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REVIEW ARTICLE

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Roles of organic anion transporters (OATs) and a urate transporter (URAT1) in the pathophysiology of human disease

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Abstract
Renal proximal and distal tubules are highly polarized epithelial cells that carry out the specialized directional transport of various solutes. This renal function, which is essential for homeostasis in the body, is achieved through the close pairing of apical and basolateral carriers expressed in the renal epithelial cells. The family of organic anion transporters (OATs), which belong to the major facilitator superfamily (*SLC22A*), are expressed in the renal epithelial cells to regulate the excretion and reabsorption of endogenous and exogenous organic anions. We now understand that these OATs are crucial components in the renal handling of drugs and their metabolites, and they are implicated in various clinically important drug interactions, and their adverse reactions. In recent years, the molecular entities of these transporters have been identified, and their function and regulatory mechanisms have been partially clarified. Workers in this field have identified URAT1 (urate transporter 1), a novel member of the OAT family that displays unique and selective substrate specificity compared with other multispecific OATs. In the OAT family, URAT1 is the main transporster responsible for human genetic diseases. In this review, we introduce and discuss some novel aspects of OATs, with special emphasis on URAT1, in the context of their biological significance, functional regulation, and roles in human disease.

Key words Organic anion transporter · Urate (uric acid) · Urate transporter · Organic cation transporter

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Introduction

In the kidney, the renal proximal and distal tubules have two important roles: they remove waste products from the blood to the urine, and at the same time they regulate the blood levels of many important molecules. These important function of the tubules is achieved by a cohort of transporters and channels expressed in the apical and basolateral membranes of the cells. Expressing specifically in the appropriate domains of cell membranes, the transporters and channels carry out bidirectional transport of their essential substrates across the tubular epithelial cells, which regulates the concentration and the equilibrium of these substrates.

Research into the molecular basis of transporters has progressed over the past 10 years. Among diverse transport systems in the kidney, the organic anion transport system has been the focus of intense scientific and medical interest because of its roles in the excretion of many clinically important pharmaceuticals.[1–3] In recent years, molecular cloning approaches have identified several members of "multispecific" organic anion transporters (OATs) which belong to the amphiphilic solute transporter family (*SLC22A*) with organic cation transporters (OCTs) (Fig. 1).[4,5] To date, five members of the OAT family (OAT1 – OAT5) have been identified and functionally characterized.[6–14] One of the hallmarks of the OAT family is their ability to accept a wide variety of organic compounds (multispecific), which requires only a hydrophobic backbone and a negative charge in the structures of their substrates.[15] Studies of the substrate selectivity and specificity of OATs revealed that a wide variety of drugs are good substrates for OATs. They include antibiotics, nonsteroidal antiinflammatory drugs (NSAIDs), loop and thiazide diuretics, angiotensin converting enzyme (ACE) inhibitors, anticancer drugs, and antivirals, suggesting that these transporters are regulators of the blood concentrations of the drugs by manipulating their excretion and reabsorption in the kidney[1–3] (Fig. 1).

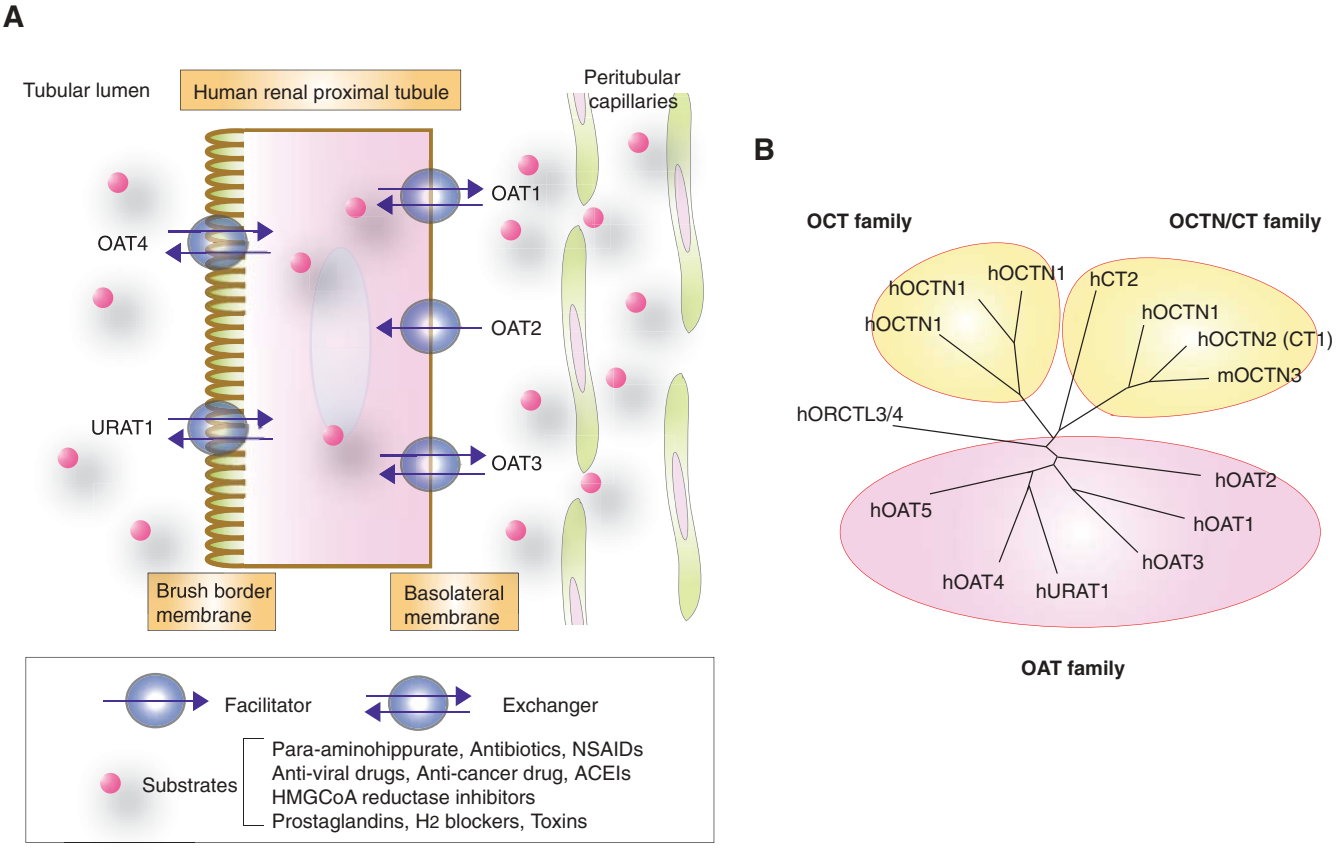

Fig. 1. **A** Transepithelial transport of organic anions by the organic anion transporter (OAT) family in human renal tubular cells. Basolateral entry of organic anions from the peritubular capillaries into the cells occurs through OAT1, OAT2, and OAT3, which are expressed in the basolateral membrane, followed by apical exit of organic anions through OAT4. OAT1 and 3 mediate the transport of their substrates through exchange for intracellular anions such as dicarboxylates.[1–3,74] It has been shown that OAT4 may contribute not only to the apical efflux but also to reabsorption of its substrates from the tubular lumen through dicarboxylate exchange.[75] Urate transporter 1 (*URAT1*) reabsorbs urate in the lumen through exchange with intracellular anions such as lactate and nicotinate (see Fig. 3). **B** Phylogenetic tree of transporters (*SLC22A* family) that are homologous to OATs. The amino-acid sequences of transporters that are homlogous to OATs are aligned using CLUSTALW, and the unrooted phylogenetic tree was created with TreeView. Similarities were calculated from the matrix of the pairwise similarity scores. *NSAID*, nonsteroidal anti-inflammatory drug; *ACEI*, angio tension converting enzyme inhibitor; *OCT*, organic cation transporter; *OCTN*, novel organic cation transporter

Although the roles of the OAT family in the renal handing of various drugs have been understood, the biological importance of each member in a whole tissue or organ and its responsibility for human disease have been largely unknown because of a lack of generation of the corresponding knockout mice, and no discovery of a human genetic disease in which genes encoding OATs are impaired. The substrate specificity for OAT family members overlaps considerably, which makes it difficult to assess the individual contribution of each transporter to the tissue transport capacity as a whole. Recently, the generation and characterization of OAT3 knockout mice has been reported, which described for the first time the biological roles of OATs in vivo.[16] This paper describes the contribution of OAT3 in tissue function, and supports the notion that genetic alterations such as mutations or single nucleotide polymorphisms (SNPs) in the OAT genes may be involved in the etiology and pathophysiological processes of human diseases. Recently, it was confirmed that hereditary renal hypouricemia is caused by mutations in a gene that codes URAT1, a uric acid transporter 1, which is a new member of the OAT family.[17] This emerging evidence confirms the important roles of the transporters in human genetic or common (non-genetic) diseases. Here, we first describe the function and regulation of URAT1 that is essential for urate homeostasis, and then focus on the roles of URAT1 and OATs in the pathophysiology of human diseases such as renal hypouricemia and chronic renal failure.

Function of URAT1

Urate transport in the human kidney

In vertebrates, urate (uric acid) is an intermediate product of purine metabolism, which is oxidized to water-soluble

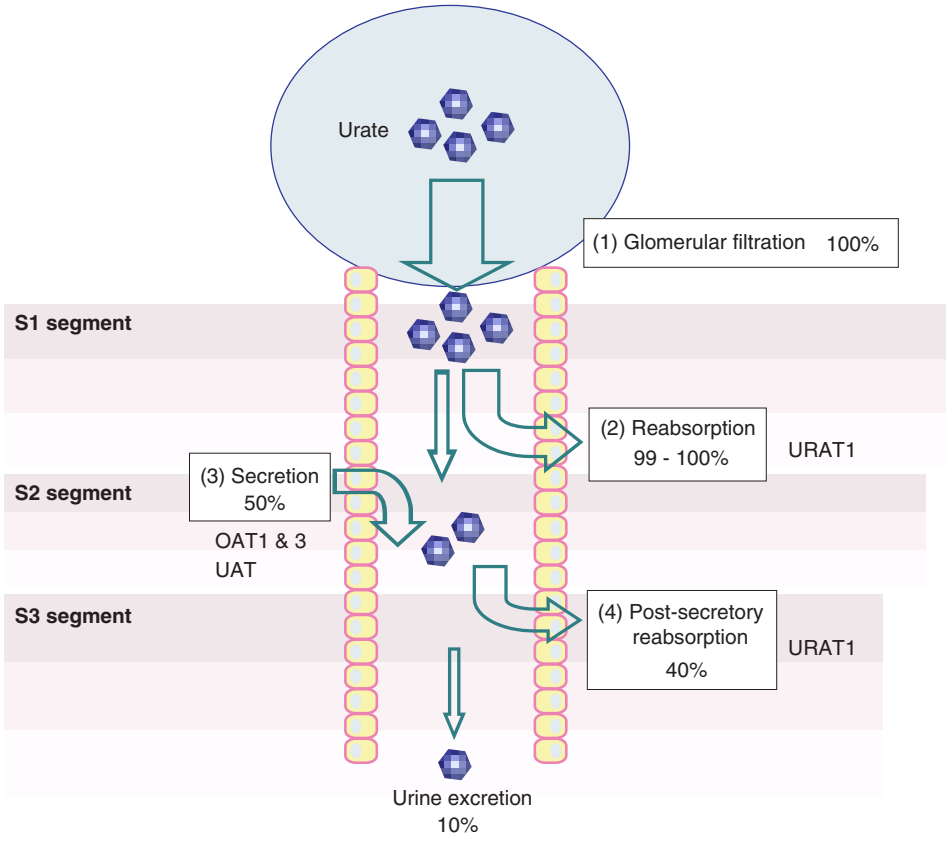

Fig. 2. 4-component model of urate handling in the human kidney. (1) Filtration, (2) reabsorption, (3) secretion, and (4) postsecretory reabsorption in the S1, S2, and S3 segments of the proximal tubules. The *arrows* denote the predominant direction of net transport. Reabsorption and postsecretory reabsorption are mediated by URAT1, whereas secretion is mediated by OAT1, 3, and the urate channel (*UAT*)[74,76,77]

allantoin by hepatic uricase (urate oxidase).[15] However, for higher primates such as humans or humanoids who have lost the uricase activity by mutational silencing, urate becomes a final breakdown product of purine metabolism,[18,19] and an accumulation of urate in the blood sometimes poses special problems for humans because of its limited solubility. Therefore, it is important to understand the urate handling mechanism in the kidney because the under-excretion of urate has been implicated in the development of hyperuricemia that leads to problems such as gout, hypertension, and cardiovascular diseases. Since the 1950s, it has been known that urate transport mechanisms in the mammalian kidney are complicated[20] because urate is transported bidirectionally, in contrast to other organic solutes.[15] This model, called the "4-component theory," is based on micropancture studies using experimental animals, and includes glomerular filtration, reabsorption, secretion, and postsecretory reabsorption (Fig. 2).[15,21,22] This complexity has made it difficult to identify the molecular mechanisms of the urate transport system in the kidney.[15] Moreover, urate handling is highly species-dependent.[15,22] For example, humans actively reabsorb urate in the tubular lumen, so that the fractional excretion of urate (FE_{urate}) is very low (10%) (net absorption). In contrast, pigs and rabbits excrete more urate than is filtered through the glomerulus and have a fractional urate excretion in excess of the filtered load of urate (net secretion). Thus, the plasma urate concentration is much higher in humans (about 300 μM) than in other mammals.

Identification and function of URAT1

We have recently identified a new member of the OAT family, URAT1 (urate transporter 1), as a transporter molecule for urate reabsorption,[17] Because urate exists primarily as a weak acid at physiological pH, we speculated that the urate transporter belongs to the OAT family. We started by searching the human genome sequence[23] for genes related to OAT genes, and found the URAT1 gene (*SLC22A12*) (in silico cloning). URAT1 is expressed only in the kidney, where the protein is located at the apical (luminal) membrane of the epithelium of proximal tubules but not of distal tubules (Fig. 3A). Functional expression experiments using *Xenopus* oocytes showed that URAT1 has the transport activity of urate but not of various typical substrates of OATs and OCTs, indicating that the URAT1 pathway constitutes a specific mechanism for the reabsorption of urate from the tubular lumen (extracellular) to the cytosol (intracellular) at the proximal tubules (Fig. 3B). Inhibition experiments revealed that the transport pathway is shared selectively by monovalent anions such as lactate, nicotinate, acetoacetate, and hydroxybutyrate. Pyrazinecarboxylic acid (PZA), nicotinate (a structural analogue of PZA), and orotate, which are monocarboxylates with aromatic structures similar to that of urate and having pyrimidine and imidazole structures, inhibited URAT1 more effectively. Various uricosuric substances[15] known to reduce hyperuricemia, such as probenecid, fenylbutazone, sulfinpyrazone, NSAIDs, and diuretic drugs, effectively *cis-*

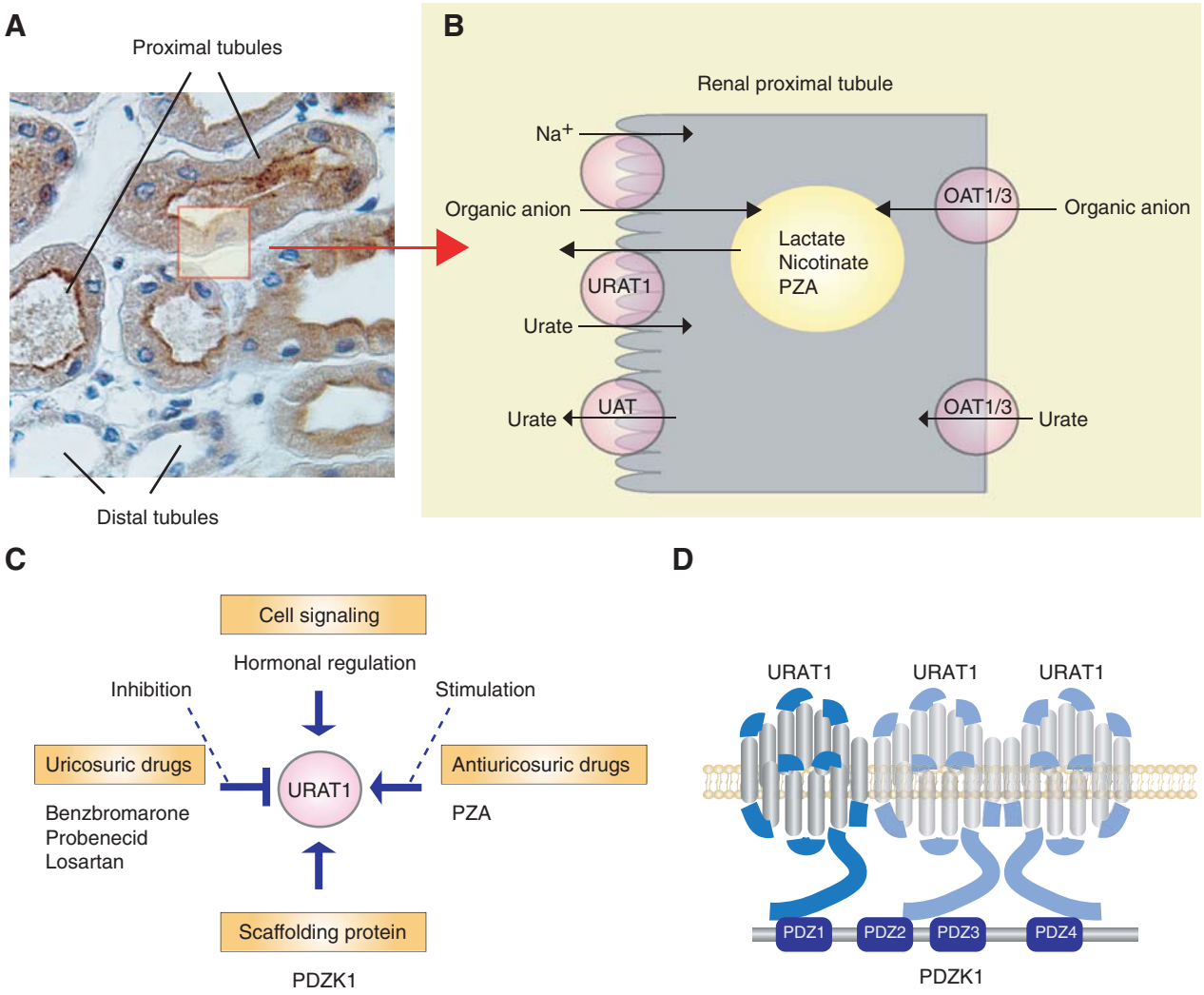

Fig. 3. **A** Immunohistochemical detection of URAT1. Brown denotes URAT1 protein that localizes in the luminal membrane of the proximal tubules but not of the distal tubules in human kidney. **B** Urate uptake via URAT1 was *trans*-stimulated by intracellular organic anions. A model of the indirect coupling of sodium and urate transport via URAT1. The coupling of anions to sodium uptake along the luminal membrane, and the later exchange of the anions for urate by URAT1 in the proximal tubule. The organic anions that are actively pumped into the proximal cells from the apical or basolateral sides, or produced in the cells, should favor urate reabsorption by leaving the cells in exchange for luminal urate. The transporters responsible for the secretion of urate are basolateral OAT1, OAT3, and luminal UAT.[74,76,77] Electrogenic Na^+/anion cotransporter(s) that express in the luminal membrane have not yet been identified. **C** Regulation of URAT1 by various compounds, cell signaling and scaffolding proteins. Drugs or agents with an affinity for URAT1 (probenecid etc.) are uricosuric (i.e., stimulating urate excretion by the inhibition of URAT1) when acting from the lumen, but PZA is antiuricosuric (which affects urate excretion) by driving the urate influx by acting from the intracellular space. Other components such as signaling molecules and scaffolding proteins are potent regulators of URAT1. **D** Schematic presentation of the association between URAT1 and PDZK1. PDZK1 possesses four PDZ domains. A yeast two-hybrid assay and an in vitro binding assay indicated that the first, second, and fourth PDZ domains are associated with the intracellular C-terminal region of URAT1 that contains a PDZ domain-binding motif[46]

inhibited URAT1. Benzbromarone (a uricosuric agent used clinically) was the most potent, completely inhibiting the urate uptake via URAT1. Interestingly, losartan, an antagonist of the angiotensin II receptor that produces a transient uricosuria (a decrease in urate level) and a concomitant decrease in the blood urate concentration in healthy volunteers and hypertensive patients,[24] also inhibited the urate uptake, indicating that the uricosuric property of losartan is due to the inhibition of urate reabsorption by acting from the lumen in the proximal tubules. The driving force for urate transport via URAT1, which is the exchange of luminal urate and intracellular organic anions (Fig. 3B), was determined.[17] The intracellular accumulation of the organic anions for which URAT1 has an affinity will favor the uphill reabsorption of urate in exchange for these anions, which move down their electrochemical gradients into the tubular lumen (Fig. 3B). Intracellular anions are made available by their uptake from the glomerular filtrate across the luminal membrane, by uptake from peritubular capillaries by basolateral transporters (OATs), and by cell metabolism.[17,25] Thus, drugs or agents with an affinity for URAT1 will be uricosuric when acting from the lumen, whereas they

will be antiuricosuric, by driving the influx of urate, when acting from the intracellular space, consequently regulating blood urate levels (Fig. 3C). This model explains the elevation of blood urate levels in patients treated with pyrazinamide, a widely used antituberculosis agent.[26,27] As shown above, PZA, an active metabolite of pyrazinamide, has a high affinity for URAT1 and *trans*-stimulates urate uptake when acting from the intracellular space on the luminal membrane. Consequently, it drastically reduces the rate of renal urate clearance to less than 1% in the humans, resulting in hyperuricemia. The effect of PZA clearly demonstrates that urate reabsorption in the human kidney is considerable.

URAT1 is a novel target of drugs against hyperuricemia

Therapeutics that are designed to modify URAT1 transport activities might be useful in treating pathologies that are associated with hyperuricemia, such as gout and kidney stones. As described above, URAT1 displays selective substrate specificity compared with other multispecific transporters, making it an attractive target of drugs that prevent urate reabsorption. Continuing studies into the pathways via URAT1 hold the promise for the development of new, more effective therapeutics for hyperuricemia.

URAT1 and human disease

Kikuchi et al.,[28] Igarashi et al.[29] and others[30,31] have previously described patients with idiopathic renal hypouricemia (Mendelian inheritance in man 220150)[32] with exercise-induced acute renal failure and chronic renal dysfunction. Renal hypouricemia is a hereditary disease characterized by increased renal urate clearance caused by an isolated inborn error in membrane transport for urate in the renal proximal tubule.[33–37] The majority of reported cases have been in Japanese and non-Ashkenzai Jews.[33] Patients with renal hypouricemia manifest low levels of serum urate levels (less than 2.0 mg/dl) without any underlying renal or systemic diseases such as Fanconi syndrome, Wilson disease, or drug-induced tubulopathy. They are usually asymptomatic except for the development of nephrolithiasis or exercise-induced acute renal failure (described below) . It has been reported that the fractional excretion of urate of these patients is 95 ± 10%, (normal range less than 10%), and they show only mild or no response in pyrazinamide and benzbromarone loading tests, indicating that they have selective defects in the urate reabsorption mechanism in their kidney.[33–37] We[17,28,29] have analyzed *SLC22A12* (the URAT1 gene) in selected patients and found that some patients with renal hypouricemia have defects in *SLC22A12*. Mutational analyses in a more systemic and larger populations have been reported by Komoda et al.[38] and Tanaka et al.[39] Recently, Ichida et al.[40] elucidated the correlation between the clinical and genetic features of patients with renal hypouricemia, and described the significance of URAT1 in the regulation of serum urate levels in vivo using 32 unrelated patients. They detected *SLC22A12* mutations in 54 of 64 alleles, and ten mutations were identified, which included two nonsense mutations, six missense mutations, one splice-site mutation, and a 5-bp deletion. The most frequent mutation (74.1%) was G → A transition at nucleotide 774 within exon 4 of *SLC22A12*, changing tryptophan-258 (TGG) to a stop codon (TGA) and producing premature truncated proteins that lack the half of the protein (W258X).[40] Expression of the mutant cDNA in *Xenopus* oocytes revealed that the truncated protein could not be targeted on the cell membrane, suggesting that the mutant lost transport function completely. Interestingly, patients harboring the W258X mutation have been found in wide areas of Japan and also in Korea. We speculate how the allele was distributed throughout such wide areas. These findings, obtained from the genetic studies, revealed a pivotal role of URAT1 in the accumulation of higher urate levels in humans than in other mammals, and bolster the case put forward that URAT1 is a key regulator of blood urate levels.

In addition to renal hypouricemia, it would be interesting to determine whether urate nephrolithiasis[41] is also associated with the defects in *SLC22A12*. We also speculate that some common polymorphisms (SNPs or natural person-to-person variations) in *SLC22A12* may be associated with the heterogeneity of circulating urate levels among humans, and also with the tendency for hypouricemia in patients with gout.

There has been no report of genetic diseases in which genes encoding the OAT family are impaired. In the OAT family, URAT1 is the first found to be responsible for a genetic disorder, and it is suggested that defects of genes encoding other members of the OAT family also have important roles in the etiology of human diseases or development.

Regulation of URAT1

Regulation of the OAT family by phosphorylation

Far from being static, some functions of renal epithelial cells are highly regulated. To date, putative mechanisms for the functional regulation of the OAT family have been implicated.[1,2] OAT1 possesses several potential phosphorylation sites for various protein kinases in their intracellular domains.[1,2,42] Previous studies have indicated that the intracellular activation of protein kinase C (PKC) inhibits the transport activity of *para*-amino hippurate (PAH) in proximal opossum kidney cells.[43] You et al.[44] reported that the activation of PKC by phorbol-12-myristate-13-acetate (a commonly used phorbol ester) leads to a decrease in V_{max} (the maximum velocity of transport) without changes in the K*m* (Michaelis constant) of mouse OAT1 transport activity, suggesting some inhibition mechanisms of active OAT1 such as internalization or degradation. This phenomenon also indicates that the membrane targeting of the trans-

porter may be actively regulated by intracellular signaling pathways. However, the phosphorylation of OAT1 has not yet been detected directly in vitro or in cells, and no direct association between the kinases and OAT1 has been demonstrated. More importantly, upstream signals that trigger the phosphorylation of transporters (receptor tyrosine kinases or G-protein coupled receptors) should be determined. Accordingly, the importance and functional role of these putative sites has not been elucidated.

Regulation of URAT1 by a scaffolding protein PDZK1

As described above, genetic studies of patients with renal hypouricemia indicate that URAT1 regulates blood urate levels and vice versa, i.e., to control blood urate levels, the URAT1 transport function should be tightly regulated. Ichida et al.[40] reported a novel genetic alteration of *SLC22A12* in a patient with renal hypouricemia. This was a 5-bp deletion (1639–1643del) that caused a frameshift and amino acid sequence modification near the URAT1 extreme intracellular C-terminal region, suggesting that this region is important for the function of URAT1. Interestingly, the region at the C-terminal end of URAT1 contains a binding motif for PDZ (PSD-95, DglA, and ZO-1) domain-containing proteins which is known to participate in protein–protein interaction,[45] and it was found that the PDZ-binding motif disappeared with the deletion mutation. What is the regulatory mechanism of the C-terminus of URAT1? Anzai et al.[46] tackled this issue using a yeast two-hybrid approach to investigate the putative URAT1 C-terminus-associated proteins that modulate its transport function. They identified the multivalent PDZ domain-containing protein PDZK1[47] as an apparent partner of URAT1 in the kidney in vivo. An in vitro binding assay revealed that this interaction requires the PDZ motif of URAT1 in its C-terminal region, and coexpression experiments demonstrated that URAT1 transport activity is increased by PDZK1/URAT1 interactions (Fig. 3C, D).[46] This results suggested that the function of PDZK1 may be as a scaffolding protein that may be a physiological regulator of the function of URAT1. The interaction of URAT1 with PDZK1 was also identified by the reciprocal approach,[48] which subjected a single PDZ domain derived from PDZK1 to a yeast two-hybrid assay. This study demonstrated that PDZK1 associates with an Na^+-dependent phosphate cotransporter (NaPi-I and NaPi-IIa), an Na^+/H^+ exchanger (NHE-3), and a novel organic cation transporter 1 (OCTN1).[48] In addition, other regulatory factors such as a protein kinase A (PKA)-anchoring protein (D-AKAP2) and an Na^+/H^+ exchanger regulator factor (NHERF-1) were also colocalized with PDZK1,[48] suggesting an extended network beneath the apical membrane to which membrane proteins and regulatory components are anchored.

The precise mechanism of PDZK1 modulation in URAT1 transport activity remains to be determined. One speculation is derived from studies of cystic fibrosis transmembrane conductance regulator (CFTR) channel interactions with the multi-PDZ domain protein CAP70 (a mouse homologue of PDZK1), in which PDZ domains in CAP70 play a modulatory function by directly affecting CFTR channel gating.[49] PDZ domain-containing proteins were initially thought simply to organize many molecules as their scaffolds, but they are now known to be involved in integrating cell structure and cell function with signal transduction. Therefore, the complex formation between URAT1 and PDZK1 not only stabilizes URAT1 in cell membranes, but also exerts its effect on transport activity via allosteric modulation, as was demonstrated in the CFTR/CAP70 interaction.

Sex differences in the expression of URAT1

Hippocrates (born 460 BC) wrote about the symptoms and features of gout, and said, "A woman does not take the gout, unless her menses be stopped," and "Eunuchs do not take the gout, nor become bald." As he suggested, it is well known that men are usually more susceptible to gout than women. The sex differences in urate levels in human blood is one of the typical sex differences in endocrinological data (male > female), and is caused by a sex-dependent difference in the probenecid-sensitive urate reabsorption in the kidney.[15] Hosoyamada et al.[50] identified a mouse homologue of URAT1 (also called RST), and also found a sex difference in the amount of protein and the mRNA levels of mouse URAT1 in the kidney. The male/female ratio of the expression levels of messenger RNA was 2.3, indicating that the transcription is regulated in sex-dependent manner. Further investigation is required into the details of the hormonal regulation of URAT1 transcription and promoter analysis.

Significance of urate in humans

Urate is a scavenger of free radicals in vivo

Information from the studies cited above leads us to ask why humans require such an effective urate reabsorption mechanism in their kidney, and why humans lost hepatic uricase by mutational silencing. Given that the tight regulatory mechanisms of URAT1 function in the renal tubular cells, what is the purpose of regulating URAT1-mediated urate transport activity? Conventionally, urate is considered to be an inert end product of purine breakdown in humans without any physiological value, because too much urate can cause significant health problems, leading to kidney stones and gout when insoluble urate crystals accumulate in the kidney and the joints. In addition, urate is a mediator of renal disease and its progression,[51] and its pathogenic roles in hypertension and cardiovascular disease have been discussed and reevaluated.[52] In contrast, many studies have shown that urate can be tremendously beneficial because it is a potent scavenger of harmful free radicals such as reactive oxygen species. Urate has been shown in vitro to scavenge the radicals such as peroxides and hypochlorous acid.[53,54] Although there had been few indica-

tions of such a role for urate in animals or humans in vivo, sequential studies by Hooper and co-workers[55–57] reported that urate potentially inhibits peroxynitrite-mediated reactions which are implicated in the pathogenesis of multiple sclerosis (MS) and other neurodegenerative disorders. They found that serum urate levels were inversely associated with the incidence of MS in humans because MS patients have low serum urate levels and individuals with hyperuricemia rarely develop the disease. The administration of urate is therapeutic in experimental allergic encephalomyelitis (EAE), which is an animal model of MS,[56,57] indicating the therapeutic potential of elevating urate levels in MS patients. Hellsten et al.[58] demonstrated that high metabolic stress to skeletal muscles, induced by intensive exercise, leads to an oxidation of urate in the exercised muscles. Using muscle samples obtained from healthy male subjects after exhaustive exercise, they showed that urate is oxidized to allantoin in the muscles during exercise, probably due to the generation of free radicals. Collectively, these findings clearly demonstrate and support the suggested importance of urate as a free radical scavenger in vivo.

Exercise-induced acute renal failure in renal hypouricemia

However, a distinct mechanism for the exercise-induced acute renal failure that threatens patients with renal hypouricemia is disputed.[28–31] Murakami et al.[31] suggested that because of their low blood levels of urate, these patients are susceptible to an increase in reactive oxygen species produced in the body, which then damage the tubular epithelial cells in the kidney. It is plausible that the renal epithelial cells are susceptible to the toxic effects of oxygen-free radicals, which are rescued by urate in the cells or the blood. However, not all patients with the disease develop exercise-induced acute renal failure, suggesting that other mechanism(s) are involved.[31]

Other biological roles of urate

In addition to its role as a free radical scavenger, it was recently revealed that urate is a principle endogenous "danger signal" in the immune system and is released from injured and dying cells.[59] Urate released from these cells stimulates dendritic cell and T cell maturation, where it acts as a molecular link between cell injury and immunity, and is implicated in vaccines, autoimmunity, and inflammation.[59] Finally, urate might contribute to human longevity.[60,61] Several antioxidant compounds that protect against biological oxidants such as vitamins A, C, and E are important factors in the lengthening of primate lifespans.[60] Urate is another example, and blood urate levels in humans are about six times those of vitamin C, suggesting that urate may be a primary antioxidant in humans.[61] The function of URAT1 and the loss of uricase activity was presumably important in producing the high levels of urate in humans. Thus, we should consider the multivalence of urate in humans. It has many beneficial effects in some cases, but harmful effects in other cases, in particular selective evolutionary advantages resulting from the loss of uricase, and disadvantages resulting from hyperuricemia. These are mutually exclusive. So it may be true that urate levels in the blood should be tightly controlled.

OAT and human disease

OAT and the progression of chronic renal failure

There is little evidence that the functions of the OAT family are involved in the pathophysiology of human diseases. We found that indoxyl sulfate, one of the uremic toxins, is a novel physiological substrate for the OAT family, and its accumulation within the renal tubules via OATs induces a decrease in cell viability (Fig. 4).[62] Indoxyl sulfate is a metabolite of indole that is derived from dietary protein, and

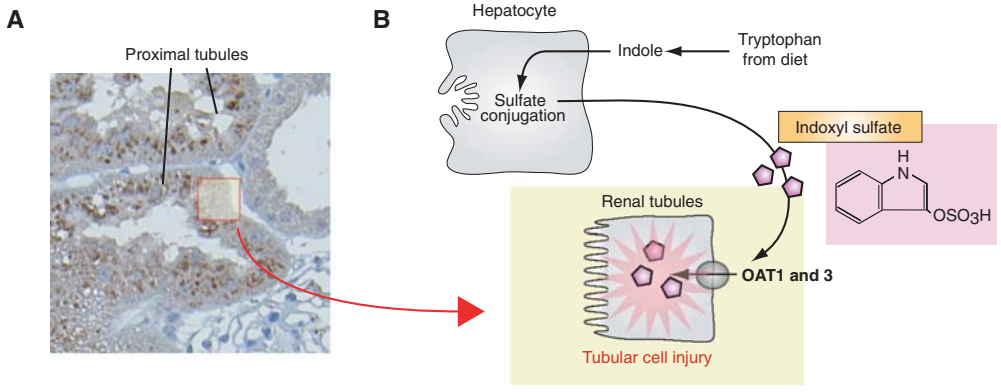

Fig. 4. **A** An accumulation of indoxyl sulfate within the tubules in a rat uremic kidney. Brown denotes indoxyl sulfate that accumulates within the proximal tubules. **B** Nephrotoxicity induced by indoxyl sulfate is mediated by OATs. Indoxyl sulfate is synthesized in the liver from indole absorbed from the intestines, and is excreted into urine via OATs in the ranla tubules. In the progression of chronic renal failure, a loss of functioning intact nephrons results in an overload of indoxyl sulfate in the remnant nephrons, especially in tubular epithelial cells.[63,65] In these cells, accumulated indoxyl sulfate decreases the cell viability[62]

its plasma levels are markedly increased in uremic patients with chronic renal insufficiency.[63–65] Studies by Niwa and coworkers[63–65] indicated that indoxyl sulfate promotes the progression of chronic renal failure in 5/6-nephrectomized uremic rats and uremic patients. The administration of indoxyl sulfate to uremic rats stimulates the expression of transforming growth factor (TGF)-β-1, tissue inhibitor of metalloproteinase (TIMP)-1, and pro-α-1(I)collagen in the kidneys, leading to glomerular sclerosis and interstitial fibrosis,[66] but the mechanisms of how indoxyl sulfate exerts these adverse effects on renal function have largely been unknown. We were aware that indoxyl sulfate is a small and relatively hydrophobic organic anion that possesses the typical chemical structure accepted by OATs. Immunohistochemical analysis showed that indoxyl sulfate accumulation in 5/6-nephrectomized rats was marked in the renal proximal tubules, where rat OAT1 and rat OAT3 were also localized, suggesting that the increased serum indoxyl sulfate concentration leads to an accumulation of indoxyl sulfate within the renal tubules by its uptake via OATs.[62] This hypothesis was confirmed by in vitro experiments which demonstrated that OAT-expressing cell lines exhibited uptake of indoxyl sulfate with a carrier-mediated transport process. An MTT colorimetric assay showed that the accumulated indoxyl sulfate within the cells induced significant toxicity.[62] Among various endogenous substrates transported by OATs, indoxyl sulfate is the first reported to be toxic for renal function.

OATs mediate the transport of various uremic toxins

Other uremic toxins, such as 3-carboxy-4-methyl-5-propyl-2-furanpropionate (CMPF), indoleacetate (IA), and hippurate (HA), are also accumulated to a high degree in uremic plasma. Deguchi et al.[67] recently reported that rat/human OAT1 and OAT3 are responsible for the renal uptake of these uremic toxins on the basolateral membrane of the proximal tubules. These findings indicate that OAT1 and OAT3 are potential sites of interaction between uremic toxins and endogenous substrates or exogenous drugs. Therefore, the study by Deguchi et al. prompts us consider the physiological role of the kidney as a detoxifying system, and help us to improve the treatment of uremic toxins. Many uremic toxins that are OAT substrates would be expected to interact with other substrates such as drugs owing to competition for OAT transport, and might thus decrease their renal secretion. Consequently, this interaction results in an increase in the half-life and extra-renal toxicity of the drugs. It has been shown that the administration of AST-120 (Kremezin), an oral absorbent for removing circulating uremic toxins from the digestive tract, decreases the intensity of indoxyl sulfate staining in the proximal tubules as well as its serum concentration.[63] The effective elimination of uremic toxins from the blood and subsequent decreases in competition for OAT transport may be one of mechanisms by which AST-120 attenuates the progression of chronic renal damage.

Life without OATs

A recent report by Sweet et al.[16] described for the first time the effects of a targeted disruption of the mouse OAT3 gene (*slc22A8*) for the assessment of its contributions to detoxification, development, and disease. Although OAT3-null mice were surviving healthy and showed no morphological tissue abnormalities, they showed a distinct decrease in organic anion transport capacity in the kidney and the choroids plexus. The uptake of typical substrates of mouse OAT3, such as taurocholate, estrone sulfate, and *para*-aminohippurate (PAH), was greatly reduced in renal slices from OAT3-null mice. In contrast, no significant difference in the uptake of these compounds was observed between hepatic slices from wild-type and OAT3-null mice. Sweet et al.[16] also found that the cellular transport of fluorescein in the choroids plexus, which is sensitive to inhibition by organic anions such as PAH and probenecid, was remarkably reduced in OAT3-null mice. These results suggest that mouse OAT3 does not play a major role in hepatic organic anion transport, but it is a key component in the detoxification and systemic homeostasis of several organic anions in the kidney and the choroids plexus. Thus, as well as increasing knowledge of OAT substrates and expression patterns, the development of OAT knockout mouse lines provides further tools for establishing a greater understanding of the contribution of each transporter to homeostasis, detoxification, and drug–drug interactions.[68] It should be noted that the function and localization of OAT3 largely overlaps with OAT1 in the kidney and the choroids plexus. Double knockouts of OAT1 and OAT3 genes might overcome such redundancy and clarify the important and unexpected roles of OATs in development.[68]

OCT/OCTN and human diseases

Function of the OCT/OCTN family

The organic cation transporter/novel organic cation transporter (OCT/OCTN) family is another subgroup in the *SLC22A* family, which includes OCT subtypes 1–6 and OCTN 1–3 (see Fig. 1).[4] OCTs have the ability to transport organic cations, including weak bases. The typical substrates of OCTs are tetraethylammonium (TEA) and N-methylquinine, xenobiotic drugs such as desipramine, acyclovir, ganciclovir, and metformin, and endogenous compounds such as serotonin and prostaglandins.[4] Members of the OCTN family show a substrate selectivity similar to that of OCTs, and they also transport carnitine, a ubiquitous and essential component for lipid metabolism. Cellular carnitine acts as an obligatory factor for mitochondrial fatty acid β-oxidation and the subsequent generation of ATP in cells. Carnitine (β-hydroxyl-γ-trimethylaminobutyric acid) is a highly polar quaternary amine that exists as a zwitterion under physiological conditions, and it has been shown that members of the OCTN family accept it in a sodium ion-dependent manner.

Relevance of the OCT/OCTN family to human disease

In contrast to OATs, there have been several reports that described the links between mutations/polymorphisms in genes encoding OCT/OCTNs and human diseases. The first report,[69] by Nezu et al., identified mutations in a gene encoding OCTN2 (*SLC22A5*) in patients with systemic carnitine deficiency (SCD). SCD is an autosomal recessive disorder characterized by progressive cardiomyopathy, skeletal myopathy, hypoglycemia and hyperammonaemia. In mammals, OCTN2 is expressed in the kidney, where it reabsorbs carnitine from urine by circulating blood to maintain its plasma levels. The report by Nezu et al.[69] suggested that a loss of OCTN2 function in the kidney results in excessive loss of carnitine into the urine, leading to low carnitine levels in plasma.

In addition to SCD, the function of the OCTN family has been linked to some unexpected diseases, which gives us further fundamental insights into OCTN function. It was reported that *SLC22A4* (a gene encoding human OCTN1) is a susceptibility gene for rheumatoid arthritis (RA) through a case-control association study with SNPs.[70] Tokuhiro et al.[70] characterized the expression of OCTN1 in hematopoietic cells, as well as the functional SNP that has an allele-specific effect on its expression. This SNP is located in a RUNX1 (an essential hematopoietic transcription factor in the hematopoietic system)-binding sequence in *SLC22A4*, which affects the expression of *SLC22A4* by altering RUNX1 binding affinity. They also found an SNP in the RUNX1 gene which is also strongly associated with RA, indicating an epistatic effect of the two genes on RA. More recently, a paper by Peltekova et al.[71] reported that two SNPs in the OCTN gene form a haplotype associated with a susceptibility to Crohn disease, a chronic inflammatory disease of the gastrointestinal tract. These SNPs included a missense substitution in *SLC22A4* and a transversion in the *SLC22A5* (a gene encoding human OCTN2) promoter. Collectively, these observations suggest that the function of the OCTN family is involved in the pathogenesis of diseases associated with inflammation and autoimmunity such as RA and Crohn disease, presumably through the transport of carnitine or other organic cations.

Human CT2, a novel carnitine transporter in human testes

We have recently identified a novel high-affinity carnitine transporter named human carnitine transporter 2 (hCT2).[72] hCT2 is only expressed in the testis, where it is localized to Sertoli cells and epithelial cells of the epididymal tracts. hCT2 has greater substrate selectivity than OCT/OCTNs in so far as it interacts with carnitine and betaine (a precursor of L-carnitine synthesis), but not with typical substrates for OCT/OCTNs such as TEA. The function of hCT2 seems to be essential for the secretion of L-carnitine into the lumen of the epididymal tract, which is required for the maturation and viability of spermatozoa. Previous studies have demonstrated that there is a significant positive correlation between the carnitine concentration in the genital tract, the number of spermatozoa, and the percentage of motile normal spermatozoa.[72,73] Furthermore, evidence indicates that the clinical administration of L-carnitine or acetyl-L-carnitine to infertile male patients was followed by an increase in sperm number and motility.[73] Therefore, it is plausible that hCT2-mediated carnitine transport is required for the maturation of spermatozoa, and that the hCT2 gene is a potential target for male infertility screening and treatment.

Conclusions and future directions

In this review, we have tried to illustrate the extent of the involvement of OATs in human diseases. Since their discovery in 1997,[6,7] OATs have been shown to have essential roles in the elimination and absorption of a broad range of substrates that include diverse clinical drugs and their metabolites. Expressed in the apical or basolateral cell membranes in the renal epithelial cells, they regulate the concentrations of the substrates in the body by bidirectional transepithelial transportation. These features of OATs as "multispecific drug transporters" and their implications in drug–drug interactions and adverse drug reactions have not been discussed here (reviewed in refs. 1–3).

Although the substrate selectivity of each transporter has been clarified, it is still not clear how its expression and function are regulated in response to stimuli in the environment, or how they are coupled to intracellular signaling pathways. We also lack knowledge about the individual contribution of each member of the OAT family to the whole tissue transport, except for that of mouse OAT3.[16] In addition, emerging new evidence has revealed the involvement of SNPs in OCTN function and the etiology of human diseases,[69,70] suggesting that pathogenic SNPs or mutations in genes encoding OATs might be discovered in the future. The analysis of URAT1, which we focused on here, hinted at the pathogenic roles of other members of the OAT family, which warrants further investigation. Furthermore, over the past few years, research has focused on the establishment of a systematic evaluation of the affinity between newly developed therapeutics and OATs using cell lines stably expressing OATs. This strategy makes it possible to estimate drug–drug interactions and adverse drug reactions for new therapeutics ahead of their clinical uses. Finally, continuing research into the fundamental roles of OATs will certainly shed light on the highly regulated dynamics of renal tubular function.

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