Chapter 13
Roles of Zinc Transporters in Cellular Transport of Cadmium and Manganese

Seiichiro Himeno and Hitomi Fujishiro

Abstract As cadmium (Cd) is a nonessential metal, there is no specific transport system for cellular entry of Cd in the organisms. The establishment of Cd-resistant cells from metallothionein-null mouse cells, application of multi-tracer technique, and microarray analyses have revealed that Cd^{2+} shares the pathway for cellular incorporation with Mn^{2+}, and the responsible transporters for this pathway were found to be ZIP8 (Zrt- and Irt-related protein 8) and ZIP14. Although other transport systems for iron or calcium are also utilized for cellular incorporation of Cd^{2+} and Mn^{2+}, characterization of ZIP8 and ZIP14 has demonstrated important physiological and pathological roles of these transporters in metal transport. We show here the significant roles of ZIP8 in segment-specific transport of Cd in proximal tubule of the kidney and the roles of ZIP14 and ZnT10 in Mn transport in neuronal cells in the presence of cytokine. Recently, critical roles of Mn transport systems have been highlighted by the findings of human diseases related to the mutation in ZIP8 and ZnT10. This chapter summarized historical background and recent advances in the studies on the roles of ZIP8 and ZIP14 in the transport of Cd^{2+} and Mn^{2+}.

Keywords Cadmium • Manganese • Zinc transporter • ZIP8 • ZIP14 • ZnT10 • Kidney • Brain

13.1 Introduction

Cadmium (Cd) is a heavy metal known as a causative agent for *Itai-itai* disease in Japan. This disease is characterized by painful (*Itai* in Japanese) bone damages including osteomalacia [1]. The primary cause of osteomalacia in this disease is the renal dysfunction called Fanconi syndrome which causes the loss of calcium (Ca) and phosphorus from the body due to the disturbance in reabsorption of these elements at the proximal tubule of the kidney. Cd accumulates in human

S. Himeno (✉) • H. Fujishiro
Laboratory of Molecular Nutrition and Toxicology, Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima 770-8514, Japan
e-mail: himenos@ph.bunri-u.ac.jp

Y. Ogra, T. Hirata (eds.), *Metallomics*, DOI 10.1007/978-4-431-56463-8_13

kidney after a long-term consumption of Cd-containing diets, especially rice in Japan. The biological half-life of Cd in human kidney is assumed to be more than 20 years [2]. Thus, the elucidation of mechanisms underlying the uptake, retention, distribution, and excretion of Cd in the kidney is critical for understanding pathophysiology of Cd-induced renal damage. Until now, however, molecular mechanism of Cd transport at cellular level has not been fully elucidated.

Metallothionein (MT) is a low-molecular-weight sulfhydryl-rich protein and plays important roles in the protection against heavy metal toxicity through its high affinities for a variety of metals including Cd [3, 4]. Under normal conditions, cellular level of MT is kept at minimal level, but upon the intrusion of metals into cells, the transcription of MT gene and consequently the synthesis of MT protein are quickly accelerated. The detoxification of metals by MT is achieved through the tight binding of the metals within cells, but not through the excretion of the metals. Thus, after the induction of MT, cellular Cd concentrations are kept high, but cytotoxicity of Cd is prevented because free Cd ions cannot be readily released from MT. This mechanism is indeed beneficial to cells but also contributes to the long-lasting accumulation of Cd in cells and tissues. Unfortunately, the existence of this efficient Cd-binding molecule within cells has made it difficult for Cd researchers to elucidate the influx and efflux mechanisms of Cd.

To solve this problem, we attempted to find non-MT factors involved in Cd toxicity and transport and found that ZIP8 and ZIP14, the zinc transporter ZIP (Zrt- and Irt-related protein) family members, play important roles in Cd transport. Further characterization of these metal transporters by our group and others has demonstrated wider roles of these transporters than initially expected in the handling of Cd as well as manganese (Mn) in a variety of cells and tissues.

13.2 Cadmium Transport from a Viewpoint of Metallomics

13.2.1 Multiple Candidate Transporters for Cadmium Transport

To date, several candidates for Cd transport system have been proposed. Because Cd is not an essential element, it has been presumed that the transport systems for other essential elements are used for cellular incorporation of Cd. Transporters for Ca, iron (Fe), zinc (Zn), Mn, and magnesium (Mg) have so far been proposed to be involved in Cd uptake in mammalian cells.

As Ca has an ionic radius similar to Cd, earlier studies have focused on the roles of Ca channels in the transport of Cd. In the field of pharmacology regarding Ca channels, Cd has been used as an inhibitor of Ca channels, though the concentrations of Cd used as an inhibitor were more than 100 μM, which is a lethal dose to cells. Furthermore, Ca channels are expressed highly in neuronal cells, but these cells are not the major target of Cd toxicity. On the other hand, intestine is the tissue

where Ca is efficiently absorbed from the diet. The experimental animals fed a Ca-deficient diet accumulated higher amounts of Cd from the diet, suggesting that the transport system for Ca is utilized for Cd absorption at least in the intestine [5]. Min et al. [6] showed that CAT1, calcium transporter 1, which is a member of Ca channel but behaves like a transporter, is responsible for intestinal Ca absorption.

Several studies have shown that animals fed an Fe-deficient diet accumulated higher amounts of Cd [7–9]. Intestinal Fe is known to be absorbed via DMT1, divalent metal transporter 1 also known as Nramp2, which was identified as a divalent Fe (Fe^{2+}) transporter but was found to have broad affinities for other divalent metals including Mn and Cd [10]. When an Fe-deficient diet was given to experimental animals, the expression of DMT1 was highly enhanced, suggesting the role of DMT1 in intestinal Cd absorption especially under anemic conditions [8, 11]. However, the roles of DMT1 in other tissues than the intestine remain unclear because subcellular localization of DMT1 was detected mainly in late endosomes in some cells [12].

Kidney accumulates high concentration of Cd after a long-term exposure. It has been assumed that Cd bound to MT in serum is filtered through the glomerulus of the kidney and reabsorbed in the proximal tubule cells via endocytosis [13]. The injection of Cd-MT complex directly to the blood circulation resulted in an efficient accumulation of Cd in the kidney [14, 15]. Thus, it has been considered that the primary route of renal Cd accumulation is the reabsorption of Cd-MT in the lumen into epithelial cells of proximal tubule, where Cd is retained for a long time. However, we have recently demonstrated that the Cd handling in the kidney may be more dynamic and complicated than previously expected. This will be discussed in more detail in Sect. 13.3.3.

Although fragmental information on the mechanism of Cd transport has been accumulated, molecular mechanism for cellular Cd incorporation remained unsolved. As Cd is thought to be transported via the systems for other essential metals, the identification of a metal that share the same pathway for cellular transport with Cd is required.

13.2.2 Establishment of Cadmium-Resistant Cells from Metallothionein-Null Cells

To identify endogenous factors for the protection against xenobiotic chemicals or drugs, the development of a drug-resistant cell line has been frequently utilized, and a number of transporters for drugs have been identified through this strategy. Also for Cd, a variety of resistant cells have been established and characterized. Most of the Cd-resistant cells so far established showed enhanced expression of MT, confirming an important role of MT for detoxification of Cd [16–18]. However, no information on Cd transport system has been provided by these resistant cells.

In general, the development of drug-resistant cells provides at least three types of cellular mechanisms involved in the protection against drug toxicity, reflecting the three strategic steps for acquiring resistance against the drug. The primary protective strategy for cells to survive when the drug enters cells is to reduce cellular concentrations of the drugs by expelling them out or by inhibiting their entry. A number of drug transporters have been identified by this way. If the cells could not reduce the cellular concentration of the drug, then the next strategy is to inactivate the drug within cells. Metabolic inactivation of drugs is a typical example of this strategy. Also, the trapping of metals by MT can be classified into this strategy. The third strategy is to repair the cellular damages quickly and efficiently. DNA repair enzymes could be categorized into this strategy.

According to this categorization of resistant factors, we attempted to develop a Cd-resistant cell line from the cells lacking the expression of MT, expecting the establishment of a cell line in which the alteration in Cd transport plays a primary role in acquiring Cd resistance. For this purpose, Yanagiya et al. [19] utilized SV40-immortalized MT-null cells derived from embryonic fibroblasts of MT-I and MT-II knockout mice [20]. Using these MT-null cells, Yanagiya et al. [19] established two lines of Cd-resistant MT-null cells and found that the resistance of these cells against Cd was conferred mainly by a marked decrease in Cd accumulation. These cells were the first Cd-resistant cells in which lowered Cd accumulation, but not enhanced MT expression, is the primary cause for acquisition of Cd resistance. The influx and efflux experiments revealed that a marked decrease in the initial uptake rate of Cd was the predominant reason for the decrease in Cd accumulation [19].

13.2.3 Metallomics Approach to Identify Cadmium Transporter

Since the uptake rate of Cd into cells was suppressed in Cd-resistant MT-null cells, Cd resistance of these cell lines might have been conferred by the "loss of function" of a certain metal incorporation system, which should be shared with other essential elements. To identify the element that is not efficiently incorporated into the Cd-resistant MT-null cells, we utilized the multi-tracer technique developed at RIKEN (the Institute for Physical and Chemical Research) in Japan [21, 22]. A solution of the multi-tracer is produced by the irradiation of target metal using high-energy heavy ions generated in the ring cyclotron in RIKEN (Fig. 13.1). The multi-tracer solution containing more than 20 radioisotopes was added to the media of Cd-resistant and parental cells for 120 min, and then the contents of all nuclides were determined simultaneously by a germanium detector.

By using this novel technique, we found that the incorporation of Mn^{2+} was extremely suppressed in MT-null Cd-resistant cells [23]. Although the term "metallomics" was not yet in use at that time, the multi-tracer technique is definitely

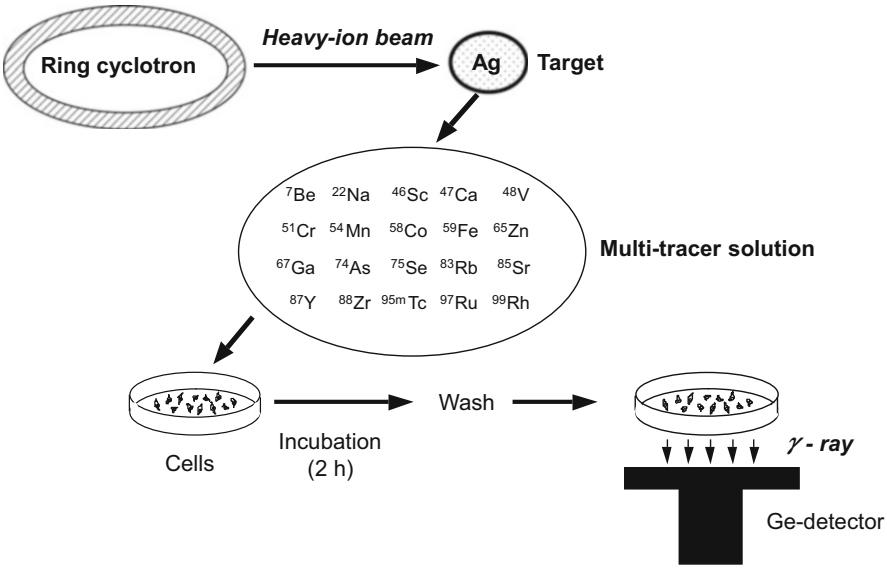

Fig. 13.1 Schematic presentation of multi-tracer technique. The solution of multi-tracer was produced by irradiation of target metal (Ag) using heavy-ion beam generated in the ring cyclotron in RIKEN. The multi-tracer solution was added to the media of cells for 120 min, and then the γ-ray emission from all nuclides can be determined simultaneously by a germanium detector

a metallomics approach and enabled us to identify the element, Mn, as the candidate metal involved in cellular Cd transport.

The decrease in Mn^{2+} uptake rate in Cd-resistant MT-null cells was also confirmed by using a single tracer of ^{54}Mn-labeled $MnCl_2$. In addition, we found that a decreased uptake of Mn^{2+} was observed only at doses less than 10 μM in Cd-resistant MT-null cells, suggesting that a high-affinity transport system for Mn^{2+} is involved in Cd^{2+} transport. In a competition assay in parental cells, the uptakes of Cd^{2+} and Mn^{2+} were mutually inhibited by each other, but not by other divalent metals such as Co^{2+}, Ni^{2+}, Fe^{2+}, or Cu^{2+}. Interestingly, the uptakes of Cd^{2+} and Mn^{2+} were significantly inhibited by Zn^{2+}. No mutual inhibition of Cd^{2+}, Mn^{2+}, and Zn^{2+} uptakes was observed in Cd-resistant MT-null cells, suggesting that the transport system having affinities for Mn^{2+}, Cd^{2+}, and probably Zn^{2+} is suppressed in Cd-resistant cells. Since mutual inhibition of Cd^{2+} and Mn^{2+} was observed also in several lines of cultured cells (HeLa, PC12, and Caco-2 cells), this transport system may exist commonly among mammalian cells.

The next question was, therefore, to identify the transporter which has affinities for Cd^{2+}, Mn^{2+}, and Zn^{2+} and is suppressed in Cd-resistant MT-null cells.

13.3 The Roles of ZIP8 and ZIP14 in Cadmium Transport

13.3.1 Identification of ZIP8 and ZIP14 as Cadmium Transporters

To identify the genes responsible for Cd resistance in Cd-resistant MT-null cells, we performed a microarray and subsequent real-time RT-PCR analyses and found that the expression of *Slc39a8* encoding ZIP8 and *Slc39a14* encoding ZIP14 was downregulated in MT-null Cd-resistant cells [24, 25]. Furthermore, the introduction of shRNA of ZIP8 into parental cells resulted in an approximately 60 % reduction in cellular Cd accumulation, whereas shRNA of DMT1, which also have affinities for Cd^{2+}, Mn^{2+}, and Zn^{2+}, did not alter Cd accumulation [26]. Among ZIP family members, ZIP8 and ZIP14 have a similarity in amino acid sequence, suggesting that both transporters have similar functions and affinities for metals [27]. These results suggest that ZIP8 and ZIP14 may be involved in the transport of Cd^{2+}, and the suppressed expression of these transporters resulted in lowered Cd accumulation in Cd-resistant MT-null cells.

During the time when we were attempting to identify the transporter involved in Cd transport, another group led by Prof. Nebert in Cincinnati University in the USA was also searching for the gene responsible for the strain difference in Cd-induced testicular hemorrhage in mice. It has been well known that injection of Cd into mice acutely causes testicular hemorrhage, but some strains of mice such as C57Bl/6 and A/J are resistant to Cd-induced hemorrhage even when very high dose of Cd was injected [28]. Dalton et al. [29] identified *Slc39a8* as the responsible gene determining the strain difference in sensitivity to Cd-induced testicular hemorrhage. Thus, two independent approaches have identified the same genes, *Slc39a8*, as a determinant of Cd resistance and transport [30].

Nebert and coworkers have shown that ectopic expression of ZIP8 in cultured cells resulted in an efficient uptake of Cd^{2+} as well as Mn^{2+} with Km values less than 10 μM for both Cd^{2+} and Mn^{2+} [31]. Similarly, ZIP14A and ZIP14B, the products of alternative splicing of *Slc39a14* gene, were expressed in mouse fetal fibroblast cells, and the uptakes of both Cd^{2+} and Mn^{2+} were found to be highly enhanced with low Km values [32]. These results demonstrated that ZIP8 and ZIP14 are the transporters that have high affinities for Cd^{2+} and Mn^{2+} in addition to Zn^{2+}.

13.3.2 Characterization of ZIP8 and ZIP14 as Cadmium Transporters

Wang et al. [33] produced transgenic mice in which BAC clone containing *Slc39a8* was expressed and determined whether ZIP8 is actually involved in Cd uptake into the target tissues of Cd in vivo. The results indicated that ZIP8 is highly expressed

in the vasculature of the testis and apical membrane of proximal tubules in the kidney in ZIP8-transgenic mice. Administration of $CdCl_2$ caused severe testicular hemorrhage in ZIP8-transgenic B6 mice, but not in wild-type B6 (C57Bl/6J) mice, a strain resistant to Cd-induced testicular hemorrhage. Thus, the overexpression of ZIP8 in the kidney and testis actually contributed to the enhancement of Cd toxicity in vivo.

The roles of ZIP8 and ZIP14, especially ZIP8, in the transport of Cd^{2+} and Mn^{2+}, have been confirmed further by additional establishment of Cd-resistant cells. We developed Cd-resistant cells using immortalized mouse embryonic fibroblast cells derived from MT+/+ mice [34]. Although the expression of MT was highly induced in Cd-resistant MT+/+ cells, the accumulation of Cd was found to be less than 50 % of parental cells. Furthermore, Cd-resistant MT+/+ cells showed a cross-resistance to Mn^{2+} accompanied by the reduced accumulation of Mn in cells. The expression of ZIP8 was suppressed in Cd-resistant cells, confirming the important role of ZIP8 for the transport of both Cd^{2+} and Mn^{2+}. In addition to ZIP8, the expression of DMT1 and L-type Ca channel subunit ($Ca_V1.2$) was also suppressed in Cd-resistant MT+/+ cells. Since IC_{50} value (about 200 μM) of Cd in Cd-r MT+/+ cells was about 10 times higher than that of Cd-resistant MT-null cells, it is presumable that the suppression of multiple transporters in addition to ZIP8 was required for the survival of cells during the selection process of resistant cells with high concentration of Cd in the medium.

Further evidence for the roles of ZIP8 in Cd^{2+} and Mn^{2+} transport has been obtained by the study on RBL-2H3 (rat basophilic leukemia) cells [35]. In the screening of Cd-sensitive or resistant cell lines, we found that the IC_{50} value of $CdCl_2$ for RBL-2H3 cells was as low as 9 μM, close to that of MT-null mouse embryonic fibroblast cells (4 μM). In addition, RBL-2H3 cells showed about tenfold higher sensitivity to $MnCl_2$ compared with other cell lines. We found that RBL-2H3 cells showed the highest expression of ZIP8, the highest accumulation of Cd and Mn at 24 h, and the highest uptake efficiency of Cd^{2+} and Mn^{2+} among the rat cell lines examined. No difference was found in the excretion efficiency. Thus, RBL-2H3 cells may serve as a good model for studying the roles of ZIP8 in cellular transport of Cd^{2+} and Mn^{2+}.

By using RBL-2H3 cells, we examined the roles of HCO_3^- in transport of Cd^{2+} and Mn^{2+} via ZIP8. Liu et al. [36] reported that the addition of HCO_3^- to the medium enhanced the uptake of Cd^{2+}, and the addition of DIDS, which is an inhibitor of HCO_3^- uptake, resulted in a decrease in Cd^{2+} uptake, suggesting that Cd^{2+} is transported with HCO_3^- via ZIP8. To test whether ZIP8 is actually involved in HCO_3^--induced enhancement of Cd^{2+} and Mn^{2+} uptakes, Fujishiro et al. [35] examined the effects of siRNA knockdown of ZIP8 in RBL-2H3 cells, in which the addition of HCO_3^- in the medium also enhanced the uptakes of Cd^{2+} and Mn^{2+}. The downregulation of ZIP8 resulted in the decrease in the uptakes of Cd^{2+} and Mn^{2+} only in the presence of HCO_3^-, confirming the involvement of HCO_3^- in the transport of these metals by ZIP8. The involvement of HCO_3^- on ZIP14-mediated metal transport was also observed in *Xenopus* oocytes in which ZIP14 gene was expressed [37]. However, the downregulation of ZIP14 in RBL-2H3 cells did not

alter the uptakes of Cd^{2+} and Mn^{2+} probably due to the compensatory effects of ZIP8 which is highly expressed in this cell line [35].

To further examine the roles of ZIP8, Fujishiro et al. [38] developed Cd-resistant RBL-2H3 (RBL-Cdr) cells and characterized their sensitivity to and transport of Cd^{2+} and Mn^{2+}. As expected, the expression of MT was upregulated. In addition to MT upregulation, the mRNA and protein levels of ZIP8 were markedly lowered in RBL-Cdr cells. Reflecting the low ZIP8 expression, RBL-2H3 cells showed suppressed accumulation of Cd in parallel with the decrease in Cd^{2+} uptake efficiency. Interestingly, RBL-Cdr cells showed a cross-resistance to $MnCl_2$ due to the reduced Mn accumulation in parallel with reduced efficiency of Mn^{2+} uptake efficiency. No change was observed in the expression of ZIP14 or DMT1, suggesting an important role of ZIP8 in the transport of both Cd^{2+} and Mn^{2+} in RBL-2H3 cells.

13.3.3 The Roles of ZIP8 and ZIP14 in Cadmium Transport in the Kidney

It has long been known that the target organ of Cd toxicity is the kidney, especially proximal tubule epithelial cells (PTECs). The next question, therefore, was whether ZIP8 and ZIP14 play a role in accumulation and toxicity of Cd in renal PTECs. Accumulating evidence has already shown that Cd-MT is deeply involved in renal Cd accumulation [3, 14, 15, 39]. As the molecular size of Cd-MT is about 7000, it has been shown that Cd-MT in blood circulation is readily filtered through the glomerulus, and the Cd-MT in the lumen of proximal tubule is reabsorbed by PTECs by megalin-dependent endocytosis in S1 and S2 segments of proximal tubule [13], where Cd accumulation and toxicity is clearly recognized. Indeed, this is the primary route of Cd accumulation in the kidney. However, the mechanisms of renal Cd accumulation other than Cd-MT endocytosis have been poorly understood.

To test the roles of ZIP8 in renal Cd accumulation, in vivo studies have been conducted in the mice in which ZIP8 is overexpressed. Wang et al. [33] produced ZIP8-transgenic mice using a BAC clone containing *Slc39a8* gene and showed that ZIP8 is highly expressed in the apical membrane of proximal tubules in the kidney. Administration of $CdCl_2$ caused enhanced renal damage in ZIP8-transgenic mice than in control mice. Schneider et al. [40] also produced ZIP8-transgenic mice with different copy numbers of *Slc39a8* genes and found that renal Cd accumulation was higher in transgenic mice after oral administration of Cd. These in vivo studies, however, did not elucidate molecular mechanisms of higher Cd accumulation by overexpression of ZIP8.

In an in vitro study, Fujishiro et al. [41] examined the roles of metal transporters by using immortalized mouse PTECs, named PT cells. In this study, a trans-well culture system was utilized to examine the efficiency of apical and basolateral

transport of metals separately. Apical and basolateral uptakes of metals into PT cells in a trans-well culture system mimic the metal incorporation from the lumen to PTECs and that from blood to PTECs in the kidney. The results showed that the uptake of Cd^{2+} from apical side was higher than that from basolateral side. The apical uptake of Cd^{2+} was dose-dependently inhibited by Mn^{2+} and that of Mn^{2+} was similarly inhibited by Cd^{2+}, suggesting that the transporter(s) having affinity for both Cd^{2+} and Mn^{2+} is involved in the uptake of ionic forms of Cd and Mn at the apical surface of PTECs. To test which transporter is responsible for the uptake of Cd^{2+} and Mn^{2+}, siRNAs for ZIP8, ZIP14, and DMT1 were introduced into PT cells, and then the metal uptake was examined after the cells were transferred to and cultured in the trans-well culture system. The transfection of ZIP8 and ZIP14 siRNAs reduced the apical uptake of Cd^{2+} by about 50 %. The transfection of DMT1 siRNA also reduced both Cd^{2+} and Mn^{2+} uptakes, but to a less extent. These data suggest that ZIP8 and/or ZIP14 may play a significant role in the uptake of ionic forms of Cd and Mn in the lumen to PTECs in the kidney.

The application of the trans-well system also enabled the determination of Cd excretion from PT cells to the medium of apical or basolateral side [41]. The results showed that the efficiency of Cd excretion into the apical side was much higher that into the basolateral side. These data suggest a possibility that at least a part of Cd could be excreted into the lumen from PTECs in the kidney. As the excretion efficiency was examined in PT cells 1 h after the exposure to Cd, it seems unlikely that Cd was excreted in the form of Cd-MT, which could not be synthesized within 1 h.

To further examine the roles of ZIP8 and ZIP14 in the transport of Cd in the kidney, an in situ hybridization of ZIP8, ZIP14, and DMT1 was carried out in mouse kidney [41]. The results showed that the expression of ZIP8 was observed in PTECs especially in the boundary of cortex and outer medulla, where S3 segment of proximal tubule is located. On the other hand, expression of ZIP14 and DMT1 was observed diffusively in the whole kidney.

Based on the abovementioned in vitro and in vivo evidence, we proposed a hypothesis of dynamic handling of Cd in the kidney. As shown in Fig. 13.2, the Cd complexed with MT passes through the glomerulus into the lumen and is absorbed from the apical side of PTECs in S1 and S2 segments where megalin-dependent endocytosis occurs. Previously, the fate of the accumulated Cd in PTECs has not been examined based on the notion that Cd bound to MT remains in PTECs for a long time. By using a trans-well culture system, however, a novel possibility was proposed that a part of Cd accumulated in PTECs may be released into the lumen in the form of ionic Cd, which may then be absorbed into PTECs in S3 segment where ZIP8 is highly expressed.

Pharmacodynamics studies of urate have already shown that the urate filtered through the glomerulus into the lumen will be absorbed into PTECs in S1 and S2 segments, then released into the lumen, absorbed again in PTECs in S3 segment, and released again into the lumen [42]. In the case of Cd, this kind of multi-compartment model of renal handling has not been anticipated to happen because Cd was believed to be stably deposited in PTECs after the endocytosis of Cd-MT.

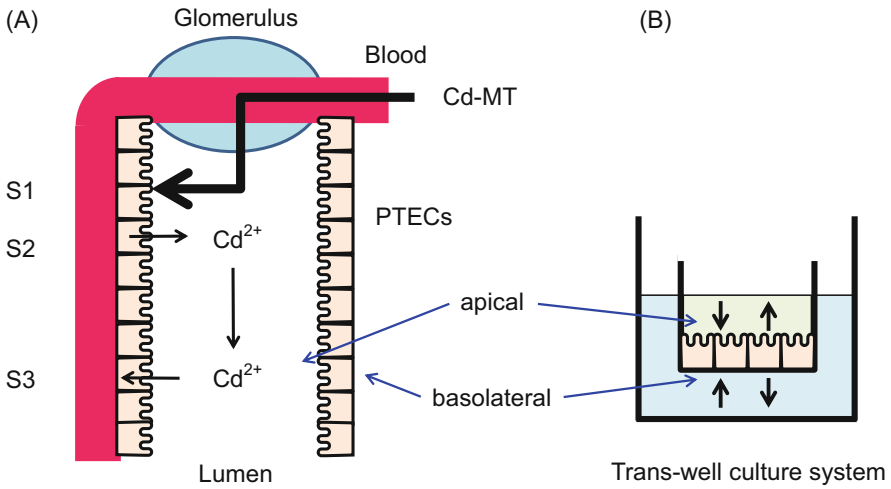

Fig. 13.2 Schematic presentation of hypothesis on Cd transport in the proximal tubule of the kidney. (**a**) Cd-MT in serum is filtered through the glomerulus and reabsorbed by endocytosis in PTECs in S1 and S2 segments. A part of Cd, possibly in the form of Cd^{2+}, is released into the lumen and then absorbed again in PTECs in S3 segment where the expression of ZIP8 is high. (**b**) To mimic the apical and basolateral transport of Cd in PTECs, a trans-well culture system was used. The cells seeded on the cup will gradually form tight junctions and apical and basolateral sides will be polarized. The influx and efflux of Cd in both sides can be determined by this culture system

However, a study on Cu and Cu-MT metabolism in the kidney has already suggested a possibility similar to our notion. Okabe et al. [43] showed that after the intravenous injection of Cu-MT, CuMT protein incorporated by endocytosis was detected in PTECs in S1 and S2 segments, but the mRNA synthesis of MT gene was detected later in PTECs in S3 segment, and suggested that Cu ions were released from the S1 and S2 segments into the lumen possibly via ATP7A or ATP7B, then reabsorbed into S3 segment, and induced MT mRNA transcription there. To test whether similar mechanisms are involved in Cd handling in the kidney or not, we are currently investigating the transport of Cd in S1, S2, and S3-derived PTECs.

13.4 The Roles of ZIP8 and ZIP14 in Manganese Transport

13.4.1 Interactions of Cd and Mn Transport in Nonmammalian Species

Before the discovery of ZIP8 and ZIP14 as a Cd/Mn co-transporter, no researchers in the field of Cd toxicity have considered that Cd is transported via the pathway for

Mn in mammals. However, in nonmammalian species including bacteria, yeast, and plants, accumulating evidence has shown the existence of the transporters having affinities for both Cd and Mn.

In bacteria, the Mn^{2+} transporter MntH in *Bacillus subtilis* [44] and MntA in *Lactobacillus plantarum* [45] were shown to have affinity for Cd^{2+}. The nucleotide sequence of MntH is homologous to Nramp family transporter and that of MntA is homologous to P-type cation-translocating ATPase family.

In yeast, SMF1 is a well-known metal transporter having affinities for various metals including Mn^{2+}, Cu^{2+}, Co^{2+}, and Cd^{2+}. The disruption of *smf1* gene resulted in a decrease in Mn^{2+} uptake, while the overexpression of *smf1* gene resulted in an increase in Mn^{2+} uptake [46]. The amino acid sequence of SMF1 showed a homology to mammalian Nramp family proteins.

Since Mn plays a crucial role in the photosynthetic reaction, this element is essential for the survival of plants and photosynthetic organisms. To identify the gene for Mn^{2+} transport in the plants, *smf1* null strain of *S. cerevisiae* was utilized. Expression of IRT1, the Fe^{2+} transporter gene of *Arabidopsis thaliana* in *smf1* null yeast restored the uptake of Mn^{2+} [47]. The enhanced uptake of Mn^{2+} by IRT1 in *smf1* null mutant yeast was inhibited by the addition of Fe^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}, suggesting that plant Fe^{2+} transporter IRT1 has broad affinities for metals including Mn^{2+} and Cd^{2+}. IRT1 has no homology to P-type ATPase or Nramp family proteins. It should be noted here that mammalian ZIP transporter was named Zrt- and Irt-related protein (ZIP) according to the similarity of the functions and structure to Irt.

Recently, a new piece of evidence demonstrated an important role of Mn^{2+} transport system for Cd^{2+} uptake in rice. Two groups in Japan independently found that Nramp5 in rice is responsible for the uptakes of Mn^{2+} and Cd^{2+} from the root. Sasaki et al. [48] showed that rice Nramp5 is localized at the distal side of both exodermis and endodermis cells of the root and knockout of Nramp5 resulted in a marked decrease in Mn accumulation in the grains and shoot of rice. Cd uptake in Nramp5−/− rice was almost completely suppressed. Ishikawa et al. [49] used a technique to induce mutations in rice grain by using carbon ion beam irradiation and selected two lines of mutated rice, in which Cd accumulation was extremely low even when cultured in Cd-contaminated soil. The mutated gene responsible for the lowered Cd uptake was identified as Nramp5 gene. Thus, two independent studies revealed that the uptake system for Mn by Nramp5 is used for Cd accumulation in rice. The disruption of Nramp5-mediated Cd transport in rice may pave a novel way to reduce the body burden of Cd in humans, especially among rice-eating Asians who accumulate considerable amounts of Cd in their kidney.

13.4.2 RBL-2H3 Cell Line as a Model of Cellular Manganese Transport

As mentioned in 3.2., RBL-2H3 cell line showed a high sensitivity to both Cd^{2+} and Mn^{2+} due to high accumulation of both metals via highly expressed ZIP8. To further elucidate the mechanisms underlying the sensitivity of RBL-2H3 cells to Mn cytotoxicity, Fujishiro et al. [38] developed an Mn-resistant cell line from parental RBL-2H3 cells by a stepwise increase in $MnCl_2$ concentration in the medium. The established Mn-resistant cell line (RBL-Mnr) showed a decrease in ZIP8 expression accompanied by a decrease in Mn accumulation. RBL-Mnr also showed a resistance to $CdCl_2$ cytotoxicity due to reduced accumulation of Cd. Thus, RBL-Mnr showed a cross-resistance to Cd, and RBL-Cdr showed a cross-resistance to Mn as mentioned in Sect. 13.3.2, both due to the decrease in metal accumulation by the suppression of ZIP8 expression.

In addition to the suppression of ZIP8 expression, there should be other changes in gene expression in RBL-Mnr. A microarray assay identified several genes downregulated or upregulated in RBL-Mnr cells compared with parental RBL-2H3 cells. Fujishiro et al. [50] found that the expression of S100A9 was markedly reduced in RBL-Mnr cells. S100A9 is the member of S100 protein and is highly expressed in neutrophils. Recent advances in the studies on the roles of S100 proteins in the protection against bacterial infection suggest that S100A9 together with S100A8 has an ability to bind Zn^{2+} and Mn^{2+} and causes bacteriocidal effects by sequestering these metals [51, 52]. Although the roles of the suppression of S100A9 expression in the acquisition of Mn tolerance remains unknown, further elucidation of interactions of these Mn-binding proteins and Mn cytotoxicity may shed light on novel roles of S100A9 proteins in cellular Mn handling.

13.4.3 Manganese Transport in Neuronal Cells

Mn is essential in many organisms, but excess amount of Mn is toxic to the nervous system. Occupational exposure to Mn dusts and fumes has long been known to cause manganism, a Parkinsonism-like syndrome [53]. However, the precise mechanism of Mn accumulation in the brain remains unsolved. Previously it was considered that the predominant chemical form of Mn in the blood circulation is Mn^{3+}, which is bound to transferrin similarly to Fe^{3+}, and is transported into neuronal cells via the endocytosis by transferrin receptor. However, speciation analyses of Mn in the serum and cerebrospinal fluid (CSF) showed that considerable amounts of Mn exist in the form of Mn-citrate especially in CSF [54]. Citrate can form a complex with Mn^{2+} [55]. As to the responsible transporter for neuronal Mn^{2+} uptake, many studies have considered DMT1 as a primary transporter [56, 57]. Indeed, DMT1 is known to have a high affinity for Mn^{2+} [10], and the enhanced expression of DMT1 in haemochromatosis showed increased

accumulation of Mn [58]. To date, however, little information is available for the roles of ZIP8 or ZIP14 in the transport of Mn in the nervous system.

Fujishiro et al. [59] examined the roles of DMT1, ZIP8, and ZIP14 in the uptake of Mn^{2+} by using SH-SY5Y cells, a model cell line for dopaminergic cells. As expected, the transfection of DMT1 siRNA into SH-SY5Y cells resulted in the reduction of Mn^{2+} uptake. On the other hand, no effects of ZIP8 siRNA was observed probably due to a very low expression level of ZIP8 at basal level in this cell line. Interestingly, the transfection of ZIP14 siRNA resulted in a decrease in Mn^{2+} uptake by about 50 % even in the presence of DMT1, suggesting that ZIP14 may be at least partly involved in Mn^{2+} uptake in SH-SY5Y cells.

An earlier study showed that the expression of ZIP14 in the liver is enhanced by interleukin-6 (IL-6), and consequently the serum Zn is taken up by the liver, resulting in higher hepatic Zn and lower serum Zn [60]. These results explained the observation of low serum Zn levels at the time of inflammation. If the expression of ZIP14 is enhanced by IL-6 also in the nervous system, the incorporation of Mn^{2+} into neuronal cells by ZIP14 may be enhanced. To test this hypothesis, Fujishiro et al. [59] examined the effect of IL-6 on the expression of ZIP14 and Mn accumulation in SH-SY5Y cells. As expected, both mRNA and protein levels of ZIP14 were elevated by IL-6 treatment and accumulation of Mn at 24 h was increased to almost threefold in SH-SY5Y cells. On the other hand, expression of DMT1 was not changed by IL-6. Thus, although DMT1 may contribute significantly to the uptake of Mn^{2+} under normal conditions, the contribution of ZIP14 to Mn accumulation into neuronal cells may be enhanced in the presence of IL-6 at the time of inflammation.

These results provided new insights into the interactions of cytokines and metal accumulation in the brain of neurodegenerative diseases. It has been well documented that brain or CSF levels of cytokines including IL-6 are enhanced in a variety of neurodegenerative diseases such as Parkinsonism and Alzheimer's disease [61]. The enhanced production of cytokines including IL-6 in these patients may induce ZIP14 expression also in the brain and affect the metal deposition in the brain. Since recent studies showed that ZIP14 may also play a role in Fe transport [62], the upregulation of ZIP14 may affect the deposition of not only Zn and Mn but also Fe in neuronal cells.

On the other hand, exposure to Mn itself induces IL-6 expression in the liver [63] and in microglia [64]. Thus, it seems likely that during the process of brain damage development by occupational exposure to Mn, the neuronal Mn accumulation is further accelerated by IL-6-induced ZIP14. More attention should be paid to the interaction of cytokine-induced ZIP14 upregulation with aberrant metal deposition in the brain of neurodegenerative disease patients as well as workers exposed to Mn.

13.5 Human Diseases Related to Disturbances in Manganese Transport

13.5.1 ZnT10 Mutation and Hyperaccumulation of Manganese

In 2012, a new piece of evidence was provided on the roles of metal transporter in the development of brain diseases. Two independent studies have demonstrated that ZnT10 mutation is the cause for the hyperaccumulation of Mn in the liver and brain, resulting in manganism syndromes characterized by neurological disturbances similar to Parkinsonism [65, 66]. ZnT (encoded by *SLC30a* genes) is a family of Zn transporters involved in cellular transport of Zn especially from cytosol to the organelle or extracellular spaces [27]. Although the precise mechanism of Mn transport via ZnT10 remains to be clarified, the human cases of ZnT10 mutation and aberrant accumulation of Mn suggested that ZnT10 is a transporter responsible for Mn excretion from cells to extracellular spaces.

Currently, little information is available on the regulation mechanism of ZnT10 expression including the effects of cytokines. As mentioned in 4.3., treatment of SH-SY5Y cells with IL-6 enhanced cellular Mn accumulation to about threefold at 24 h [59]. However, the initial Mn uptake rate was increased to only to about 30 %. Therefore, there is a possibility that not only the uptake but also the excretion of Mn may be altered by IL-6 treatment. To test this possibility, the effects of IL-6 treatment on mRNA and protein levels of ZnT10 in SH-SY5Y cells were examined [59]. The results showed that IL-6 reduced the expression of ZnT10 by about 50 %, suggesting that the increase in 24-h Mn accumulation by IL-6 treatment was achieved not only by the increase in initial Mn uptake rate caused by ZIP14 upregulation but also by the decrease in the excretion of Mn caused by downregulation of ZnT10. It is noteworthy that the suppression of ZnT10 expression was found in the frontal cortex of Alzheimer's disease patient [67]. However, further studies are required to elucidate the regulatory mechanism of ZnT10 and its involvement in aberrant Mn accumulation in the brain.

13.5.2 ZIP8 Mutation and Disorders of Glycosylation

Recently, a clinical case of congenital disorders of glycosylation was found to be related to the mutation in ZIP8 [68]. An infant showing multiple metabolic disorders was diagnosed with congenital disorders of glycosylation as evidenced by abnormal glycosylation pattern of serum transferrin with the loss of galactose residues. Supplementation of a large dose of galactose was effective in curing the symptoms. The known important transporter for glycosylation is UDP-galactose transporter, encoded by *SLC35A2*, responsible for the Golgi import of UDP-galactose. However, no mutation was detected in *SLC35A2* gene. Whole

exome sequencing was performed, and finally the mutation in *SLC39A8* encoding ZIP8 was identified as the likely candidate for causing defective glycosylation.

The transfer of galactose to acceptor proteins is carried out by a group of enzymes called galactosyltransferases (GalT) located in the Golgi apparatus. GalT activation is completely dependent on metal ions, and Mn^{2+} was shown to be the most potent activator. Since the metal-binding site of GalT has a high affinity for Mn^{2+} (Km value of 0.4 μM), the loss of Mn^{2+} leads to a marked decrease in enzyme activity. The serum and urinary concentrations of Mn in this patient were non-detectable levels, although the concentrations of Zn and other metals were within normal ranges. No biochemical changes, however, were observed in other Mn-dependent enzymes such as arginase or glutamine synthetase. The mutation of ZIP8 in this patient was identified as ^{340}Ile to Asn, which is located in the transmembrane domain V of this protein.

Similar cases of glycosylation disorder were found in Egypt with no detectable Mn in serum [69]. Currently, however, no explanation is available why ZIP8 mutation resulted in undetectable serum Mn level. Further studies are required for the elucidation of the link between ZIP8 mutation and severe deficiency of Mn leading to a specific disturbance in GalT activity.

13.6 Conclusion

More than a decade ago, we started a study to solve a simple question "how Cd enters cells." This simple question led us to the finding of Mn as a candidate metal that shares the pathway for cellular incorporation with Cd. This was achieved by using a multi-tracer technique, a powerful tool for metallomics studies. Genetic studies have enabled us to identify ZIP8 and ZIP14 as the transporters responsible for the cellular uptake of Cd^{2+} and Mn^{2+}. Recent studies showed that ZnT10 is a transporter for the excretion of Mn from cells. A number of studies have demonstrated that ZIP and ZnT family transporters play important roles as the regulators of Zn ions across the plasma membranes of cells as well as between the organelle and cytosol [70]. However, the important roles of ZIP8 and ZIP14 as well as ZnT10 were highlighted by their affinities for multiple metals. Recently, the human diseases related to the disturbances in Mn metabolism have been found to be caused by mutations in ZnT10 and ZIP8. These findings will further accelerate the studies on molecular mechanisms of regulation, subcellular localization, and pathophysiological roles of these multi-metal transporters.

Novel findings in the important roles of ZIP8 and ZIP14 in the transport of Cd and Mn have also accelerated the mechanistic studies in the field of heavy metal toxicology. As the toxicity manifestation of metals largely depends on the amounts of metals deposited in the tissues, cells, and organelles, further studies on the roles of metal transporters in dynamic handling of metals not only between cells and extracellular fluids but also between the organelles and cytosol are required in a future study.

References

1. Yanaga M, Enomoto S, Hirunuma R, Furuta R, Endo K, Tanaka A, Ambe S, Tozawa M, Ambe F (1996) Multitracer study on uptake and excretion of trace elements in rats. Appl Radiat Isot 47(2):235–240
2. Yanagiya T, Imura N, Enomoto S, Kondo Y, Himeno S (2000) Suppression of a high-affinity transport system for manganese in cadmium-resistant metallothionein-null cells. J Pharmacol Exp Ther 292(3):1080–1086
3. Schneider SN, Liu Z, Wang B, Miller ML, Afton SE, Soleimani M, Nebert DW (2014) Oral cadmium in mice carrying 5 versus 2 copies of the *Slc39a8* gene: comparison of uptake, distribution, metal content, and toxicity. Int J Toxicol 33(1):14–20
4. Fujishiro H, Doi M, Enomoto S, Himeno S (2011) High sensitivity of RBL-2H3 cells to cadmium and manganese: an implication of the role of ZIP8. Metallomics 3(7):710–718
5. Que Q, Helmann JD (2000) Manganese homeostasis in *Bacillus subtilis* is regulated by MntR, a bifunctional regulator related to the diphtheria toxin repressor family of proteins. Mol Microbiol 35(6):1454–1468
6. Hayden JA, Brophy MB, Cunden LS, Nolan EM (2013) High-affinity manganese coordination by human calprotectin is calcium-dependent and requires the histidine-rich site formed at the dimer interface. J Am Chem Soc 135(2):775–787
7. He L, Girijashanker K, Dalton TP, Reed J, Li H, Soleimani M, Nebert DW (2006) ZIP8, member of the solute-carrier-39 (SLC39) metal-transporter family: characterization of transporter properties. Mol Pharmacol 70(1):171–180
8. Matzapetakis M, Karligiano N, Bino A, Dakanali M, Raptopoulou CP, Tangoulis V, Terzis A, Giapintzakis J, Salifoglou A (2000) Manganese citrate chemistry: syntheses, spectroscopic studies, and structural characterizations of novel mononuclear, water-soluble manganese citrate complexes. Inorg Chem 39(18):4044–4051
9. Boycott KM, Beaulieu CL, Kernohan KD, Gebril OH, Mhanni A, Chudley AE, Redl D, Qin W, Hampson S, Küry S, Tetreault M, Puffenberger EG, Scott JN, Bezieau S, Reis A, Uebe S, Schumacher J, Hegele RA, McLeod DR, Gálvez-Peralta M, Majewski J, Ramaekers VT, Care4Rare Canada Consortium, Nebert DW, Innes AM, Parboosingh JS, Abou Jamra R (2015) Autosomal-recessive intellectual disability with cerebellar atrophy syndrome caused by mutation of the manganese and zinc transporter gene SLC39A8. Am J Hum Genet 97 (6):886–893
10. Fukada T, Kambe T (2011) Molecular and genetic features of zinc transporters in physiology and pathogenesis. Metallomics 3(7):662–674
11. Wang B, Schneider SN, Dragin N, Girijashanker K, Dalton TP, He L, Miller ML, Stringer KF, Soleimani M, Richardson DD, Nebert DW (2007) Enhanced cadmium-induced testicular necrosis and renal proximal tubule damage caused by gene-dose increase in a *Slc39a8*-transgenic mouse line. Am J Physiol Cell Physiol 292(4):C1523–C1535
12. Grady DL, Moyzis RK, Hildebrand CE (1987) Molecular and cellular mechanisms of cadmium resistance in cultured cells. Exp Suppl 52:447–456
13. Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL, Hediger MA (1997) Cloning and characterization of a mammalian proton-coupled metal-ion transporter. Nature 388(6641):482–488
14. Guilarte TR (2010) Manganese and Parkinson's disease: a critical review and new findings. Environ Health Perspect 118(8):1071–1080
15. Kim J, Buckett PD, Wessling-Resnick M (2013) Absorption of manganese and iron in a mouse model of hemochromatosis. PLoS One 8(5):e64944
16. Klaassen CD, Liu J, Diwan BA (2009) Metallothionein protection of cadmium toxicity. Toxicol Appl Pharmacol 238(3):215–220
17. Liuzzi JP, Lichten LA, Rivera S, Blanchard RK, Aydemir TB, Knutson MD, Ganz T, Cousins RJ (2005) Interleukin-6 regulates the zinc transporter Zip14 in liver and contributes to the hypozincemia of the acute-phase response. Proc Natl Acad Sci U S A 102(19):6843–6848

18. Korshunova YO, Eide D, Clark WG, Guerinot ML, Pakrasi HB (1999) The IRT1 protein from Arabidopsis thaliana is a metal transporter with a broad substrate range. Plant Mol Biol 40 (1):37–44
19. Quadri M, Federico A, Zhao T, Breedveld GJ, Battisti C, Delnooz C, Severijnen LA, Di Toro ML, Mignarri A, Monti L, Sanna A, Lu P, Punzo F, Cossu G, Willemsen R, Rasi F, Oostra BA, van de Warrenburg BP, Bonifati V (2012) Mutations in SLC30A10 cause parkinsonism and dystonia with hypermanganesemia, polycythemia, and chronic liver disease. Am J Hum Genet 90(3):467–477
20. Suzuki T, Momoi K, Hosoyamada M, Kimura M, Shibasaki T (2007) Normal cadmium uptake in microcytic anemia *mk*/*mk* mice suggests that DMT1 is not the only cadmium transporter *in vivo*. Toxicol Appl Pharmacol 227(3):462–467
21. Hao Z, Chen S, Wilson DB (1999) Cloning, expression, and characterization of cadmium and manganese uptake genes from *Lactobacillus plantarum*. Appl Environ Microbiol 65 (11):4746–4752
22. Yu CW, Chen HC, Lin LY (1998) Transcription of metallothionein isoform promoters is differentially regulated in cadmium-sensitive and -resistant CHO cells. J Cell Biochem 68 (2):174–185
23. Ishikawa S, Ishimaru Y, Igura M, Kuramata M, Abe T, Senoura T, Hase Y, Arao T, Nishizawa NK, Nakanishi H (2012) Ion-beam irradiation, gene identification, and marker-assisted breeding in the development of low-cadmium rice. Proc Natl Acad Sci U S A 109(47):19166–19171
24. Fujishiro H, Yano Y, Takada Y, Tanihara M, Himeno S (2012) Roles of ZIP8, ZIP14, and DMT1 in transport of cadmium and manganese in mouse kidney proximal tubule cells. Metallomics 4(7):700–708
25. Sabolic I, Ljubojevic M, Herak-Kramberger CM, Brown D (2002) Cd-MT causes endocytosis of brush-border transporters in rat renal proximal tubules. Am J Physiol Renal Physiol 283(6): F1389–F1402
26. Enomoto S (2005) Development of multitracer technology and application studies on biotrace element research. Biomed Res Trace Elements 16(3):233–240
27. Nebert DW, Gálvez-Peralta M, Hay EB, Li H, Johansson E, Yin C, Wang B, He L, Soleimani M (2012) ZIP14 and ZIP8 zinc/bicarbonate symporters in *Xenopus* oocytes: characterization of metal uptake and inhibition. Metallomics 4(11):1218–1225
28. Roth JA, Garrick MD (2003) Iron interactions and other biological reactions mediating the physiological and toxic actions of manganese. Biochem Pharmacol 66(1):1–13
29. Horiguchi H, Aoshima K, Oguma E, Sasaki S, Miyamoto K, Hosoi Y, Katoh T, Kayama F (2010) Latest status of cadmium accumulation and its effects on kidneys, bone, and erythropoiesis in inhabitants of the formerly cadmium-polluted Jinzu River Basin in Toyama, Japan, after restoration of rice paddies. Int Arch Occup Environ Health 83(8):953–970
30. Himeno S, Yanagiya T, Fujishiro H (2009) The role of zinc transporters in cadmium and manganese transport in mammalian cells. Biochimie 91(10):1218–1222
31. Bosomworth HJ, Adlard PA, Ford D, Valentine RA (2013) Altered expression of ZnT10 in Alzheimer's disease brain. PLoS One 8(5):e65475
32. Park JH, Hogrebe M, Grüneberg M, DuChesne I, von der Heiden AL, Reunert J, Schlingmann KP, Boycott KM, Beaulieu CL, Mhanni AA, Innes AM, Hörtnagel K, Biskup S, Gleixner EM, Kurlemann G, Fiedler B, Omran H, Rutsch F, Wada Y, Tsiakas K, Santer R, Nebert DW, Rust S, Marquardt T (2015) SLC39A8 deficiency: A disorder of manganese transport and glycosylation. Am J Hum Genet 97(6):894–903
33. Valberg LS, Sorbie J, Hamilton DL (1976) Gastrointestinal metabolism of cadmium in experimental iron deficiency. Am J Physiol 231(2):462–467
34. Squibb KS, Pritchard JB, Fowler BA (1984) Cadmium-Metallothionein nephropathy: relationships between ultrastructural/biochemical alterations and intracellular cadmium binding. J Pharmacol Exp Ther 229(1):311–321
35. Chen P, Chakraborty S, Mukhopadhyay S, Lee E, Paoliello MM, Bowman AB, Aschner M (2015) Manganese homeostasis in the nervous system. J Neurochem 134(4):601–610

36. Vallee BL (1995) The function of metallothionein. Neurochem Int 27:23–33
37. Fujishiro H, Yoshida M, Nakano Y, Himeno S (2014) Interleukin-6 enhances manganese accumulation in SH-SY5Y cells: implications of the up-regulation of ZIP14 and the down-regulation of ZnT10. Metallomics 6(4):944–949
38. Okabe M, Nakayama K, Kurasaki M, Yamasaki F, Aoyagi K, Yamanoshita O, Sato S, Okui T, Ohyama T, Kasai N (1996) Direct visualization of copper-metallothionein in LEC rat kidneys: application of autofluorescence signal of copper-thiolate cluster. J Histochem Cytochem 44 (8):865–873
39. Kobayashi K, Kuroda J, Shibata N, Hasegawa T, Seko Y, Satoh M, Tohyama C, Takano H, Imura N, Sakabe K, Fujishiro H, Himeno S (2007) Induction of metallothionein by manganese is completely dependent on interleukin-6 production. J Pharmacol Exp Ther 320(2):721–727
40. Nordberg GF, Goyer R, Nordberg M (1975) Comparative toxicity of cadmium-metallothionein and cadmium chloride on mouse kidney. Arch Pathol 99(4):192–197
41. Tabuchi M, Yoshimori T, Yamaguchi K, Yoshida T, Kishi F (2000) Human NRAMP2/DMT1, which mediates iron transport across endosomal membranes, is localized to late endosomes and lysosomes in HEp-2 cells. J Biol Chem 275(29):22220–22228
42. Schäfer SG, Schwegler U, Schümann K (1990) Retention of cadmium in cadmium-naive normal and iron-deficient rats as well as in cadmium-induced iron-deficient animals. Ecotoxicol Environ Saf 20(1):71–81
43. Sasaki A, Yamaji N, Yokosho K, Ma JF (2012) Nramp5 is a major transporter responsible for manganese and cadmium uptake in rice. Plant Cell 24(5):2155–2167
44. Dalton TP, Miller ML, Wu X, Menon A, Cianciolo E, McKinnon RA, Smith PW, Robinson LJ, Nebert DW (2000) Refining the mouse chromosomal location of *Cdm*, the major gene associated with susceptibility to cadmium-induced testicular necrosis. Pharmacogenetics 10 (2):141–151
45. Kondo Y, Yanagiya T, Himeno S, Yamabe Y, Schwartz D, Akimoto M, Lazo JS, Imura N (1999) Simian virus 40-transformed metallothionein null cells showed increased sensitivity to cadmium but not to zinc, copper, mercury or nickel. Life Sci 64(11):PL145–PL150
46. Fujishiro H, Okugaki S, Yasumitsu S, Enomoto S, Himeno S (2009) Involvement of DNA hypermethylation in down-regulation of the zinc transporter ZIP8 in cadmium-resistant metallothionein-null cells. Toxicol Appl Pharmacol 241(2):195–201
47. Michalke B, Lucio M, Berthele A, Kanawati B (2013) Manganese speciation in paired serum and CSF samples using SEC-DRC-ICP-MS and CE-ICP-DRC-MS. Anal Bioanal Chem 405 (7):2301–2309
48. Filipov NM, Seegal RF, Lawrence DA (2005) Manganese potentiates in vitro production of proinflammatory cytokines and nitric oxide by microglia through a nuclear factor κB-dependent mechanism. Toxicol Sci 84(1):139–148
49. Yanagiya T, Imura N, Kondo Y, Himeno S (1999) Reduced uptake and enhanced release of cadmium in cadmium-resistant metallothionein null fibroblasts. Life Sci 65(14):PL177–PL182
50. Washko P, Cousins RJ (1977) Role of dietary calcium and calcium binding protein in cadmium toxicity in rats. J Nutr 107(5):920–928
51. Min KS, Ueda H, Tanaka K (2008) Involvement of intestinal calcium transporter 1 and metallothionein in cadmium accumulation in the liver and kidney of mice fed a low-calcium diet. Toxicol Lett 176(1):85–92
52. Smith JA, Das A, Ray SK, Banik NL (2012) Role of pro-inflammatory cytokines released from microglia in neurodegenerative diseases. Brain Res Bull 87(1):10–20
53. Fujishiro H, Okugaki S, Kubota K, Fujiyama T, Miyataka H, Himeno S (2009) The role of ZIP8 down-regulation in cadmium-resistant metallothionein-null cells. J Appl Toxicol 29 (5):367–373
54. Park JD, Cherrington NJ, Klaassen CD (2002) Intestinal absorption of cadmium is associated with divalent metal transporter 1 in rats. Toxicol Sci 68(2):288–294

55. Dalton TP, He L, Wang B, Miller ML, Jin L, Stringer KF, Chang X, Baxter CS, Nebert DW (2005) Identification of mouse SLC39A8 as the transporter responsible for cadmium-induced toxicity in the testis. Proc Natl Acad Sci U S A 102(9):3401–3406
56. Liu XF, Supek F, Nelson N, Culotta VC (1997) Negative control of heavy metal uptake by the *Saccharomyces cerevisiae* BSD2 gene. J Biol Chem 272(18):11763–11769
57. Tuschl K, Clayton PT, Gospe SM Jr, Gulab S, Ibrahim S, Singhi P, Aulakh R, Ribeiro RT, Barsottini OG, Zaki MS, Del Rosario ML, Dyack S, Price V, Rideout A, Gordon K, Wevers RA, Chong WK, Mills PB (2012) Syndrome of hepatic cirrhosis, dystonia, polycythemia, and hypermanganesemia caused by mutations in SLC30A10, a manganese transporter in man. Am J Hum Genet 90(3):457–466
58. Fujishiro H, Kubota K, Inoue D, Inoue A, Yanagiya T, Enomoto S, Himeno S (2011) Cross-resistance of cadmium-resistant cells to manganese is associated with reduced accumulation of both cadmium and manganese. Toxicology 280(3):118–125
59. Dantzler WH (2005) Challenges and intriguing problems in comparative renal physiology. J Exp Biol 208(Pt 4):587–594
60. Fujishiro H, Okugaki S, Nagao S, Satoh M, Himeno S (2006) Characterization of gene expression profiles of metallothionein-null cadmium-resistant cells. J Health Sci 52 (3):292–299
61. Wolff NA, Abouhamed M, Verroust PJ, Thévenod F (2006) Megalin-dependent internalization of cadmium-metallothionein and cytotoxicity in cultured renal proximal tubule cells. J Pharmacol Exp Ther 318(2):782–791
62. Elinder CG, Lind B, Kjellström T, Linnman L, Friberg L (1976) Cadmium in kidney cortex, liver, and pancreas from Swedish autopsies. Estimation of biological half time in kidney cortex, considering calorie intake and smoking habits. Arch Environ Health 31(6):292–302
63. Cigliano S, Remondelli P, Minichiello L, Mellone MC, Martire G, Bonatti S, Leone A (1996) Analysis of metal-regulated metallothionein and heat shock gene expression in HeLa-derived cadmium-resistant cells. Exp Cell Res 228(2):173–180
64. Jenkitkasemwong S, Wang CY, Coffey R, Zhang W, Chan A, Biel T, Kim JS, Hojyo S, Fukada T, Knutson MD (2015) SLC39A14 is required for the development of hepatocellular iron overload in murine models of hereditary hemochromatosis. Cell Metab 22(1):138–150
65. Clark HL, Jhingran A, Sun Y, Vareechon C, de Jesus CS, Skaar EP, Chazin WJ, Calera JA, Hohl TM, Pearlman E (2016) Zinc and manganese chelation by neutrophil S100A8/A9 (calprotectin) limits extracellular *Aspergillus fumigatus* hyphal growth and corneal infection. J Immunol 196(1):336–344
66. Liu Z, Li H, Soleimani M, Girijashanker K, Reed JM, He L, Dalton TP, Nebert DW (2008) Cd $^{2+}$ versus Zn^{2+} uptake by the ZIP8 HCO_3^--dependent symporter: kinetics, electrogenicity and trafficking. Biochem Biophys Res Commun 365(4):814–820
67. Fukada T, Kambe T (eds) (2014) Zinc signals in cellular functions and disorders. Springer, Tokyo
68. Fujishiro H, Ohashi T, Takuma M, Himeno S (2013) Down-regulation of S100A9 and S100A10 in manganese-resistant RBL-2H3 cells. J Toxicol Sci 38(5):753–757
69. Girijashanker K, He L, Soleimani M, Reed JM, Li H, Liu Z, Wang B, Dalton TP, Nebert DW (2008) *Slc39a14* gene encodes ZIP14, a metal/bicarbonate symporter: similarities to the ZIP8 transporter. Mol Pharmacol 73(5):1413–1423
70. Fujishiro H, Ohashi T, Takuma M, Himeno S (2013) Suppression of ZIP8 expression is a common feature of cadmium-resistant and manganese-resistant RBL-2H3 cells. Metallomics 5 (5):437–444