structurally related or unrelated compounds than physiological receptors [5]. They also have multiple transmembrane regions that form a transport channel and exhibit extensive substrate exclusivity [6].

As we all know, oral drugs first need to survive in the gastrointestinal (GI) [7], then penetrate the intestinal epithelium, bypass blood clearance and are eventually transported to the target site [8]. Although effective oral absorption can be achieved by modifying the molecular structure of the drug or by interfering with the GI environment (pH modulators, osmotic enhancers) [9], most of the orally absorbed drugs face shortcomings in the physiological conditions of the GI [10], unstable physicochemical properties of the drug (low solubility or poor permeability), *etc.* [11,12], thus, how to achieve effective oral drug delivery is a significant challenge for current research efforts [13–15]. The latest research reports found that a variety of drug transporters are expressed in intestinal epithelial cells with high affinity for drugs, drug absorption in the intestine is accomplished not only by simple free diffusion, but more often by using transporters to medi-

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ate transporter absorption, the use of transporter targeting allows loaded drugs to cross the intestinal barrier into the systemic circulation more efficiently [16]. Interestingly, the regional distribution of the transporter in the intestine is characterized by an association with the “absorption window”, *i.e.*, its present only at specific intestinal sites, and its substrate drugs show specific effective absorption sites [17]. If we lengthen the retention time of the drug at the site of absorption and simultaneously shorten the time it takes for the drug to be secreted and metabolized in the intestines, we can maximize drug absorption [18]. Existing evidence indicates that there are differences in the expression levels of transporters in different regions of the intestine [19], such as the mRNA and protein expression levels of oligopeptide transporter 1 (PepT1) in rats increased gradually from the proximal to the distal part of the small intestine [20]. Therefore, intestinal transporter-mediated drug absorption also determines the bioavailability of orally administered drugs [21]. The Human Gene Nomenclature Committee (HGNC) has identified gene codes for the transporter family, on the basis of their different transport mechanisms and directions [22], transporters can be classified into two categories: solute carriers (SLC) transporters and ATP-binding cassette (ABC) transporters, among them, SLC are mainly involved in the uptake process of drugs, which is an uptake transporter, while ABC mediates cellular efflux of drugs and is an efflux transporter [23]. Transporters act as “transport vehicle”, mediating the transfer of molecules or ions across biological membranes, and have the following advantages: (1) high transit rate and wide GI distribution [24]; (2) broad substrate specificity and overlap [25]; (3) influencing the *in vivo* process and efficacy of drugs, adverse drug reactions, *etc.*, can intermediate drug-drug interactions (DDI) for accurate analysis [26,27]; (4) improving oral absorption bioavailability of insoluble drugs with substrate-modified nano-formulations [28]; (5) targeting transporters to treat clinical diseases (tumor diseases, immune diseases) [29].

Currently, a systematic review of the application of transporters in improving oral absorption is lacking. Herein, we outline the recent advances in the types and distribution characteristics of transporters, particular attention was paid to the GI transporters' drug delivery rule, summarizing the possible mechanisms by which various approaches or strategies utilize intestinal transporter-mediated to enhance oral absorption, and discusses the current challenges faced by intestinal transporters, effective solutions, we hope that this review can provide some reference in the field of GI transporters to effectively improve the design of drug oral absorption and delivery systems.

2. The types and properties of intestinal transporters

Transporters as the structures and molecules that move substances in living organisms, which transit substances across membranes *via* transporter proteins on top of the cell membrane [30,31]. In general, the major modes of transmembrane transport of drugs in the body are passive, active and membrane-activated transport, among them, passive transport includes three modes, namely, simple diffusion, filtration and ease of diffusion [32]. However, transporter is a transmembrane transporter protein, which is one kind of drug carrier can be found in the body's tissues and organs [33]. Its primary mode of transport is active transport. As shown in Fig. 1, it is widely distributed in the brain, liver, and kidney, especially in the intestinal tract, where the highest percentage of distributed expression is present. Several transporters have been detected in human cells at the molecular and functional levels, according to the mechanism and direction of transport, they have been categorized as SLC and ABC transporters [34]. In particular, the effects on the ADME processes in the human body were inti-

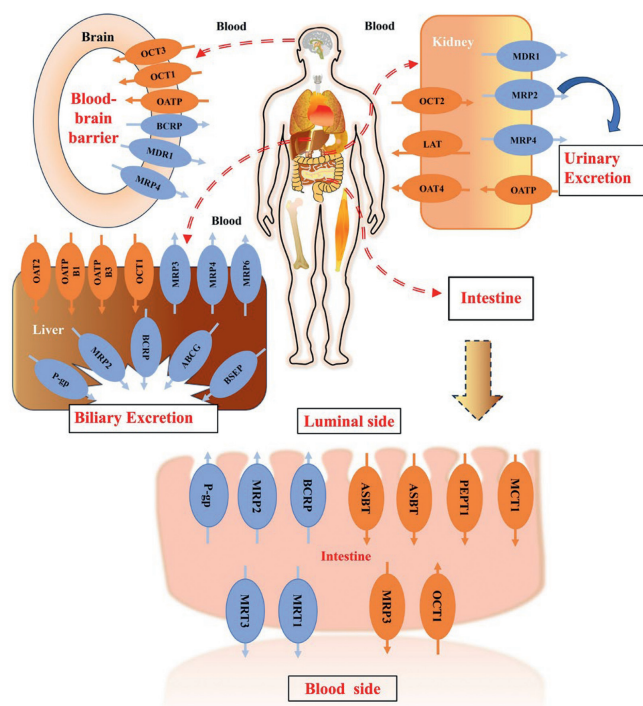


Fig. 1. The distribution and types of transporters in tissues and organs throughout the body, including the brain, liver, kidneys, and small intestine.

mately connected with drug efficacy [35], DDI [36], adverse drug reactions (ADR) [37], and so on.

2.1. SLC transporters

SLC transporters are mainly involved in the process of drug uptake, as an uptake-type transporter, and their main function is to promote the intracellular transport of drugs, metabolic substances, ions, *etc.*, to achieve transcellular membrane transport [38]. Based on sequence similarity studies can be categorized into 52 subfamilies with more than 400 SLC transporters [39]. The secondary structure of SLC family transporters shows twelve trans-membrane helical polypeptide chains [40], which can form a tertiary structure through the arrangement of the secondary structure in three-dimensional space, it has glycosylated regions outside the membrane, with the amino and carboxy ends usually inside the cell [41]. Studies have reported that, for example, the organic anion transporting polypeptide (OATP) of the vascular lateral membrane of hepatocytes can transport pravastatin for uptake into hepatocytes [42]. PepTs located in the epithelial cells of the small intestinal mucosa are one of the most well-studied and widely used of drug transporters [43]. There are mainly two types of PepT1 and PepT2, which the highest expression in the GI tract is PepT1, mediating the oral absorption of dipeptides, tripeptide compounds and some peptidomimetic drugs, and playing an essential role in the intestinal transport of oligopeptides and peptidomimetic drugs [44]. In addition, sodium-dependent glucose transporters (SGLTs) [45], sodium-independent facilitated diffusion GLUTs [46], monocarboxylate transporter (MCT; SLC16A) [47], equilibrative nucleoside transporter (ENT; SLC28A) [48], plasma membrane monoamine transporter protein (PMAT; SLC29) [49], carnitine/organic cation transporter 1 (OCTN1), *etc.* are also classified as such transporters [50].

In recent years researchers and scholars have revealed that the SLC family could be an emerging class of drug targets for the treatment of diabetes [51], as shown in Fig. 2A, the potential SLC targets

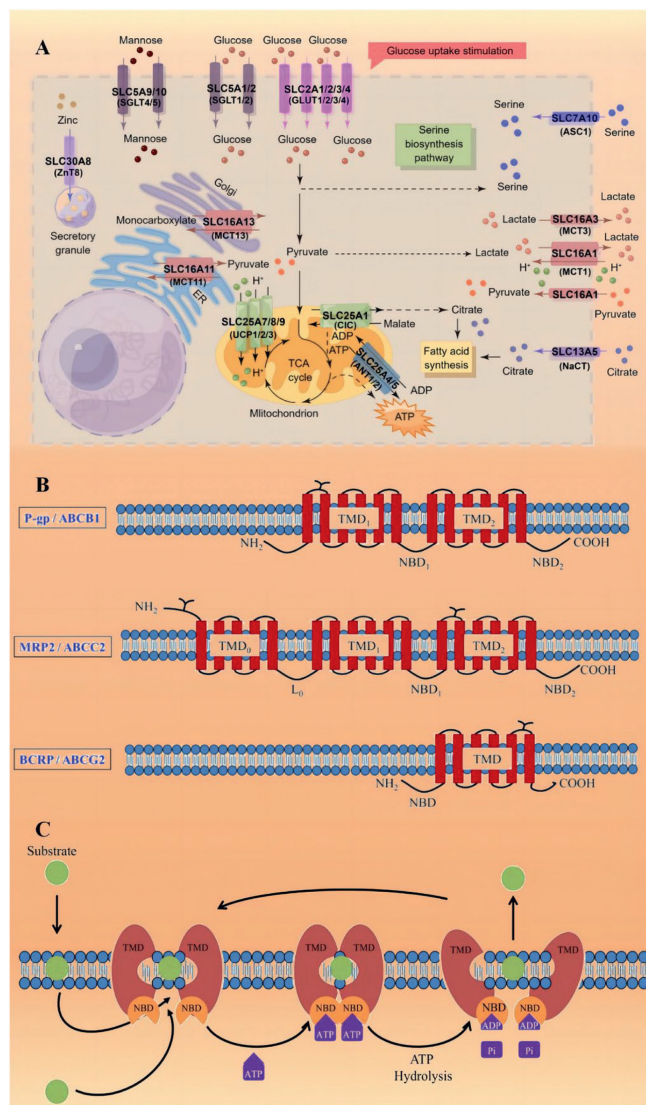


Fig. 2. Multiple SLC and ABC transporter family types and schematic diagrams of transport. (A) Transit patterns and localization of SLC transporters for the treatment of type 2 diabetes mellitus. Reproduced with permission [51]. Copyright 2024, Elsevier Science Ltd. (B) Secondary structure models of the ABC transporters, such as P-gp/ABCB1, MRP2/ABCC2, BCRP/ABCG2. (C) Conformation changes of the ABC transporters during the transport of substances (substrate binding and ATP hydrolysis). Reproduced with permission [55]. Copyright 2016, Elsevier Science Ltd.

are found in the plasma membrane and various intracellular membranes and are involved in the regulation of glucose metabolism, while the arrows indicate the direction of their transport, such as, SLC2A1/2/3/4 and SLC5A1/2 are isotropic transport proteins that facilitate the influx of glucose into the cytoplasm for reabsorption in renal tubular epithelial cells and SLC25A7/8/9 transport protons to the mitochondrial matrix, that shows that SLC transporters have potential in the treatment of diseases.

2.2. ABC transporter

ABC transporter as an efflux transporter [52], the main function can use the energy of hydrolyzed ATP to realize the transmembrane transport and the transport of drugs and a variety of endogenous substances, and mediating cellular the efflux of drugs, limiting drug uptake and absorption (similar to an efflux pump) [53]. All ABC transporter have two transmembrane structural domains (TMDs) and two nucleotide-binding structural domains (NBDs),

and the substrates appear to bind at high-affinity sites within the TMDs (Fig. 2B) [54]. As shown in Fig. 2C, the ABC transporter consists of 12 transmembrane polypeptide chains running through the cell membrane, forming a pore-like cavity structure that is a channel for substance membrane transport, and the NBD region binds ATP for hydrolysis, which causes a conformational change in the TMD region and thus opens up the transporter pathway for substance transport [55]. Medical practitioners made the first discovery from the study of nutrient uptake by bacteria. Subsequently, it has been further investigated in the clinical therapeutic field because of its multidrug resistance (MDR) that can be generated by the extracellular expulsion of exogenous substances in a counter-concentration gradient [56,57]. Thus far, more than 100 ABC transporter proteins have been identified by research, scientists have discovered 49 ABC transporter proteins in the human genome [58]. Such as P-glycoprotein (P-gp) [59], multidrug resistance-associated proteins (MRPs) [60], bile salt efflux pump (BSEP) [61], breast cancer resistance protein (BCRP) [62]. One of them, P-gp is encoded by MDR1 and is the most widely studied efflux transporter, specifically, it is highly expressed at the terminal end of the apical side of intestinal epithelial cells, which efflux drugs into the intestinal epithelium and into the intestinal lumen, thus resulting in a decreased oral bioavailability of medicine, and enhancing oral drug absorption by reducing P-gp efflux is also a hot research topic in the field of pharmacy [63].

3. Distributional properties of intestinal transporters

Transporters are broadly expressed in the cells of a variety of organs in the body, including epithelial cells of major organs such as the liver, intestines, and kidneys, and organs with barrier functions (brain, testis, placenta, *etc.*) [64]. The intestine is the major digestive and absorptive organ of the human body [65], and a highly enriched expression of different types of transporters on the membranes of intestinal epithelial cells, which can transport glucose amino acids and other nutrients into the body circulation to meet the body's high demand for a lot of various nutrients [66]. Special attention was paid to the intestinal segments that included the duodenum, jejunum, and ileum, with distributional variability in each part of the intestinal segment area [67]. As shown in Table 1 [68–87], the expression varies according to the uptake type or efflux type distribution. A detailed review of the distribution characteristics of intestinal transporters at different sites during their uptake and efflux, including a comparison of the variability present in animals and humans, is provided in the below.

3.1. Intestinal distribution and transport characteristics of the SLC family transporters

3.1.1. PepTs

PepTs mediated substrate specificity, transports dipeptides and tripeptides, and has a high affinity for oligopeptides containing L-amino acid residues [88]. As shown in Fig. 3A, taking the topology of mammalian peptide transporter as an example, PepT has more than 12 transmembrane polypeptide chains, the transport activity is increased by the free amino group at the N-terminus, while the transport activity is unaffected by a variety of group modifications at the C-terminus, both terminals are located intracellularly, with a large hydrophilic extracellular loop consisting of 204 amino acids and five N-glycosylation sites between the 9th and 10th membrane polypeptide chains [89]. Beale *et al.* revealed that the crystal structure of the PepT1 extracellular structural domain is in tandem with two immunoglobulin-like folds, which also offers structural insights into mammalian peptide transport, as shown in Fig. 3B. PepT mostly consist of PepT1 and PepT2, the former of which is extensively expressed in intestinal epithelial cells' parietal membrane

Table 1
Types and distribution characteristics of ABC and SLC intestinal transporter families.

Transporter code	Gene family code	Protein family	Type	Intestinal distribution	Localization	Substrate	Substrate specificity/transit characteristics	Inhibitor	Ref.
PepT1	SLC15A1	Oligopeptide transporter	SLC	Duodenum jejunum, ileum, colon	Parietal membrane, basolateral membrane	Dipeptide, tripeptide, peptide analog	(1) Broad substrate specificity, (2) stereoselectivity, (3) high affinity for oligopeptides containing L-amino acid residues	Gly-Sar, JBP485, lisinopril, zinc ions	[68]
GLUT2	SLC2A2	GLUT	SLC	Proximal intestine	Basolateral membrane	Glucose, galactose, fructose	(1) Broad substrate specificity, (2) regulated by the body's internal environment and signaling molecules, small molecule factors, metabolites, extensively involved in signaling pathways, (3) a strong association with diabetes	STF-31, flavone, phloretin hesperidin, adindine alkaloids	[69]
GLUT5	SLC2A5	GLUT	SLC	Proximal intestine	Parietal membrane	Fructose	Fructose-specific exclusive transporter protein	MSNBA	[70]
SGLT1	SLC5A1	GLUT	SLC	Proximal intestine	Parietal membrane	Glucose, galactose	Sodium ion-dependent sequential active transport	Phloretin canagliflozin, dapagliflozin, empagliflozin	[71,72]
OCTN1	SLC22A4	Novel organic cation transporter	SLC	Small intestine	Parietal membrane	Quinidine, verapamil, pyrilamine, L-carnitine, ergothioneine	Delivery of oral cationic drug	Cimetidine, ondansetron, ergometrine	[73]
OCTN2	SLC22A5	Novel organic cation transporter	SLC	Small intestine	Parietal membrane	Choline, imatinib, verapamil, pyrilamine, ipatropium, valproic acid, cephaloridine	(1) Sodium ion-dependent transport of carnitine, (2) high affinity	Mildronate (MET-88)	[74]
OATP1A2	SLC21A9	Organic anion transporter	SLC	Small intestine	Parietal membrane	Sodium, atenolol, enalapril, pravastatin, fexofenadine, opioid peptides, N-methyl quinine	Possible mediation of drug-food interactions in gastrointestinal absorption	PEG 400, probenecid, naringenin	[75]
MCT1	SLC16A	Monocarboxylic acid transporter	SLC	Colon	Parietal membrane, basolateral membrane	Lactate, pyruvate, foscarnet, acetoacetate, salicylic acid, aminohippuric acid	(1) MCT1 knockdown is able to affect multiple metabolic phenotypes, which are sex-specific, (2) mediates lactate transport in the gut and regulates metabolic homeostasis of mouse in a sex-dimorphic pattern	AZD-3965, AR-C15585, alpha-cyano-4-hydroxycinnamic acid	[76]
PCFT	SLC46A1	Proton-coupled folate transporter	SLC	Small intestine	Parietal membrane	Folic acid	Anti-tumor as a folate antagonist	EC131	[77]

(continued on next page)

Table 1 (continued)

Transporter code	Gene family code	Protein family	Type	Intestinal distribution	Localization	Substrate	Substrate specificity/transit characteristics	Inhibitor	Ref.
SMVT	SLC5A6	Sodium ion dependent multivitamin transporter	SLC	Small intestine	Parietal membrane	Biotin, pantothenic acid, zinc sulfate	Biotin undergoes calcium-dependent transport across the intestinal wall	Sotalol	[78]
LAT1	SLC7A5	L-Type amino transporter	SLC	Small intestine	Basolateral membrane	Amino acid, thyroid hormones	(1) Intracellular and extracellular substrate selectivity, (2) tendency to transport larger uncharged amino acids	KMH-233, LAT1-IN-1	[79]
ASBT	SLC10A2	Apical sodium-dependent bile acid transporter	SLC	Ileum	Parietal membrane	Cholates, deoxycholate, glycocholate	(1) Narrow substrate profile, (2) mediates bile salt intestinal reabsorption and its expression is negatively regulated by the ileal FXR	Maralixibat, odevixibat, GSK2330672	[80,81]
ATB ^{0/+}	SLC6A14	Sodium-coupled neutral amino acid transporter	SLC	Colon	Parietal membrane	L-Carnitine, amino acids	(1) Low spatial resistance, (2) low immunogenicity, (3) good <i>in vivo</i> stability, (4) active tumor targeting	Valganciclovir	[82,83]
MDR1/P-gp	ABCB1	P-glycoprotein	ABC	Duodenum, jejunum	Parietal membrane	Reserpine, alkaloids, paclitaxel, colchicine, saquinavir, vincristine, doxorubicin, grepafloxacin	(1) Broad substrate specificity, (2) transport amphipathic hydrophobic compounds, or cationic molecules	PEG, Tween80, verapamil, cyclosporin, SDZPSC833, salvianolic acid B	[84]
MRP1	ABCC1	Multidrug resistance-associated protein	ABC	Small intestine, large intestine	Basolateral membrane	Etoposide, vincristine, mitoxantrone	Transport hydrophobic drugs, the anionic tripeptide GSH, glucuronic acid	Probenecid, schisandrin B	[85]
MRP2	ABCC1	Multidrug resistance-associated protein	ABC	Duodenum, jejunum	Parietal membrane	Colchicine, leukotriene, GSH, methotrexate, grapefruit juice	(1) Overlap with P-gp substrate, (2) transport leukotrienes C4, D4 and E4 and various GSH conjugates, unconjugated organic anions	MK-571, valsopodar, indomethacin, probenecid	[86]
BCRP	ABCC2	Breast Cancer Resistance Protein	ABC	Small intestine, large intestine	Parietal membrane	SN-38, lactone, gefitinib, simvastatin, methotrexate	(1) Overlap with MRP2 substrate, (2) transport hydrophilic conjugated organic anions	17- β -Estradiol, dipyridamole, fumitremorgin C	[87]

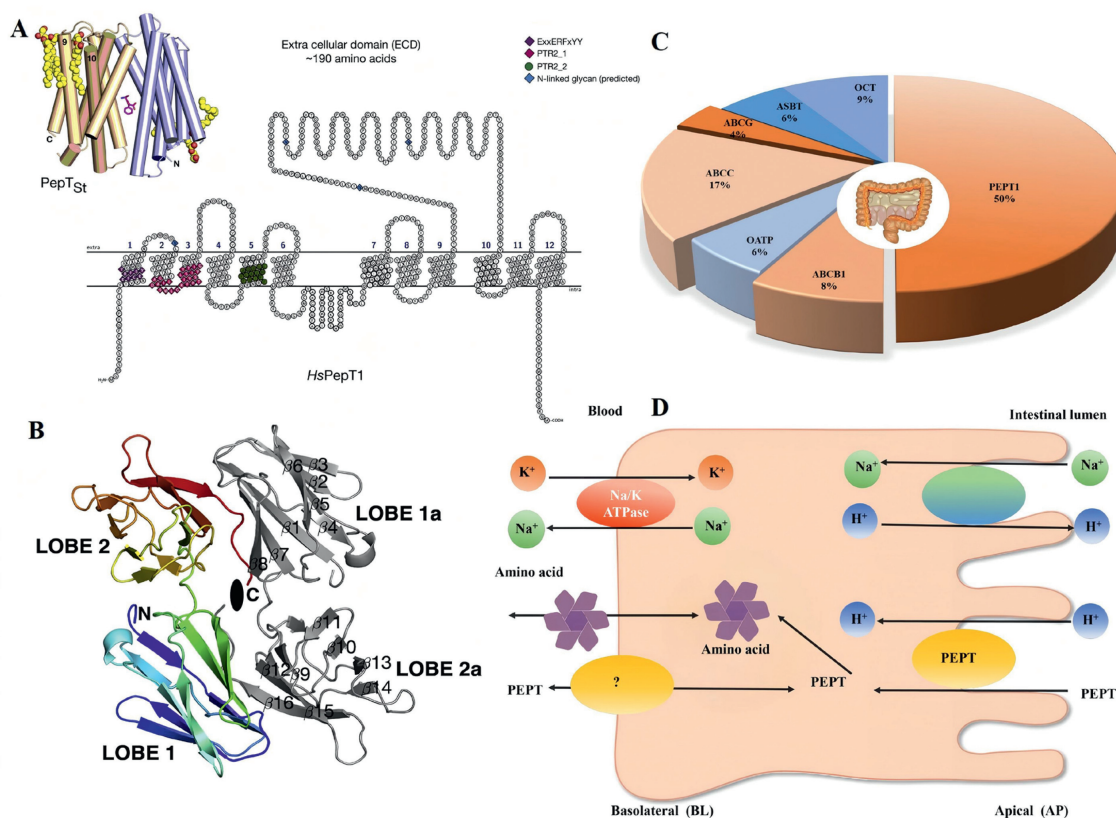


Fig. 3. Transport characteristics of PepT. (A) Topology diagram of the human plasma membrane peptide transporter PepT1. (B) Crystal structure of PepT1 extracellular domain, two monomers are generated through a twofold amorphous axis of symmetry (black ellipse), which are primarily iridescent and gray. Reproduced with permission [89]. Copyright 2015, Elsevier Science Ltd. (C) The average percentage of *in vivo* transporter expression in 6 healthy subjects. (D) The transport mechanism of PepT1.

and the latter of which is expressed in various bodily areas like the kidney and lungs, among others [90]. We focus on the intestinal transporter PepT1, in general, the quantification of human intestinal physiological transporter protein expression was based on diagnostic endoscopic biopsies or tissue specimens obtained from patients with intestinal diseases [91]. Drozdziak *et al.* [92] summarized the mRNA expression of PepT1 in the small intestine by reverse transcription polymerase chain reaction (RT-PCR) measurements in six healthy subjects occupying 50% of the total mRNA expression, whereas the percentage of expression of the other types of transporters was low, and the results are shown in Fig. 3C. In addition, it is also distributed in the apical plasma membrane of enterocytes from rats, mice, rabbits, and sheep, revealing a high degree of homology between species [93]. Investigators have been demonstrated that PepT1 has a differentiated regional distribution of intestinal segments, which is not lower than that of the jejunum and ileum in the duodenum region, but very thin in the colon, in the following order: duodenum > jejunum > ileum > colon [94]. Meier *et al.* [95] identified that in the adult intestine, PepT1 is less expressed in the ascending colon, transverse colon, and descending ileum, with only about 3%–6% of the expression in the duodenum or ileum.

PepT1 is a secondary active transporter protein coupled with H⁺, Fig. 3D illustrates the transport mechanism, which is mainly exchanges Na⁺/K⁺-adenosine triphosphatase (ATPase) at the basement membrane of small intestinal epithelial cells, and pumps Na⁺ out of the cell through Na⁺/K⁺ exchange [96], generating an intra and extracellular concentration gradient, and then the low intracellular Na⁺ concentration then drives the Na⁺/H⁺ exchange transporter proteins located on the apical membrane side of the cell and transports the H⁺ to the outside of the cell [97]. As the H⁺ con-

centration rises, a concentration difference between the inside and outside of the cell and a negative membrane potential is generated which supplies energy that can be used to transport oligopeptides or drugs into the cell [98]. Du *et al.* [99] demonstrated that the introduction of L-valine-valine and different molecular weights of polyoxyethylene stearate into PepT1-targeting polymers modified nanoparticles significantly increased the oral bioavailability of docetaxel (DTX) [100]. Therefore, PepT1 mediates the intestinal absorption of peptide-mimetic and nonpeptide substrates the peptide derivation of amino acid-related drugs is applicable to improve their intestinal absorption [101].

3.1.2. GLUTs

GLUTs are mediated transmembrane transport of glucose by facile diffusion [102,103]. GLUT1 is the most well-studied of the family, it is composed of twelve transmembrane helical regions (Fig. 4A), with green and white denoting the N and C structural domains, respectively, and the intracellular helical (ICH) structural domain colored orange [104]. And its isoforms, SGLT1, GLUT2, GLUT5, and GLUT7, are expressed predominantly in the intestine and are generally localized on the basolateral side of the enterocyte, where they can aid in the transmigration of substrates out of the cell [105]. Glucose is the energy for life activities, and its digestion and absorption in the intestine is critical for its utilization [106], which is mainly dependent on SGLT1 and GLUT2 and is transported in a carrier-dependent manner [107]. SGLT1 was mostly expressed in the villous epithelium of the small intestine of adult animals and also efficiently expressed in crypt cells, with expression levels in the order of jejunum > duodenum > ileum, and was not expressed in the large intestine [108]. Whereas information on GLUT2 was first obtained from mouse and human liver

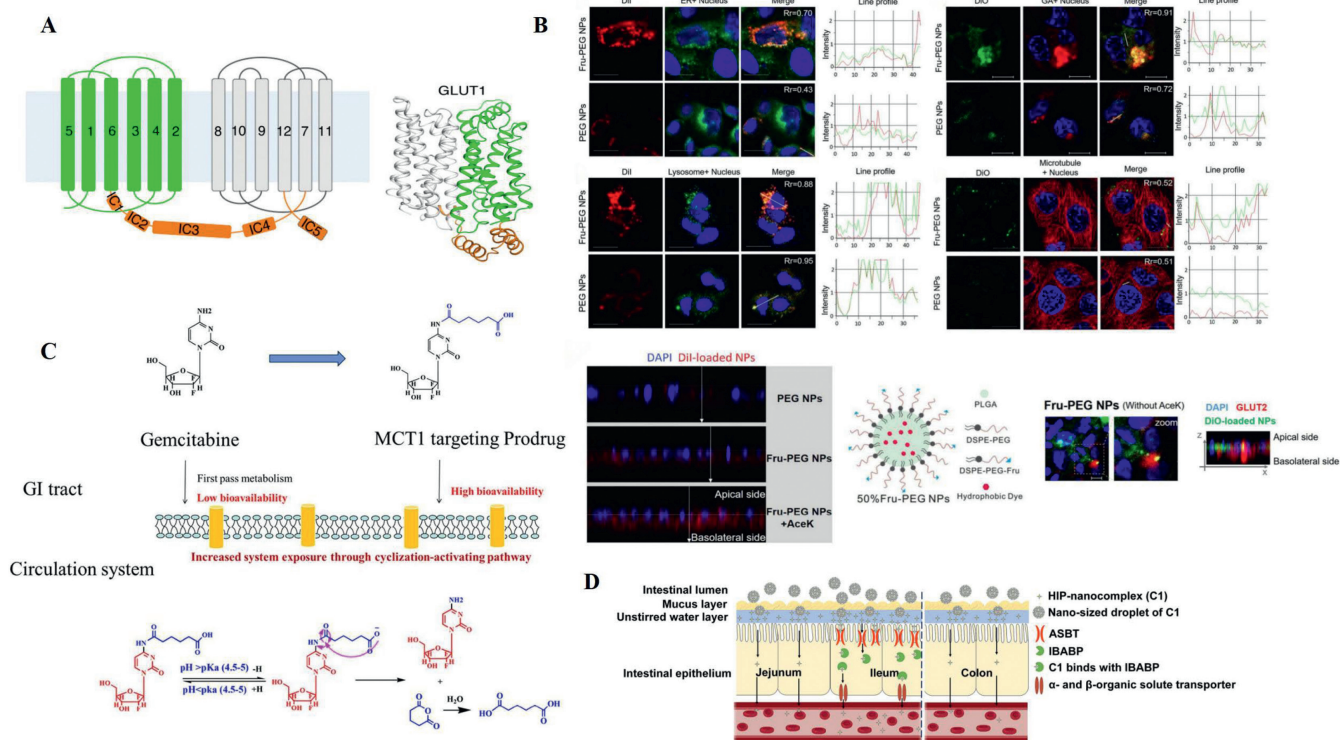


Fig. 4. Transporter properties of the SLC transporter family. (A) The structure of the GLUTs, right hand side shows the structure of human GLUTs. Reproduced with permission [104]. Copyright 2016, Wiley-Blackwell (B) Confocal laser scanning microscopy (CLSM) images of the localization distribution of DiO-loaded PEG NPs and Fru-PEG NPs translocated to different organelles (ER, GA, lysosome, microtubule) in Caco-2 cells, with transcytosis and exocytosis images of NPs shown at the bottom of the figure, respectively, scale bar: 20 μ m. Reproduced with permission [111]. Copyright 2020, Elsevier Science Ltd. (C) A flow chart of modified Gem prodrugs targeting MCT1 to enhance oral absorption. Reproduced with permission [116]. Copyright 2020, Elsevier Science Ltd. (D) Schematic illustration of proposed absorption mechanism for hydrophobic ion-pairing (HIP)-nanocomplex (C1) following bile acid pathway. Copied with permission [124]. Copyright 2021, Elsevier Science Ltd.

cDNA libraries, both of which mediate the transport of monosaccharides [109]. After oral administration of the drug, the intestinal lumen glucose concentration increased dramatically, SGLT1 reaches the maximum transport rate, and GLUT2 with high transport capacity is recruited to the parietal membrane of intestinal epithelial cells for a short period of time to participate in the absorption of glucose [110]. Wu *et al.* [111] demonstrated that fructose-modified polyethylene glycol-coated nanoparticles (Fru-PEG NPs) are transported by GLUT2-dependent processes of endocytosis and basolateral exocytosis [112], as shown in Fig. 4B, using DiO-loaded PEG NPs and Fru-PEG NPs transported to different organelles, it was revealed that Fru-PEG NPs were transported via multiple pathways (*i.e.*, endoplasmic reticulum (ER), Golgi apparatus (GA), lysosome pathway, and microtubule-dependent shuttlin), and we can also observe its distribution in the basolateral side and the localization of fluorescent signals of GLUT2 (red) and NPs (green) in the cell monolayer, and bright yellow dots were observed, which suggests that the co-localization of GLUT2 and NPs can effectively promote the transmembrane transport for oral absorption. Thus, the glycosylation of peptide or drug molecules, as well as monosaccharides may promote biofilm permeation via SGLT1, and glycosylation may improve organ targeting specificity and increase metabolic stability [113].

3.1.3. MCT1

MCT1 (SLC16A) is highly expressed in the apical large/small intestine [114]. Non-endogenous monocarboxylate and β -lactam antibiotics have been demonstrated to be absorbed via the MCT1 intestine [115]. Wang *et al.* explored a facile di-acid mono-amidation strategy to target MCT1 for oral chemotherapy, gemcitabine (Gem) was monoamidated with various acids to develop a prodrug ex-

hibiting stronger affinity and improve its poor solubility [116], it showed higher MCT1 affinity, better GI stability and oral bioavailability (Fig. 4C). Moreover, MCT1 has been noted to have higher transport activity in the colon and rectum [117], and if ligands of MCT1 and carnitine/OCTN2 are introduced simultaneously into the parent drug or carrier, oral bioavailability will be improved [118].

3.1.4. Apical sodium-dependent bile acid transporter (ASBT)

Food digestion in the human intestines requires 12–32 g of bile acids (BAs) per day, and about 95% of the BAs are recycled through the enterohepatic cycle [119]. ASBT, an ileal Na^+ -dependent transporter, with high expression on the apical brush border membrane of terminal ileal enterocytes and relatively low expression in the apical membrane of the proximal tubule and the epithelium of the bold tubule in the kidney [120,121]. In the last several decades, BAs and ASBT have been investigated for oral administration in various forms, of which the main types are BA conjugated prodrugs, BA/drug electrostatic complexation, and BA-modified nanocarriers [122]. Tolle-Sander *et al.* [123] conjugated valacyclovir to phenol deoxycholate and showed that the uptake of acyclovir by hASBT-COS cells was increased more than 10-fold compared to acyclovir solution, and through ASBT-mediated translocation. Bashyal *et al.* [124] prepared hydrophobic ion pair (HIP)-nanocomposites can to overcome the intestinal barrier and improve oral absorption, pass through the jejunal anion exchange mechanism and the ASBT uptake mechanisms of the distal ileum, the absorption mechanism is shown in Fig. 4D. In summary, the intestinal specificity of BAs re-absorption via ASBT could be an effective strategy to increase oral absorption [125,126].

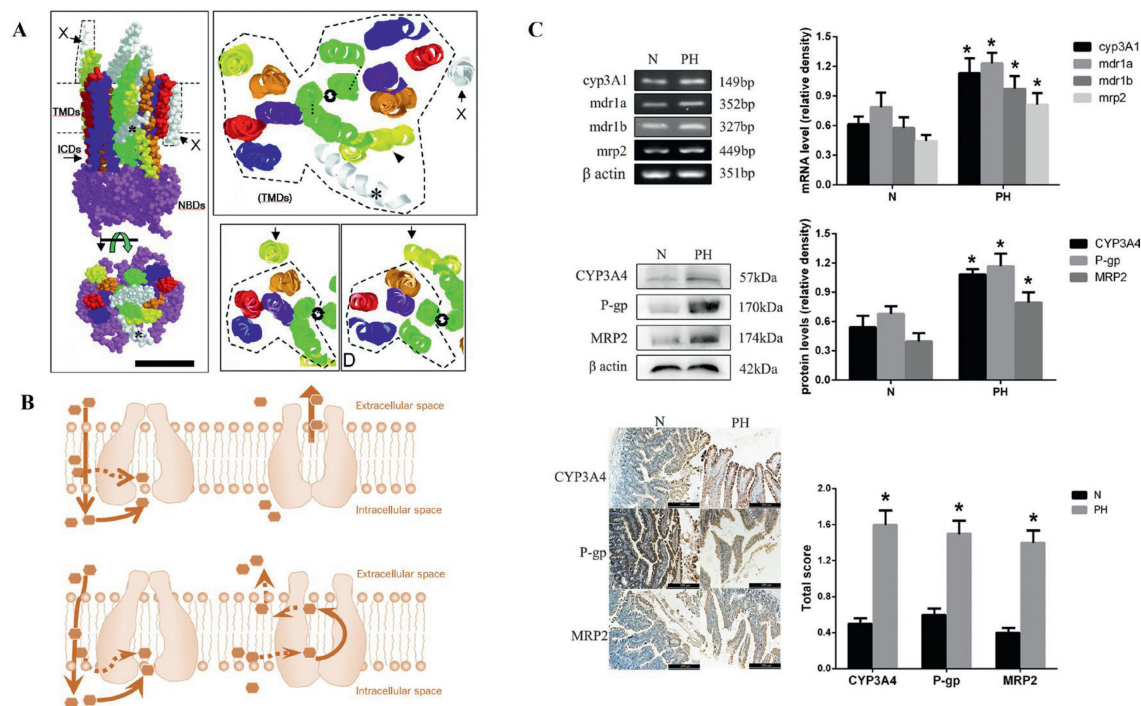


Fig. 5. Transporter properties of the ABC transporter family. (A) Three-dimensional structure of P-gp. Reproduced with permission [129]. Copyright 2005, Elsevier Science Ltd. (B) Model of P-gp transport mechanisms (hydrophobic vacuum cleaner mode and flippase model), arrows indicate the direction of substrate transport. (C) Location and extent of protein expression in the rat intestine after administration of P-gp/MRP2/CYP3A4 inhibitors. Reproduced with permission [137]. Copyright 2021, Elsevier Science Ltd.

3.2. Intestinal distribution and transport properties of ABC family transporters

3.2.1. P-gp

P-gp is the most intensively studied class of the ABC family, with a wide range of substrates, inhibitors, and inducers, and competitive or noncompetitive effects at the level of the P-gp molecule affecting efficacy are frequently observed during co-administration of drugs. Three heterozygous genes (MDR1a, MDR1b, MDR2) were expressed in rodents, whereas humans had two (MDR1, MDR3), of which, the MDR1 gene had high interspecies homology. P-gp is expressed less proximally than distally in the small intestine and excretes drugs that enter the intestinal epithelium into the intestinal lumen [127]. For endogenous cytotoxic metabolites and substances such as exogenous drugs, they can be pumped out of the cell and have a protective effect on the cell [128]. With special attention to the high-resolution three-dimensional structure determination of P-gp (Fig. 5A), significant progress has now been made, and a higher resolution structure of eukaryotic P-gp has been accomplished using the Cryo-EM method, and then the NBD is an ATP-binding site and a unique and representative NBD site of the ABC transporters, and the study of these resolved conformations facilitates our mechanistic studies in drug transport processes [129]. In small intestinal epithelial cells, P-gp is ideally positioned to limit the oral absorption of compounds by driving substrate back into the lumen, revealing that its secretion varies across different segments of the intestine [130]. Sultan *et al.* [131] detected that the intestinal absorption of adriamycin in the rabbit jejunum and ileum was increased about twofold with the addition of P-gp efflux inhibitors, like piperine and dipyrindamole, while there was no significant increase in the duodenum, and this study verified that the expression of P-gp in the rabbit duodenum was lower than that in the jejunum and ileum. P-gp has two sides for the human body, In normal tissues, it has a protective effect on the body, preventing foreign substances harmful to the body from entering the

body, and plays an import barrier role in the blood-brain, blood-testis and blood-fetal barriers, nevertheless, in tumor tissues, P-gp leads to MDR overexpression in many tumor cells can pump antitumor drugs out of the cells thereby reducing the drug concentration in the tumor cells to a less than therapeutic level, and can also inhibit apoptosis in tumor cells by regulating endogenous and exogenous apoptotic pathways, thereby reducing drug efficacy.

Presently, there are two models of the transport mechanism that might explain how P-gp transports substrates out of the cell, the hydrophobic luminal wash model and the flip-flop model, respectively (Fig. 5B) [132]. For reducing the exocytosis of P-gp and enhancing the oral absorption of drugs is currently a hot research issue in the field of pharmacy [133], and there are three classic ways to decrease P-gp efflux, (1) adding P-gp efflux inhibitors (calcium channel blockers, immunosuppressants, and medicinal excipients), (2) wrapping the drug to decrease the affinity of P-gp for the drug, and (3) altering the structure of the drug to avoid the recognition of the drug by P-gp. Guo *et al.* [134] prepared adriamycin-curcumin, nanomicelles using D- α tocopherol polyethylene glycol 1000 succinate (TPGS, a P-gp efflux pump inhibitor) [135] and polyamides to fabricate precursor materials with MDR-reversing and long-circulating properties, which promoted MDR reversal and achieved higher antitumor activity [136].

3.2.2. MRP

MRP1, 4, and 5 are located in the basolateral membrane of intestinal epithelial cells, while MRP2 is expressed in the apical part of intestinal epithelial cells, and MRP3 is currently found to be the most highly expressed transporter protein in the intestine, with the exception of the terminal ileum, where transporter protein gene expression shows the following order: MRP3 > MDR1 > MRP4/MRP5 > MRP1 > MRP2. MRP/ABC transporter proteins are all capable of transporting organic anions, such as glutathione (GSH), sulfate, or glucuronic acid-coupled drugs coupled to GSH or glucuronide. Sun evaluated the bioavailability of intestinally ab-

sorbed octreotide (OCT) by comparing normal rats to rats with portal hypertension that had been administered P-gp/MRP2/CYP3A4 inhibitors, the location and extent of protein expression in the intestines of each group of rats are shown in Fig. 5C, which demonstrated that inhibition of P-gp, MRP2, and CYP3A4 significantly reduced the first-pass effect of OCT, and enhanced protein expression levels and decreasing portal vein pressure in rats with portal hypertension [137].

3.2.3. BCRP

BCRP is present in the luminal membrane of intestinal epithelial cells and has been demonstrated to limit intestinal absorption of anticancer drugs (doxorubicin, methotrexate, etc.), Jani predicted that the BCRP topology diagram consists of only one carbon-terminal transmembrane region membrane-spanning domain (6 transmembrane α -helical polypeptide chains), and NBD [138]. Real-time fluorescence quantitative PCR for gene expression BCRP mRNA revealed that expression was greatest in the duodenum and declined with the continued distribution of intestinal segments [139,140].

3.3. Other intestinal transporters

Besides the previously discussed high expression of intestinal transporters, moreover, a handful of other transporters are also lowly expressed in the intestines. Organic cation/carnitine transporters (OCTN1, OCTN12) were highly expressed in the basolateral small intestine, which are absorbed in the intestine and transported into the bloodstream is mainly dependent on sodium ion endocytosis, and sodium ion is synergistically transported with carnitine [141]. However, the majority of the literature on OCT transporter function has been conducted in the liver and kidney, whereas the role in the GI has rarely been discussed [142,143]. Organic anion transporters (OATs) are sodium ion non-dependent transporters, which were first isolated from rats, have a broad distribution in the small intestine and are involved in the absorption of many drugs [144]. Recently, nine human OATs and eleven rodent OATs have been isolated, it is noteworthy that grape and orange juices inhibit OAT1 activity and reduce the intestinal absorption of thyroxine, β -blockers, and ciprofloxacin hydrochloride [145,146]. Including the sodium-dependent multivitamin transporter (SMVT) situated in the parietal membrane of the small intestine, the proton-coupled folate transporter (PCFT) is most highly expressed in the duodenum, and its potent target for promoting drug absorption upregulates PCFT expression [147]. Thus, it has an impact on the transport and distribution of drugs in the human body by investigating diverse types of transporters.

4. Oral absorption enhancing strategies based on intestinal transporters

The lack of efficient intracellular transport route hinders the transport of numerous drugs, even they are loaded by delivery systems, such as nanodelivery system [148,149], the interaction of the substrate with the transporter can be utilized to extend its residence time at the surface of the intestinal membrane and to pass through the enterocytes *via* an osmotic mechanism [150]. We mainly summarized the current strategies on enhancing the oral absorption of drugs based on the representative intestinal transporters with high expression levels, such as PepT1, P-gp.

4.1. Construction of the small molecular prodrug delivery system

The design of prodrugs targeting transporters distributed on intestinal epithelial cells has now emerged as a new direction to improve drug absorption and pharmacokinetic parameters [151,152].

Specifically, the appointed functional groups are attached to the active moiety of the parent drug [153], so that it can be specifically recognized by the transporter during *in vivo* transport as a means of altering the membrane permeability and thereby improving the poor physicochemical properties of the parent drug [154–156]. Among them, the most extensively and typically studied is the intestinal PepT1, which are abundantly expressed in the apical membrane of small intestinal absorptive epithelial cells. The primary idea of prodrug design that targets PepT1 is to attach the drug to a peptide or amino acid that has a particular spatial structure, a process known as peptidomimetic design, it has an elevated affinity for PepT1 [157,158]. Owing to the multi-substrate specificity of PepT1, the modified drug has enhanced affinity for the transporter and better carried out to achieve transmembrane transport [159,160]. Sun *et al.* synthesized amino acid esters and aminoamide prodrugs using paramivir as the parent drug and discovered that oral amino acid esters (paramivir-(CH₂)₂-L-Val) or aminoamide (paramivir-L-Ile)-prodrugs to rats increased the bioavailability of paramivir from 4.1% to 65.3% and 37.3%, respectively [161]. Sufficiently, PepT1-mediated prodrugs were shown to have high affinity. For instance, the antiviral drug oseltamivir carboxylate and amino acid-conjugated acyloxy(alkyl)ester prodrugs showed a 30-fold increase in affinity for PepT1, suggesting a high degree of recognition between the prodrugs and the corresponding transporters [162]. The FDA has authorized 178 precursor medications as of right now, LY354740 (alanine prodrug) from Eli Lilly and Company has entered the clinical phase, but still faces the disadvantages of its hydrophilicity, poor small intestinal membrane permeability, and poor pharmacokinetic properties, in view of this, the researchers acylated LY354740 with alanine to obtain the prodrug LY544344 and performed membrane permeability studies, which resulted in a 10-folds increase in the permeability coefficient of the prodrug LY544344 by approximately 50% targeting of PepT1 into cells for the treatment of osteoporosis and hypercalcemia [163,164]. What needs to be attended that when we design the structure of a modified drug, not only do we make it easily recognisable by the transporter, but we also need to consider whether or not it is a substrate for P-gp, with the effect of the presence of efflux.

Therefore, some researchers have found that if multiple transporters are introduced simultaneously into the parent drug or carrier to synthesise a dual-targeted prodrug, intestinal uptake time will be improved and prolonged and bioavailability will be enhanced [165,166]. Although the design of prodrugs targeting PepT1 improves the bioavailability of more orally available drugs, there is no clear clarification of the specific binding mechanism of PepT1 to the substrate, efforts are still needed to resolve.

4.2. Systems of drug delivery mediated by transporter substrate modification

In the clinical use of oral formulations, their bioavailability may be affected by intestinal transporters [167]. Whereas some illuminating reports pointed out that by targeting design using endogenous apical uptake or basolateral efflux-type transporters within the intestinal epithelium, the substrate modification of intestinal transporters is used to construct novel drug delivery systems that either by the selection of transporters for specific binding to cross the enterocyte in an endocytosis manner [168], or by exploiting the specific interactions of the substrate with the transporter that prolonged retention at the surface of the intestinal membrane and crossed the enterocytes by an osmotic mechanism [169]. The current strategies for transporter substrate modification of delivery systems include covalent binding [170], electrostatic interactions [171], and hydrophobic interactions [172], the modification is

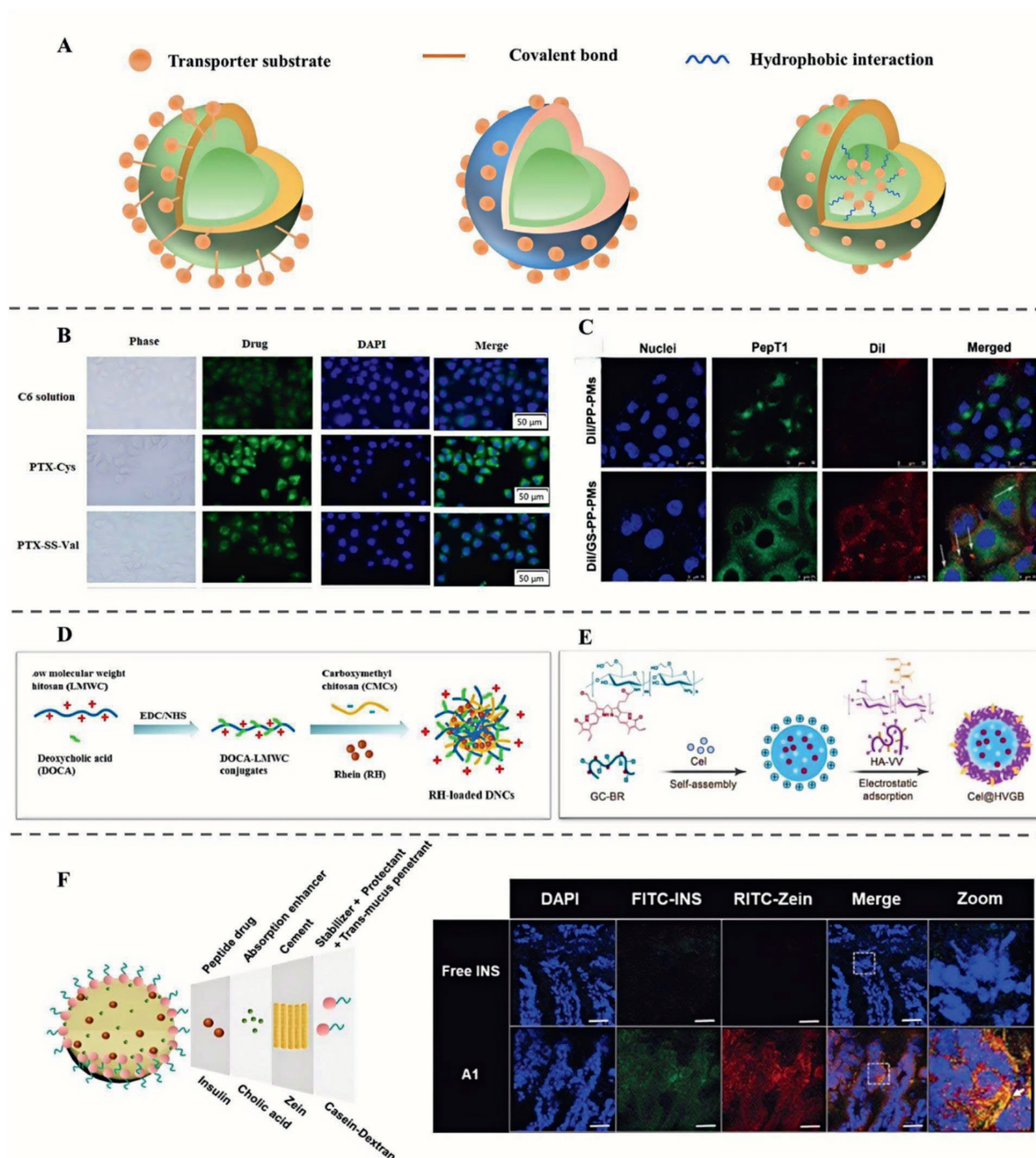


Fig. 6. Transporter substrate-modified nano-formulations as strategy to enhance oral absorption. (A) Three strategies for transporter substrate modification, from left to right, are covalent binding, electrostatic interactions, and hydrophobic interactions. (B) Intensity of cellular uptake fluorescence by C6-loaded NPs of PTX-Cys, PTX-SS-COOH, and PTX-SS-Val after 4 h. Reproduced with permission [174]. Copyright 2021, Elsevier Science Ltd. (C) The role of PepT1 in the cellular uptake of DiI/GS-PP-PMs, DiI (red), PepT1 immunofluorescent stained with Alexa Fluor®488 (green), yellow fluorescent signals (arrows), indicating interaction with PepT1. Reproduced with permission [178]. Copyright 2019, Royal Society of Chemistry. (D) RH-DNCs prepared by electrostatic interactions and internalised into the intestinal epithelium via ASBT-mediated endocytosis. Reproduced with permission [186]. Copyright 2021, Elsevier Science Ltd. (E) Cel@HVGB encapsulated by electronic interactions for the treatment of ulcerative colitis. Reproduced with permission [188]. Copyright 2023, Elsevier Science Ltd. (F) The diagram on the left shows the constituent NPs and their corresponding functions and the corresponding functions, and the right panel shows images of CLSM of mouse ileum sections after 2 h of oral administration of free FITC-INS, FITC-INS-prepared A1, scale bar: 40 μ m. Reproduced with permission [190]. Copyright 2021, Royal Society of Chemistry.

schematically shown in Fig. 6A, all of which may offer the possibility of transmembrane drug delivery for poorly absorbed drugs.

4.2.1. Covalent binding or coupling

Covalent binding refers to direct covalent binding of the transporter substrate to the drug delivery system or indirect binding via a linker (such as polyethylene glycol) [173]. In order to achieve responsive release from the tumor site, Li *et al.* [174] interconnected L-valine (a substrate of PepT1) with PTX-succinic anhydride-cysteamine via amide bond to obtain small molecule pa-

clitaxel precursor drug nanoparticles (PTX-SS-Val), which can be recognized by transporters as a substrate of PepT1, due to the peptide analogs formed by the drug-amino acid linkage, increasing PTX intestinal absorption and oral bioavailability [175], by loading coumarin 6, high fluorescence intensity was found using covalently bound PTX-SS-Val (Fig. 6B) [176]. In addition, PepT1-based modification-mediated drug delivery system also includes polymeric micelles, the construction of PepT1-mediated micelles by grafting valine or phenylalanine onto the surface of TPGS revealed that the uptake and transport of curcumin was effectively fac-

itated by PepT1-mediated means [177]. Jin *et al.* [178] revealed that PepT1-mediated transcellular transport of polymer micelles is more completely studied (Fig. 6C), these specifically modified micelles are internalized into the cell together with PepT1 binding in the presence of other endocytosis. In the cytoplasm, micelles are mainly transported to the basolateral side via the endoplasmic reticulum-golgi pathway and finally enter the somatic circulation via the lymphatic transport pathway [179–181].

4.2.2. Electrostatic interaction

The complexes that result from the binding of transporter substrates and macromolecular substances that use electrostatic interactions as a means of forming shells around the surface of the drug delivery system are known as electrostatic interactions, whereas the transporters mediated in this manner are dominated by ABST and PepT1 [182]. We have learned that BA or deoxycholic acid (DOCA) in the human body is mediated by ASBT located in the apical part of the ileal cells for transportation, which are suitably hydrophobic [183]. When DOCA is introduced into the polymer, the conjugate does not form hydrophobic aggregates until it induces electrostatic interactions with oppositely charged hydrophilic polymers [184], and DOCA can bind directly to the carboxyl and hydroxyl groups of the BA binding site of ASBT [185]. Yao *et al.* used amide bonding to link the amino group in the chitosan derivative LMWC with DOCA, subsequently produced self-assembled nanoparticles loaded with rhein through electrostatic interactions between positively charged DOCA-LMWC and negatively charged CMC [186], its internalized DOCA-modified nanoparticles into the intestinal epithelium through ASBT-mediated endocytosis, which significantly increased the oral bioavailability of the encapsulated drug (Fig. 6D) [187]. Chen *et al.* [188] used bilirubin-chitosan self-assembled into nanoparticle structures, the anti-inflammatory drug celecoxib loaded into the hydrophobic core, and the PepT1 substrate L-valine-valine (VV) using coupled hyaluronic acid is further coated to form the PepT1 and CD44 dual targeting nanoparticles (Cel@HVGB) by electronic interaction, that normalize the intestinal microenvironment by modulating cell-specific activity for the treatment of ulcerative colonic inflammation (Fig. 6E). So, the modification of the ASBT and PepT1 substrates could also be used as a potential target to enhance the absorption of biopharmaceutics classification system (BCS) class III/IV drugs [189].

4.2.3. Hydrophobic interaction

Transporter substrates can be embedded within or on the surface of drug delivery system through hydrophobic interactions. Bao *et al.* [190] embedded casein-dextran coupling (CN-DEX) shelled corn alcohol protein nanoparticles with tryptophan and BA CA via hydrophobic interactions. When the encapsulated nanoparticles were progressively eroded by the enzyme, the embedded BA molecules were successively exposed on the surface, CA, as a substrate of ABST, can promote the uptake of nanoparticles in ileum and liver (Fig. 6F), it was observed that the ileal segment A1 group showed fluorescein isothiocyanate-conjugated insulin (FITC-INS) and Rhodamine B isothiocyanate (RITC)-Zein fluorescence signals uniformly distributed and overlapped from the surface to the interior and the fluorescence intensity was significantly higher than that of the free insulin group [191]. Moreover, L-carnitine (LC), as the endogenous substrate of OCTN2, is a utilizable ligand for OCTN2-targeted carrier system improving the intestinal absorption of drug [192,193], using chitosan-stearic acid (CS-SA) as a carrier and coupling LC-SA via hydrophobic interactions, LC-SA/CS-SA micelles encapsulating PTX will pass through enterocytes through OCTN2-mediated transport, confirming that facilitated intestinal absorption of PTX [194].

4.3. Strategies to inhibit P-gp efflux

4.3.1. Suppression by surfactants

P-gp-mediated efflux transport limits drug bioavailability [195]. As we know that the nonionic surfactant have solubilization and stabilization effects, the main reason for which is to reduce mucosal viscosity by disrupting cell membranes and altering the integrity of tight junctions [196,197]. The vitamin E TPGS 1000, Cremophor EL, and Tween 80 could inhibit the efflux of P-gp and MRP2, which implies that surface-active substances enhance the intestinal absorption of proteins and peptides and play an essential role in improving oral bioavailability [198,199]. Several *in vitro* assays have been utilized to investigate the P-gp inhibitory properties of these surfactants, using cells that over-express P-gp, a fluorescence-based efflux test (e.g., calcein) or measuring the effect of surfactant on substrate uptake and/or secretory transport, *etc.* on cells or excised intestinal segments of animals [200]. Hence, the inclusion of surfactant with P-gp inhibitory properties may be favorable for enhancing substrate transport across biological membranes [201].

Of interest, different perspectives have been proposed on the mechanism by which nonionic surfactants promote drug uptake by inhibiting P-gp-mediated cellular efflux [202]. Furthermore, the previously mentioned transporter-targeted prodrug design also avoids P-gp-mediated exocytosis, and we revealed that when a substrate of P-gp binds to a transporter protein, which may trigger a change in its conformation and its release into the cytoplasm. Once in the cytoplasm, the prodrug is subjected to enzymes that cleave the linkage bonds and release the parent drug, that *in vivo* transport is shown schematically in Fig. 7A, the transporter is shown in the inward-open conformation [160]. As shown in Fig. 7B, work from Seelig *et al.* [203] have suggested that the efflux inhibition may occur through the entry of the hydrophobic tail of the surfactant into the cell membrane, while the formation of hydrogen bonds between the hydrogen bond acceptor groups in the hydrophilic group of the surfactant and the hydrogen bond donor groups in the TMD of the protein. And then the ability of non-ionic surfactant to bind to phospholipids on cell membranes and reduce viscosity, thereby causing changes in the fluidity of the cell membrane bilayer may also indirectly inhibit the activity of ATPase [204]. Wei and his colleagues believed that the primary mechanism of Pluronic P123/F127 is owing to the depletion of intracellular ATPase [205]. So, investigations regarding the effects of surfactants on the oral absorption of different P-gp substrates may be helpful in the design and execution of human clinical studies [206].

4.3.2. P-gp inhibitor

P-gp has a wide range of substrate, inhibitor, and inducer specificity, when administering medications in combination, competitive or noncompetitive drug interactions at the P-gp molecular level frequently occur and affect pharmacological effectiveness, and the different mechanisms for inhibiting P-gp are thought to be through several ways: blocking drug-binding site either competitively or non-competitively; interfering ATP hydrolysis and altering integrity of cell membrane lipids [207].

The hypothesis that inhibition of P-gp enhances the bioavailability of drugs that act as substrates for transporter proteins is widely accepted, the drug delivery system can cross the membrane and inhibit P-gp efflux through multiple routes (Fig. 7C) [208]. As the inhibitor HM30181 developed by foreign researchers has been shown to increase its bioavailability greatly when combined with paclitaxel [209]. Efflux transporters have the role of restricting endocytosis and promoting efflux to prevent intracellular accumulation of their own substrate compounds [210,211]. Especially in tumor cells, these functions enable MDR to various anticancer

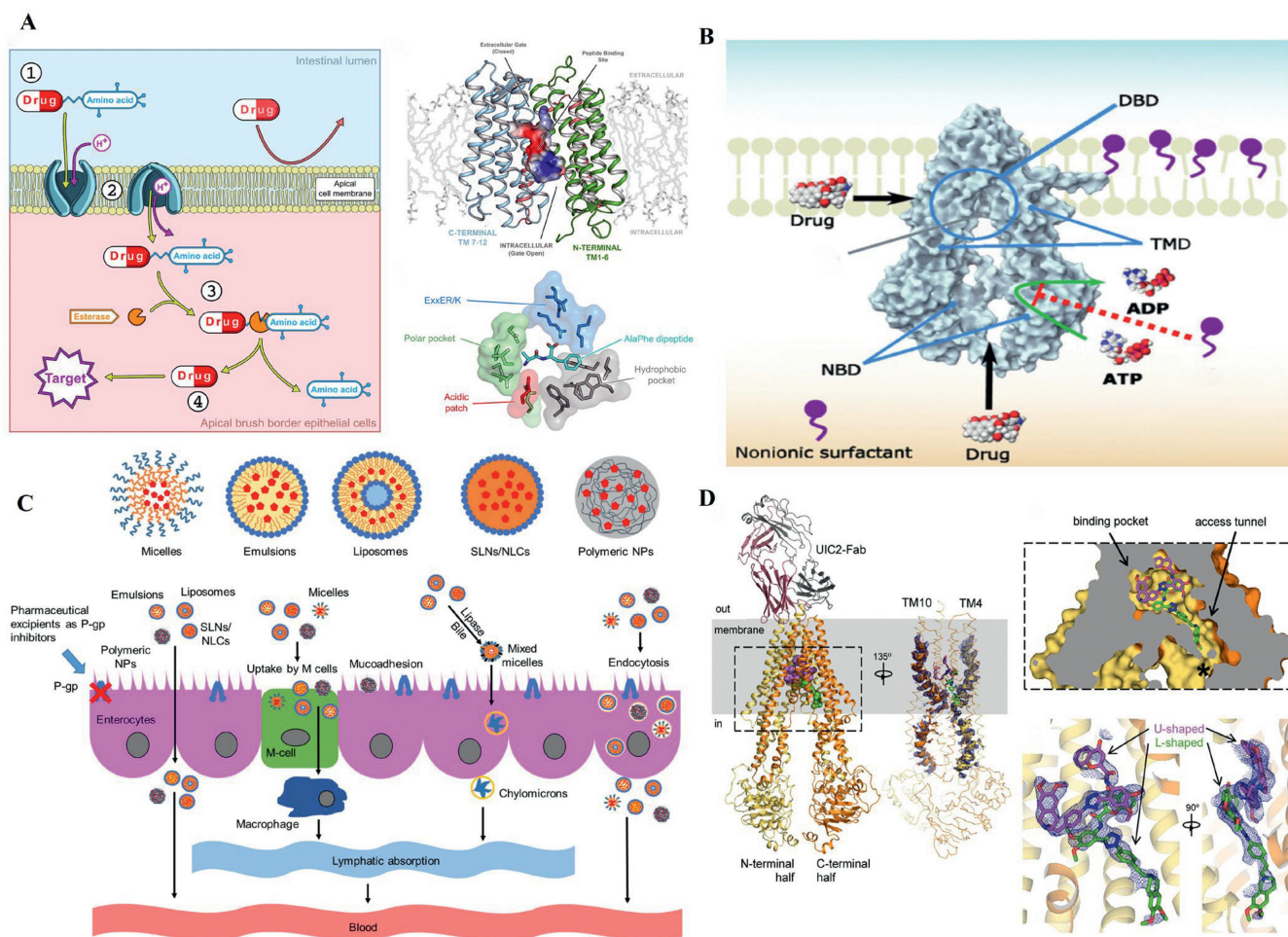


Fig. 7. Schematic representation of several mechanisms or strategies to enhance oral absorption by modification into precursor drugs, inhibition of P-gp efflux and exploitation of transporter-CYP3A interactions. (A) Transport of small molecule precursor drugs *in vivo* and PePT-binding sites. Reproduced with permission [160]. Copyright 2020, Elsevier Science Ltd. (B) Nonionic surfactants inhibit the P-gp mechanism, where NDB, TMD and DBD stand for nucleotide binding domain, transmembrane binding domain and drug binding domain, respectively. Reproduced with permission [204]. Copyright 2022, Elsevier Science Ltd. (C) Inhibit P-gp efflux through multiple routes. Reproduced with permission [208]. Copyright 2021, Multidisciplinary Digital Publishing Institute. (D) Cryo-EM structure of encephalid bound to human P-gp (PDB 7O9W). Copied with permission [236]. Copyright 2022, Elsevier Science Ltd.

agents [212,213]. With the emergence of new P-gp inhibitors in recent years, they can improve the efficacy of chemotherapeutic drugs and promote the effective absorption of drugs in the intestine [214]. P-gp inhibitors require good affinity and selectivity, combined with the ability to inhibit P-gp, other efflux transporters, and metabolic enzymes. [215]. Verapamil is the earliest known substrate of P-gp, but toxicity concerns limit application, while newer inhibitors have been exploited to address the problems posed by previous generations, allowing for better selection of specific ABC transporters high affinity for P-gp, and the reversal of multi-drug resistance at low doses [216], for example, Elacridar (GF120918) [217], OC144–093 (ONT-093) [218].

In general, most investigators on transporter regulation have concentrated on individual transporters [219]. Whereas fourth-generation P-gp inhibitors using natural compounds and dual regulation by P-gp have been progressively used in clinical studies [220]. Lee *et al.* extracted topotecan (TPT), a natural compound with anticancer properties, as a substrate for P-gp and BCRP, and when the dual inhibitor was co-administered orally with TPT, it increased the bioavailability of TPT, while significantly enhancing tumor growth inhibition in xenograft mice [221]. Accordingly, we speculate that the induction or inhibition of transporters or

metabolic enzymes by Chinese medicines and their active ingredients will alter the activity or expression of the transporters, thereby changing the pharmacokinetics of the drugs, which in turn will affect the ADME of the drugs in the body, and ultimately influence the safety and efficacy of the drugs [222]. The low bioavailability of natural flavonoids (genistein, soy flavonoids, quercetin, etc.) are the major reason for limiting their clinical application, which are closely associated with their intestinal absorption and metabolism; however, they contain multiple hydroxyl groups in their structure, which can regulate efflux-type transporters, and their bound metabolites are all substrates of efflux transporters BCRP and MRP2, which can be used as a class of non-toxic P-gp inhibitors [223]. When the binding of P-gp to 7-O-geranylquercetin (7-O-geranylquercetin, GQ) increased the antitumor effect of adriamycin and decreased the expression of P-gp in cells, so the flavonoid is effective in reversing P-gp-mediated adriamycin resistance in breast cancer [224]. Moreover, it also includes the alkaloid berberine hydrochloride, which is a substrate of the efflux transporter P-gp and its inhibitor, and is able to bi-directionally regulate P-gp and the cytochrome P4503A4 enzyme [225], and cucurbitacin E (Cu E), an oxidized tetracyclic triterpenoid, has been shown to be strongly associated with MDR in tumor through the

study of P-gp, BCRP and ATP-binding cassette member B5 (ABCB5) in the ABC transporter family, and Cu E has also been revealed to be a substrate for P-gp and BCRP and a potent inhibitor of ABCB5 [226]. Taking the above into consideration, inhibiting intestinal efflux transporters not only increases drug absorption, but also reduces drug metabolism, thereby significantly increasing drug bioavailability.

4.4. Interaction with CYP3A

CYP3A is the most dominant cytochrome P450 enzymes (CYP) subfamily in humans and rodents, with a content of about 80% of the total intestinal CYP [227], which can metabolize drugs passing through the intestinal epithelium, leading to an intestinal first-pass effect of some of the drugs, and affecting drug absorption and efficacy [228,229]. Despite the low levels of CYP3A4 in the gut compared to the liver, the first metabolism of drugs through CYP3A in the gut affects numerous drugs [230]. Many scholars at home and abroad have investigated the existence of coupling between metabolic enzymes and efflux transporters (e.g., P-gp, MRPs, BCRP), which become a barrier to the absorption of orally administered drugs, and there is a significant overlap in the substrates between them, so that the interaction between CYP3A and efflux transporters (coupling effect) may significantly affect its pharmacokinetics and pharmacodynamics [231]. Wang *et al.* supposed that CYP3A4 can be coupled with P-gp the mechanism of action may be oral drugs into the intestinal epithelial cells metabolized by CYP3A4 [232], meanwhile, P-gp can be continuously exocytosis of the drugs inside the intestinal epithelial cells to the intestinal lumen, so that the drugs in the intestinal epithelial cells and intestinal lumen and the formation of a continuous and repeated cycle between: intestinal cycle, that allows CYP3A4 to be repeatedly exposed to metabolized drugs, which reduces the entry of drugs into the circulation and avoids saturation of CYP3A4 metabolism, eventually leading to poor bioavailability [233]. Noteworthy is the fact that there might also be an effect of the distributional properties and transport behavior of transporters in the gut, we speculate that owing to the distance between P-gp (located in the apical membrane) and CYP3A4 (located in the endoplasmic reticulum) in the enterocytes leads to a decrease in blood flow to the intestinal mucosa. What is more, the formation of the intestinal circulation allows for an increase in metabolizing enzymes, reducing the drug entry into the somatic circulation and decreasing its bioavailability. Not only that, the researchers revealed that it is also affected by drug structure modifications, transporter substrates, *etc.* So, the question is that, whether can we reduce intestinal P-gp, on the number of times the drug circulates between cells and the intestinal lumen, and reduce CYP3A4 on drug metabolism to improve oral bioavailability of the drug [234].

Extensive studies of oral DTX using transgenic mouse models with intestinal/hepatic CYP3A knockouts have established the metabolizing power of intestinal CYP3A4, after administration of the P-gp inhibitor elacridar and the CYP3A inhibitor ritonavir, a significant increase in oral bioavailability of paclitaxel analogues [235]. Urgaonkar and his colleagues reported a dual-targeting strategy of P-gp and CYP3A4 based on Enequidar (the least absorbed P-gp inhibitor), showing a frozen-EM structure that binds to human P-gp. Enequidar has the structural characteristics of high-efficiency P-gp inhibitors and can interact with proteins, which provides a reference for us in the structure-activity relationship (Fig. 7D) [236]. However, the oral bioavailability of P-gp and CYP3A4 substrates is largely determined by the affinity of the substrate for these proteins, and the biotransformation of drugs entering the organism can generate multiple metabolites, whether this process affects the interaction with the efflux transporter remains unknown; there-

fore, the use of transporter-CYP3A interactions to improve oral absorption still needs to be addressed.

4.5. Molecular simulation strategies

The entry of drugs into the circulation is influenced by the transporters, and the knowledge of their detailed 3D structural information, combined with an in-depth knowledge of the function and mechanism, which will be of immense value in aiding rational drug design [237]. Whereas, it was revealed that computer modelling techniques can help to further understand the transporter-substrate interactions, directly predict the 3D structure of the transporter, comprehend the drug transport process, and better optimize the design of drugs based on transporter guidance [238].

For molecular modelling requiring access to the 3D structure of a protein, Yan's team has investigated the crystal structure of the human GLUT1, an unprecedented scientific achievement that initially reveals its working mechanism and the pathogenesis of related diseases [239]. GLUT1 is embedded in the cell membrane, opening a door in the hydrophobic cell membrane to transport glucose from the extracellular to the intracellular compartment, and exhibited the classical major facilitator super (MFS) family folding, which consists of 12 transmembrane helices that form the N- and C-terminal domains, the structure presents an inward-opening conformation. Most notably, Park used a similar method to construct two human GLUT1 homology models (Fig. 8A), in which the interface between the TMD and the ICH can interact (Fig. 8B), using directed molecular dynamics, we can observe the movement of D-glucose to the inside of the cell (Fig. 8C) [240]. The GLUT secondary molecular structure is shown in Fig. 8D, unlike other MFS transporters, the two structural domains of GLUT1 are connected by an ICH consisting of four short α -helices [241], a large intracellular loop between helices VI and VII, and an N-linked glycosylation site on the extracellular loop, as yet the development of such GLUT1 target-based drugs has become a new hotspot in tumor therapy.

Guo *et al.* studied the affinity of three aspergillus derivatives for GLUTs by a computer-assisted molecular docking method [242], and showed that the SGLT1 promoted the intestinal absorption of salicin, arbutin, and 4-methoxyphenyl- β -D-glucoside (4-MG), and that there are subtle differences in the chemical structure that have an impact on the pathway of the drugs through the GLUTs. Nevertheless, there are still some transporters that still lack a 3D structure due to the crystallization problem of multiple hydrophobic transmembrane structural domains, and experimentally unavailable, and we can use homology modeling to obtain parameter or information files, including atomic coordinates, secondary structure assignments. Among them, molecular docking is the most commonly modeled way to study the interaction between drugs and transporters, for example, non-covalent interactions between drug-ABC transporter protein superfamily are explored to develop new anticancer drugs targeting MDR [243]. It is capable of searching for ligand binding-induced changes in the 3D structure of proteins and applying computers to perform quantitative structure-effect relationship (QSAR) studies of binding sites related to pathogenic mechanisms [244]. Just like P-gp is known to be overexpressed in many MDR cancer cell lines (Fig. 8E) which contains 2 TMD and 2 NBDs [245], within these regions, two binding packages important for drug development studies are the ATP-binding site and the drug binding site [246,247]. The computer simulation of drug development focuses on identifying target locations on receptors or transporters where molecules will interact, and subsequent homology modeling studies of the binding package, which allows for high-precision detection of drug-transporter interactions [248].

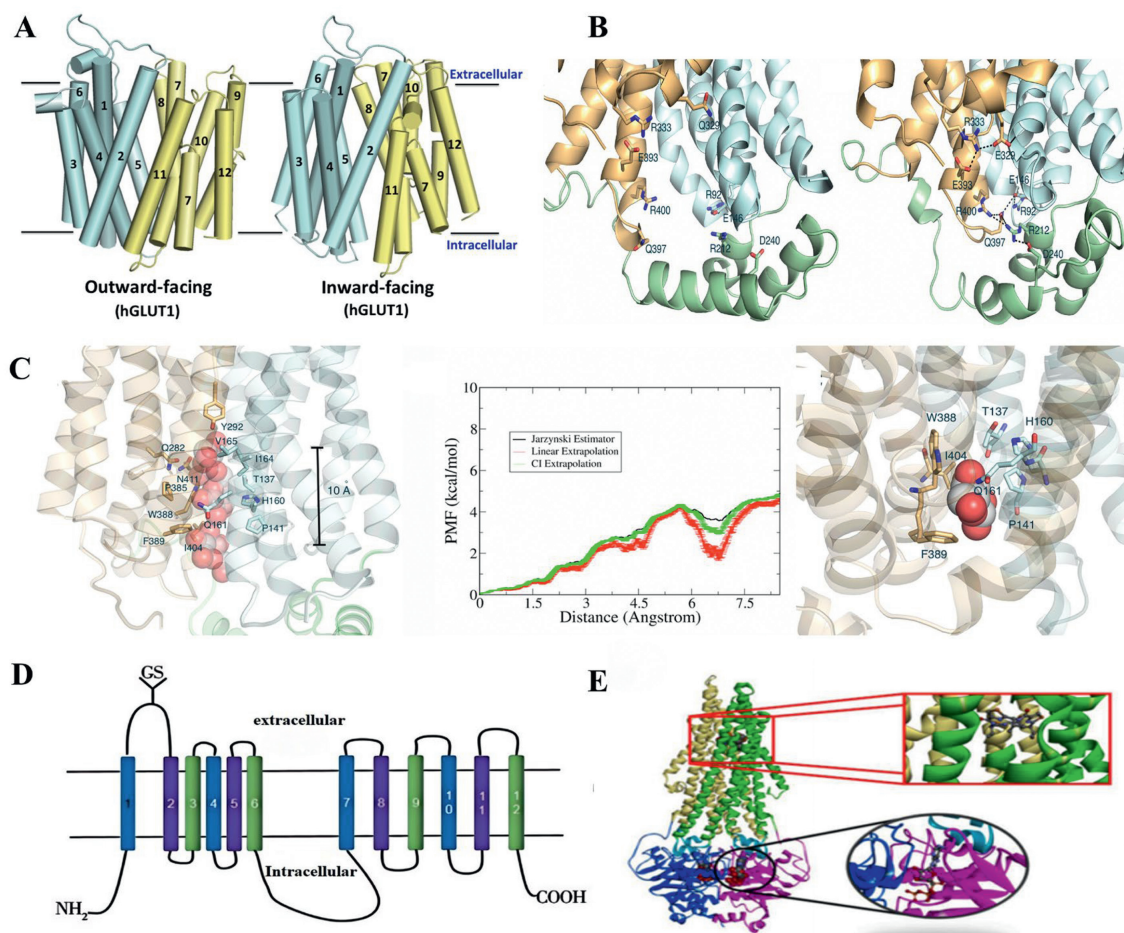


Fig. 8. Utilizing molecular modeling to determine the transporter's crystal structure and potential drug binding locations. (A) Two human GLUT1 homology models. (B) Comparison of interactions between TM domains and ICH domain of GLUT1. (C) The movement of D-glucose toward the intracellular side by steered molecular dynamics (SMD) simulations. Reproduced with permission [240]. Copyright 2015, Public Library of Science. (D) Secondary molecular structure of GLUT1. (E) ABCB1 transporter proteins and its binding packages, ABCB1 with an inhibitor, OJZ, (PDB ID: 4M2S) in red rectangle and ATP (PDB ID: 6C0V) in black circle. Copied with permission [245]. Copyright 2021, Elsevier Science Ltd.

5. The challenges and opportunities of transporters for oral absorption

For compounds with low permeability, enhancing the role of uptake transporters and reducing the influence of efflux-type transporters may be a very beneficial strategy. Multiple transporters on the enterocyte membrane are also excellent targets for oral drug delivery system, and then a large number of studies have conclusively confirmed the potential of intestinal transporters in the field of oral drug delivery systems. However, the examination of transcellular transport mechanisms of transporter-mediated drug delivery system is not sufficiently comprehensive, and there may be off-targeting of the designed transporter-targeted delivery systems, as most of the transporters are also expressed in other tissues of the human body; and secondly, another underestimated aspect is the possible interactions between intestinal enzymes and exocytosis and uptake of the transporters in the intestine. So far, only the CYP3A4-P-gp interaction in the intestine has been described in detail. Chinese medicines, as an important part of contemporary Chinese specialty pharmacology, have the advantages of lower toxicity, better multi-target biological activity and synergistic effects, compared with synthetic drug, instead, there is a relative lack of research on transporter-targeted delivery system for encapsulating the active ingredients of Chinese medicines.

Therefore, in order to solve the current limitations, some suggestions are made for the future direction of development: first, emphasize transporter research and expand the development of other transporter-mediated drug delivery systems. Secondly, in-depth research on intracytoplasmic transport and basolateral cytosolic pathways should be carried out, so that more ideal formulations can be designed only with a comprehensive understanding of the whole transport process. Third, rational design of drug delivery systems to prevent off-targeting, and the design of dual or multiple targeting can also be considered. Fourth, based on traditional Chinese medicine (TCM) theory, we focus on developing transporter-mediated drug delivery systems containing active ingredients of TCM, not only targeting TCM monomers, but also multi-active ingredients of TCM, and integrating novel targeting strategies and technologies into the research of TCM, in order to better promote the oral efficacy and application of TCM in the clinic, which will be an important direction of development of modern TCM formulations, with a very far-reaching prospect.

6. Conclusions and outlook

Growing numbers of intestinal drug transporters are being used to accomplish drug delivery through their mediated mechanisms thanks to the extensive use of molecular pharmacology, molecular

biology, and genetic engineering techniques. We are investigating its role, mechanism, and tissue distribution in intestinal drug absorption, which will be helpful in enhancing drug bioavailability, avoiding drug-drug interactions, and developing new drug targets for diseases. In this paper, we reviewed the distributional properties of intestinal transporters and analyze various strategies to promote oral absorption. Indeed, the complexity of the body environment and the process of comprehensive transport is unclear. In the future, we would need to continue to strengthen the study of intestinal transporters with an insight to the design and development of targeted novel drug delivery systems, and to search for and develop safe and effective modulators of transporters to enhance the targeted distribution of drugs and to improve the efficacy of the drugs, so as to guide the clinic in a more sensible and rational use of medication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ju Wang: Writing – review & editing, Writing – original draft. **Yongbing Sun:** Visualization, Validation. **Lingbang Meng:** Formal analysis, Conceptualization. **Jianfang Feng:** Supervision. **Meng Cheng:** Supervision. **Liangxing Tu:** Writing – review & editing, Supervision, Funding acquisition.

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