HHS Public Access

Author manuscript

Pharmacol Ther. Author manuscript; available in PMC 2022 January 01.

Published in final edited form as:

Pharmacol Ther. 2021 January ; 217: 107647. doi:10.1016/j.pharmthera.2020.107647.

Regulation of organic anion transporters: role in physiology, pathophysiology, and drug elimination

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Abstract

The members of the organic anion transporter (OAT) family are mainly expressed in kidney, liver, placenta, intestine, and brain. These transporters play important roles in the disposition of clinical drugs, pesticides, signaling molecules, heavy metal conjugates, components of phytomedicines, and toxins, and therefore critical for maintaining systemic homeostasis. Alterations in the expression and function of OATs contribute to the intra- and inter-individual variability of the therapeutic efficacy and the toxicity of many drugs, and to many pathophysiological conditions. Consequently, the activity of these transporters must be highly regulated to carry out their normal functions. This review will present an update on the recent advance in understanding the cellular and molecular mechanisms underlying the regulation of renal OATs, emphasizing on the post-translational modification (PTM), the crosstalk among these PTMs, and the remote sensing and signaling network of OATs. Such knowledge will provide significant insights into the roles of these transporters in health and disease.

Keywords

Drug transporter; Organic anion transporter; Drug disposition; Post-translational modification; Regulation

1. Introduction

Organic anion transporters (OATs), a subfamily of the solute carrier 22 (SLC22) transporters, are localized on the physiological barriers of multiple tissues, such as kidney, liver, brain, placenta, retina, and olfactory mucosa (He, et al., 2014; S. K. Nigam, et al., 2015). They are the key players for the translocation of various substances into and out of cells, such as signaling molecules, toxins, and a diverse array of important clinical therapeutics, including antivirals, anti-cancer drugs, antibiotics, anti-hypertensives, and anti-inflammatories. (Ahn & Nigam, 2009; Cha, et al., 2000; Dantzler & Wright, 2003; He, et al.,

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

2014; Pritchard, 1990; Srimaroeng, Perry, & Pritchard, 2008; Taki, Nakamura, Miglinas, Enomoto, & Niwa, 2006; Terada & Inui, 2007; Vallon, Eraly, et al., 2008; VanWert, Gionfriddo, & Sweet, 2010; You, 2002). Therefore, OATs are not only critical for physiological and pathological processes in the body, but also critical in absorption, distribution, metabolism, and elimination (ADME) of clinical therapeutics, thus affecting the pharmacokinetics and pharmacodynamics of the drug profile. Among the tissues involved in the ADME of clinical therapeutics, the kidney is one of the vital organs responsible for drug elimination after its administration. Renal drug transporters are in charge of the transfer of the drugs between blood and proximal tubule lumen. Among various renal drug transporters, OATs, mainly interacting with organic anionic molecules, are expressed at both the basolateral membrane and apical membrane of the proximal tubule cells and are responsible for the excretion of numerous endogenous and exogenous substances. Because of the importance of OATs in disposition of many important clinical drugs and in various physio-pathological processes, numerous efforts have been made to uncover molecular and cellular mechanisms that contribute to the regulation of OATs. In this review, we discussed the recent advance in understanding the regulation of OATs, highlighting the regulation at the level of post-translational modification and the regulatory network of the remote sensing and signaling.

2. OAT expression, structure, and function

The OAT family consists of a group of transmembrane proteins with around 540-560 amino acids. The OATs have been identified in the epithelia barriers such as kidney, liver, brain, placenta, and intestine. These OATs play significant roles in modulating the movement of organic anion molecules across cell membranes. One of the characteristics of OATs are their wide range of substrate recognition including both physiological/endogenous substrates and their metabolites and xenobiotic molecules such as environmental toxins and therapeutic drugs, which make them important players in body homeostasis and pharmacological responses (VanWert, et al., 2010). Understanding the relationships between the molecular features of OATs and their functions provided significant insights into their influences on clinical drug elimination, efficacy, and toxicity. (Emami Riedmaier, Nies, Schaeffeler, & Schwab, 2012; Koepsell, 2013; S. K. Nigam, et al., 2015; L. Wang & Sweet, 2013b; D. Xu, Wang, & You, 2016b).

Kidney is responsible for eliminating substances from the blood and reabsorbing certain compounds back into the circulation. In this way it keeps essential nutrients in the circulation while removing harmful metabolites and therapeutic agents from the body (L. Wang & Sweet, 2013b). There are three major events in this process: glomerulus filtration, tubule secretion, and reabsorption. Unlike the passive filtration processes occurring in kidney glomeruli, much of the active exchange of compounds happen in the kidney proximal tubule, where a number of important transporters are expressed (Fig. 1). Proximal tubule cells polarize into apical membrane, which faces the urine, and basolateral membrane, which faces the blood vessel. The OAT members are expressed in renal proximal tubule, both on the basolateral and on the apical membrane. They stand as important mediators in the active process of renal elimination and reabsorption (Emami Riedmaier, et al., 2012; Motohashi, et al., 2013).

In human, OAT1, OAT2, OAT3, OAT4, OAT10, and URAT1 have been detected in kidney samples. It is worth to mention that in rodents certain Oats have different expression profiles from those in human. These comparisons could help to understand the differences of OAT functions and regulations between rodent models and clinical studies. (S. K. Nigam, et al., 2015; L. Wang & Sweet, 2013b). OAT1, OAT2, and OAT3 have been located on the basolateral membrane of the proximal tubule cells in human kidney, which is the blood side of these cells (Fig. 1). These three OATs facilitate the transfer of organic anions from blood into the proximal tubule cells driven by a tertiary transporting mechanism. Across basolateral membrane, the cells utilize the sodium gradient generated by Na^+-K^+-ATPase to indirectly drive the influx of organic anions into the proximal tubule cells. OAT4 and URAT1 (another membrane of OAT family) are expressed on the apical membrane or the urine side of the proximal tubule cells (Fig. 1). OAT4 mediates reabsorption of organic anions from urine back into the tubule cells. OAT10, with higher expression in kidney proximal tubule and small intestine, has been proved to transport nicotine and uric acid. URAT1 is mainly responsible for the reabsorption of urate via monocarboxylates exchange. (S. K. Nigam, et al., 2015; L. Wang & Sweet, 2013b).

Through computer modeling and site-directed mutagenesis studies, it has been revealed that OAT family shares a similar structure feature consisting of intracellular N- and C-termini, 12 α-helical transmembrane domains (TMDs), a large extracellular loop between TMD1/2, and a central intracellular loop between TMD6/7 (Anzai, Kanai, & Endou, 2006; F. Zhou & You, 2007; Zhu, et al., 2015). Three highly conserved regions are mainly responsible for protein functions: the large extracellular domain between TMD1/2, the central intracellular loop between TMD6/7, and TMD9/10. These three regions work together to ensure substrate specificity and proper transport activity of OATs (Koepsell, 2013; Zhu, et al., 2015). Furthermore, the large extracellular domain between TMD1/2 contains multiple glycosylation sites, which are important for OAT trafficking to cell surface (F. Zhou, et al., 2005). The C-terminus and central intracellular loop between TMD6/7 contain potential phosphorylation sites, which are involved in regulations of transport function and expression (Zhu, et al., 2015). OAT1 formed homo-oligomers on plasma membrane when expressed in cell culture, which were confirmed via coimmunoprecipitation and gel filtration chromatography (Hong, et al., 2005). TMD6 is important to homo-oligomerization of OAT1 on plasma membrane, which plays a significant role on transporter expression and activity (P. Duan, Li, & You, 2011). In addition, TMD12 has been shown to play a critical role in OAT maturation and stability (Hong, Li, Zhou, Thomas, & You, 2010).

3. OATs and drug-drug interaction (DDI)

Clinically observed DDIs can be dated back to more than 50 years ago with the co-administration of probenecid and penicillin. Probenecid, an inhibitor for organic anion transport system, inhibited the renal clearance of penicillin, consequently increased the efficacy of penicillin (Gibaldi & Schwartz, 1968). With progress in molecular cloning and characterization of individual OATs, molecular and cellular mechanisms underlying OAT-mediated DDIs have been revealed. Due to the vast range of substrate recognition of OATs, multiple therapeutic agents, when taken together, may mutually affect each other's pharmacokinetic profiles through interacting with the same transporters, either in a

competitive or non-competitive manner, which causes drug-drug interaction (Huo & Liu, 2018; L. Wang & Sweet, 2013b). It would be impractical to assess all the DDI possibility of approved drugs and new drug candidates only in clinical studies. Thus, cell line-based in vitro DDI assays are proven to be valuable research tools (Giacomini, et al., 2010; Huo & Liu, 2018). In recent years, the US Food and Drug Administration (FDA) with International Transporter Consortium have issued guidance for the assessment of transporter-mediated drug–drug interactions during drug development process. Among the important drug transporters, OAT1 and OAT3 were listed as potential targets for DDI assessment (Giacomini, et al., 2010; Hillgren, et al., 2013).

Numerous in vitro OAT-mediated DDI studies of therapeutic drugs, such as antituberculosis drugs, anti-viral drugs, and anti-cancer drugs, have been reported (Maeda, et al., 2014; Parvez, Kaisar, Shin, Jung, & Shin, 2016; Toh, et al., 2016; L. Wang, Pan, & Sweet, 2013; L. Wang & Sweet, 2013a). In a recent study, OAT4-expressing cells were utilized to screen a panel of anti-cancer drugs. Epirubicin hydrochloride and dabrafenib mesylate showed cis-inhibitory effect on OAT4 uptake activity. Furthermore, it was discovered that dabrafenib mesylate exerted competitive inhibition while inhibition by epirubicin hydrochloride was in a noncompetitive manner, and epirubicin hydrochloride had a higher chance causing clinical DDI (C. Liu, Zhang, & You, 2019). In a rat study both mizoribine and bezafibrate were found to be Oat1 and Oat3 substrates. The co-administration of mizoribine and bezafibrate increased the accumulation of bezafibrate in the circulation, causing enhanced toxicity and increased risk of developing rhabdomyolysis (Feng, et al., 2016). In addition to approved drugs, indoxyl sulfate, a uremic toxin, was confirmed as a substrate of OAT1, OAT3, and OAT4 in cultured cells (Hsueh, et al., 2016). In a rat study, the blood concentration of indoxyl sulfate increased with lower renal clearance when administrated with quinapril, whose metabolite quinaprilat was also a substrate of Oat3 (Fujita, et al., 2012; Yuan, et al., 2009). To connect in vitro studies with in vivo research, Mathialagan, et al. applied transporter data obtained from OAT-expressing human cells to quantitatively predict in vivo renal elimination and total renal clearance. Their results concluded that renal transport mediated by OAT3 played a predominant role in renal elimination for a majority of drugs they tested (Mathialagan, et al., 2017).

Recognized as a powerful organic anion transport inhibitor, probenecid was used with clinical therapies to prolong half-life of drugs therefore enhance therapeutic effects (L. Wang & Sweet, 2013b). In healthy volunteers, probenecid inhibited the clearance of mesna, an OAT substrate, increasing its protective effect against the toxicity of cyclophosphamide and cisplatin (Cutler, et al., 2012). However, inhibition of OATs works as a double-edged sword, which may cause higher systematic toxicity when exposing whole body to a drug for an extended time (Takahara, et al., 2013). In a clinical study of patients with non-small cell lung cancer, hematologic toxicity caused by pemetrexed was amplified when lansoprazole was co-administrated. Cell line-based study later confirmed that lansoprazole inhibited OAT3, thus decreased the renal uptake and elimination of pemetrexed, and eventually increased hematologic toxicity in patients (Ikemura, et al., 2016). A clinical study found that AK106–001616, a cytosolic phospholipase A2 inhibitor, inhibited OAT1 and OAT3 and increased the area under curve of methotrexate in rheumatoid arthritis patients (Kozaki, et al., 2015). In cancer patients who received methotrexate, a drug for arthritis and cancer, with

proton pump inhibitors, their plasma concentrations of methotrexate were significantly higher than those in patients taking methotrexate alone, which was consistent with another cell-based study using cultured cells expressing OAT1 and OAT3. It was found that proton pump inhibitors like omeprazole significantly decreased the uptake of methotrexate through inhibiting OAT1 and OAT3 (Chioukh, et al., 2014; Narumi, et al., 2017). In summary, with expanding knowledge and discoveries, OAT-mediated DDIs continue to play significant roles in the pharmacokinetic profiles, efficacy, and toxicity of a wide range of clinical therapeutic agents.

4. OATs in kidney injury and diseases

Clinical observations between kidney diseases and renal OATs are often complex and intertwined. On one hand, kidney injury and diseases could directly affect renal OAT expression, function, and localization. On the other hand, direct damage on proximal tubule cells and OATs could also change various renal functions, leading to kidney disease progression. Plentiful animal and clinical studies have revealed possible correlations between them (Huo & Liu, 2018; Schwenk & Pai, 2016; L. Wang & Sweet, 2013b; D. Xu, Wang, & You, 2016b).

Acute kidney injury (AKI) is a common and complex condition especially for patients in the intensive care units. The well-recognized causes of AKI are drug/toxicant-induced renal toxicity and renal ischemia/reperfusion (L. Wang & Sweet, 2013b). Renal ischemia/reperfusion often decreases Glomerular filtration rate (GFR) and damage tubular functions like secretion and reabsorption (Bischoff, Bucher, Gekle, & Sauvant, 2014b). In ischemic rat kidneys, the mRNA and protein expression levels of Oat1 and Oat3 were both reduced (Bischoff, et al., 2014b; Schneider, et al., 2015). Anti-inflammatory drugs meclofenamate, quercetin, and resveratrol reduced indoxyl sulfate accumulation during AKI and ameliorated the reduction of Oat1 and Oat3 protein expression in ischemic AKI rats (Saigo, et al., 2014; Saito, et al., 2014). Prostaglandin E2 decreased the mRNA levels of Oat1 and Oat3 through E prostanoid receptor type 4 in rats with ischemic-induced AKI (Bischoff, Bucher, Gekle, & Sauvant, 2014a; Preising, Schneider, Bucher, Gekle, & Sauvant, 2015). A wide range of clinical therapeutics could cause renal toxicity and induce AKI, such as aminoglycosides antibiotics and angiotensin-converting-enzyme inhibitors (Pannu & Nadim, 2008). Previous research revealed that gentamicin can cause necrosis of proximal tubule cells, which would inhibit protein synthesis in kidney and induce AKI. Furthermore, gentamicin was able to increase the levels of superoxide anion and hydrogen peroxide in renal cortical cells, which would also contribute to renal toxicity (Baliga, Ueda, Walker, & Shah, 1999). In a rat model of gentamicin-induced AKI, both plasma creatinine and blood urea nitrogen levels were increased, indicating reduced renal function and toxicity. In this AKI model, both the mRNA and protein expressions of Oat1 and Oat3 were significantly decreased. It was possible that gentamicin-caused toxicity down-regulated kidney Oat1 and Oat3 expression, which contributed to the reduced renal function and accumulated endogenous substances (X. Guo, et al., 2013). Resveratrol, an anti-inflammatory and antioxidant agent, reduced methotrexate-induced renal toxicity in rats via decreasing Oat-mediated kidney elimination of methotrexate. This reduced toxicity was mainly due to direct inhibition by resveratrol on Oat1 and Oat3 (Jia, et al., 2016).

In patients with chronic kidney failure (CKF), the glomerular filtration rate and renal clearance decline gradually and continuously, which would cause endogenous metabolites, uremic toxins, and therapeutic agents to accumulate in the circulation and often lead to renal failure (Naud, et al., 2011). In the process, uremic toxins exert effects on OATs in two aspects. On one hand, uremic toxins could regulate OAT expression. In a rat model of adenine-induced CKF, the mRNA and protein expression levels of Oat1 and Oat3 were significantly reduced (Komazawa, et al., 2013). In a CKF rat model by 5/6 nephrectomy, Oat1, Oat2, and Oat3 mRNA and protein expression levels were reduced. Interestingly, incubating human proximal tubule cells with sera from CKF rats caused a similar decreasing effect on human OATs (Naud, et al., 2011). The authors hypothesized that the decreasing effects on OAT/Oat were possibly due to accumulated metabolites and uremic toxins in the sera of CKF rats, although regulatory mechanisms were not clearly revealed. In support, p-cresyl sulfate, a uremic toxin, reduced Oat1 expression in a separate animal study. Oat1 protein expression was decreased by 40% after p-cresyl sulfate administration by oral gavage in CKF rats (Jansen, et al., 2019).

In addition to the regulation on OAT expression, accumulated metabolites and uremic toxins could also inhibit OAT transport activity and cause OAT-mediated drug-drug interactions (DDI) (Huo & Liu, 2018). Accumulated indoxyl sulfate (IS) and hippuric acid decreased renal clearance of morinidazole metabolites, substrates of Oat1 and Oat3, through Oat-mediated DDIs in CKF rats. Thus the plasma concentrations of morinidazole metabolites were significantly elevated (Kong, et al., 2017; Zhong, et al., 2014). In CKF rats, green tea metabolites further reduced kidney clearance and increased the plasma levels of IS and p-cresyl sulfate by inhibiting the functions of Oat1 and Oat3 (Peng, et al., 2015). The complex relationship between uremic toxins and OATs has also been reported in Oat1 and Oat3 knockout mice (A. K. Nigam, et al., 2020; W. Wu, Bush, & Nigam, 2017). In Oat1 knockout mice, the levels of IS, kynurenine, and xanthurenic acid were increased in the plasma, and these toxins could inhibit Oat1 function in vitro (Wikoff, Nagle, Kouznetsova, Tsigelny, & Nigam, 2011). Indoleacetate and p-cresyl sulfate were significantly increased in the plasma of Oat3 knockout mice, and their interactions with Oat3 were also confirmed by in vitro data (W. Wu, et al., 2017). In conclusion, the interaction between uremic toxins and renal OATs is complicated under CKF conditions. The accumulated metabolites and uremic toxins due to decreased expression and function of OATs in CKF would in turn further reduce OAT function, which forms a positive feedback loop between uremic toxins and OATs.

A wide range of other pathological conditions were also revealed to affect functions and expressions of renal OATs in animal studies. The expression and function of Oats under diabetic conditions have been studied in various animal models. One animal study showed that the activity and protein level of Oat3 were decreased in streptozotocin-induced diabetic rats, which could be restored by insulin treatment (Phatchawan, Chutima, Varanuj, & Anusorn, 2014). In another study using Ins2Akita mouse, a model for diabetes, the mRNA and protein expression levels of Oat1, Oat2, and Oat3 were all reduced (C. Xu, et al., 2015). Furthermore, the mRNA level of Oat2 were decreased in both Ob/Ob obese mice and Db/Db diabetic mice (Cheng, et al., 2008). In obese rats with high fat diet (HF), it was reported that Oat3 transport function and protein expression were decreased. Atorvastatin or vildagliptin treatment in HF rats partially reversed the impaired renal Oat3 function (Pengrattanachot, et

al., 2020). In addition to diabetes, cholestasis, a liver disease, in which the flow of bile from liver is reduced or obstructed, was reported to affect renal Oats in animal models. Administration of alpha-naphthyl isothiocyanate (ANIT) to induce biliary obstruction in rats resulted in reduced protein expression of Oat1 and Oat3 (T. Liu, et al., 2012). In rats with bile duct ligation (BDL), protein expression of Oat1 was decreased while Oat3 expression was increased (Brandoni, Anzai, Kanai, Endou, & Torres, 2006). The method of BDL-induced biliary obstruction animal model is different from that of ANIT-induced intrahepatic cholestasis model, which possibly contributes to the difference in the variation of OAT3 expression in both reports. In a rat model of bilateral ureteral obstruction, a disease that blocks the flow of urine from kidney to bladder, the transport activity and protein expression levels of Oat1 and Oat3 in the kidney were reduced (Villar, Brandoni, & Torres, 2008).

In addition to animal models, OAT expression was investigated in multiple clinical studies. In patients with metastatic colorectal cancer, higher OAT2 expression was detected in tumor tissues after 5-fluorouracil/leucovorin/oxaliplatin (FOLFOX) treatment. And higher OAT2 level was significantly correlated with good objective tumor response which could serve as an independent predictor of good FOLFOX treatment outcome, possibly due to the roles of OAT2 in uptake of the FOLFOX drugs (Tashiro, et al., 2014). In hepatocellular carcinoma (HCC) patients who received curative local ablation therapy, those with reduced OAT2 expression had significantly higher rates of multifocal recurrence than those with normal OAT2 expression. In addition, the decreased level of OAT2 was significantly correlated with future development of HCC in chronic hepatitis C virus infected patients (Yasui, et al., 2014). The mRNA level of OAT1 was significantly lower in kidney biopsy specimens from patients with renal diseases compared to normal kidney cortex tissues, while the levels of OAT2/4 mRNA seemed to increase slightly (Sakurai, et al., 2004).

In recent years, adjusting drug dosage became necessary in patients with renal injury and diseases. The changes in GFR as well as renal OAT expression and functions should be taken into consideration to achieve an effective therapy. Thus, further studies of mechanistic connections between OATs and various diseases are required to improve therapeutic efficacy and reduce possible toxicity in patients with altered renal functions.

5. Genetic polymorphisms of OATs and clinical impact

In recent years, correlation analysis between OATs polymorphisms and diseases in clinical studies were performed to further validate the physiological function of OATs. A human study including normal subjects and patients with chronic kidney disease (CKD) demonstrated that patients with CKD had a higher frequency of the −475 single nucleotide polymorphisms (SNP) in the 5’ regulatory region in OAT1 than normal subjects. Moreover, −475 SNP in OAT1 with T to G transversion reduced the binding of hepatoma-derived growth factor (HDGF, a known transcription repressor), and HDGF can down-regulate OAT1 protein expression, suggesting an increase of OAT1 expression and renal uptake of toxins, and nephrotoxicity with the −475 SNP (Sun, et al., 2018). In HEK293 cells, the OAT3-Ile305Phe variant had a reduced maximum transport activity for cefotaxime, a substrate of OAT3 without affecting the Michaelis-Menten constant value of OAT3, and a significantly decreased surface expression of OAT3. As OAT3-Ile305Phe variant accounts

for about 3.5% allele frequency in Asians, a clinical study showed that OAT3-Ile305Phe variant significantly suppressed the renal clearance of cefotaxime, in healthy volunteers (Yee, et al., 2013). Besides, a SNP (Position at chromosome11: 64088038, A/G) of OAT4 was associated with renal underexcretion type gout by analysis of OAT4 gene in gout patients and healthy volunteers, suggesting that OAT4 expressed at apical membrane of renal proximal tubule cells contributed to urate transport in humans (Kolz, et al., 2009; Sakiyama, et al., 2014). Cho et al found that five new SNPs in the human URAT1 gene were significantly associated with uric acid concentration in blood by analyzing subjects with normal uric acid level and subjects with hyperuricemia (Cho, Kim, Chung, & Jee, 2015). Among the five SNPs, rs75786299 had the highest association with hyperuricemia, followed by rs7929627 and rs3825017, while rs11602903 and rs121907892 were negatively correlated with hyperuricemia. OAT1 and OAT3 at the basolateral membrane of the kidney proximal tubule cells may affect secretion of uric acid rather than reabsorption like URAT1 at the apical membrane, which was confirmed in Oat1 knock-out (Oat1KO) and Oat3 knock-out (Oat1KO) mice (Eraly, et al., 2008). It is interesting that there are few SNPs of OATs in uric acid-related human diseases (such as hyperuricemia or hypouricemia) and other types of diseases according to genome-wide association studies, which is possibly due to the overall low-frequency genetic variants of OATs compared with other transporters with high polymorphisms (Lipkowitz, 2012; Lozano, et al., 2018). One study found that a SNP (rs3793961) of OAT3 had association with lower serum uric acid levels among men with CKD (Bhatnagar, et al., 2016). Besides, intestinal secretion of uric acid by ATP-binding cassette transporter G2 (ABCG2), a key luminal intestinal secretory urate transporter may play a complementary role for renal excretion (Ichida, et al., 2012). In the 5/6 nephrectomy rats with CKD, the serum uric acid did not increase despite the urine uric acid excretion in the kidney significantly decreased; under such condition, overexpression of Abcg2 in intestine was observed, suggesting that Abcg2 in intestine possibly rescued uric acid excretion in renal failure (Yano, Tamura, Kobayashi, Tanemoto, & Uchida, 2014). Consistent with the rat model of renal failure, Bhatnagar et al found that there was a significant association between serum uric acid and a SNP (rs4148157) on ABCG2 in intestine in patients of European with CKD, further supporting that intestinal-expressed ABCG2 remotely compensates to maintain uric acid homeostasis in human with renal decline (Bhatnagar, et al., 2016). These clinical studies showed that genetic variants in drug transporters can cause individual differences in drug effectiveness, drug toxicity, and some diseases.

6. Roles of OATs in the handling of endogenous substances and their metabolites

A variety of endogenous substances and their metabolites are eliminated by renal OATs to avoid the systemic toxicity, and to maintain the body’s homeostasis. Using Oat1 knock-out (Oat1KO) and Oat3 knock-out (Oat1KO) mice permits to investigate the physiological role of OAT1 and OAT3 without the interference from other functionally redundant transporters, (Bush, Wu, Lun, & Nigam, 2017; Eraly, et al., 2006). Nigam’s group showed that the levels of more than 100 metabolites in the plasma were altered which were involved in key metabolic pathways such as in vivo metabolism of gut microbiome products, flavonoids, bile

acids, nutrients, amino acids, and lipids (Bush, et al., 2017; Eraly, et al., 2006; A. K. Nigam, et al., 2020; S. K. Nigam, 2018; Rosenthal, Bush, & Nigam, 2019). Among those metabolites, many of them are endogenous substrates of OATs, such as bile acids (cholic acid and taurocholic acid) are substrates of OAT3, and Indoxyl sulfate is substrate of OAT1 and OAT3 (Chen, Terada, Ogasawara, Katsura, & Inui, 2008; Lin, et al., 2018). Whether other metabolites are endogenous substrates of OATs needs further to be validated. Besides, many metabolites are active signaling molecules, which will be discussed in detail in section of "Remote Sensing and Signaling Hypothesis of OATs". Therefore, the abnormity of OATs under certain kidney diseases may impact the handling of these endogenous substances and their metabolites.

7. Regulations of OATs

Given the crucial roles of OATs in physiological and pathological processes and in determining the therapeutical efficacy and toxicity of many clinical drugs, elucidating the cellular and molecular mechanisms underlying OAT regulation is of great significance. The regulations of OATs can take place at multiple levels, such as at the levels of transcription, post-transcription, translation, and post-translation, and numerous signaling pathways are involved in these regulations.

Several transcription factors have been identified to be involved in the regulations of OATs. For example, in hepatocyte nuclear factor 1α (HNF1α)-null mice, the levels of renal Oat1, Oat3, and Urat1 mRNA were markedly reduced as compared to those in wild-type mice, and HNF1α overexpression enhanced OAT1, OAT3, and URAT1 promoter activity in vitro (Kikuchi, et al., 2007; Kikuchi, et al., 2006; Maher, et al., 2006; Saji, et al., 2008). In ex vivo experiments with kidney organ culture, HNF4α antagonist attenuated the expression of Oat1 and Oat3 mRNA (Martovetsky, Tee, & Nigam, 2013). In addition, HNF4α transactivated OAT1 promoter through DR-2 and IR-8 elements in vitro (Ogasawara, Terada, Asaka, Katsura, & Inui, 2007). Furthermore, B-cell CLL/lymphoma 6 (BCL6) increased OAT1 promotor activity dependent on HNF1α element and HNF1α protein in vitro (Wegner, Burckhardt, & Henjakovic, 2014). Besides, cAMP responsive element binding protein 1 (CREB1) and activating transcription factor 1 (ATF1), and the corresponding DNA binding sequence motifs on OATs were also involved in the regulation of OATs (Ogasawara, Terada, Asaka, Katsura, & Inui, 2006). Several great review articles have already covered the regulations of OATs at the levels of transcription, post-transcription, and translation (Burckhardt, 2012; S. K. Nigam, et al., 2015; Terada & Inui, 2007; L. Wang & Sweet, 2013b). Therefore, we will place our focus on the post-translational modifications of OATs in the following discussion.

Post-translational modifications (PTMs), the alternations on the amino acids of the target protein after its synthesis, refer to a process of the covalent attachment of various functional group(s) to the amino acid side chain, terminal amino, or carboxyl group of the target protein (G. Duan & Walther, 2015; Spoel, 2018). Most of the PTMs are dynamic and reversible processes which can be catalyzed by specific enzymes to promote or demote the modification. These modifications influence the expression, cellular localization, stability, structure, activity, or substrate specificity of the target proteins. PTMs provide complexity to

the proteome for diverse functions of the proteins. Various PTMs can modify different parts of the target proteins individually or simultaneously. As a result, the functional diversities of the target proteins much exceed their molecular diversities. Various PTMs of OATs have been described in details in our previous review articles (P. Duan & You, 2010; D. Xu, Wang, & You, 2016b; D. Xu & You, 2017). In this review article, we will update the recent progress in uncovering the new PTMs of OATs, the relationship among these PTMs, and the regulatory network on OAT through remote sensing and signaling.

7.1. Regulation of OATs by direct phosphorylation

Phosphorylation process is a crucial PTM which adds negative charged phosphoryl group(s) to the target protein catalyzed by protein kinases, and the addition happens to a serine, threonine, or tyrosine residue (Czuba, Hillgren, & Swaan, 2018). Phosphorylation is an important regulatory mechanism for various membrane proteins including receptors, channels, and transporters through a direct or indirect manner, and induces the change in protein conformation, protein activity, cellular localization of protein, protein stability, or protein-protein interaction.

Many membrane proteins are the substrates of protein kinase-induced direct phosphorylation (Aromolaran, Chahine, & Boutjdir, 2018; Mayati, et al., 2017). And many protein kinases have been reported to phosphorylate various transporters. (Cetinkaya, et al., 2003; Foster & Vaughan, 2017). It is recently demonstrated that Protein Kinase A (PKA) activation by Bt2-cAMP induced a significant increase in OAT3 phosphorylation, which was correlated with an enhanced OAT3 transport activity in cultured cells. Moreover, Insulin-like growth factor 1 (IGF-1), an upstream hormone of PKA signaling, increased OAT3 phosphorylation, and the stimulatory effect was abrogated by H89 (a selective PKA inhibitor). IGF-1-stimulated OAT3 phosphorylation was also correlated with enhanced OAT3 transport activity and protein expression, and the up-regulation effect was abrogated by PKA inhibitor H89. Therefore, PKA activation by Bt2-cAMP and IGF-1 up-regulated OAT3 expression and transport activity possibly by directly phosphorylating OAT3 in COS-7 cells (J. Zhang, Yu, & You, 2020).

Dephosphorylation, countering phosphorylation, refers to a process which removes phosphoryl group(s) from the target proteins, catalyzed by phosphatases. Phosphorylation and dephosphorylation form an opposing regulatory network, thus affecting a variety of cellular processes in health and disease (Ardito, Giuliani, Perrone, Troiano, & Lo Muzio, 2017; Vitrac, Mallampalli, & Dowhan, 2019). Phosphatase inhibitor okadaic acid inhibited Oat1-mediated transport of para-aminohippurate (PAH) in cultured cells, which was correlated with an increased phosphorylation of Oat1 (You, Kuze, Kohanski, Amsler, & Henderson, 2000).

7.2. Regulation of OATs by indirect phosphorylation

Other than directly phosphorylating OATs, protein kinases could also regulate OATs through phosphorylating OAT-interacting proteins. For example, Nedd4-2, a ubiquitin ligase, is an OAT-interacting partner. Ubiquitination of OATs, catalyzed by Nedd4-2, led to the internalization of OATs from cell surface to intracellular endosomes and subsequent

degradation. Several protein kinases, hormones, and chemicals excreted their regulation on OATs through phosphorylating Nedd4-2 at different sites, which either weakened or strengthened the protein-protein interaction between OATs and Nedd4-2, and led to either stimulating- or inhibiting OAT activity (H. Wang, Liu, & You, 2018; H. Wang, Xu, Toh, Pao, & You, 2016; H. Wang & You, 2017; H. Wang, Zhang, & You, 2019b; J. Zhang, Liu, & You, 2018).

Our published and unpublished results indicated PKC activation decreased OAT expression and transport activity through Nedd4-2 phosphorylation instead of directly phosphorylating OAT itself in cultured cells (D. Xu, Wang, & You, 2016a; D. Xu, Wang, Zhang, & You, 2016; D. Xu, et al., 2017; You, et al., 2000; Q. Zhang, Li, Patterson, & You, 2013). Wolff et al further confirmed such observation by site mutagenesis assay: mutagenesis of five canonical PKC phosphorylation sites individually and in combination resulted in mutants that were insensitive toward specific PKC activator dioctanoylglycerol in cultured cells (Wolff, et al., 2003). The short-term PKC/Nedd4-2 activation increased OAT ubiquitination, leading to an accelerated endocytosis of OATs and a reduction of its cell surface expression and transport activity in cultured cells. In addition, the prolonged PKC/Nedd4-2 activation resulted in the endocytosed OAT to target to proteolytic system for degradation (Fig. 2). (D. Xu, Wang, & You, 2016a; D. Xu, Wang, Zhang, et al., 2016; D. Xu, et al., 2017). Angiotensin II, an endogenous hormone, inhibited OAT1 and OAT3 transport activity through the activation of PKC/Nedd4-2 pathway (P. Duan, Li, & You, 2010; S. Li, Duan, & You, 2009). Nedd4-2 phosphorylation happens not only on serine/threonine residues, but also on tyrosine residues. AG490, a specific inhibitor of the Janus tyrosine kinase 2 (JAK2), reduced OAT3 cell surface expression and transport activity in cultured cells. The reduced transport activity resulted from an enhanced OAT3 ubiquitination, following a reduced Nedd4-2 tyrosine phosphorylation and an enhanced interaction between OAT3 and Nedd4-2. The inhibition effect of AG490 on OAT3 was abrogated by knocking down the endogenous Nedd4-2 using Nedd4-2-specific siRNA (J. Zhang, et al., 2018).

Some modulators of OATs reduce OAT transport activity through Nedd4-2 phosphorylation, whereas others enhance OAT function through phosphorylating Nedd4-2 at different sites. Overexpression of serum and glucocorticoid-regulated kinase 1 (Sgk1) stimulated OAT3 transport activity in cultured cells. It was shown that Sgk1 phosphorylated Nedd4-2 on Ser327, which weakened the interaction between OAT3 and Nedd4-2, and therefore decreased OAT3 ubiquitination (Fig. 2) (H. Wang & You, 2017). Furthermore, Dexamethasone, an upstream hormone of Sgk1, stimulated OAT3 expression and transport activity through Nedd4-2 phosphorylation in cultured cells (H. Wang, et al., 2018). Overexpression of Sgk2, an isoform of Sgk1, enhanced the surface expression, total protein expression, and transporter activity of OAT1 and OAT4 through impairing the binding between OATs and Nedd4-2 and decreasing OAT ubiquitination in cultured cells (Fig. 2). overexpression of Nedd4-2/C821A, a ligase-dead mutant of Nedd4-2, or Nedd4-2 knockdown by Nedd4-2-specific siRNA abrogated the stimulatory effect of Sgk2 on OATs, indicating Sgk2 regulated OATs possibly through Nedd4-2 phosphorylation (H. Wang, et al., 2016; D. Xu, Huang, Toh, & You, 2016). Insulin, an endogenous hormone, increased OAT4 transport activity resulting from an increased OAT4 cell surface. Furthermore, insulin up-regulated OAT4 through phosphorylating Nedd4-2 on Ser327, leading to the impaired

association between OAT4 and Nedd4-2. The up-regulation effect was abrogated by knocking down the endogenous Nedd4-2 by Nedd4-2-specific siRNA (H. Wang, et al., 2019b). In summary, the dynamic phosphorylation of Nedd4-2, a central switch, at different sites exerts opposite regulations of OATs through different modulators, followed by distinct conformational changes of Nedd4-2 and distinct associations between Nedd4-2 and OATs, which leads to the change in OAT transport activity and expression.

7.3. Regulation of OATs by ubiquitin-proteasome system

Ubiquitin, an 8.6 kDa protein, consists of 76 amino acids. The addition of ubiquitin to the lysine residue(s) of a substrate protein is called ubiquitination, and ubiquitination could occur in different types of conjugation including monoubiquitination (conjugation of one single ubiquitin to one single lysine on the substrate), multi-ubiquitination (conjugation of several monoubiquitin molecules to multiple lysine residues on the substrate), and polyubiquitination. Ubiquitin itself has seven lysine residues and an N-terminal methionine residue including K6, K11, K27, K29, K33, K48, K63, and M1, and a polyubiquitin chain is formed between a glycine residue of one ubiquitin molecule and a lysine residue or N-terminus of another ubiquitin molecule. The addition of a polyubiquitin chain to a single lysine residue of the target protein is called polyubiquitination (Komander & Rape, 2012; Pickart & Eddins, 2004). Ubiquitination regulates the target proteins by affecting their activity, stability, cellular location, and their interactions with other proteins.

The ubiquitin-proteasome system (UPS) is a major protein degradation system. Ubiquitination occurs in a sequence of three enzymatic steps and ubiquitinated proteins are targeted to the 26S proteasome for degradation (Bence, Sampat, & Kopito, 2001; Gong, Radulovic, Figueiredo-Pereira, & Cardozo, 2016; Schwartz & Ciechanover, 2009). The UPS has been reported to be involved in modulating OATs via altering cellular location and protein stability (Fig. 3).

7.3.1. Regulation of OATs by ubiquitination and deubiquitination—

Ubiquitination is a PTM that conjugates ubiquitin molecules to target proteins, catalyzed by ubiquitination enzymes, including ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin ligase (E3). Nedd4-1 and Nedd4-2, the two E3 ubiquitin ligases, have been identified as important regulators for OATs (Fig. 3). Phorbol 12-Myristate 13-Acetate (PMA), a PKC activator, inhibited OAT transport activity and expression in cultured cells. Such inhibition resulted from an increased OAT ubiquitination, following PKC-promoted interaction between OATs and Nedd4-2, which led to an accelerated internalization of OATs from cell surface to early endosomes and subsequent degradation (S. Li, Zhang, & You, 2013; D. Xu, Wang, & You, 2016a; Q. Zhang, Suh, Pan, & You, 2012). Overexpression of Nedd4-1 or Nedd4-2 decreased the OAT1 protein expression and transport activity following the enhanced OAT1 ubiquitination in cultured cells. Knocking down endogenous Nedd4-2 with Nedd4-2-specific siRNA or overexpression of Nedd4-2/C821A, a ligase-dead mutant of Nedd4-2, abrogated the PKC-induced change in OAT1 ubiquitination, expression, and transport activity in cultured cells. And PKC-dependent changes in OAT1 ubiquitination, expression, and transport activity were not affected by knocking down endogenous Nedd4-1 or overexpression of Nedd4-1/C867S, a ligase-dead

mutant of Nedd4-1, in cultured cells (D. Xu, Wang, Zhang, et al., 2016). In summary, ubiquitin conjugation to OATs, catalyzed by Nedd4-1 or Nedd4-2, triggers the internalization of OATs from plasma membrane to intracellular endosomes and subsequent degradation in proteolytic systems. As a result, the amount of OATs at the cell surface is reduced, and OAT transport activity is subsequently decreased. Nedd4-1 is mainly involved in the constitutive OAT ubiquitination, whereas Nedd4-2 is largely involved PKC-regulated OAT ubiquitination.

Countering the ubiquitination is a process called deubiquitination that removes ubiquitin molecules from the target proteins by deubiquitinating enzymes (DUBs) (Amerik & Hochstrasser, 2004; Komander, Clague, & Urbe, 2009). Ubiquitination and deubiquitination form an opposing network and are related to a variety of physiological and pathological processes (Cai, Culley, Zhao, & Zhao, 2018; Lee, et al., 2006; Y. Wu, et al., 2018; Zheng, et al., 2016).

To date, approximately 100 human DUBs has been found, and DUBs can be classified into six families including the ubiquitin-specific proteases (USPs), the ubiquitin C-terminal hydrolases (UCHs), the Josephin family, the ovarian tumor proteases (OTUs), Zn-dependent JAB1/MPN/MOV34 metalloprotease DUBs (JAMMs), and the motif interacting with ubiquitin (MIU)-containing novel DUB family (MINDYs). (Abdul Rehman, et al., 2016; Clague, et al., 2013). A variety of membrane proteins, such as channels, receptors, and transporters, are regulated through deubiquitination process by DUBs (Butterworth, et al., 2007; Mines, Goodwin, Limbird, Cui, & Fan, 2009; L. Zhang, et al., 2012; R. Zhou, et al., 2013).

The investigations on the regulation of OATs by DUBs demonstrated that overexpression of USP8 decreased OAT1 ubiquitination, leading to an increased OAT1 expression at the cell surface and an increased OAT1 transporter activity in cultured cells (Fig. 3). No significant difference in OAT1 expression and transport activity was observed in cells transfected with an inactive mutant of USP8 as compared to those in control cells. Furthermore, knocking down the endogenous USP8 in COS-7 cells by USP8-specific siRNA led to an increase in OAT1 ubiquitination which correlated with a reduced OAT1 transport activity (J. Zhang, Liu, & You, 2017).

7.3.2. Regulation of OAT transport activity by proteasome inhibitors—The ubiquitin-proteasome system is the major proteolytic machinery that degrades the majority of ubiquitinated intracellular proteins and certain ubiquitinated membrane proteins in eukaryotic cells (Alam, Farasyn, Crowe, Ding, & Yue, 2017; Jandial, et al., 2009; Ogura, et al., 2011). Cancer cells have enhanced nuclear factor kappa B (NF-kB) activity and are dependent on this signaling pathway for cell survival and proliferation. Proteasome inhibition can down-regulate NF-*k*B-dependent gene expression and lead to an arrest of tumor growth. Besides, proteasome inhibition can stabilize tumor suppressor proteins and repress cell cycle progression. As many cancer cells are highly sensitive compared with normal cells for proteasome inhibition, therefore proteasome has become an important drug target for cancer therapy (Lenos & Vermeulen, 2016; Thibaudeau & Smith, 2019). The 26S proteasome is comprised of a proteolytic 20S core particle (20S proteasome) and one or two

capped 19S regulatory particles for recognizing the ubiquitinated proteins (Voges, Zwickl, & Baumeister, 1999). Bortezomib, carfilzomib, and ixazomib, approved by FDA for the treatment of patients with multiple myeloma, are reversible or irreversible 20S proteasome inhibitors with suppression of chymotrypsin-like activity. The pharmacodynamic study showed carfilzomib significantly inhibited the 20S proteasome activity in the kidney of Sprague-Dawley rats (FDA, 2012a). As ubiquitination is one essential post-translational modification which mediates the regulation of OATs, the alteration of proteasome activity induced by proteasome inhibitors can also potentially affect the transporter function.

At clinically therapeutic concentrations, incubation of OAT1-expressing HEK293 with bortezomib or carfilzomib led to a significant accumulation of ubiquitinated OAT1, suggesting that ubiquitinated OAT1 degraded through proteasomes (Fan, Wang, & You, 2018; Fan & You, 2020). Bortezomib and carfilzomib significantly stimulated transport activity, and there was a positive correlation between the degree of proteasomal inhibition by bortezomib and augmentation of OAT1 transporter activity. Bortezomib- and carfilzomib-induced increase in OAT1 surface expression and transport activity were mainly attributed to a decrease of OAT1 degradation rate (Fig. 3). Therefore, proteasome inhibitors can provide a novel tool to reverse the ubiquitination-induced downregulation of OATs expression and transport activity, indicating their potential influence on the renal OATs-mediated drug disposition and drug-drug interactions. Besides, other proteasome inhibitors are currently in clinical trials, and their influences on the kidney OATs could be attentioned.

7.4. Regulation of OATs by SUMOylation and deSUMOylation

SUMOylation and deSUMOylation, a pair of opposing and dynamic PTMs, refer to the process, which adds SUMO to or removes SUMO from lysine residue of the target protein, catalyzed by specific enzymes. SUMOylation and deSUMOylation create an on and off switch which is essential for biological regulations and are involved in various cellular processes in health and disease (Flotho & Melchior, 2013; C. Guo & Henley, 2014; Zhao, 2007).

7.4.1. Regulation of OATs by SUMOylation—SUMOylation is another type of post-translational modification known as a crucial regulatory mechanism of protein function on both nuclear proteins and cellular membrane proteins (Gareau & Lima, 2010; Gill, 2004; Kang, Saunier, Akhurst, & Derynck, 2008; Plant, et al., 2010; Rajan, et al., 2015; Rajan, Plant, Rabin, Butler, & Goldstein, 2005; Ulrich, 2005, 2008). Till now three functional isoforms (SUMO1-3) have been identified in mammals, and all three isoforms are expressed in a wide range of tissues, such as brain, lung, liver, Pancreas, and kidney. SUMO2 and SUMO3 are usually written as SUMO2/3 since they share 97% identity in their amino acid sequences, while SUMO2/3 only shares 50% homology with SUMO1. Although SUMO proteins are considered as the member of the ubiquitin-like protein family, they only share approximately 18% identity with ubiquitin, and all three SUMO proteins are polypeptides of ~12 kDa. Like ubiquitination, the conjugation of SUMO to target proteins also involves a series of enzymatic steps. The inactive precursors of SUMO proteins are initially processed by members of the SUMO1/sentrin specific peptidase (SENP) family to truncate a ten amino acid long fragment from the C terminus, therefore exposing a C-terminal diglycine motif to

mature the SUMO proteins. Then the SUMO-activating enzyme (E1) catalyzes the ATP-dependent formation of a thioester bond between the C terminus of matured SUMO and the active cysteine residue of a SUMO-activating enzyme (E1). The activated SUMO is later transferred to a SUMO-conjugating enzyme (E2). Eventually, SUMO is attached to the specific lysine residue on the target protein with the facilitation of SUMO protein ligase (E3). A SUMO substrate can be modified by various types of SUMO conjugation: monoSUMOylation (conjugation of one single SUMO to one single lysine on the target protein), multiSUMOylation (conjugation of several monoSUMO molecules to multiple lysine residues on the target protein), and polySUMOylation (extended polySUMO chain). Majority of SUMO substrates contain the consensus motif, Ψ-K-x-D/E (where ψ is a large hydrophobic residue, K is the lysine conjugated to SUMO, x is any amino acid, E is a glutamic acid, and D is an aspartic acid). SUMO2 and SUMO3 contain internal SUMO consensus motifs, and therefore are capable of forming polySUMO chains, whereas SUMO1 does not share such property.

Recently published work showed that in COS-7 cells, OAT3 transport activity and expression at the plasma membrane were increased by short term PKA activation. Such increase resulted from the enhanced rate of OAT3 recycling with no change in the rate of OAT3 internalization. In addition, OAT3 was identified as a SUMO substrate, and the conjugation of SUMO2/3 to OAT3 was also PKA-dependent. PKA activation enhanced OAT3 SUMOylation, and such enhancement can be abrogated by the presence of PKA-specific inhibitor H-89 (Fig. 4) (H. Wang, Zhang, & You, 2019a). In Oat3 knockout mice, bile acids such as cholic acid and taurocholic acid accumulated. Both substances are endogenous substrates of Oat3 and can activate G protein-coupled receptors (GPCRs) (Deutschmann, et al., 2018; Duboc, Tache, & Hofmann, 2014). The activation of GPCRs elevated the cAMP level leading to PKA signaling pathway activation. PKA enhanced OAT3 SUMOylation, recycling rate, and transport activity. Thus, the accumulation of bile acids contributed by OAT3 reduction could potentially result in the upregulation of OAT3 expression and function to form a negative feedback loop. This connection between OAT endogenous substrates and PTMs of OATs is an interesting area to explore. We now know OAT3 is the substrate of SUMOylation. However, which lysine residues on OAT3 are responsible for SUMO2/3 conjugation is still unknown. Further investigations of mapping the SUMO conjugation sites on OAT3 is needed.

7.4.2. Regulation of OATs by deSUMOylation—SUMOylation is a dynamic and reversible event, and SUMO is removed from target protein by SUMO-specific proteases including members of ubiquitin-like specific protease (Ulp, in yeast) and SENP family (in mammals) (Han, Feng, Gu, Li, & Chen, 2018; Hannoun, Greenhough, Jaffray, Hay, & Hay, 2010; Miura & Hasegawa, 2010; Ulrich, 2005). So far, six human Senp proteins have been isolated and shown to have the ability to de-conjugate SUMO. Among them (Yeh, 2009), Senp2 is identified to travel between the nucleus and the cytoplasm, modulating the activities of some plasma membrane proteins including receptors, channels, and transporters (Benson, et al., 2007; Itahana, Yeh, & Zhang, 2006; Qi, et al., 2014; Tan, et al., 2017).

It was recently revealed that in COS-7 cells, overexpression of Senp2, a member of the SENP family, resulted in a decreased OAT3 SUMOylation, which paralleled well with a

reduced OAT3 expression and transport activity. Furthermore, knocking down the endogenous Senp2 with Senp2-specific siRNA led to an enhanced OAT3 SUMOylation, which paralleled well with an increased OAT3 expression and transport activity. Coimmunoprecipitation experiments revealed that Senp2 directly interacted with OAT3/Oat3 both in COS-7 cells and in rat kidneys (Fig. 4) (H. Wang, You, G., 2019).

Senps have been reported as key regulators in upholding a balance between SUMOylated and unSUMOylated proteins that are crucial for physiological homeostasis. Many investigations indicated the alterations in the amount of Senps under pathophysiological conditions, and Senps were associated with the progress of a number of diseases, especially cancer. For example, the level of Senp2 was decreased in bladder cancer and hepatocellular carcinoma (HCC) tissues, and the hyperexpression of Senp2 resulted in the suppressions on both bladder cancer metastasis and HCC development (Shen, Zhu, Yang, & Ji, 2012; Tan, et al., 2017). Other than cancers, the overexpression of Senp2 played an important role in the development of congenital heart defects and cardiac dysfunction by enhancing deSUMOylation (Kim, et al., 2012). Thus, Senps have received increasing recognition as interesting targets for drug discovery. 1,2,5-Oxadiazoles were developed as a new class of Senp2 inhibitors, which could have the therapeutic potential for many diseases (Kumar, Ito, Takemoto, Yoshida, & Zhang, 2014). Further studies exploring the effects of Senp2 inhibitors on OAT transport activity, surface expression, and SUMOylation would be very exciting.

7.5. Regulation of OATs by Glycosylation

Glycosylation, a common and complex PTM of proteins, is the covalent attachment of carbohydrates to specific residues of a target protein, which expands the proteasome complexity and modulates the target protein via changes in cellular location, protein stability, protein structure, and protein activity (Eichler, 2019). Glycosylation are classified into several different protein-sugar linkages, such as N-glycosylation, O-glycosylation, C-glycosylation, S-glycosylation, and P-glycosylation. N-glycosylation and O-glycosylation are predominantly found in eukaryotes, and N-glycosylation accounts for more than half of the protein glycosylation in eukaryotes. N-glycosylation refers to the attachment of a glycan to the asparagine residue of the target protein within a consensus peptide sequence (Asn-X-Ser/Thr, X can be any amino acid except proline). For membrane proteins, endoplasmic-Golgi pathway, glycosidases, and glycosyltransferases are involved in the glycosylation process (Christiansen, et al., 2014). Many membrane proteins have been identified as the substrates of glycosylation, and glycosylation plays a critical role in regulating the function and activity of those membrane proteins (L. B. Li, et al., 2004).

OAT1 has been reported as the substrate of glycosylation. Asp-39 on OAT1/Oat1 is crucial for substrate recognition of glycosylation, and glycosylation is essential for the targeting of OAT1/Oat1 onto the plasma membrane (Tanaka, Xu, Zhou, & You, 2004). Furthermore, mutagenesis of glycosylation sites on OAT4 and treatment of tunicamycin, a glycosylation inhibitor, resulted in a non-glycosylated OAT4 and the failure of targeting OAT4 onto the plasma membrane in cultured cells. In addition, OAT4 expressed in CHO-Lec1 cells, carrying oligosaccharides bearing mannose-rich intermediates, had reduced binding affinity

towards the substrates compared with OAT4 in CHO wild-type cells, and it was concluded that processing of added oligosaccharides from mannose-rich type to complex type was important for modulating OAT4 substrate binding affinity (F. Zhou, et al., 2005). Congenital disorders of glycosylation (CDG), an ever-expanding disease, are a group of inherited metabolic disorders, which affecting glycosylation process. As many steps and enzymes are involved in the glycosylation process, CDG patients commonly are deficient of one or more enzymes for one or more glycosylation steps and show variable clinical symptoms including multi-organ dysfunction (Bryant, et al., 2020; Ferreira, et al., 2018). Kidney disfunction, nephropathy, and lesion of proximal tubule, where OATs are mainly expressed, are symptoms of CDG, and glycosylation is critical for modulating transport activity of OATs. Thus, OAT function and expression in CDG patients would be interesting and exciting to investigate.

7.6. Crosstalk between various PTMs

During the past decade, evidence for comprehensive crosstalk between different PTM types has stacked up, especially the interplay between the PTMs occurring on the same type of amino acid residue(s). One of the examples is the crosstalk between ubiquitination and SUMOylation, in which both ubiquitin and SUMO covalently conjugate to the lysine residue (s) of a substrate protein. One scenario is that ubiquitin and SUMO modify the same lysine residue(s) in a target protein through competitive manner. On the other hand, SUMO and ubiquitin may modify different lysine residues in a substrate protein. In such case conjugation of SUMO may potentially mask a nearby ubiquitin conjugation site. Under both circumstances, SUMOylation may preclude the ubiquitin-mediated degradation of target protein (Hunter & Sun, 2008). It was demonstrated that the enhancement of OAT3 SUMOylation by PKA activation paralleled with a decrease in OAT3 ubiquitination in cultured cells. Therefore, SUMOylation and ubiquitination may coordinately regulate OAT3 through crosstalk (H. Wang, et al., 2019a).

Other PTMs, which also occur on lysine residue(s) of the target protein, could potentially be involved in the crosstalk with both ubiquitination and SUMOylation. Besides the direct competitions on the same lysine residue(s) among these modifications, underlying mechanism can be more complex and may not follow a uniform rule (Appikonda, et al., 2018; Caron, Boyault, & Khochbin, 2005; Gareau & Lima, 2010). In addition, the interplay could also happen between two or more PTMs modifying different types of amino acid residues (Hietakangas, et al., 2003; Muller, Matunis, & Dejean, 1998; Nguyen, Kolch, & Kholodenko, 2013; Rajan, et al., 2015; Yang, Jaffray, Senthinathan, Hay, & Sharrocks, 2003). It would be interesting to explore new potential crosstalk among various PTMs of OATs.

7.7. Remote Sensing and Signaling Hypothesis of OATs

In the recent years, systems biology studies together with the investigations of OAT knockout mice have indicated that the roles of OATs in different organs may form a network, and this network allows the intercellular and inter-organ communication. Such communication between cells, as well as between organs, regulates the local and whole-

body homeostasis. This hypothesis is called remote sensing and signaling (Ahn & Nigam, 2009; S. K. Nigam, 2018; S. K. Nigam, et al., 2015).

The overlapping of substrate specificities among different OAT isoforms, the wide tissue distributions of different OAT isoforms (e.g., kidney, liver, brain, placenta, retina, olfactory mucosa, etc.), and the various regulations of OAT expression and function mediated by signaling molecules secreted from remote tissues into the body fluid contribute to the complicated communication network of OATs (Burckhardt, 2012; Roth, Obaidat, & Hagenbuch, 2012; You, 2002). For example, it was reported that indoxyl sulfate, a gut microbiome-derived metabolite and endogenous OAT substrate, up-regulated OAT1 via AhR and EGFR signaling under the control of miR-223 in cultured cells, and the up-regulation on OAT1/Oat1 was to react to the elevated indoxyl sulfate level and to maintain homeostasis through inducing renal secretion. This phenomenon was observed in cultured cells, rat kidneys, and human kidneys (Jansen, et al., 2019). Oat3 was involved in the regulation of blood pressure through the remote sensing and signaling. The blood pressure of Oat3 knockout mice was 15% lower than that of control mice. Metabolomic analysis indicated that plasma concentrations of Oat3 substrates were increased in Oat3 knockout mice, and some endogenous Oat3 substrates could serve as vasodilators, such as thymidine, cAMP, and cGMP, to reduce the blood pressure (Vallon, Eraly, et al., 2008; Vallon, Rieg, et al., 2008). In addition, the renal excretion of vasodilators, the substrates of Oat3, was reduced responding to the elevated blood pressure, caused by internally and externally environmental changes. And then the accumulated vasodilators subsequently decreased the blood pressure thereby maintaining homeostasis (Ahn & Nigam, 2009; Eraly, 2008).

Hormones and growth factors produced and released from the original organ under the internal and external stimuli arrive at the target organ and regulate the OATs in the target organ through binding to the receptors and activating the downstream signaling pathways. For example, Oat3 expression and transport activity were impaired in the streptozotocin-induced type 1 diabetic rats compared with those in wild-type rats, and insulin treatment abolished the effects. Streptozotocin was used to damage insulin-producing beta cells in the pancreas to induce diabetes in animal models. Protein kinase C alpha (PKCα) and phospho-PKCα expression were increased in diabetic rats, and insulin treatment reversed the effects (Phatchawan, et al., 2014). In addition, insulin stimulated OAT4 expression and transport activity through impairing the interaction between Nedd4-2 and OAT4, and Nedd4-2 knockdown abolished the stimulation effect of insulin on OAT4 in cultured cells (H. Wang, et al., 2019b). IGF-1, produced in the liver under stimuli, up-regulated renal OAT3 function through PKA following binding to its receptor in cultured cells, which was abrogated by PKA inhibitor H89 and linsitinib (J. Zhang, et al., 2020). Besides, it was demonstrated that Angiotensin II, produced in adrenal gland, reduced OAT1 and OAT3 function through PKC in cultured kidney cells (P. Duan, et al., 2010; S. Li, et al., 2009). Furthermore, parathyroid Hormone, produced in parathyroid glands in the neck, enhanced OAT4 activity through a PKA independent pathway in cultured kidney cells (P. Duan, Li, & You, 2012). In remote sensing and signaling model, OATs play an essential role in intercellular and inter-organ communication and in maintaining local and whole-body homeostasis. Such complex and dedicated communication is carried out by hormones, small molecules and cell signaling.

8. Conclusion

Elucidating the mechanisms by which OATs are regulated contributes significantly to our knowledge of the processes involved in physiology, pathology, and drug disposition. It has become increasingly clear that OATs are regulated by a variety of extracellular factors originated from different organs, by multiple intracellular signaling pathways, and by various PTMs. Our overview of several important OAT regulators provides insights into the complexity of this process. Most studies have examined these regulatory factors in isolation. How they work in concert to modulate OAT function, to improve OAT-related medical treatment, and to keep body homeostasis are important questions that continue to be addressed.

Acknowledgements

This work was supported by grants (to Dr. Guofeng You) from National Institute of General Medical Sciences (R01-GM079123, R01-GM097000 and R01-GM127788).

Abbreviations

OAT	organic anion transporter
TMD	transmembrane domain
DDI	drug–drug interaction
Nedd4-1/Nedd4-2	neural precursor cell expressed developmentally down-regulated 4-1/4-2
PTM	post-translational modification
DUB	deubiquitinating enzyme
Sgk	serum- and glucocorticoid-inducible kinase
PKA	protein Kinase A
PKB	protein Kinase B
PKC	protein Kinase C
USP8	ubiquitin-specific protease 8
SENP	SUMO1/sentrin specific peptidase
IGF-1	Insulin-like growth factor 1

Reference

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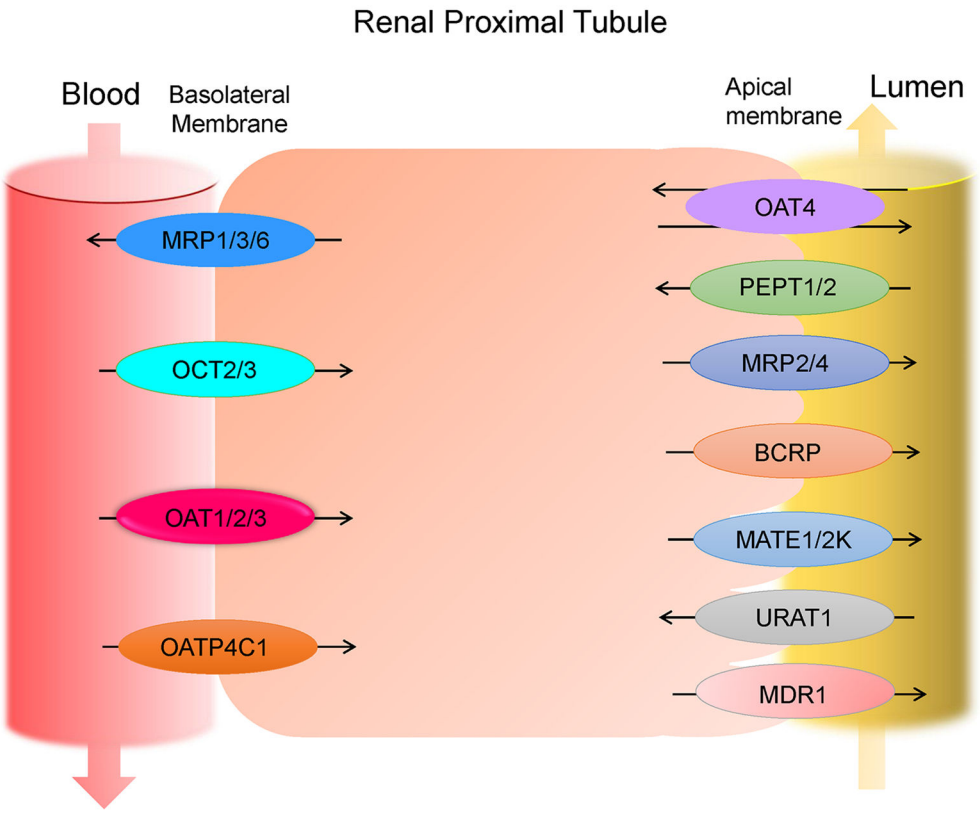

Fig. 1.

Major drug transporters expressed in human renal proximal tubule cells. MRP: multidrug resistance-associated protein, OCT: organic cation transporter, OAT: organic anion transporter, OATP: organic anion-transporting peptide, MATE: multidrug and toxin extrusion protein, PEPT: peptide transporter, BCRP: breast cancer resistance protein, MDR: multidrug resistance mutation, URAT: urate transporter.

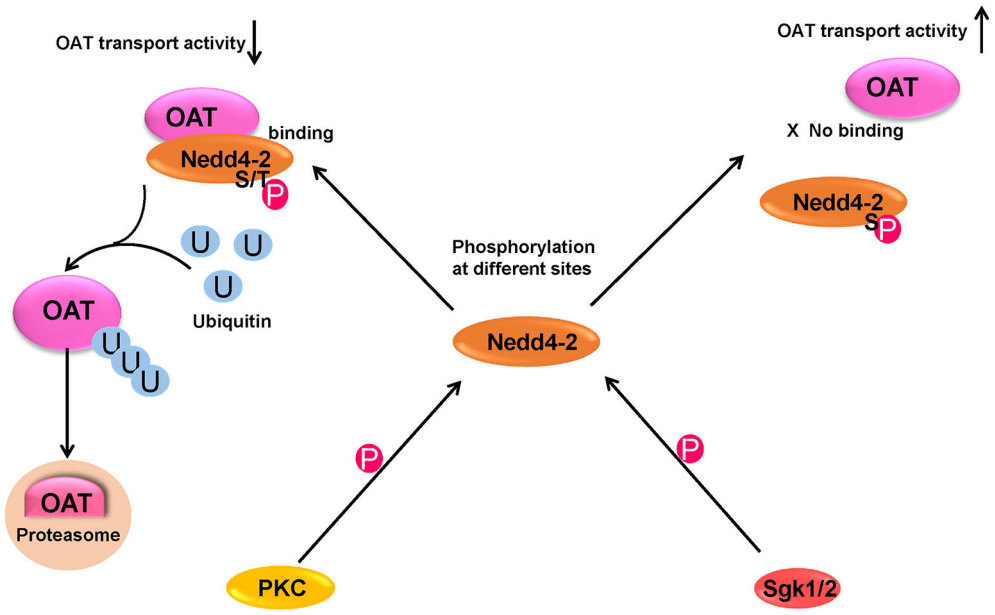

Fig. 2.

Phosphorylation of Ubiquitin ligase Nedd4-2 mediates the regulation of OAT transport activity by various kinases. U: ubiquitin, P: Phosphoryl group, S: Serine, T: Threonine, PKC: Protein kinase C, Sgk1/2: Serum- and glucocorticoid-inducible kinase 1/2, Nedd4-2: Neural precursor cell expressed developmentally down-regulated 4-2.

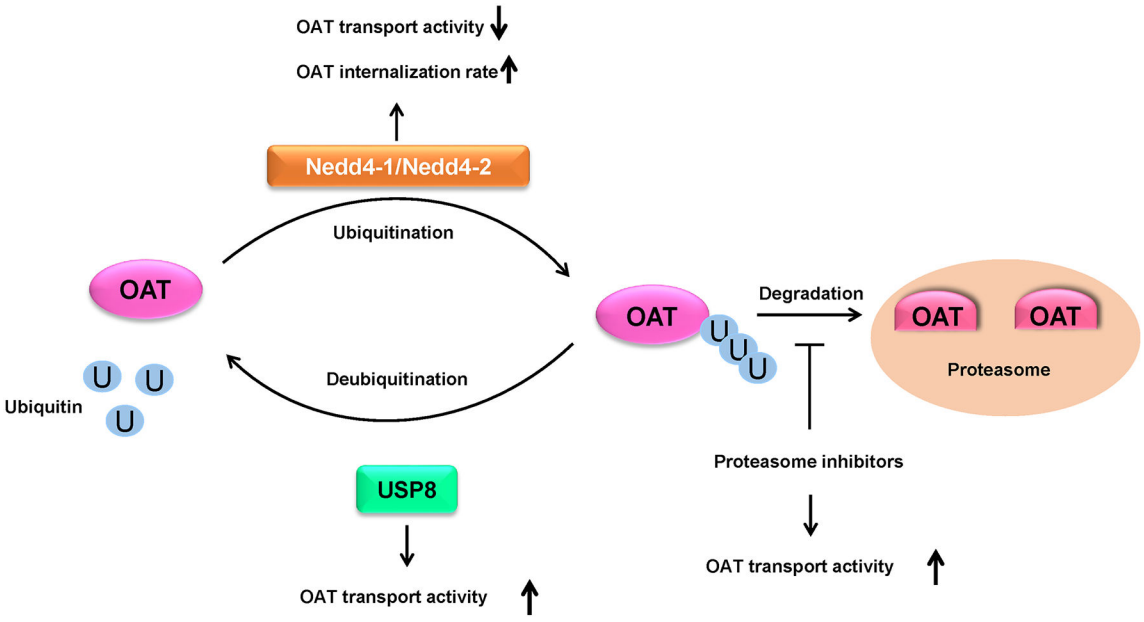

Fig. 3.

Regulation of OATs by Ubiquitination, deubiquiting enzyme USP8, and proteasome inhibitors. U: ubiquitin, USP8: ubiquitin-specific proteases 8, Nedd4-1/Nedd4-2: Neural precursor cell expressed developmentally down-regulated 4-1/4-2.

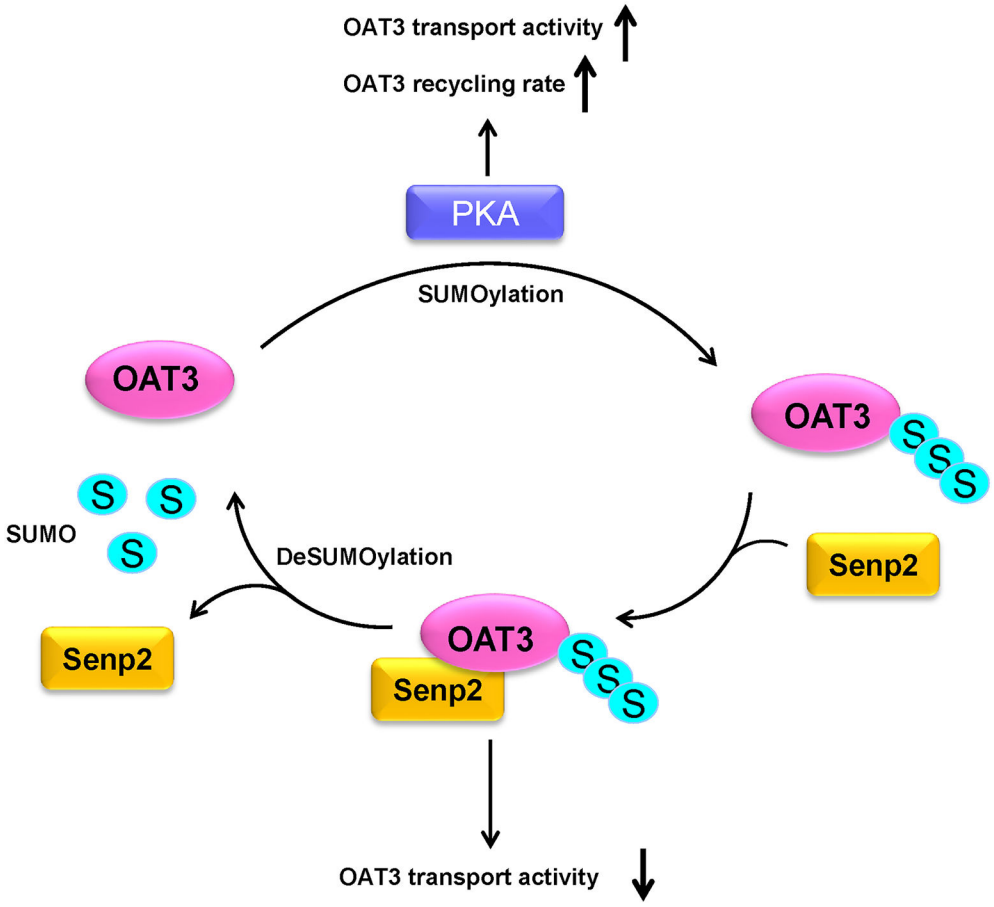

Fig. 4.

Regulation of OATs by SUMOylation and deSUMOylation enzyme Senp2. S: SUMO, Senp2: SUMO1/sentrin specific peptidase 2, PKA: Protein kinase A.