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Post-translational Regulation of Organic Anion Transporters by Ubiquitination: Known and Novel

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Synopsis

Organic anion transporters (OATs) encoded by solute carrier 22 (SLC22) family are localized in the epithelia of multiple organs, where they mediate the absorption, distribution, and excretion of a diverse array of negatively charged environmental toxins and clinically important drugs. Alterations in the expression and function of OATs play important roles in intra- and inter-individual variability of the therapeutic efficacy and the toxicity of many drugs. As a result, the activity of OATs must be under tight regulation so as to carry out their normal functions. The regulation of OAT transport activity in response to various stimuli can occur at several levels such as transcription, translation, and posttranslational modification. Posttranslational regulation is of particular interest, because it usually happens within a very short period of time (minutes to hours) when the body has to deal with rapidly changing amounts of substances as a consequence of variable intake of drugs, fluids, or meals as well as metabolic activity. This review article highlights the recent advances from our laboratory in uncovering several posttranslational mechanisms underlying OAT regulation. These advances offer the promise of identifying targets for novel strategies that will maximize therapeutic efficacy in drug development.

Keywords

Membrane transporter; Drug transporter; Posttranslational modification; regulation

1. Introduction

The organic anion transporter (OAT) family of 10 membrane proteins belongs to the SLC22 (solute carrier 22) subfamily of the major facilitator superfamily (MFS); the SLC22 subfamily also includes the organic cation transporters (OCTs) and organic carnitine (zwitterion) transporters (OCTNs)^{1–5}. Computer modeling and biochemical studies revealed that OAT family members have 12 putative membrane spanning segments and multiple sites for posttranslational modifications such as glycosylation, phosphorylation, and ubiquitination^{6–8}. The major physiological functions of OATs 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 these transporters is not strictly constrained to their physiological substrates in that exogenous drugs that bear similar

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structural features can also be recognized and transported. OATs are thus referred to the term of “drug transporters”⁹.

OAT family members play critical roles in the handling of common drugs (antibiotics, antivirals, diuretics, nonsteroidal anti-inflammatory drugs), toxins (mercury, aristolochic acid), and nutrients (vitamins, flavonoids)^{1–5}. OAT isoforms are expressed in many tissues, including kidney, liver, brain, and placenta. In the kidney, OAT1 and OAT3 utilize a tertiary transport mechanism to move organic anions/drugs across the basolateral membrane into the proximal tubule cells for subsequent exit across the apical membrane into the urine for elimination. This tertiary transport mechanism involves three transport systems: Na⁺/K⁺-ATPase actively pumps Na⁺ out of the proximal tubule cells, establishing an inwardly directed (blood to cell) Na⁺ gradient. The potential energy stored in this Na⁺ gradient is used by a second transport protein, Na⁺/dicarboxylate cotransporter, to import the dicarboxylate α-ketoglutarate (α-KG), maintaining an outwardly directed (cell to blood) α-KG gradient. α-KG then serves as the physiological counterion for the third transport protein in this chain, a dicarboxylate/organic anion exchanger, namely OAT, which translocates organic anion substrates into the cells^{10–13}. Once inside cells, the organic anions then move across the apical membrane into the urine for secretion via other transporters, such as the multidrug resistance-associated proteins MRP2/MRP4, OAT4, and urate transporter 1⁷ (Figure 1).

Recent studies from our laboratory using pharmacophore modeling identified two common structural features associated with inhibitors for OAT1 and OAT3, viz., an anionic hydrogen-bond acceptor atom, and an aromatic center separated by ~5.7 Å¹⁴. Because of the characteristics of OATs in 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 United States Food and Drug Administration (FDA) issued guidance/recommendations for the assessment of OAT- and several other transporter-mediated drug-drug interactions during drug development^{15,16}.

Alterations in OAT expression and function have been observed with several disease states (Table 1), which can have a significant impact on OAT-mediated drug disposition and therefore affect drug efficacy and toxicity. A number of relevant studies have been conducted in animal models. The decreased rOat1 and rOat3 levels and function have been reported in rats with chronic renal failure^{17,18} and acute renal failure^{19,20}. In a rat model of bilateral ureteral obstruction (BUO), a disease that blocks the urine to pass from kidney to bladder, the function and expression of renal rOat1/3 were also decreased²¹. In addition, the relationship between OATs and diabetes has been extensively studied and the results seem disparate with species difference²². Studies conducted in type I diabetic rats indicated a decreased function and expression level of rOat3, but not rOat1²³, but mRNA and protein expression of mOat1, mOat2 and mOat3 were all found to be decreased in type I diabetic mice²⁴. At the same time, type II diabetic rat showed elevated protein expression of rOat2²⁵, however, mRNA expression of mOat2 was found to be reduced in type II diabetic mice^{26,27}. In some cases, liver impairment directly affected renal functions with varying OAT expression; however, the reported results were not consistent, which may be due to the use

of different methods and models²⁸. Cholestasis rat model induced by administration with alpha-naphthylisothiocyanate (ANIT) or by bile duct ligation (BDL), both showed reduced protein expression for rOat1, while rOat3 was reduced in ANIT model but increased in BDL model^{28,29}.

Given the critical roles OATs play in determining the effects of therapeutics and toxic chemicals, understanding the molecular and cellular mechanisms underlying OAT regulation is of clinical and pharmacological importance.

2. Regulation of Organic Anion Transporters OATs

OAT activity must be delicately controlled in order to carry out their normal activity. Like other proteins, OAT activity can be regulated at multiple levels from gene to protein, including transcriptional regulation (when and how often a gene encoding the transporter is transcribed), post-transcriptional regulation (how the primary RNA transcript for the transporter is cleaved or processed), translational regulation (which mRNA for the transporter in the cytoplasm is translated by ribosomes), and post-translational regulation (how the transporter is covalently modified and assembled after its synthesis).

The regulations at the levels of transcription, post-transcription, and translation are usually referred to as long-term or chronic regulation, which happens within hours to days. Long-term regulation usually occurs when the body undergoes massive change, for example, during development or the occurrence of disease. Several excellent review articles have described the long-term regulation of drug transporters^{1,4,22,30}. While post-translational modifications belong to short-term or acute regulation and the time frame ranges from minutes to hours. 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 new functional group(s) are conjugated to the amino acid side chains in a target protein through reversible or irreversible biochemical reactions. The common modifications include glycosylation, phosphorylation, ubiquitination, sulfation, methylation, acetylation, and hydroxylation³¹. Post-translational modification contributes significantly to the structural complexity and functional diversity of the proteins beyond the coding capacity of the genome, affecting the physical and chemical properties of the transporters, their folding, conformation, distribution, trafficking, stability, and activity.

During the past couple of years, our laboratory has uncovered several mechanisms underlying the post-translational regulation of OATs. We have reported that OAT activity can be regulated by glycosylation, phosphorylation, and ubiquitination-mediated membrane trafficking. Glycosylation is a process, in which a sugar chain is covalently added to the NH₂ group on the side chain of an asparagine residue (N-linked glycosylation) of the target protein. Our mutagenesis study combined with biochemical analyses showed that simultaneous elimination of all the glycosylation sites resulted in OAT being trapped in an intracellular compartment, suggesting that glycosylation plays a critical role in the targeting

of these transporters to the plasma membrane^{32,33}. Phosphorylation was another important mechanism in the regulation of OAT activity. Phosphorylation is a process in which a negatively charged phosphate group is added to the target protein, consequently influencing the conformation and charge of the target protein, thereby also its activity. We showed that the treatment of OAT-expressing cells with okadaic acid resulted in an increased level of phosphorylation in OAT, which paralleled, in time and concentration, the decrease of OAT1-mediated transport of para-aminohippurate (PAH), a prototypical organic anion³⁴. Okadaic acid is a potent inhibitor of protein phosphatase 1 and protein phosphatase 2A, and it enhances the phosphorylation of many cellular proteins by preventing dephosphorylation³⁵. The detailed description of OAT regulation by glycosylation and phosphorylation has been reviewed previously^{5,36–38}. Here, we will focus on the recent progress made from our laboratory in delineating the molecular mechanism underlying ubiquitination-mediated OAT trafficking and transport activity.

3. Regulation of OAT by Ubiquitination

3.1 Constitutive and Regulated Membrane Trafficking of OAT

The amount of OATs at the cell surface is critical for their drug transport activity. Rather than being statically resident plasma membrane proteins, our laboratory has shown that members of OAT family constitutively internalize from and recycle back to cell surface. The rate of OAT internalization is equal to the rate of OAT recycling being ~ 10% per 5 min³⁹. The dynamic characteristic of OAT rather than a static state would make it more effective for the transporter to initiate trafficking in response to stimuli such as activation of protein kinases, therefore providing quick and efficient fine-tuning in response to environmental changes. We further demonstrated that the activation of protein kinase C (PKC) inhibits OAT transport activity by accelerating the internalization of these transporters from cell surface to EEA1-positive early endosomes, without changes in the rate of OAT recycling. As a result, the amount of OAT at the cell surface is reduced and OAT transport activity is decreased^{39,40}. Prolonged PKC activation results in the degradation of OAT in both proteasome and lysosome⁴⁰. Evidence from our studies also pointed out that both constitutive and PKC-modulated OAT internalization occurred partly through a dynamin- and clathrin-dependent pathway^{39,41,42}. PKC-induced direct phosphorylation has been reported for other membrane proteins^{43–46}. Interestingly, our results showed that a range of PKC activators failed to elevate the phosphorylation level of OATs under various experimental conditions³⁴. This suggests that direct phosphorylation of OATs is unlikely to be the cause for PKC-induced OAT trafficking and inhibition of OAT activity.

3.2 Ubiquitination of OAT

Modification of receptors and channels by ubiquitin conjugation has emerged as the major regulatory mechanism of internalization, intracellular sorting, and turnover of many membrane proteins^{47,48}. Ubiquitin moieties can be recognized by the components of plasma membrane internalization and endosomal sorting machinery.

Ubiquitin is a highly conserved 8-kDa polypeptide. Ubiquitination occurs in a sequence of three enzymatic steps. The initial step is the formation of a thioester bond between the

COOH terminus of ubiquitin and the active cysteine residue of an ubiquitin-activating enzyme (E1). Next, the activated ubiquitin is transferred to an ubiquitin-conjugating enzyme (E2). The E2 enzymes are catalytically similar to E1 in that a thioester bond is formed with ubiquitin. The third and last step in ubiquitination is a reaction catalyzed by an ubiquitin-protein ligase (E3) in which an isopeptide bond between the COOH-terminal glycine of ubiquitin and the amino group of a lysine residue on the target protein is formed. It is the E3 that is responsible for substrate recognition. An ubiquitin molecule itself has seven lysine residues (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63) that can serve as a base for chain elongation. Therefore, a substrate can be modified by different types of ubiquitin conjugation: monoubiquitination (conjugation of one single ubiquitin to one single lysine on the substrate), multiubiquitination (conjugation of several monoubiquitin molecules to multiple lysine residues on the substrate), and polyubiquitination (extended polyubiquitin chain)^{49,50}. In addition, a polyubiquitin chain can bear different linkages such as Lys48 linkage (Lys48 in the ubiquitin serves as a base for chain elongation) and Lys63 linkage (Lys63 in the ubiquitin serves as a base for chain elongation). It has been shown that different type and linkage of ubiquitination have different physiologic outcome for the ubiquitinated substrates^{51–54}. Recent evidences have shown that proteins that are ubiquitinated in the plasma membrane are internalized into early endosomes, where these proteins are then deubiquitinated by deubiquitinating enzymes and recycle back to the plasma membrane. Alternatively, these ubiquitinated proteins can interact with the endosomal sorting complexes required for transport machinery and are sorted to late endosomes, and ultimately, to the lysosomes for degradation^{50,55}. Thus, the balance between ubiquitination, mediated by E3 ligases and deubiquitination, mediated by deubiquitinating enzymes, regulates the abundance of membrane proteins on plasma membrane.

Our investigation of OATs in cultured cells and in rat kidney slices demonstrated that the PKC-induced acceleration of OAT internalization is due to an elevation in OAT ubiquitination⁴². Ubiquitination-mediated functional regulation has been observed with other members of solute carrier (SLC) family of drug transporters^{56–58}. Mass spectroscopy analysis revealed that the ubiquitination of OAT occurs through the conjugation of a lysine 48-linked polyubiquitin chain to the transporter⁴². Three important ubiquitin-accepting lysine residues Lys297, Lys303, and Lys315 were identified within the large intracellular loop between transmembrane domains 6 and 7 of OAT1⁵⁹. These lysine residues play a synergistic role in PKC-regulated OAT1 ubiquitination, as mutating any one of the three lysines prevented the ubiquitin conjugation to the other two lysines⁵⁹. Our unpublished results also indicated that lysine 48-linked polyubiquitin chain plays an important role in the long-term PKC regulation of OAT1 stability.

3.3 Ubiquitination Enzymes for OAT

As mentioned above, ubiquitination involves the sequential reaction catalyzed by a cascade of enzymes, including the ubiquitin-activating enzyme E1, the ubiquitin-conjugating enzyme E2, and the ubiquitin-protein ligase E3. It is the E3 ubiquitin ligase that is responsible for the substrate recognition. There are two major families of E3 ubiquitin ligases: RING (Really Interesting New Gene) and HECT (Homology to E6AP C-Terminus) E3 ligases. Nedd4 (neural precursor cell expressed, developmentally down-regulated 4) is a member of the

HECT family of E3 ligases⁶⁰. It was first identified in a set of genes with developmentally down-regulated expression in the mouse brain⁶¹. There are nine members in Nedd4 subfamily, including Nedd4-1 (Nedd4), Nedd4-2 (Nedd4L), Itch, Smurf1, Smurf2, WWP1, WWP2, Nedl1, and Nedl2, of which Nedd4-1 is the prototype^{62–64}. Structurally, Nedd4 family members contain three functional domains: catalytic HECT domain in their carboxyl termini, the C2 (Ca²⁺/lipid binding) domain at the amino termini, and 2–4 protein-protein-interacting WW domains, each of which contains two highly conserved tryptophan residues⁶⁵. The Nedd4 ubiquitin ligases bind to their substrates via WW domains that recognize the (L/P)PxY motif on the substrates^{66,67}. However, exception also exists that many Nedd4 family substrates do not contain this motif and interact with the E3 ligases either through other mechanism or indirectly via an adaptor protein containing (L/P)PxY sequences, like the arrestin-related trafficking (ART) proteins^{68,69}.

Many studies suggested that Nedd4 family members are involved in the PKC regulation of membrane transporters, such as dopamine transporter (DAT)^{70,71}, cationic amino acid transporter (CAT-1)⁷², and glutamate transporter GLT-1⁷³. Recent investigation from our laboratory identified ubiquitin ligases Nedd4-1 and Nedd4-2 as important regulators for OAT1⁷. Overexpression of Nedd4-1 or Nedd4-2 enhanced OAT1 ubiquitination, reduced OAT1 expression at the cell surface, and suppressed OAT1 transport activity. Furthermore, we discovered that PKC-dependent changes in OAT1 ubiquitination, expression, and transport activity were significantly blocked in cells transfected with the ligase-dead mutant of Nedd4-2 (Nedd4-2/C821A) or with Nedd4-2-specific siRNA to knockdown endogenous Nedd4-2, but not in cells transfected with the ligase-dead mutant of Nedd4-1 (Nedd4-1/C867S) or with Nedd4-1-specific siRNA to knockdown endogenous Nedd4-1. These observations demonstrated that both Nedd4-1 and Nedd4-2 are important regulators for OAT1 ubiquitination, expression, and function. Yet they play distinct roles, as Nedd4-2 but not Nedd4-1 can block the response of OAT1 to PKC-induced ubiquitination, expression, and transport activity. An interesting question that remains to be addressed is how PKC discriminates between the two very similar ubiquitin ligases? The study carried out by Garcia-Tardon, et al⁷³ showed that PKC activation induces Nedd4-2 phosphorylation and the formation of GLT-1·Nedd4-2 complexes. Our unpublished work also revealed that activation of PKC by phorbol 12-myristate 13-acetate (PMA) induced phosphorylation of Nedd4-2 on serine and/or threonine residues. Thus, we propose that PKC exerts its effect through directly phosphorylating Nedd4-2 but not Nedd4-1. Supporting such a hypothesis is our preliminary analysis, which indicates that Nedd4-2 contains several conserved PKC phosphorylation sites (Ser-325, Ser-327, Thr-352 and Ser-417) that are not present in Nedd4-1. This area of investigation is currently underway in our laboratory.

In addition to OAT1, our recent study also adds OAT3 to the list of membrane transporters that are recognized and ubiquitinated by Nedd4-2⁸. Overexpression of Nedd4-2 enhanced OAT3 ubiquitination, decreased its expression at the cell surface, and inhibited its transport activity, while the ubiquitin ligase-dead mutant Nedd4-2/C821A or Nedd4-2 siRNA had a dominant negative effect. OATs do not have conventional WW domain-interacting (L/P)PXY motifs, yet we observed physical interaction between Nedd4-2 and OATs (OAT1⁷/OAT3⁸/OAT4⁷⁴), suggesting that other unidentified motif(s) or adaptor proteins containing (L/P)PxY sequences must be present to interact with Nedd4-1/Nedd4-2. This is in line with

the previous reports that Nedd4-1 has physical association with β_2 -adrenergic receptor (β_2 AR)⁷⁵ and mammalian Na^+/H^+ exchanger (NHE)⁷⁶, and Nedd4-2 could directly interact with dopamine transporter (DAT)⁷¹, all of which do not contain the conventional WW domain-binding motifs.

3.4 SGK Regulates OATs through Nedd4-2

In addition to PKC, we recently demonstrated that the serum- and glucocorticoid-inducible kinase 2 (sgk2) also regulates OAT activity via Nedd4-2⁷⁴. The sgk family of protein kinases has three isoforms: sgk1, sgk2, and sgk3 that are involved in controlling diverse cellular processes including sodium Na^+ homeostasis, osmoregulation, cell survival, and cell proliferation^{77–82}. Sgk1 and sgk3 are ubiquitously expressed, whereas sgk2 expression seems to be restricted primarily to liver, kidney, pancreas, and brain. It has been shown that sgk2 within the mammalian kidney has distinct expression, regulation, and role from sgk1 and sgk3⁸³. Unlike sgk1 and sgk3, sgk2 expression in the kidney was not subjected to the regulation by aldosterone. Renal expression characterized by immunocytochemistry localized sgk1 protein to distal convoluted tubule, cortical, and medullary collecting duct, whereas sgk2 protein was highly expressed in kidney proximal tubule cells⁸³. Based on the distinct characteristics of sgk2, we investigated whether OATs, also highly expressed in proximal tubule cells, are regulated by sgk2. We showed that in contrast to the inhibitory effect of PKC, sgk2 stimulated OAT1 and OAT4 transport activity. Such stimulation mainly resulted from an increased cell surface expression of the transporter, kinetically revealed as an increased maximal transport velocity V_{max} without significant change in substrate-binding affinity K_m ^{74,84}. We further showed that the regulation of OAT4 activity by sgk2 was mediated by ubiquitin ligase Nedd4-2. Overexpression of Nedd4-2 enhanced OAT4 ubiquitination and inhibited OAT4 transport activity, whereas overexpression of ubiquitin ligase-dead mutant Nedd4-2/C821A or siRNA knockdown of endogenous Nedd4-2 had dominant negative effects on sgk2 stimulation of OAT4 activity. Our co-immunoprecipitation experiment revealed that sgk2 weakened the association between OAT4 and Nedd4-2. Therefore, sgk2 stimulates OAT4 transport activity by abrogating the inhibition effect of Nedd4-2 on the transporter⁷⁴.

Studies from other laboratories showed that Sgk1, an isoform of sgk2, stimulates the activity of the epithelial sodium channel ENaC by phosphorylating Nedd4-2 at Ser221, Thr246, and Ser327^{85,86}. Such phosphorylation leads to the inhibition of the Nedd4-2-ENaC interaction and further results in the loss of the ability of Nedd4-2 to ubiquitinate and to suppress ENaC activity⁸⁷. Our unpublished data showed that sgk2 also enhanced Nedd4-2 phosphorylation possibly at same sites as that in response to sgk1.

3.5 Ubiquitin Ligase Nedd4-2 may Serve as A Central Switch for Protein Kinase-Regulated OAT Expression and Transport Activity

Our published and unpublished work showed that the inhibitory effect of PKC vs. stimulatory effect of sgk2 on OAT expression and transport activity seem to converge on the same molecule: the ubiquitin ligase Nedd4-2. This observation suggests that the opposite regulation of OATs by PKC (negative regulation) and sgk2 (positive regulation) is exerted through the dynamic phosphorylation at distinct sites on Nedd4-2, a central switch, thereby

inducing distinct conformational changes of Nedd4-2 and modifying its binding (association/dissociation) to OAT, which leads to a change (promotion/demotion) in OAT ubiquitination, trafficking, and transport activity. Our laboratory is currently testing this novel mechanism (Figure 2), the outcome of which will provide significant insights into our understanding of how protein kinase-Nedd4-2 signaling network transduces diverse physiological stimuli to OAT-mediated drug transport *in vivo*. Indeed, we and others have previously shown that OAT activity was suppressed by physiological hormones such as angiotensin II and parathyroid hormone via activation of PKC^{88–90}. We therefore speculate that Nedd4-2-mediated OAT ubiquitination plays critical role in these pathways.

4. Conclusion and future perspectives

In this review, we updated recent advances from our laboratory in uncovering the loops and layers of posttranslational mechanisms controlling OAT expression and function (Table 2). Ubiquitination is one of the post-translational modifications that has attracted more and more attention recently, and its role in affecting the trafficking and stability of drug transporters is evident at least for OATs as revealed by our laboratory. Ubiquitination is a highly dynamic process that can be reversed by the action of specialized enzymes known as deubiquitinases, which are a large group of proteases that remove monoubiquitin and polyubiquitin chain from target proteins. Identification of the specific deubiquitinases in the regulation of OATs will be an interesting research area to pursue. In clinics, several anti-cancer drugs target the ubiquitin-proteasome system. Bortezomib (Velcade; Millennium Pharmaceuticals) is the first approved proteasome inhibitor and used for multiple myeloma and mantle cell lymphoma⁹¹. Carfilzomib (Kyprolis; Onyx Pharmaceuticals)⁹² and Marizomib (NPI-0052; Nereus Pharmaceuticals)⁹³ are examples of second generation proteasome inhibitors which provide irreversible proteasome inhibition with sustained response. These drugs are used for the treatment of hematologic tumors, with possible extensive application to solid tumors⁹³. It is therefore interesting to investigate whether these drugs can interfere with the regulation of OATs by ubiquitination. Thorough understanding of the posttranslational modifications of OATs will provide significant insights into the regulation of OAT-mediated drug transport in various physiological and pathophysiological conditions.

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Figure 1.
Simplified model of organic anion transport in the kidney proximal tubule cells.

Figure 2.
Hypothetical model of ubiquitin ligase Nedd4-2 as a central switch for protein kinase-regulated OAT activity.

Table 1

Disease status and OAT expression

Disease status	Models	Transporters	RNA/protein	Change	Ref
Chronic renal failure (CRF)	5/6 Nephrectomized rat	rOat1, rOat2, rOat3	mRNA/protein	↓	17,18
Acute renal failure (ARF)	Rat with renal ischemia	rOat1, rOat3	mRNA/protein	↓	19,20
Bilateral ureteral obstruction (BUO)	Rat with bilateral obstruction of the proximal ureters	rOat1, rOat3	Surface protein	↓	21
Diabetes	streptozotocin (STZ) induced diabetic rat	rOat3	protein	↓	23
	Ins2Akita mouse	mOat1, mOat2 mOat3, mOat5	mRNA/protein	↓	24
	Ob/Ob (Obese) mouse	mOat2	mRNA	↓	26
	Db/Db (diabetic) mouse	mOat2	mRNA	↓	27
	STZ induced diabetic rat with high-fat diet	rOat2	protein	↑	25
Cholestasis	ANIT induce intrahepatic cholestasis rat	rOat1, rOat3	mRNA/protein	↓	28
	Bile duct ligation (BDL) rat	rOat1	protein	↓	29
		rOat3	protein	↑	

Table 2

Post-translational modifications (PTM) of OATs

Type of PTM	OATs	Details	Ref.
Phosphorylation	mOat1	Treatment with phosphatase inhibitor okadaic acid promotes serine/threonine phosphorylation and inhibits mOat1 transport activity.	34
Glycosylation	mOat1 hOAT1 hOAT4	Treatment with N-linked glycosylation inhibitor, tunicamycin, or mutagenesis of all glycosylation sites impairs the targeting of OATs to the plasma membrane and inhibits OAT transport activity.	32,33,94
	hOAT4	Expression of OAT4 in cells defective in processing of oligosaccharides from mannose-rich type to complex type decreases the affinity of OAT4 for its substrates.	33
Ubiquitination	rOat1 hOAT1 hOAT3	Treatment of OAT-expressing cells or rat kidney slides with protein kinase C activator, PMA, increased OAT ubiquitination, while PKC inhibitor staurosporin blocked OAT ubiquitination.	8,42
	hOAT1 hOAT3 hOAT4	Overexpression of Nedd4-2 increased OAT ubiquitination and decreased OAT activity. siRNA knockdown of endogenous Nedd4-2 abolished PKC-stimulated OAT1/OAT3 ubiquitination, reversed PKC-induced decrease of OAT activity. Knockdown of endogenous Nedd4-2 also abolished sgk2-stimulated OAT4 activity.	7,8,74
	hOAT1	LC-MS/MS detected Lys48-linked polyubiquitin peptides conjugated to OAT. Ubiquitin mutant Ub-K48R prevents the formation of polyubiquitin chains, abolishes PKC-stimulated OAT1 ubiquitination, internalization and PKC-induced decrease in OAT expression at the cell expression.	42
		Mutagenesis of Lys297, Lys303 and Lys315 abolished PKC-stimulated OAT1 ubiquitination.	59
		Overexpression of Nedd4-1 increased OAT1 ubiquitination and decreased OAT transport activity.	7