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Toxicometallomics of Cadmium, Manganese and Arsenic with Special Reference to the Roles of Metal Transporters

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Abstract

The transport systems for metals play crucial roles in both the physiological functions of essential metals and the toxic effects of hazardous metals in mammals and plants. In mammalian cells, Zn transporters such as ZIP8 and ZIP14 have been found to function as the transporters for Mn(II) and Cd(II), contributing to the maintenance of Mn homeostasis and metallothionein-independent transports of Cd, respectively. In rice, the Mn transporter OsNramp5 expressed in the root is used for the uptake of Cd from the soil. Japan began to cultivate OsNramp5 mutant rice, which was found to accumulate little Cd, to prevent Cd accumulation. Inorganic trivalent arsenic (As(III)) is absorbed into mammalian cells via aquaglyceroporin, a water and glycerol channel. The ortholog of aquaporin in rice, OsLsi1, was found to be an Si transporter expressed in rice root, and is responsible for the absorption of soil As(III) into the root. Since rice is a hyperaccumulator of Si, higher amounts of As(III) are incorporated into rice compared to other plants. Thus, the transporters of essential metals are also utilized to incorporate toxic metals in both mammals and plants, and understanding the mechanisms of metal transports is important for the development of mitigation strategies against food contamination.

Key words: Cadmium, Manganese, Arsenic, Silicon, Zinc, Transporter

ROLES OF METAL TRANSPORTERS IN TOXICOMETALLOMICS

Proteins, nucleic acids, and lipids are essential for the lives of all organisms. From the viewpoint of elemental composition, however, they are composed of a limited number of elements such as C, H, O, N, S, and P. On the other hand, all prokaryotes and eukaryotes require a variety of metals such as Ca, Mg, Na, K, Fe, Zn, Cu, Mn, and so on, for survival. These metals constitute important components or cofactors of versatile biomolecules, and influence the functions of proteins, nucleic acids, and lipids. Metallomics is a field of science that aims to elucidate all features of the actions, interactions, structures, transports and roles of metals in biological systems (1).

Although organisms can biosynthesize most proteins, nucleic acids, and lipids, they cannot create metal elements within cells. They must incorporate metals from the extracellular environment via transporters. Therefore, metal transporters are essential to maintaining appropriate amounts of metals within tissues, cells, and organelles for the survival of all organisms. For example, human cells contain 24 types of Zn transporters, which regulate the influx and efflux of Zn at the plasma membrane and organelles, and the expression of each Zn transporter is distinctly regulated in a tissue-, cell-, and organelle-specific manner (2). Dysfunctions of metal transporters may lead to disturbances in metal homeostasis, resulting in the syndromes of metal deficiency or excess.

It has long been known that metals such as Hg, As, Cd, and Pb play no essential role in organisms, and incorporation of excess amounts of these toxic metals causes a variety of diseases in plants, animals, and humans. During the process of evolution, organisms did not develop any specific transporters for cellular incorporation of these toxic metals. Consequently, when toxic metals enter the cells of animals and plants, they utilize the transporters developed

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for other essential metals. This can happen because most metal transporters have a broad spectrum of affinities for multiple metals. To elucidate the mechanisms of cellular accumulation of toxic metals, the identification and characterization of the transporters involved in the transport of both toxic and essential metals are required. From a viewpoint of toxicometallomics, which is a part of metallomics and focuses on the actions, metabolisms, and the effects of chemical forms of toxic metals as well as the interactions of toxic metals with essential metals, elucidation of the transport mechanisms of both toxic and essential metals is crucial for understanding the accumulation modes of toxic metals and for solving the metal contaminations in plants, animals, and humans.

In this review, we briefly describe the roles of metal transporters involved in the transport of Cd and Mn, as well as those involved in the transport of As, water, and Si in mammals and plants.

TRANSPORTERS FOR CELLULAR INCORPORATION OF Cd AND Mn IN MAMMALS

It has long been considered that metallothionein (MT), a cysteine-rich metal-binding protein, plays several crucial roles in the transport and accumulation of Cd in mammalian cells (3). MT can efficiently bind Cd, as well as other metals such as Zn, Cu, Bi, Hg, and Ag, and protect against cytotoxicity of these metals. In the kidney, which is the target organ of chronic toxicity of Cd, the Cd bound to MT in the blood circulation is readily filtered through the glomerulus, due to its low molecular weight (about 7,000), and then reabsorbed from the lumen to the proximal tubule epithelial cells (PTECs) via megalin-dependent endocytosis (4,5). Since the Cd ions released from the absorbed MT protein within PTECs may continuously induce the synthesis of MT, leading to the binding of Cd ions to the newly synthesized MT protein, the biological half-life of Cd in the kidney was thought to be very long (6). However, the modes of cellular incorporation of Cd via pathways other than Cd-MT endocytosis have not been fully elucidated.

To identify non-MT factors, including non-MT transport systems involved in Cd resistance, we have developed Cd-resistant cell lines from mouse embryonic fibroblast (MEF) cells obtained from MT-I, II knockout mice via stepwise increases in Cd concentrations in the media (7). The MT-null Cd-resistant cells showed a markedly reduced accumulation of Cd, primarily caused by extremely reduced Cd uptake rates (7). Since there is no specific transporter for Cd uptake in mammalian cells, it is assumed that expression of the transporter that functions for the uptake of other essential metals, and also has an affinity for Cd, was suppressed in the Cd-resistant MT-null cells. To identify which metal was involved in this suppression of metal uptake, we applied a multi-tracer technique (8). Among 20 metals examined by this technique, Mn(II) showed a markedly lower uptake rate in Cd-resistant MT-null cells, suggesting that Cd(II) and Mn(II) share the same pathway for cellular incorporation (8).

The transport of Cd(II) and Mn(II) by the same transporter was also supported by competitive inhibition of the uptakes of Cd(II) and Mn(II) (8,9). Subsequent microarray analysis revealed that the expression of ZIP8, a Zn transporter coded by *Slc39a8*, was suppressed in Cd-resistant MT-null cells compared with parental MT-null cells (10). The role of ZIP8 in Cd transport was confirmed by the reduced uptake of Cd(II) in the parental MT-null cells transfected with shRNA of ZIP8 (11). The suppressed expression of ZIP8 in Cd-resistant MT-null cells was not due to the mutation of *Slc39a8* but to epigenetic hypermethylation of the *Slc39a8* gene, because the treatment of Cd-resistant MT-null cells with 5-aza-cytidine, an inhibitor of DNA methyltransferase, led to the recovery of ZIP8 expression, the enhanced uptake of Cd, and consequently, the enhanced cytotoxicity of Cd (12).

To further examine the role of ZIP8 in Cd resistance, we also established Cd-resistant cell lines from MT-expressing MEF cells and from rat basophilic leukemia RBL-2H3 cells. The latter showed extremely high sensitivity to Cd. Both Cd-resistant cell lines showed suppressed expression of ZIP8 with lowered uptake rates of Cd (13,14). Interestingly, both Cd-resistant cell lines showed cross-resistance to $MnCl_2$ due to the lowered uptake rates of Mn(II). As the parental RBL-2H3 cells showed higher expression of ZIP8 and higher sensitivity to $MnCl_2$ cytotoxicity than the other rat cell lines examined (15), we also developed Mn-resistant cells from RBL-2H3 cells. As expected, the Mn-resistant RBL-2H3 cells showed a cross-resistance to Cd, accompanied by the suppression of ZIP8 expression and lower uptake rates of both Mn(II) and Cd(II) (14). Collectively, the results obtained from Cd-resistant and Mn-resistant cells indicate that ZIP8 plays an important role in the transport of Cd(II) and Mn(II) in addition to Zn(II), and that the lack of ZIP8 expression results in the reduced accumulation and cytotoxicity of Cd and Mn.

In addition to ZIP8, ZIP14, which has the highest similarity to ZIP8 in its amino acid sequence among ZIP family transporters, was also shown to be involved in the transport of Cd(II) and Mn(II) (Fig. 1) in our studies and those of other groups (16,17). The affinities of ZIP8 and two isoforms of ZIP14, ZIP14A and ZIP14B, for Cd(II), Mn(II), and Zn(II) have been compared in the mammalian cells and *Xenopus* oocytes in which these transporters were ectopically expressed (17). The Km values for the uptake of Cd(II) (0.62 μM, 0.10 μM, and 0.14 μM for ZIP8, ZIP14A, and ZIP14B, respectively) were lower than those of Mn(II) (2.2 μM, 18 μM, and 4.4 μM for ZIP8, ZIP14A, and ZIP14B, respectively) in mammalian cells,

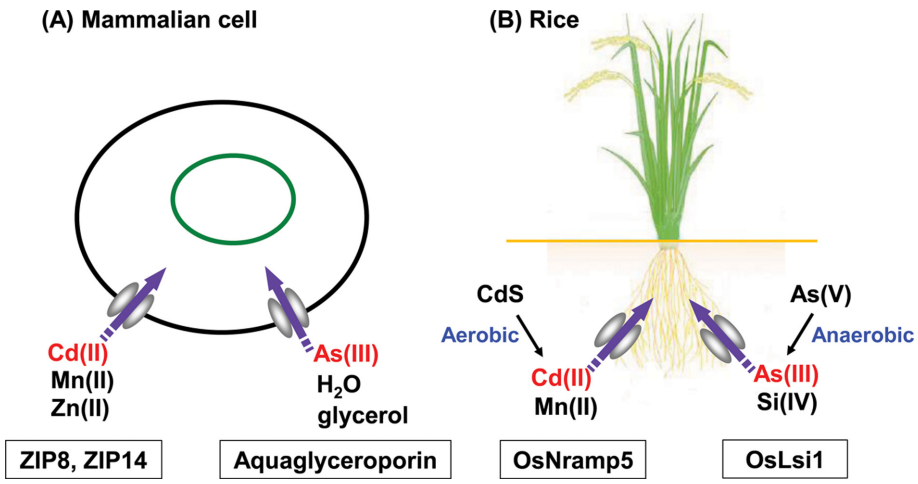

Fig. 1. Schematic illustrations of Cd and As(III) uptake via metal transporters in mammalian cells (A) and rice root (B). (A): Among the ZIP family members of Zn transporters, ZIP8 and ZIP14 participate in the transports of Cd(II) and Mn(II) as well as that of Zn(II). The functionalities of ZIP8 and ZIP14 for the transports of Cd(II) and Mn(II) have been recognized in a variety of mammalian cells including hepatic (15), renal (21,23), cardiac (15), neuronal (16), intestinal (19), and uterine (8) cells. Cellular uptake of As(III) in mammalian cells is mediated by aquaglyceroporin, a water channel used also for the uptake of glycerol (34-37). (B): In the root of rice, the uptake of Cd(II) is mediated by the Mn(II) transporter, OsNramp5 (29), and that of As(III) is mediated by the Si(IV) transporter OsLsi1 (44), which is an ortholog of aquaporin in mammals. Since rice is a hyperaccumulator of Si (42), higher amounts of As(III) is accumulated in rice than other plants. The microenvironment of soil and water surrounding the rice root affect the release of soluble Cd(II) and As(III) from the soil in opposite ways. Under aerobic conditions, the insoluble CdS in soil, which is stable under anaerobic conditions, is oxidized to $CdSO_4$, leading to the release of soluble Cd(II). In contrast, under anaerobic conditions, the complex of As(V)-Fe(III), which is stable under aerobic conditions, is reduced to As(III) and Fe(II), leading to the release of soluble As(III). To achieve the mitigation of Cd and As contaminations in rice by the management of irrigation water, this trade-off problem should be solved (46-49).

whereas the Km values for the uptake of Cd(II) (0.48 μM and 0.46 μM for ZIP8 and ZIP14A, respectively) were roughly similar to those of Zn (0.26 μM and 0.38 μM for ZIP8 and ZIP14A, respectively) in *Xenopus* oocytes (17). These data indicate the high affinity of ZIP8 and ZIP14 for Cd(II).

A divalent metal transporter 1 (DMT1) also has affinities not only for Fe(II) but also for Mn(II) and Cd(II) (18). Since the expression of DMT1 is high in the intestine (11,18,19), and that of ZIP14 is high in the liver (20), these transporters may play tissue-specific roles in the transport of Cd(II) and Mn(II) in different tissues of the body. We found high expression of ZIP8 in the S3 region (straight part) of kidney proximal tubules (21-23). Since it is known that Cd-MT in the glomerular filtrate is mainly reabsorbed in the S1 and S2 regions of proximal tubules (4,5), where megalin-dependent endocytosis largely contributes to the reabsorption of low-molecular-weight proteins, ZIP8 may play a different role in the reabsorption of Cd not bound to MT in the lumen (24).

To explore the possibility that ZIP8 is involved in the reabsorption of Cd(II) from the apical side of PTECs, especially at the S3 region, we carried out an *in vitro* experiment using immortalized S1, S2, and S3 cells derived from each region of mouse proximal tubules (23). When Cd(II) was added to the apical side of monolayer S1, S2, and S3 cells cultured on the membrane of the trans-well, the highest uptake of Cd(II) was detected in S3 cells, and the uptake of Cd(II) from the apical side of S3 cells was competitively inhibited by Mn(II) and Zn(II) (23). This suggests that ZIP8 plays a role in the reabsorption of luminal Cd(II) in the S3 region of proximal tubules. However, further *in vivo* studies are needed to clarify the roles of ZIP8 in renal handling and accumulation of Cd.

Recently, accumulating evidence has shown the role of ZIP8 as an Mn(II) transporter in humans. In Germany and Egypt, congenital glycosylation dysfunctions in infants were found to be related to mutations of human *SLC39A8* coding ZIP8 (25,26). Blood Mn levels of these infants were extremely low or undetectable. Since the galactosyl transferase, which is essential for the glycosylation process in the body, is highly dependent on Mn as a cofactor, disturbed metabolism of Mn might have led to the whole-body disorder of glycosylation. Since the excretion rate of Mn from the liver into bile is markedly high, the role of ZIP8 in the epithelial cells of the bile ducts in reabsorbing the Mn excreted to the bile has been considered (27). The ZIP8 expressed in the S3 region of renal proximal tubules may also be involved in the renal reabsorption of Mn (23), but the physiological roles of ZIP8 in the regulation of

whole-body Mn homeostasis remain to be elucidated.

Recently, the results of the genome-wide association study (GWAS) showed the links between the SNP of ZIP8 causing amino acid change (p.Ala391Thr) and a variety of conditions such as hypertension, schizophrenia, and other common diseases (28). Future studies will be needed to clarify whether disordered Mn metabolism is involved in these diseases. Thus, greater attention is currently being paid to the roles of ZIP8 not only in Cd transport but also in Mn transport in humans.

ABSORPTION OF SOIL Cd IN RICE VIA A Mn TRANSPORTER

For Asian people who eat large amounts of rice as a staple food, the contamination of rice with toxic metals such as Cd or As is an important issue, and the scientists in the fields of plant physiology and soil sciences are attempting to mitigate the metal contamination of rice. Since plants cannot move like animals, they evolved a variety of transport systems for acquiring necessary metals from the soils. One plant physiology study revealed that OsNramp5 located in the outer membrane of rice root is the transporter responsible for the uptake of Mn into rice (29). Intriguingly, OsNramp5 also has an affinity for Cd (Fig. 1), and the knockout of the *OsNramp5* gene resulted in almost complete loss of Cd absorption into the roots and consequently grains of rice (29). Since Mn constitutes the catalytic centers of the water-oxidizing complex of photosystem II in plants, it is inevitable that rice will absorb significant amounts of soil Cd via the transporter for Mn. Thus, the transport systems for Mn play an important role in Cd incorporation in rice as well as in mammals.

Recently in Japan, mutant rice, in which the function of OsNramp5 is lost and therefore Cd is not accumulated, was developed and has begun to be cultivated as a countermeasure against Cd contamination of rice. This mutant rice was developed using an ion-beam irradiation that can generate a variety of mutations with high specificity in plants (30). Approximately 3,000 rice seeds were irradiated with an ion beam, and three mutants obtained in the next generation seeds were found to produce rice that accumulated no detectable levels of Cd in their grains even when they were cultivated in Cd-contaminated soil. All three mutant rice varieties were found to have a mutation in the *OsNramp5* gene (30). The mutant rice also showed low levels of Mn, but did not show apparent Mn deficiency symptoms such as growth defects, possibly because of the compensatory uptake of Mn by other pathways.

TRANSPORTERS FOR CELLULAR INCORPORATION OF As IN MAMMALS

Human As poisonings have been extensively reported in Asian countries, especially in Bangladesh, India, and China, where groundwater is contaminated by inorganic As (iAs) from the earth's crust (31). On the other hand, arsenic trioxide is clinically used for the treatment of acute promyelocytic leukemia (32). Among As compounds, iAs such as arsenite (As(III)) and arsenate (As(V)) are highly toxic compared with the methylated forms of As. Therefore, elucidation of the transport systems for iAs is important for understanding the metabolic fates and pharmacological or toxicological effects of As compounds.

The search for the transport systems for As(III) in eukaryotes was triggered by a report showing that the *Saccharomyces cerevisiae* strain that lacks the expression of glycerol transporter Fps1p exhibited a resistance to As(III) (33). The yeast Fps1p is a protein homologous to the mammalian aquaglyceroporins AQP7 and AQP9. Microinjection of mammalian AQP7 or AQP9 into *Xenopus* oocytes (34) and that of AQP9 into mouse hepatocytes (35) resulted in enhanced uptake of As(III). AQP3 and AQP10 have also been shown to transport As(III) in mammalian cells (36,37). Aquaglyceroporins (AQP3, 7, 9, and 10) are members of the aquaporin superfamily, which is basically a water channel. However, aquaglyceroporins can also permeate electrically neutral small molecules such as glycerol and urea in addition to water molecules. Since AQP9 is highly expressed in the liver, AQP9 may play an important role in the absorption of As(III) into the liver, the major organ for the metabolism of ingested As compounds (38).

The expression of AQP9 is also detected in human leukemia cell lines such as HL-60 (39). Recently, we found that treatment of HL-60 cells with all-*trans* retinoic acid, the first-line drug for treatment of acute promyelocytic leukemia, significantly increased the expression of AQP9, leading to an enhanced cellular uptake of As(III) (39). This suggests that co-treatment of leukemic cells with all-*trans* retinoic acid and arsenic trioxide may enhance the therapeutic efficacy of arsenic trioxide.

As(V), another form of iAs, is absorbed by phosphate transporters because arsenate has physicochemical properties similar to those of phosphate. In particular, it has been suggested that the intestinal phosphate transporter NaPiIIb is involved in the absorption of As(V) into the body (40).

ABSORPTIONS OF As VIA Si TRANSPORTER IN RICE AND ITS TRADE-OFF PROBLEM WITH Cd

In areas where As poisoning is prevalent, not only groundwater for drinking and cooking but also the rice cultivated with irrigation water derived from As-contaminated groundwater is the source of As ingestion in humans (41). Since As is not an essential element for plants, the absorption of As into rice is mediated by other essential elements, similar to Cd absorption via the Mn transporter.

The rice accumulates higher amounts of As than other plants because of its specific nature as a hyperaccumulator of Si. The content of Si in rice is as high as 10% of the total weight, and Si deficiency causes detrimental effects on the rigidity and stress resistance of rice (42). The absorption of Si in the form of $Si(OH)_4$ from the soil to rice root is mediated by the OsLsi1 transporter expressed in rice root (43). Intriguingly, OsLsi1 is an ortholog of mammalian AQP, which is involved in As(III) uptake. It was found that OsLsi1 has the ability to transport As(III) in addition to Si into rice root (Fig. 1) (44). Thus, rice, as a hyperaccumulator of Si inevitably becomes a hyperaccumulator of As(III). Actually, 60-80% of As in rice grains is in the form of As(III) (45). Since OsLsi1 plays an essential role in the absorption of Si, its inactivation by mutation or knockout rice could not be achieved.

The solubilities of As(III) and As(V) from soil are affected by the oxidizing/reducing conditions of the microenvironment of soil and water around the rice root (46-48). For cultivation of rice, the management of the irrigation water supply is important; water is supplied to (flooding) or drained from (non-flooding) the paddy field depending on the growth periods of rice. The "flooding" causes reducing (anaerobic) conditions in the microenvironment of soils and water surrounding the rice roots due to the blockage of air, while the "non-flooding" causes oxidizing (aerobic) conditions. Under the oxidizing conditions, As and Fe form a stable and insoluble complex in the form of As(V)-Fe(III)$(OH)_3$, whereas under the reducing conditions the reductions of Fe(III) to Fe(II) and As(V) to As(III) occur, leading to the release of soluble As(III) from the soil. Thus, the flooding of rice fields facilitates the release of the soluble form of As(III), and therefore the non-flooding of fields is preferable to minimize the absorption of As(III) into rice, especially at harvest time (45).

Unfortunately, however, the release of soluble Cd from insoluble CdS in the soil via the formation of $CdSO_4$ is facilitated under the oxidizing conditions and suppressed under the reducing conditions (47,49). This opposite behavior of As and Cd depending on environmental conditions produces a trade-off problem in the mitigation strategy against metal contaminations of rice. Especially in Japan, flooding rice fields at harvest time is recommended as a water management strategy for reducing Cd accumulation in rice in areas where there is soil contamination of Cd (49,50); this strategy may, however, increase As accumulation in rice. Since the Codex Alimentarius Commission of FAO/WHO has recommended that iAs concentration in polished rice grains be less than 0.2 mg/kg (51), a solution for this trade-off problem is urgently needed, especially in areas where soil Cd contamination is detected. The utilization of the low-Cd rice cultivar (30,52) may be one of the warranted solutions for this problem.

CONCLUSIONS

In this review, recent advances in the study of the transport systems for Cd and As in mammals and plants are briefly summarized. In both mammals and plants, transporters originally evolved for the uptake of essential elements such as Mn, Zn, and Si are used for the uptake of toxic metals such as Cd and As. To facilitate the mitigation of metal contamination in plants and animals, a more detailed understanding of the whole profiles of transporting systems for essential and toxic metals is needed.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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