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Loops and layers of post-translational modifications of drug transporters☆

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ABSTRACT

Drug transporters encoded by solute carrier (SLC) family are distributed in multiple organs including kidney, liver, placenta, brain, and intestine, where they mediate the absorption, distribution, and excretion of a diverse array of environmental toxins and clinically important drugs. Alterations in the expression and function of these transporters play important roles in intra- and inter-individual variability of the therapeutic efficacy and the toxicity of many drugs. Consequently, the activity of these transporters must be highly regulated to carry out their normal functions. While it is clear that the regulation of these transporters tightly depends on genetic mechanisms, many studies have demonstrated that these transporters are the target of various post-translational modifications. This review article summarizes the recent advances in identifying the posttranslational modifications underlying the regulation of the drug transporters of SLC family. Such mechanisms are pivotal not only in physiological conditions, but also in diseases.

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1. Introduction

The major physiological functions of membrane transporters are to facilitate the transfer of nutrients or endogenous necessities across the cell membrane, such as endogenous metabolites and signaling molecules. However, the specificity of some transporters is not strictly constrained to their physiological substrates in that exogenous drugs that bear similar structural features can also be recognized and transported. These transporters are thus referred to the term of "drug transporters". The solute carrier (SLC) family of drug transporters includes but is not limited to organic anion transporters (OAT), organic cation transporters (OCT), organic zwitterion/cation transporters (OCTN), organic anion transporting polypeptides (OATP), monocarboxylate transporters (MCT), nucleoside transporters (CNT/ENT), bile acid transporters (NTCP/ASBT), and multidrug and toxin extrusion transporters (MATE). These transporters are distributed in multiple organs including kidney, liver, placenta, brain, and intestine, and translocate substrates through either secondary or tertiary mechanisms, which require the movement of a co-substrate (such as an ion) or are indirectly linked to the hydrolysis of ATP. The basic properties of these transporters such as their substrate specificities, tissue and membrane localizations, and transport mechanisms have been described in several excellent review articles [1–8].

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The SLC family of drug transporters plays critical roles in the handling of common drugs, environmental toxins, signaling molecules, and nutrients [9–13]. Because of their wide range of substrate recognition, co-administered drugs may compete for the same transporters, causing serious side effects through drug–drug interaction, and therefore affecting the pharmacokinetics and pharmacodynamics of the drug profile. In recognizing these facts, the International Transporter Consortium in conjunction with the US Food and Drug Administration (FDA) issued guidance/recommendations for the assessment of transporter-mediated drug–drug interactions during drug development [14,15]. Among these transporters are the organic anion transporting polypeptide 1B1 and 1B3 (OATP1B1/OATP1B3, SLCO1B1/SLCO1B3), organic cation transporter 2 (OCT2, SLC22A2), and organic anion transporters 1 and 3 (OAT1/OAT3, SLC22A6/SLC22A8), and multidrug and toxin extrusion transporters (MATEs, SLC47A).

Alterations in the expression and function of SLC family of drug transporters have been observed with several disease states, which can have a significant impact on drug disposition and therefore affect drug efficacy and toxicity. For example, Na^+ taurocholate cotransporting polypeptide (NTCP), organic anion transporting polypeptide (OATP) 1B1, and OATP1B3 are the major transporters responsible for bile acids uptake on the sinusoidal membrane in liver [16]. In patients with progressive familial intrahepatic cholestasis, the protein level of NTCP and both the mRNA and protein levels of OATP1B1 and OATP1B3 were found to be reduced in their liver samples [17]. Similarly, in liver biopsies of patients with primary sclerosing cholangitis, a chronic cholestatic liver disease, the level of OATP1B1 mRNA was decreased nearly by half [18]. The decreased level of expression and function of rOat1/3 have been reported in rats with chronic renal failure [19,20] and acute renal failure [21,22]. In a rat model of bilateral ureteral obstruction (BUO), a disease that blocks urine to pass from the kidney to the bladder, the function and expression of renal rOat1/3 were also decreased [23]. OCT family and MATE family are two SLC transporter subfamilies that work in concert to play pivotal roles in the clinical profile of metformin for type II diabetic patients. Genetic polymorphisms of OCT1 [24], OCT3 [25] and MATE1 [26] in the liver are associated with the altered uptake and pharmacological action of metformin, while the genetic variation of OCT2 [27], MATE1 [28] and MATE2-K [29] in the kidney is directly related to variable metformin clearance and therapeutic response.

Given the critical roles the drug transporters of SLC family play in determining the effects of therapeutics and toxic chemicals, understanding the molecular and cellular mechanisms underlying the regulation of these transporters is physiologically and clinically important.

2. Regulation of SLC family of drug transporters

The activity of drug transporters must be delicately controlled in order to carry out their normal activity. Like other proteins, drug transporters can be regulated at multiple levels from gene to protein, including transcriptional regulation, post-transcriptional regulation, translational regulation, and post-translational regulation. The regulations at the levels of transcription, post-transcription, and translation happen within hours to days, and are therefore called long-term or chronic regulation. Long-term regulation usually occurs when the body undergoes massive change, for example, during growth or the development of disease. In contrast, post-translational regulation happens within minutes to hours, and is therefore called short-term or acute regulation. Short-term regulation often takes place when the body has to deal with rapidly changing amounts of substrates in the case of variable intake of drugs, fluids, ions, meals, and metabolism processes.

Post-translational modification is a process where the amino acid side chains in a target protein are modified by conjugating new functional group(s) through reversible or irreversible biochemical reactions. The common modifications include glycosylation, phosphorylation, ubiquitination, sumoylation, sulfation, methylation, acetylation, nitrosylation, palmitoylation and hydroxylation [30]. Post-translational modification affects the folding, conformation, distribution, trafficking, stability, and activity of the proteins and therefore contributes significantly to the structural complexity and functional diversity of the proteins beyond the coding capacity of the genome. Over the last decade, our laboratory and other laboratories have discovered several mechanisms underlying the post-translational regulation of SLC family of drug transporters. In the following, we will focus on these discoveries that are pivotal not only in physiological conditions, but also in pathophysiological states.

2.1. Phosphorylation

Phosphorylation is defined as the reversible addition of a negatively charged phosphate group to a protein substrate, typically to a serine, threonine, or tyrosine residue. The presence of this heavily charged group is important for changing the hydrophobicity and electric charge of a protein region and, therefore, it can result in a change in the protein conformation, cellular localization, or interactions with other proteins. The phosphorylation state of a target protein is dynamically controlled by protein kinases and protein phosphatases, which act in an exact opposite fashion to remove phosphate, making phosphorylation a reversible process [31].

The functional effects of phosphorylation were observed with many drug transporters. For example, treatment of the mouse organic anion transporter 1 (mOat1)-expressing cells with phosphatase inhibitor okadaic acid promotes serine/threonine phosphorylation of the transporter and inhibits mOat1-mediated transport of para-aminohippurate (PAH), a prototypical organic anion. Activation of protein kinase C (PKC) enhances the phosphorylation of the rat organic cation transporter 1 (rOct1), and substitutions of PKC-sites on rOct1 with alanine suppressed PKC-induced stimulation of rOct1 transport activity [32,33]. Another member of the OCT family human organic cation transporter 2 (hOCT2) was subject to tyrosine kinase-induced increase in phosphorylation and transport activity. PKC activation or treatment with phosphatase inhibitor okadaic acid also results in an increased phosphorylation and a functional inhibition of organic anion-transporting polypeptides OATP2B1, OATP1B3, and rOatp1a1, which correlated with an accelerated internalization of these transporters from cell surface. The phosphorylation and transport activity of sodium taurocholate cotransporting polypeptide (rNtcp) was also influenced by protein kinase A (PKA), PKC, and hyperosmolarity. Similarly, interleukin-1β promoted degradation of apical sodium-dependent bile acid transporter rAsbt through JNK-regulated phosphorylation. PKA-regulated phosphorylation of monocarboxylate transporter rMct1 and serum- and glucocorticoid-inducible kinase 1 (SGK1)-regulated phosphorylation of peptide transporter PEPT2 both exerted functional consequences on these transporters. The details on the functional regulation of these transporters by phosphorylation can be found in Table 1.

2.2. Glycosylation

Glycosylation is a modification that involves the addition of oligosaccharides to secretory and membrane proteins. There are two main types of glycosylation, with sugar moiety added on to NH2 group of asparagine (N-linked) and on to the OH group of serine/threonine (O-linked) [34–36]. N-linked glycosylation initiates co-translationally at rough endoplasmic reticulum and further processes in the Golgi apparatus, while O-linked glycosylation occurs post-translationally in the Golgi apparatus [37,38]. Glycosylation is one of the most common forms of post-translational protein modification and rapidly emerges as a fundamental mechanism not only controlling the proper folding of nascent transporter proteins but also their subcellular localization, and function.

Studies from our laboratory showed that members of organic anion transporter (OAT) family are heavily glycosylated under normal condition. When all of the potential glycosylation sites localized in the large extracellular loop between transmembrane domains 1 and 2 of OAT