

• Reviews •

Delineation on Therapeutic Significance of Transporters as Molecular Targets of Drugs

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Abstract: Transporters are membrane proteins mediating permeation of organic and inorganic solutes through the plasma membrane and membranes of intracellular organella. They play essential roles in the epithelial absorption and cellular uptake of nutrients as well as absorption, distribution, metabolism, and excretion of drugs. Because transporters contribute to determining the distribution of compounds in the body in concert with metabolic/synthetic enzymes, the drugs that affect the functions of transporters are expected to alter the distribution of compounds in the body and to ameliorate disrupted homeostasis. In this context, drugs targeting transporters have been used clinically. Such drugs include antidepressants targeting monoamine transporters, diuretics targeting inorganic ion transporters of renal tubules, and uricosuric agents targeting renal urate transporters. Now new transporter-targeting drugs designed based on post-genome drug development strategy have been in the process of clinical trials or basic/clinical researches. For example, the inhibitors of renal Na⁺/glucose cotransporter SGLT2 have been proved for their efficacy in the treatment of diabetes mellitus. The cancer L-type amino acid transporter 1 (LAT1) has been considered as a target of cancer diagnosis and therapeutics. The transporter-targeting drugs are expected to provide new rationale in the therapeutics of various diseases.

Key words: absorption; distribution; excretion; metabolism; molecular target; transporter

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Introduction

Transporters are membrane proteins that mediate permeation of organic and inorganic solutes through the plasma membrane and membranes of intracellular organella. They are essential for the epithelial absorption and cellular uptake of nutrient such as sugars, amino acids, lipids, vitamins, and minerals as well as absorption, distribution, metabolism, and excretion of drugs. Because nutrients and metabolites

of most drugs, including chemical, natural, and biotech drugs are hydrophilic, they are less permeable to biomembranes including plasma membrane and membranes of intracellular organella. Therefore, special machineries are required for their permeation through the membranes. Transporters are responsible for such selective permeability to enable hydrophilic compounds to pass through the membrane in a selective manner (Hediger *et al.*, 2004).

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Transporters were originally studied in the field of enzymology (Hediger and Rhoads, 1994). Apparently, the transporter-mediated transport of solutes follows the Michaelis-Menten kinetics or Hill equation. Therefore, the binding of the transported solutes to the catalytic center of the transporter proteins was supposed to be the rate-limiting process and to promote the conformational changes leading to the translocation of the solutes to the other side of membrane (Fig. 1) (Hediger and Rhoads, 1994). The transported solutes were, thus, called “substrate” following the terminology in enzymology. The sites to which the substrate binds are called “substrate binding sites” that correspond to the catalytic centers of enzymes. Channels are also membrane proteins that mediate selective permeability of solutes similar to transporters. Distinct from transporters, channels are regarded as the gated “pores” which have no substrate binding structures (Hille, 2001). Once the channels open, a lot of permeable solutes pass through the pore following the electrochemical gradient regarding the solutes so that the rate of permeation through channels is very rapid. In contrast, the permeation of solutes through transporters requires multiple steps covering the binding of substrate to its substrate binding site, conformational changes of the transporter proteins corresponding to the translocation of substrates, and the release of substrates from the substrate binding site (Hediger *et al.*, 2004). In this single transport cycle, only one substrate (or the number of substrates corresponding to the number of substrate binding sites) can be transported by transporters (Fig. 1).

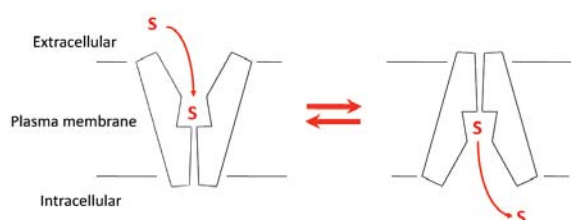


Fig. 1 A model describing transporters' function

Substrate-binding site is located in the middle of membrane. Binding of the substrate (S) to the substrate-binding site promotes the conformational changes of the transporter protein leading to the translocation of the substrate to the other side of membrane

Thus, the rate of permeation through transporters is in general slow. Recently, crystallography has been successful for bacterial transporter proteins and revealed

crystal structures of some transporters (Murakami *et al.*, 2002; Abramson *et al.*, 2003; Huang *et al.*, 2003; Yernool *et al.*, 2004; Yamashita *et al.*, 2005; Hollenstein, Frei, and Locher, 2007; Faham *et al.*, 2008; Newstead *et al.*, 2011). They have confirmed the classical view of transporters as enzymes by revealing, for example, the presence of substrate binding site, and furthermore added new insights into the transport process.

By mediating selective permeation of substrates through the plasma membrane, transporters contribute to determining the distribution of their substrate in the body. As they will be discussed in detail in this article, transporters thermodynamically determine the ratio of concentration of substrates between two sides of the membrane on which the transporters are located (Newstead *et al.*, 2011; Fei *et al.*, 1994; Zerangue and Kavanaugh, 1996). The contribution of transporters is supposed to be significant, although metabolic and/or synthetic enzymes also contribute to determining the distribution of compounds in the body. Therefore, if the drugs can affect the functions of transporters, they are expected to alter the distribution of compounds in the body by inhibiting or stimulating the functions of transporters. In many diseases, the distribution of compounds is, in fact, altered. For example, concentrations of glucose, cholesterol, and urate are elevated in blood in diabetes, hypercholesterolemia, and hyperuricemia, respectively. If it is possible to suppress or enhance the function of responsible transporters and reduce the blood concentration of those compounds by drugs, such drugs can be effective in the treatment of the diseases. In this context, drugs targeting transporters have been used clinically. Antidepressants, such as tricyclic antidepressants, tetracyclic antidepressants, selective serotonin reuptake inhibitors (SSRI), and serotonin noradrenaline reuptake inhibitors (SNRI) are the inhibitors of monoamine transporters (Andersen *et al.*, 2009). Diuretics, such as furosemide and thiazide, are inhibitors of inorganic ion transporters of renal tubules (Giménez, 2006; Ko and Hoover, 2009). Uricosuric agents, such as probenecid, benzbromarone, and losartan used for the treatment of hyperuricemia, are the inhibitors of renal urate transporters (Wright *et al.*, 2010). Inhibitors of gastric H^+ pump such as Omeprazole are used for the treatment of gastroduodenal ulcer (Robinson, 2005). Digitalis that

has been used for the treatment of cardiac insufficiency for a long period is an inhibitor of $\text{Na}^+\text{-K}^+$ pump (Lingrel, 2010). These drugs are first used clinically and then their targets of pharmacological effects have later been revealed to be transporters. Now, some drugs that target transporters have been in the process of development by post-genome drug development strategy in which the compounds affecting the functions of cloned transporters are first screened and optimized on *in vitro* transport assay systems expressing genes of transporters of interest and then evaluated for the effectiveness and toxicity in experimental animals and humans. As examples of such post-genome drug development targeting transporters, the drug targeting renal Na^+ /glucose transporters and cancer cell amino acid transporters will be described in detail in this article in terms of treatment of diabetes and cancer, respectively.

ABC and SLC transporters

Membrane proteins with mediating selective permeability of organic and inorganic solutes are roughly classified into ion channels and transporters. Transporters are furthermore divided into ion pumps, ABC transporters, and solute carrier (SLC) transporters (Fig. 2) (Hediger *et al*, 2004). Sometimes, ion pumps are not included in transporters. In such a classification, the membrane proteins with mediating selective permeability are categorized into three groups: ion channels, ion pumps, and transporters. Ion pumps are ATPases that mediate active transport of inorganic ions. They include P-type ATPases such as $\text{Na}^+\text{/K}^+$ -ATPase (sodium-potassium pump), H^+ -ATPase (proton pump), $\text{H}^+\text{/K}^+$ -ATPase (proton-potassium pump), and Ca^{2+} -ATPase (calcium pump); V-type ATPases, the vacuolar-type H^+ -ATPases, including proton pumps of lysosomal membranes; and F-type ATPases, the ATPases of bacterial plasma membranes and mitochondrial inner membranes that use a proton gradient to drive ATP synthesis (Chan *et al*, 2010; Beyenbach and Wieczorek, 2006).

ABC transporters are a group of membrane proteins that possess consensus amino acid sequences for ATP-binding called ATP-binding cassette, so that they are named as ABC transporters (Seeger and van Veen, 2009). There are 48 ABC transporters reported in

humans, which are classified into seven families (ABCA, ABCB, ABCC, ABCD, ABCE, ABCF, and ABCG). A full list of human ABC transporters can be found at <http://nutrigene.4t.com/humanabc.htm>. Many of mammalian members of ABC families (ABC proteins) are the transporters mediating active transport of organic solute utilizing free energy obtained upon the hydrolysis of ATP by the ATPase activity of the ATP-binding cassette, whereas some of the ABC proteins such as cystic fibrosis transmembrane conductance regulator (CFTR, ABCC7) and sulfonylurea receptor (ABCC8 and ABCC9) are not transporters (<http://nutrigene.4t.com/humanabc.htm>).

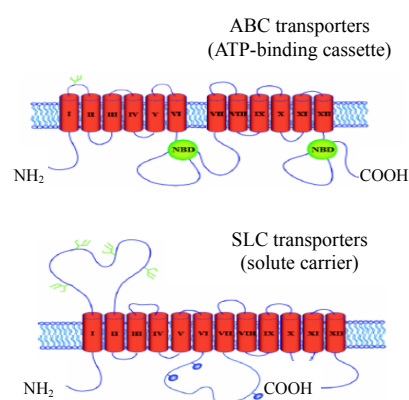


Fig. 2 ABC and SLC transporters

ABC transporters possess consensus ATP-binding cassettes in the nucleotide binding domains (NBD). SLC transporters do not have ATP hydrolysis activity

The SLC transporters are those other than ion pumps and ABC transporters (Hediger *et al*, 2004) to which most of the transporters belong. There are over 350 members of SLC transporters organized into 51 families. A full list of human SLC transporters is available at <http://www.bioparadigms.org/slc/menu.asp>. SLC transporters include uniporters, symporters, and antiporters (Fig. 3) (Hediger *et al*, 2004). Uniporters are the transporters that mediate the transport of single substrate. Substrate transport mediated by uniporters just follows the electrochemical gradient of the substrate and is called facilitated diffusion.

Symporters, also called cotransporters, couple the transport of a substrate with the transport of other substrates. Usually, symporters co-transport organic substrates with Na^+ or H^+ to utilize the free energy stored as the electrochemical gradient of Na^+ or H^+ across the plasma membrane. The examples of symporters are: SLC5 family (sodium glucose

cotransporter family) which couples the transport of organic substrate with the transport of Na^+ (Wright and Turk, 2004); SLC6 family (sodium- and chloride-dependent neurotransmitter transporter family) which couples the transport of organic substrate with the transport of Na^+ and Cl^- (Chen, Reith, and Quick, 2004); SLC1 family (high-affinity glutamate and neutral amino acid transporter family) which couples the transport of organic substrate with the co-transport of Na^+ and H^+ and the counter-transport of K^+ (Kanai and Hediger, 2004); SLC12 family (electroneutral cation-coupled Cl^- cotransporter family) which co-transport Na^+ and/or K^+ with Cl^- (Hebert, Mount, and Gamba, 2004). Although SLC transporters do not have ATPase activity, the transport of organic substrates mediated by Na^+ -coupled transporters and H^+ -coupled transporters is the energetically active process. Na^+ -coupled transporters mediate secondary active transport whereas H^+ -coupled transporters mediate tertiary active transport (Hediger *et al.*, 2004). Transporters that have ATPase activity and hydrolyze ATP to utilize free energy stored in the high-energy phosphate bonds are called primary active transporters. The transporters that use free energy stored as electrochemical gradient of solutes generated by primary active transporters are called secondary active transporters (Hediger *et al.*, 2004).

Because Na^+ /organic solute cotransporters such as Na^+ /glucose cotransporters use free energy stored as electrochemical gradient of Na^+ that is generated by Na^+/K^+ ATPase (a primary active transporter), and Na^+ /organic solute cotransporters are secondary active transporters. Furthermore, the transporters that use free energy stored as electrochemical gradient of solutes generated by secondary active transporters are called tertiary active transporters (Hediger *et al.*, 2004).

The proton oligopeptide cotransporter PepT1 of SLC15 family is an example of tertiary active transporter because it uses the free energy stored as electrochemical gradient of H^+ that is generated by Na^+/H^+ exchanger (a secondary active transporter) (Fei *et al.*, 1994). Antiporters, also called exchangers, mediate exchange transports of substrates. Because antiporters can utilize free energy stored as electrochemical gradient of counter substrates for the transport of substrates, antiporters can be secondary or tertiary

active transporters. For example, Na^+/H^+ exchangers of SLC9 family are secondary active transporters because they use Na^+ gradient to drive the transport of H^+ (Orlowski and Grinstein, 2004). Organic anion transporter 1 (OAT1, SLC22 family) that mediates the influx of organic anions in exchange for the efflux of dicarboxylates at the basolateral (BL) membrane of renal proximal tubules is a tertiary active transporter because it uses the gradient of dicarboxylates generated by a secondary active Na^+ /dicarboxylate cotransporter NaDC-3 (NaC3) of SLC13 family (Sekine *et al.*, 1997).

Transporters as determinant of compound distribution

As already mentioned, transporters are located on the biomembranes to mediate selective permeability of solutes and equilibrate the solutes of both sides of the membrane. Based on this, it is possible to calculate the concentration of solutes of each side of membrane after being equilibrated by the transporters on the membrane. When the transporter on the plasma membrane is permeable to the organic substrate X that has the electric charge of δ (Fig. 3A), the free energy change associated with the transport of X from the outside to the inside of the cells through the plasma membrane is:

$$\Delta G = RT \ln([X]_i/[X]_o) + \delta VF \quad (1)$$

$[X]_i$: intracellular concentration of X; $[X]_o$: extracellular concentration of X; R: gas constant; T: absolute temperature, V: membrane potential (difference in the electric potential between inside and outside of the membrane); F: Faraday's constant

At equilibrium ($\Delta G = 0$), the equation (1) is:

$$RT \ln([X]_i/[X]_o) + \delta VF = 0$$

Therefore,

$$[X]_i/[X]_o = \exp(-\delta VF/RT) \quad (2)$$

Due to the transporter on the plasma membrane mediating the transport of X, the compound X is distributed at equilibrium in both intracellular and extracellular compartments as described by equation (2). This indicates that transporter is a determinant of the distribution of compounds in the compartments separated by membranes on which the transporters are located. The equation (2) is to describe the distribution of a substrate determined by uniporters. Then, the distribution of substrates determined by symporters will be obtained in the same way. Now, for simplicity, we suppose that the transporter co-transport Na^+ and electrically neutral substrate X such as glucose (Fig.

3B). The free energy change associated with the co-transport of Na^+ and X from the outside to the inside of the cells through the plasma membrane is:

$$\Delta G = RT \ln([X]_i/[X]_o) + [RT \ln([Na^+]_i/[Na^+]_o) + VF] \quad (3)$$

At equilibrium ($\Delta G = 0$), the equation (3) is:

$$[X]_i / [X]_o = [Na^+]_o / [Na^+]_i \exp(-VF/RT) \quad (4)$$

This indicates that the distribution of substrate X is determined by Na^+ concentration gradient (the ratio of extracellular and intracellular concentrations). In general for symporters, when m (coupling number) molecules of substrate X with the electric charge of δ_1 are co-transported with n molecules of substrate Y with the electric charge of δ_2 (Fig. 3C), the free energy change associated with the co-transport of X and Y from the outside to the inside of the cells through the plasma membrane is:

$$\Delta G = m[RT \ln([X]_i/[X]_o) + \delta_1 VF] + n[RT \ln([Y]_i/[Y]_o) + \delta_2 VF] \quad (5)$$

At equilibrium ($\Delta G = 0$), the equation (5) is:

$$\{[X]_o/[X]_i \exp(-\delta_1 VF/RT)\}^m \{[Y]_o/[Y]_i \exp(-\delta_2 VF/RT)\}^n = 1 \quad (6)$$

This furthermore demonstrates that the distributions of co-substrates of symporters are in general determined so that they are dependent on each other. In the case of antiporter which mediates the influx of m molecules of substrate X with the electric charge of δ_1 in exchange for the efflux of n molecules of substrate Y with the electric charge of δ_2 (Fig. 3D), n of the equation (6) will be changed to -n:

$$\{[X]_o/[X]_i \exp(-\delta_1 VF/RT)\}^m \{[Y]_o/[Y]_i \exp(-\delta_2 VF/RT)\}^{-n} = 1 \quad (7)$$

Now we have gone through uniporters, symporters, and antiporters. We altogether conclude that transporter is a determinant of the distribution of compounds between compartments separated by the membrane on which the transporters are located. In the body, the metabolic and synthetic enzymes also contribute to determining the distribution of compounds as mentioned above. Furthermore, transporters are not working at equilibrium in the body because of their slow performance due to the slow flux rate as well as metabolism and stirring/agitation of substrates that makes it difficult to reach the equilibrium. Transporters are, instead, working at dynamic equilibrium in human bodies. Thus, not only the presence or absence of the transporters on the membrane separating compartments

but also the alterations in the number of transporter molecules and in their functional activity would affect the distribution of substrates in the body. If the drugs are able to alter the function of transporters, they can be the drugs that alter the distribution of substrates of the transporter in the body. In fact, the distribution of various endogenous substances changes in many diseases. The corrections of such disrupted distribution of endogenous substances are expected to cure the diseases. Therefore, transporters would be excellent therapeutic targets in such diseases.

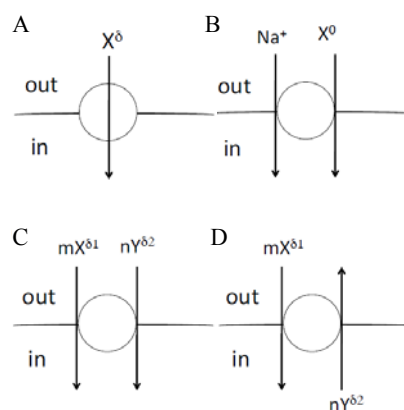


Fig. 3 Classification of SLC transporters according to transport mode

A: uniporter is transporting a substrate X with an electric charge of δ

B: the symporter is transporting Na^+ and electrically neutral substrate X

C: the symporter is transporting m molecules of substrate X with the electric charge of δ_1 and n molecules of substrate Y with the electric charge of δ_2

D: antiporter is taking up m molecules of substrate X with the electric charge of δ_1 in exchange for n molecules of substrate Y with the electric charge of δ_2

Transporter roles in health and disease in relevance to transporter-targeting drugs

To understand the significance of transporters as therapeutic targets, it is essential to discuss the roles of transporters in health and disease. As already mentioned, transporters contribute to determining the distribution of endogenous compounds by working in concert with metabolic/synthetic enzymes under the influences of many factors affecting the flux of chemical compounds in the body, just as the distribution of administered drugs is determined by transporters and metabolic enzymes as well as many other factors affecting drug metabolisms and pharmacokinetics. By contributing to such processes, transporters play critical roles in homeostasis. Physiological roles of transporters are summarized in

the following two aspects.

First, transporters are critical to maintain the survival of cells. Cells have to take up nutrients and excrete unnecessary metabolites or metabolic wastes. Because nutrients and metabolites are in general hydrophilic compounds, cells require the mechanisms to efficiently and selectively permeate such hydrophilic compounds through the plasma membrane. Obviously, transporters play such essential roles. For nutrient uptakes, the transporters for sugars, amino acids, fatty acids, dicarboxylates, monocarboxylates, vitamins, and minerals are present on the plasma membrane. For the excretion of metabolites, organic anion transporters play major roles (Hediger *et al.*, 2004). Additionally, cells are equipped with machinery to prevent themselves from the invasion of xenobiotics by extruding them from the plasma membrane (Hediger *et al.*, 2004; Seeger and van Veen, 2009). Some transporters on the plasma membrane, particularly ABC transporters, function as gate-keepers by mediating the efflux of xenobiotics (Seeger and van Veen, 2009). In summary, transporters contribute to the survival of individual cells in the body, which is the first aspect of physiological roles of transporters.

Second, transporters take the part of tissue functions. It is supposed that transporters were originally generated for the survival of cells as mentioned above in the single cell stage of the evolution. When multicellular organisms developed, transporters were incorporated into tissues and started to take the part of tissue functions. Those transporters are not functioning any more for the survival of the cells in which the transporters are present, but they contribute to the functions of tissues. For example, in the epithelia of intestine and renal tubules, distinct transporters are localized on the apical (AP) and BL membrane for the directional transport through the epithelia (Hediger and Rhoads, 1994; Robertson and Rankin, 2006; Bröer, 2008): the uptake of nutrients from lumen to blood and the excretion of unnecessary metabolites and xenobiotics from blood to lumen. In the neuronal tissues, highly concentrative Na^+ -coupled transporters are localized at the presynaptic terminals and on the glial cell membranes and function to remove released neurotransmitters from synaptic clefts by taking up neurotransmitters into the presynaptic

terminals and glial cells (Kanai and Hediger, 2003). Thus, transporters take the part of tissue functions, which is the second aspect of physiological roles of transporters.

Because of above-mentioned two physiological roles of transporters, the relevance of transporters to diseases also has to be considered from two aspects related to two roles of transporters. Classically characterized transporter diseases involving the alteration of transporter functions are in general genetic defects of transporters. This issue is related to the second aspect on the physiological roles of transporters mentioned above. There are many genetic diseases in which transporter genes are defective. Cystinuria, lysinuric protein intolerance, Hartnup disease, iminoglycinemia, and acidic amino aciduria are the defects of amino acid transporters in renal proximal tubules (Bröer, 2008). Idiopathic renal hypouricemia, renal glycosuria, and glucose-galactose malabsorption are the defects of urate transporters or Na^+ /glucose cotransporters in the renal or intestinal epithelia (Enomoto *et al.*, 2002; Matsuo *et al.*, 2008; Heuvel *et al.*, 2002; Turk *et al.*, 1991). Defects of transporters responsible for Na^+ re-absorption from renal tubules cause Bartter or Gitelman syndrome (Hebert, Mount, and Gamba, 2004; Lang *et al.*, 2005). Wilson and Menkes diseases are the defects of copper transporters related to intracellular storage and excretion of copper (La Fontaine, Ackland, and Mercer, 2010). Recently, genetic loss of function of an ABC transporter ABCG2 in the AP membrane of renal proximal tubules has been demonstrated to cause hyperuricemia leading to gout (Matsuo *et al.*, 2009). In monoamine transporters, correlation between genetic polymorphism and some psychiatric disorders has been reported (Serretti *et al.*, 2006). These experiments of nature have confirmed that transporters contribute significantly to the determination of distribution of endogenous compounds and that the alteration of transporter functions is in fact able to change the distribution of compounds. This gives us a strong support that transporters can be the therapeutic targets of the diseases in which altering the distribution of endogenous compounds is supposed to be beneficial for the treatment of the diseases.

In pathologic conditions, even though the transporters are normal, the normally functioning transporters

sometimes exacerbate the conditions. In the disease conditions in which homeostasis systems are disrupted, normal function of transporters is not appropriate any more and sometimes contributes to facilitating the progress of the diseases. This is sometimes the case with the nutrient transporters, because nutrients transporters are in general not regulated well. Because animals have endured the starvation in the course of evolution, they have developed the machinery to maintain nutrient into the body. In contrast, the protection against too much nutrient is very poor in animals. Therefore, glucose transporters in small intestine and renal proximal tubules keep absorbing glucose even though blood glucose level is high, which contributes to maintaining high blood glucose level in diabetes patients (Bailey, 2011). In the similar way, urate transporters in renal proximal tubules keep reabsorbing urate even though the blood urate level is high in the patients with hyperurecemia. In these cases, the inhibitors of the transporters are expected to reduce the absorption of substrates and to decrease their blood levels leading to subsiding of the diseases.

The pathological relevance of transporters related to the first aspect of transporter roles described above to maintain cells to survive is that some transporters provide nutrient to the cells responsible for the progress of diseases and to maintain such cells to survive and to be active. The nutrient transporters in cancer cells or inflammatory cells fall into this category. These transporters can be molecular markers for the diagnosis of the diseases and also to be therapeutic targets. The drugs specifically inhibiting such transporters are expected to suppress the biological activity of such pathogenic cells and to cure the diseases. If such transporters are specifically expressed in pathogenic cells and not present in normal cells, the significance of such transporters as diagnostic and therapeutic targets would be really high.

Drug development targeting transporters

As discussed in the last section, transporters could be excellent drug targets. In this section, first, some examples of clinically used drugs targeting transporters will be described. These drugs are those first used clinically and then their targets of the pharmacological effects have later been revealed to be transporters. Now,

some drugs targeting transporters have been in the process of development or pre-clinical or clinical researches by post-genome drug development strategy. Two such examples will be introduced and discussed: renal Na^+ /glucose cotransporter SGLT2 as a therapeutic target of diabetes mellitus (Bailey, 2011) and cancer *L*-type amino acid transporter LAT1 as a diagnostic and therapeutic target of cancers (Kaira *et al.*, 2007; 2009; Oda *et al.*, 2010). In the post-genome drug development strategy, the identification of transporter genes is the first step of the research. Then, physiological and pathological significance needs to be extensively investigated. After that, chemical compounds will be designed based on structure-activity relationship or obtained by screening the chemical library. In this process, *in vitro* assay systems involving the transporter genes or cDNAs will be used. The chemical compounds will be evaluated whether they affect the functions of recombinant transporters. In this section, such processes will be described for SGLT2 and LAT1.

Clinically used drugs targeting transporters

Antidepressants It has been clinically demonstrated that antidepressants such as tricyclic and tetracyclic antidepressants are effective in the treatment of depression (Andersen *et al.*, 2009). Because it was shown that these drugs inhibited monoamine transporters including dopamine, noradrenalin, and serotonin transporters, the inhibition of reuptake of monoamines at the monoaminergic synapses is proposed to be the rationale of the antidepressants (Andersen *et al.*, 2009). Other currently more popular antidepressants such as SSRI and SNRI are also the inhibitors of monoamine transporters (serotonin transporter for SSRI; serotonin and noradrenalin transporter for SNRI).

Diuretics Loop diuretics, such as furosemide, bumetanide, and ethacrynic acid, and thiazide diuretics such as hydrochlorothiazide are clinically used diuretics (Giménez, 2006; Ko and Hoover, 2009). Particularly, loop diuretics exhibit very strong diuretic actions. It was demonstrated that loop and thiazide diuretics were the inhibitors of $\text{Na}^+/\text{K}^+/\text{2Cl}^-$ cotransporter and Na^+/Cl^- cotransporter of SLC12 electroneutral cation- coupled Cl^- cotransporter family, respectively (Giménez, 2006; Ko and Hoover, 2009; Hebert, Mount, and Gamba, 2004). By inhibiting these transporters responsible for

Na⁺ reabsorption, such diuretics suppress the reabsorption of Na⁺ from the luminal fluid of renal tubule, resulting the increase of water excretion with Na⁺ into urine.

Uricosuric agents The uricosuric agents, such as probenecid, benzbromarone, and losartan, are the inhibitors of urate transporter UAT1, a member of SLC22 organic cation/anion/zwitterion transporter family (Wright *et al.*, 2010; Enomoto *et al.*, 2002). UAT1 is located on the AP membrane of renal proximal tubules and responsible for the reabsorption of urate from the proximal tubules (Enomoto *et al.*, 2002). Because urate reabsorption by UAT1 is a rate-limiting process to determine blood urate level as shown previously, the inhibition of UAT1 using those drugs very effectively reduces blood urate level, so that they are clinically used for the treatment of hyperuricemia.

Ezetimibe Ezetimibe is a drug that lowers blood cholesterol (Altmann *et al.*, 2004). It acts by decreasing cholesterol absorption in the intestine. The target of ezetimibe was searched and turned out to be a novel intestinal transporter Niemann-Pick C1-like 1 (NPC1L1) responsible for intestinal cholesterol absorption (Altmann *et al.*, 2004). By inhibiting cholesterol absorption through NPC1L1, ezetimibe lowers blood cholesterol level.

Inhibitors of ion pumps Digitalis has been described in medical literature for more than 200 years and used for the treatment of cardiac insufficiency. It has been proved that digitalis is an inhibitor of Na⁺/K⁺ pump (Na⁺/K⁺ ATPase) (Lingrel, 2010). Inhibitors of gastric H⁺ pump (H⁺/K⁺ ATPase) such as Omeprazole are also used clinically. They are able to efficiently suppress H⁺ secretion from gastric parietal cells into gastric juice and used for the treatment of gastroduodenal ulcer (Robinson, 2005).

SGLT2 as a therapeutic target of diabetes mellitus

Molecular identification of SGLT2 In the renal proximal tubules, glucose filtered into urine at glomerulus is reabsorbed by glucose transporters located on the AP and BL membrane of tubular epithelial cells (Hediger and Rhoads, 1994). The AP membrane transporters are Na⁺-coupled ones to utilize free energy stored as Na⁺ electrochemical gradient to take up glucose from luminal fluid to the epithelial cells,

whereas BL membrane transporters are glucose uniporters that mediate facilitated diffusion of glucose along the concentration gradient through the BL membrane into blood. There are two types of Na⁺-coupled glucose transporters known to be present on the AP membrane of renal proximal tubules. The one located in the more proximal part of proximal tubules (S1-S2 segment of proximal tubules) is with low affinity and high capacity, whereas the other one located in the distal part of proximal tubules (S2-S3 segment of proximal tubules) is with high affinity and low capacity (Hediger and Rhoads, 1994). The former is coupled with one Na⁺, whereas the latter is coupled with two. The properties of the latter are corresponded to those of SGLT1 (Na⁺/glucose cotransporter 1) identified in 1987 in the small intestine (Hediger and Rhoads, 1994; Hediger *et al.*, 1987). The former major renal proximal tubule Na⁺/glucose cotransporter with high glucose transporting capacity was identified in 1994 and named SGLT2 (Kaira *et al.*, 1994). SGLT2 as well as SGLT1 belongs to SLC5 sodium glucose cotransporter family and exhibits expected properties of low affinity/high capacity Na⁺/glucose cotransporter of the proximal tubules (Hediger and Rhoads, 1994; Wright and Turk, 2004). Upon the discovery of SGLT2, drugs specifically targeting SGLT2 have been searched for the treatment of diabetes (Bailey, 2011).

Inhibitors of SGLT2 as antidiabetic agents In both type 1 and type 2 diabetes, elevated blood glucose generates “glucose toxicity” causing many complications associated with diabetes and exacerbating the disease condition (Bailey, 2011). Decreasing blood glucose releases the β -cells of pancreatic islet from the glucose toxicity so that they recover insulin secreting ability, and at the same time, decreasing blood glucose prevents many disorders (glucose toxicity in peripheral tissues) associated with diabetes (Bailey, 2011). When phlorizin, a competitive inhibitor of Na⁺/glucose cotransporter, was administered in diabetic model animals, the inhibition of renal Na⁺/glucose cotransporter turned out to be effective in reducing blood glucose level by suppressing glucose reabsorption from renal proximal tubules and in recovering from insulin resistance and restoring insulin secreting ability (Rossetti *et al.*, 1987). Because of low bioavailability and short half-life, phlorizin itself was not considered

for clinical application (Bailey, 2011). Instead, new compounds that have better pharmacokinetic properties and inhibit SGLT2 selectively without affecting SGLT1 have been the targets of drug developments. Such drugs are expected to inhibit renal glucose reabsorption with no affecting intestinal glucose absorption and improve diabetic condition. First compound reported with such properties was T-1095 (Tsujihara *et al.*, 1999; Oku *et al.*, 1999). T-1095 is an ester-type pro-drug that is metabolized by esterase in liver to become an active form. Therefore, T-1095 is not active in the intestine when ig administered, so that it avoids intestinal Na^+ /glucose cotransporter SGLT1 and selectively inhibits renal Na^+ /glucose cotransporters (Tsujihara *et al.*, 1999; Oku *et al.*, 1999). When administered to diabetic animals, T-1095 dramatically reduced blood glucose concentration and restored insulin secreting ability, which established the concept of SGLT2 inhibitors as antidiabetic drugs (Oku *et al.*, 1999; 2000; Arakawa *et al.*, 2001). Now BI10773, Canagliflozin (TA7284), and Dapagliflozin with more metabolically stable C-glycoside structure are under clinical trials (Bailey, 2011). ASP1941, TS-071, and Tofogliflozin (CSG452) are also in the stage of clinical trial (Bailey, 2011). Recently, a randomized, double-blind, placebo-controlled phase 3 trial on Dapagliflozin has been published (Bailey *et al.*, 2010). Dapagliflozin is a metabolically stable C-glycoside. It is well absorbed from intestine with high bioavailability and exhibits long half-life due to low clearance (Bailey, 2011). Dapagliflozin is highly selective to SGLT2 (> 1000 fold selective to SGLT2 compared to SGLT1). The effectiveness of Dapagliflozin in the control of type 2 diabetes has been proved in the clinical trials (Bailey, 2011). In the untreated type 2 diabetic patients, Dapagliflozin increased urinary glucose excretion and reduced blood HbA1c levels similar to Metformin (List *et al.*, 2009). In the patients from whom blood sugar control was not successful by Metformin, co-administration of Dapagliflozin with Metformin significantly reduced blood HbA1c level. Distinct from many other antidiabetic drugs, Dapagliflozin works independently on insulin (Bailey *et al.*, 2010). As drugs with new mechanisms of action, SGLT2 inhibitors are expected to contribute to the improvement of diabetic treatment.

LAT1 as a diagnostic and therapeutic target of cancers

Amino acid transporters and their molecular identification Plasma membrane amino acid transporters are essential to supply cells with amino acids for cellular nutrition. In the epithelia of kidney and small intestine, distinct transporters are developed in the AP and BL membranes of epithelial cells to ensure the directional transport of amino acids through the epithelia (Bröer, 2008). In brain, transporters for amino acids and related neurotransmitters function to terminate synaptic transmission and to protect neurons from the toxicity of excitatory amino acids (Kanai, 1997; Billups *et al.*, 1998). A large number of amino acid transport systems in mammals distinguished based on substrate selectivity and ion-dependence, and the transporters corresponding to each transport system have been identified so far (Table 1) (Bröer, 2008). They include three Na^+ -dependent families, SLC1, SLC6, and SLC38, and three Na^+ -independent families, SLC7, SLC16, and SLC43.

The first molecular identification of amino acid transport systems was a serendipitous finding of Na^+ -independent basic amino acid transporter cationic amino acid transporter 1 (CAT1) subserving system y^+ (MacLeod *et al.*, 1994). Following CAT1, a taurine transporter with the properties of β -system, a glycine transporter with the properties similar to those of system G, and a brain specific proline transporter which could not be assigned to classically characterize amino acid transport systems were identified as members of Na^+/Cl^- -dependent neurotransmitter transporter family (SLC6) (Chen, Reith, and Quick, 2004). Later on, a transporter with the properties of Na^+ -dependent neutral and basic amino acid transport system $\text{B}^{0,+}$ was also isolated as a member of SLC6 family (Chen, Reith, and Quick, 2004). The transporter for system B^0 whose genetic defect is responsible for Hartnup disease has been identified as a member of SLC6 (Bröer, 2008). In 1992, three glutamate transporters with the properties of Na^+ -dependent acidic amino acid transport system $\text{X}^-_{\text{A,G}}$ were cloned so that a new family of amino acid transporters was established (Kanai and Hediger, 1992; 2004; Kanai, 1997). This family was further expanded to include the transporters that exhibited functional properties of Na^+ -dependent small neutral amino acid

Table 1 Amino acid transport systems

Transport system	Substrate	Transporter	Family
Neutral amino acids			
A	Ala, Pro, N-methyl amino acids	ATA1, ATA2, ATA3	^c SLC38
G	Gly, Sar	GLYT1, GLYT2	SLC6
B ⁰	broad substrate selectivity	B ⁰ AT1	SLC6
ASC	Ala, Ser, Thr, Cys,(Gln)	ASCT1, ASCT2	SLC1
Na ⁺ -dependent			
N	Gln, Asn, His	SN1, SN2	SLC38
β	β-Ala, Tau	Taut	SLC6
y ⁺ L	^a neutral and basic amino acids	^b y ⁺ LAT1/4F2hc, y ⁺ LAT2/4F2hc	SLC7
Na ⁺ -independent			
L	large neutral amino acids	^b LAT1/4F2hc, LAT2/4F2hc	SLC7
		LAT3, LAT4	SLC43
asc	Ala, Ser, Thr, Cys	^b Asc-1/4F2hc, Asc-2/?	SLC7
T	aromatic amino acids	TAT1	SLC16
b ^{0,+}	neutral and basic amino acids	^b b ^{0,+} AT/BAT1/rBAT	SLC7
Basic amino acids			
Na ⁺ -dependent			
B ^{0,+}	neutral and basic amino acids	ATB ^{0,+}	SLC6
Na ⁺ -independent			
y ⁺	basic amino acids	CAT1, CAT2, CAT2a, CAT3, CAT4	SLC7
b ^{0,+}	neutral and basic amino acids	^b b ^{0,+} AT/BAT1/rBAT	SLC7
y ⁺ L	^a neutral and basic amino acids	^b y ⁺ LAT1/4F2hc, y ⁺ LAT2/4F2hc	SLC7
Acidic amino acids			
Na ⁺ -dependent			
X _{AG} ⁻	L-Glu, L-/D-Asp	EAAC1, GLT-1, GLAST, EAAT4, EAAT5	SLC1
Na ⁺ -independent			
x _C ⁻	cystine/Glu exchange	^b xCT/4F2hc	SLC7

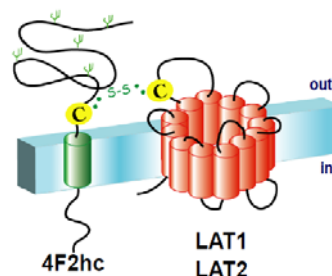
a: System y⁺L is partially dependent on Na⁺ for neutral amino acids and Na⁺-independent for basic amino acids

b: Heterodimeric transporters are composed of two subunits, ex. y⁺LAT1/4F2hc is a heterodimer of y⁺LAT1 (SLC7 family) and a type II membrane glycoprotein 4F2hc

c: SLC family is the naming of transporter families by Human Gene Nomenclature Committee

transport system ASC (SCL1 family) (Kanai and Hediger, 2004; Kanai, 1997). Four amino acid transporter families have subsequently been identified. In 1998, a heterodimeric amino acid transporter subserving Na⁺-independent neutral amino acid transport system L was cloned (Fig. 4) (Kanai *et al*, 1998; Mastroberardino *et al*, 1998). Following this, molecular nature of several amino acid transport systems was revealed as that of heterodimeric amino acid transporters in SLC7 family. Transporters for Na⁺-dependent neutral amino acid transport systems N and A were found as proteins structurally related to plant amino acid/auxin transporters and mammalian vesicular GABA transporters (SLC38 family) (Mackenzie and Erickson, 2004). In 2001, a Na⁺-independent transporter subserving system T that

transports aromatic amino acids was identified by functional expression cloning (Kim *et al*, 2001).

**Fig. 4** Heterodimeric amino acid transporters

Twelve membrane-spanning transporters of SLC7 family (LAT1 or LAT2 in the figure) link with single membrane-spanning protein of SLC3 family (4F2hc in the figure). LAT1 is a cancer cell type system L amino acid transporter, whereas LAT2 is a normal cell type system L amino acid transporter

Interestingly, the system T transporter exhibited the structural similarity to H^+ /monocarboxylate transporters (SLC16). In 2003, the identification of a novel system L amino acid transporter structurally distinct from heterodimeric amino acid transporters of SLC7 family established a new transporter family (SLC43) (Babu *et al.*, 2003).

Among amino acid transporters, heterodimeric amino acid transporters are unique because they form heterodimeric complexes with single membrane spanning protein named 4F2hc (4F2 heavy chain; CD98hc; SLC3A2) or rBAT (SLC3A1) via a disulfide bond (Fig. 4) (Verrey *et al.*, 2004; Palacin and Kanai, 2004). Six members, LAT1, LAT2, Asc-1, y^+ LAT1, y^+ LAT2, and xCT link with 4F2hc, whereas one member, $b^{0,+}$ AT is associated with rBAT (Verrey *et al.*, 2004; Palacin and Kanai, 2004). LAT1 and LAT2 are neutral amino acid transporters with the properties of 2-amino-bicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH)-sensitive system L exhibiting broad selectivity to neutral amino acids (Kanai *et al.*, 1998; Verrey *et al.*, 2004). Asc-1 is also a neutral amino acid transporter but it exhibits the properties of system Asc preferring small neutral amino acids. y^+ LAT1 and y^+ LAT2 are system y^+ L transporters that transport both neural and basic amino acids (Verrey *et al.*, 2004). xCT is an acidic amino acid transporter with the properties of system x^-_c mediating an exchange transport of glutamate and cystine. $b^{0,+}$ AT exhibits the properties of system $b^{0,+}$ and transports both neural and basic amino acids (Verrey *et al.*, 2004). Thus, heterodimeric amino acid transporters contain transporters of varieties of substrate selectivity. The finding of this family contributed greatly to the understanding of molecular basis of amino acid transport.

Amino acid transporters in malignant tumors

For continuous growth and proliferation of tumor, rapidly dividing tumor cells require more supply of sugars and amino acids. They are supported by the upregulation of transporters specialized for these nutrients (Christensen, 1990). Among the nutrient transporters, the transporters for essential amino acids are particularly important because they are indispensable for protein synthesis.

In the search for the genes unregulated in rat hepatoma cells, Thompson and co-workers identified a

tumor associated sequence designated TA1 exhibiting oncofetal pattern of expression (Sang *et al.*, 1995). TA1 expression was closely associated with the progress in rat hepatoma model, suggesting TA1 play a role in the malignant phenotype. Now it has turned out that TA1 is a partial sequence of one in the 4F2 light chains LAT1 (Kanai *et al.*, 1998). LAT1 was originally cloned from rat C6 glioma cells by functional expression cloning methodology (Kanai *et al.*, 1998). Because LAT1 is a system L-amino acid transporter which transports large neutral amino acids including several essential amino acids, LAT1 is proposed to be at least one of the amino acid transporters essential for tumor cell growth (Yanagida *et al.*, 2001). LAT1 is widely expressed in primary human cancers of various tissue origins, such as brain, colon, lung, liver, thymus, prostate, ovary, and skin as well as cancer cell lines, where it plays essential roles in growth and survival of cancer cells (Kanai *et al.*, 1998; Yanagida *et al.*, 2001; Fuchs and Bode, 2005; Nawashiro *et al.*, 2006; Kaira *et al.*, 2008a; 2008b; 2009a; Sakata *et al.*, 2009). LAT1 is upregulated in malignant tumors and its expression is associated with tumor proliferation, angiogenesis, and poor prognosis (Kaira *et al.*, 2007; 2008a; 2008b; 2009a; 2009b; 2009c; 2009d; Sakata *et al.*, 2009; Kobayashi *et al.*, 2008; Imai *et al.*, 2009; Ganapathy, Thangaraju, and Prasad, 2009). In contrast, the expression of LAT2, other isoform of system L and one of the heterodimeric transporters (Fig. 4), is not detected in cancer cells but it is widely expressed in normal tissues including epithelia of small intestine and kidney so that it is regarded as a non-cancer cell type transporter (Verrey *et al.*, 2004; Kaira *et al.*, 2010). It was found that expression of LAT1 is an independent and significant factor for predicting a poor prognosis in patients with non-small cell lung cancer (Kaira *et al.*, 2008b; 2009a; 2009e; 2010). Therefore, it is proposed that LAT1 can be a molecular target of cancer diagnosis and therapeutics.

Cancer diagnosis targeting LAT1 As indicated above, LAT1 is upregulated in cancers. Although its mRNA was reported to be widely expressed in animal body (Prasad *et al.*, 1999), its protein was only reported in blood-brain barrier (BBB) and placenta other than cancer cells (Matsuo *et al.*, 2000; Ritchie and Taylor, 2001). In BBB, the level of the expression of LAT1 protein seems to be low and LAT2 is also present

together with LAT1 in the BBB (Matsuo *et al*, 2000). Although LAT1 is highly expressed in cancers, the significance of LAT1 in the diagnosis of cancer needs to be proved *in vivo*. To prove the significance of LAT1 in the cancer diagnosis, positron emission tomography (PET) was applied to the patients with non-small cell lung cancer (Fig. 5) (Kaira *et al*, 2007; 2009b; 2009e). Fortunately, LAT1-specific PET probe was available. L-3- ^{18}F - α -methyl-tyrosine (^{18}F -FMT), an LAT1-specific amino acid, was used as a PET probe (Fig. 5D). It was shown that α -methyl aromatic amino acids are specifically transported by LAT1 but not by LAT2. FMT is specific to LAT1 due to its α -methyl moiety (Uchino *et al*, 2002; Kim *et al*, 2002; Morimoto *et al*, 2008). ^{18}F -FMT PET clearly imaged the cancers in the lung (Fig. 5A). The level of ^{18}F -FMT accumulation was correlated with the level of expression of LAT1 protein determined by immunohistochemistry of surgically removed tumors (Fig. 5C), indicating that the level of ^{18}F -FMT accumulation well predicts the level of expression of LAT1 in tumors (Kaira *et al*, 2007). In FMT-PET, the background accumulations of ^{18}F -FMT in normal tissues were low enough to detect cancer mass clearly (Kaira *et al*, 2007; Inoue *et al*, 1998; 1999). Brain background was also low, suggesting that the

expression of LAT1 in BBB be low and almost negligible compared with cancers (Kaira *et al*, 2007; Inoue *et al*, 1998; 1999).

At the moment, PET diagnosis of cancers is in general conducted using a PET probe 2- ^{18}F -fluoro-2-deoxy-*D*-glucose (^{18}F -FDG), a substrate of glucose transporter 1 (GLUT1) upregulated in cancers (Plathow and Webe, 2008). Compared with FDG-PET, FMT-PET has the following advantages. First, the accumulation of ^{18}F -FMT is specific to cancers. It is known that ^{18}F -FDG accumulates not only in cancers but also in the inflammatory lesions and granulomatous tissues (Strauss, 1996). In contrast, ^{18}F -FMT does not accumulate in inflammatory and granulomatous lesions (Kaira *et al*, 2007; 2009b). In sarcoidosis of thoracic region, ^{18}F -FMT does not accumulate in sarcoidosis lesions whereas ^{18}F -FDG shows strong accumulation in the sarcoidosis (Kaira *et al*, 2007; 2009b). Another advantage of FMT-PET is that FMT-PET can be used for the diagnosis of brain tumors due to its low background in brain (Inoue *et al*, 1999). It is well known that ^{18}F -FDG is highly accumulated in brain and cannot be used for brain tumor diagnosis (Kaira *et al*, 2007; 2009b; Strauss, 1996). These advantages of FMT-PET indicate that FMT is specific to cancers. Because FMT is an LAT1-specific substrate, it is finally confirmed that LAT1 is specific to cancers so that it is appropriate for a molecular target of the diagnosis of cancers.

LAT1 as a molecular target used in cancer therapeutics By means of FMT-PET and many histochemistry studies on cancer tissues, LAT1 has been proved to be a cancer-specific amino acid transporter. Because LAT1 is proposed to be upregulated in cancer cells to provide the cells with amino acids as nutrients and to support their continuous growth and proliferation, the inhibition of LAT1 is expected to suppress cancers. The classical system L inhibitor BCH and an antisense oligonucleotide designed against LAT1 were shown to inhibit proliferation of tumor cells (Imai *et al*, 2010; Yamauchi *et al*, 2009). In agreement with this, the inhibition of LAT1 by BCH and the antisense oligonucleotide prolongs the survival of tumor-bearing mice (Nawashiro *et al*, 2006; Kobayashi *et al*, 2008). Therefore, it is proposed that the inhibition of LAT1 could be a new rationale to antitumor therapy.

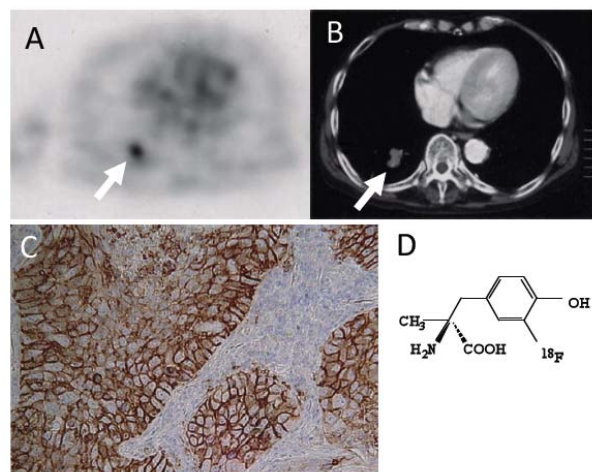


Fig. 5 ^{18}F -FMT PET of lung cancer

A patient with large cell lung cancer was subjected to ^{18}F -FMT PET (Kaira *et al*, 2007)

A: PET imaging of lung cancer (arrow)

B: CT image corresponding to PET image of A. The arrow indicates the lung cancer

C: Immunostaining of the surgically removed tumor (A and B) by anti-LAT1 antibody. LAT1 is highly expressed and localized on the plasma membrane of cancer cells

D: Chemical structure of ^{18}F -FMT. Courtesy of Dr. Kyoichi Kaira, Gunma University Graduate School of Medicine

In order to design the LAT1-specific high-affinity inhibitors, the properties of substrate recognition by LAT1 were examined (Uchino *et al*, 2002). It was found that LAT1 relied on the ionic interaction with α -carboxyl group and α -amino group and also on the hydrophobic interaction with a hydrophobic side chain of the substrates (Uchino *et al*, 2002). Based on this observation, chemical compounds were designed to obtain high affinity and LAT1-specific inhibitors. Among those compounds, a compound KYT0353 was selected, which had an affinity about 1000 fold higher than that of BCH and was highly selective to LAT1 without acting on LAT2 (Oda *et al*, 2010). In *in vitro* and *in vivo* experiments, it was proved that KYT0353 effectively suppressed tumor growth with lower doses compared with BCH, which established the concept of LAT1 inhibitors as antitumor agents (Oda *et al*, 2010). An advantage of such LAT1-specific inhibitors would be with fewer side effects due to tumor-specific expression of LAT1. Such LAT1-specific drugs can be designed by comparing the effects on LAT1 and LAT2 using *in vitro* assay systems in which LAT1 or LAT2 is specifically expressed.

Recently, LAT1 has also been of interest from the perspective of boron neutron capture therapy. Boronophenylalanine, used for the boron neutron capture therapy, is taken up by LAT1 and accumulated in cancer cells (Detta and Cruickshank, 2009). Such a borono-compound interacts with a neutron beam and produces alpha particles in the cancer cells to destroy cancer cells. Based on the analyses of interaction of chemicals with transporters using *in vitro* assays, it is possible to design and improve the borono-compounds more appropriate for cancer specific boron neutron capture therapy.

Drugs enhancing transporter functions

Most of clinically used drugs targeting transporters are the inhibitors of transporters. In some cases, enhancement of transporter functions would be appropriate to treat pathological conditions. Such examples are glutamate transporters in brain ischemia. Upon brain ischemia, local glutamate concentration increases, which damages neurons through excitotoxic action of glutamate. Glutamate transporters remove extracellular glutamate by transporting it into cells and protect neurons from excitotoxicity due to the elevation

of extracellular glutamate. If drugs enhancing the functions of glutamate transporters are available, it would be beneficial to treat brain ischemia. An ergot alkaloid bromocriptine was shown to increase the activity of a glutamate transporter subtype GLAST (Yamashita *et al*, 1995). Similarly, nicergoline, a derivative of ergot alkaloid, clinically used as an ameliorator of cerebral circulation and metabolism, was demonstrated to enhance glutamate uptake via glutamate transporters of synaptosomes as well as a subtype of glutamate transporter EAAC1 (Nishida *et al*, 2004). This enhancement of glutamate transporter functions is proposed to contribute to the clinical effect of the drug. In general, designing drugs enhancing transporter functions is not an easy task. There may be some hope searching them in herbal medicine.

Conclusion

Transporters are responsible for the selective permeability of organic and inorganic solutes through the bio-membrane and contribute to determining the distribution of compounds in the body in concert with metabolic/synthetic enzymes. Therefore, the drugs affecting transporters are expected to alter the distribution of compounds in the body and to restore homeostasis in the disease conditions. In this context, drugs targeting transporters have been used clinically. Now new transporter-targeting drugs designed based on post-genome drug development strategy have been in the process of clinical trials or basic/clinical researches. The transporter-targeting drugs are expected to provide new rationale in the therapeutics of various diseases.

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Editorial Words

Investigation on Transporters: One of Emphases for Drug Research and Development

Investigation on transporters has developed rapidly since the 1990's, and many studies on transporters have been reported in literature. Transporters, which are classified as integral membrane proteins, play essential roles in the permeation of organic and inorganic solutes through the plasma membrane. The distribution of their substrates and the ratio of intra- and extra-cellular concentration can be determined by different kinds of transporters. Meanwhile, substrate distribution can also be regulated by inhibiting or enhancing transporters' activities.

Physiological roles of transporters are critical to maintain the survival and are involved in tissue functions. Nutrients uptake and unnecessary metabolites excretion are in general hydrophilic compounds that are able to selectively permeate through cell membrane. Transporters, particularly ABC transporters, can function as "gate-keeper" to protect cells and tissues against unexpected chemicals. In multicellular organisms, transporters are incorporated into tissue and contribute to the functions of tissues.

At present many medicines are in clinical use, such as antidepressants, diuretics, uricosuric agents, and ezetimibe targeting transporters. Among those chemicals, monoamine transporters, $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransporter, Na^+/Cl^- cotransporter, and urate transporter are inhibited by antidepressants, such as diuretics and uricosuric, respectively. In addition to those, ezetimibe acts by decreasing cholesterol absorption.

As a new drug target for diabetes therapy, sodium-glucose cotransporter 2 (SGLT2) was located on the apical membrane of tubular epithelial cells. It is believed that SGLT2 might be involved in glucose re-absorption. Compounds that can selectively inhibit SGLT2 without affecting SGLT1 have been considered as a potential class of chemicals for new drug development. Such compounds can inhibit renal glucose re-absorption without affecting intestinal glucose absorption and improve diabetic condition.

Prof. Kanai is well known in the field of pharmacokinetics, in particular of drug transporter. He has carried out research on transporters since 1991. Under his guidance, the research group has completed many research projects and new technology development projects supported by Japanese government, and published hundreds of academic papers, in addition, several patents granted as well. The representative papers were published in *Nature*, *Nat Neurosci*, *Nat Genet*, *Sci Transl Med*, *J Biol Chem*, *J Am Soc Nephrol*, *Am J Hum Genet*, etc. In this review paper, Prof. Kanai illustrated the therapeutic significance of transporters as molecular targets of drugs. In this study, Prof.

Kanai identified a new transporter, *L*-type amino acid transporter 1 (LAT1). It is a membrane amino acid transporter, essential to supply amino acid for cellular nutrition, and over-expressed on most tumor cell membranes of various types of tissues, such as brain, colon, lung, liver, thymus, prostate, ovary, and skin. While the subtype *L*-type amino acid transporter 2 (LAT2) only can be found on normal tissues, including epithelia of small intestine and kidneys. Both LAT1 and LAT2 are Na⁺-independent amino acid transporters. It is proposed that LAT1 will be a novel molecular target for cancer diagnosis and treatment by selective inhibition of LAT1. Chemical compounds were designed and synthesized; Screening *in vitro* was then carried out to identify the compounds with high affinity. It was found that LAT1 mediated ¹⁴C-leucine uptake and cell growth in human colon cancer-derived HT-29 cells could be significantly inhibited by compound KYT-0353, and the affinity was almost 1000 fold higher than that of BCH and without acting on LAT2. The similar results were also observed by *in vivo* study with HT-29 tumors transplanted nude mice.

In contrast to the inhibition of transporter, enhancing glutamate transporter functions could remove extracellular glutamate into cells to protect neurons. The results suggested that transporter targeting drug may provide new approaches for clinical treatment.

Understanding the role of drug transporters is of importance in the research and development of new drugs to explain the efficacy, safety, and the mechanisms, based on efficacy, bio-availability, safety, tolerability, and convenience. The guidance of drug transporters issued by U. S. Food and Drug Administration, which highly recommended drug transporter should be included in preclinical and clinical studies of new chemical entities, has been extensively reported. The concept of U. S. Food and Drug Administration for drug transporters has issued preclinical and clinical studies involving transporters which have often been extensively reported; Therefore, a great emphasis has been placed on transporter research. As the author's conclusion in his paper, the drugs affecting transporters are expected to alter the distribution of compounds in the body and to restore homeostasis in the pathological conditions. In this context, drugs targeting transporters have been used clinically. Now new transporter-targeting drugs designed based on post-genome drug development strategy have been in the process of basic/clinical research or clinical trials. The transporter-targeting drugs are expected to provide new rationale in the therapeutics of many diseases. We greatly appreciate that Prof. Kanai and his co-authors' comment on this review published in *Chinese Herbal Medicines*.

(by YI Xiu-lin and LI Ya-zhuo)