Multidrug and Toxic Compound Extrusion Protein-1 (MATE1/SLC47A1) Is a Novel Flavonoid Transporter

Ji Hae Lee,† Jung Eun Lee,‡ Yeojin Kim,† Hojoung Lee,§ Hee-jin Jun,† and Sung-Joon Lee*,†

†Department of Biotechnology, BK21-PLUS Graduate School of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea
‡Department of Food and Nutrition, Sookmyung Women’s University, Seoul 140-742, Republic of Korea
§Division of Biotechnology, College of Life Sciences and Biotechnology, Korea University, Seoul 136-713, Republic of Korea

ABSTRACT: Dietary flavonoids have various biological functions. However, their cellular transport mechanisms are largely unknown. We have determined that the multidrug and toxic compound extrusion transporter-1 (MATE1) is a membrane transporter for flavonoids and has a high affinity for quercetin. HEK293T cells overexpressing MATE1 exhibited increased intracellular quercetin accumulation. This effect disappeared in the presence of a MATE1 inhibitor and after MATE1 gene knockdown. HepG2 cells expressed MATE1 significantly, with the uptake quercetin of which was dramatically reduced with MATE1 inhibition. On the basis of immunofluorescence analysis, MATE1 was highly expressed in peroxisomes and the endoplasmic reticulum (ER) as well as in plasma membranes in the liver and intestine, which suggests potential accumulation of quercetin in peroxisomes and the ER in these tissues. Fluorescent microscopic analysis confirmed selective accumulation of quercetin in peroxisomes. The effects of quercetin on cellular lipid reduction and glucose uptake were exaggerated with MATE1 overexpression. In conclusion, MATE1 is a membrane transporter for quercetin; its overexpression enhances the hypolipidemic activity of quercetin and cellular glucose transport. Considering the low bioavailability of quercetin, appropriate regulation of MATE1 expression may optimize cellular quercetin concentrations and promote health benefits.

KEYWORDS: flavonoid transporter, multidrug and toxic compound extrusion transporter-1, lipid metabolism, quercetin

INTRODUCTION

Flavonoids are one of the largest classes of plant secondary metabolites and are abundant in fruits and vegetables. The ubiquitous nature of flavonoids in plant-based diets has led to extensive studies of the effects of their biological activities on human health. It is well-known that flavonoids are antioxidants because of the numerous hydroxyl groups in their chemical structures and, thus, can donate electrons through resonance to stabilize the free radicals. They also possess hypolipidemic, hypoglycemic, anti-inflammatory, and antiobesity activities, which are important activities in the context of metabolic syndrome.2,3 The pharmacological activities of flavonoids have been well-established. For example, daily administration of quercetin, a representative flavonoid that is abundant in common dietary plants, ameliorated metabolic syndrome with an improvement in abdominal obesity, insulin resistance, dyslipidemia, and hypertension in obese Zucker rats.4 In a human study, an obese subject with a high risk of cardiovascular disease experienced a reduction in systolic blood pressure and plasma-oxidized low-density lipoprotein (LDL) concentration after 6 weeks of quercetin (150 mg/day) administration.5

Membrane transporters mediate the cellular uptake of most lipophilic nutrients, including cholesterol, fatty acids, and bile acids.6−8 Cellular flavonoid uptake studies also show saturation kinetics and sensitivity to protease treatment, which suggests unique transporters for uptake. However, the membrane transporter responsible for flavonoid uptake has not been clearly identified. In mammals, it has been previously reported that bilitranslocase, which takes up bilirubin, is also a flavonoid transporter, although not the sole transporter.9 Passamonti et al. reported that the intake of flavonoid-rich beverages induces vasodilation with increased concentrations of intracellular flavonoids in the vascular endothelium, which suggests that the uptake of flavonoids, particularly quercetin and malvidin-3-glycoside, is mediated by endothelial bilitranslocase.9,10 In their study, flavonoid uptake was a rapid and specific process and was inhibited by a bilitranslocase-specific antibody. In addition to bilitranslocase, the ATP-binding cassette transporters, including P-glycoprotein and multidrug resistance-associated protein-1, have been suggested as potential flavonoid transporters;11 however, the correct subtype of the membrane transporter that is responsible for flavonoid uptake is not yet known.

The bioavailability of flavonoids is often extremely low in mammals; e.g., anthocyanin absorption in human intestinal cell monolayers was between 3 and 4% of the administered dose.12 Thus, the improvement of the bioavailability of flavonoids may contribute to increased bioactivity and health benefits, including the elevation of cellular flavonoid uptake. Despite the importance of the cellular uptake mechanism of flavonoids, their transport mechanism remains largely unknown in mammalian tissues but is better understood in plants.
In plants, flavonoids are contained in most compartments of cells, including the cytosol, vacuoles, endoplasmic reticulum (ER), chloroplasts, nucleus, and extracellular space, although the sites of flavonoid biosynthesis, storage, and usage are all separate. This observation indicates the presence of efficient systems that transport flavonoids across endomembranes and plasma membranes in plants. Recent studies have implicated multidrug and toxic compound extrusion transporters (MATEs), which are major proteins involved in the transport of plant secondary metabolites, including flavonoids. MATEs are found on vacuolar membranes in plant cells and mediate flavonoid uptake using electrochemical gradients across membranes, which are generated by proton pumps, including P-type ATPase, vacuolar V-type ATPase, and H+-pyrophosphatase. MATEs have been found in the vacuolar membranes of various plant species, with various substrate specificities. For example, MATE from Arabidopsis and Medicago truncatula transports epicatechin-3-O-glucoside, whereas a grapevine MATE transports cyanidine-3-O-glucoside to the inside of the vacuole.

Interestingly, MATE orthologs are also highly expressed in humans, particularly in the kidney, adrenal gland, liver, and skeletal muscle. Human MATE1 has 95% amino acid sequence homology compared to that of Arabidopsis. To date, the major role of human MATE1 is thought to be in the transport of various organic cationic drugs, such as tetraethylammonium, 1-phenylalanine, and 1-phenylethylamine. However, some anionic compounds, including estrone sulfate, acetylcholine, and gliclizide, have also been reported to be MATE1 substrates.

The purpose of this study was to investigate whether MATE1 could take up dietary flavonoids in metabolically active mammalian cells, such as hepatocytes. To assess this, we first demonstrated the cellular localization of MATE1, in both mouse and human liver and intestine, via immunofluorescence microscopy. We then assessed intracellular flavonoid accumulation in cultured hepatocytes and HEK293 cells overexpressing MATE1, using eight representative dietary flavonoids (quercetin, kaempferol, luteolin, apigenin, and their glycosides). Experiments with MATE1 gene knockdown and MATE1 inhibitors were performed to demonstrate MATE1-specific flavonoid transport.

### MATERIALS AND METHODS

**Cell Culture.** HepG2 and HEK293T cells (Korean Cell Line Bank, Seoul, Korea) were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% heat-inactivated fetal bovine serum and 1% penicillin—streptomycin at 37 °C in 5% CO₂. To establish a MATE1-overexpressing stable cell line (hMATE1), human MATE1 plasmid DNA (Origene, Rockville, MD) in a neomycin-resistance vector was transfected into HEK293T cells. Cells that stably expressed MATE1 DNA (Origene, Rockville, MD) in a neomycin-resistance vector were used to establish an MATE1 overexpressing stable cell line (hMATE1). Human MATE1 plasmid DNA (Origene, Rockville, MD) was used to establish an MATE1-overexpressing stable cell line (hMATE1).

**Cellular Flavonoid Accumulation Assay.** Quercetin, kaempferol, luteolin, apigenin, and quercetin-3-O-glucoside were purchased from Sigma-Aldrich (St. Louis, MO). Kaempferol-3-O-glucoside was purchased from Extrasynthese (Genay, France). Luteolin-7-O-glucoside and apigenine-7-O-glucoside were purchased from the Indofine Chemical Company (Hillsborough, NJ). HEK293T and hMATE1 cells were seeded in 12-well plates at 5 × 10⁵ cells/well the day before treatment. Cells were stimulated with flavonoids for 0, 2, 4, 6, 8, and 10 min, and then the cellular flavonoid concentration was quantified using spectrophotometry as described previously. After washing cells twice with phosphate-buffered saline (PBS), cellular flavonoids were extracted with methanol (1 mL/well) at 65 °C for 1 h, followed by centrifugation at 17,000 rpm for 2 min. The supernatants were concentrated with a SpeedVac concentrator (N-Biotek, Bucheon, Korea) and then dissolved in 200 μL of 4,6-diamidino-2-phenylindole, which contained dimethyl sulfoxide (DMSO), in a fluor 96-well plate. The intensity of the fluorescence signal was measured with an excitation/emission of 485/535 nm using a Victor X2 multilabel plate reader (PerkinElmer, Waltham, MA). The flavonoid concentrations were calculated using a standard curve that was generated with known concentrations of the examined flavonoids.

**Effects of MATE1 Gene Knockdown, Overexpression, and Inhibitors on Cellular Quercetin Accumulation.** siRNA of the MATE1 gene was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). HEK293T cells were transfected with MATE1 siRNA (20 pmol/5 × 10⁵ cells) and incubated for 1 day before experiments. The knockdown of MATE1 gene expression was confirmed by immunoblot analysis. In the assays with transporter inhibitors, phenylmethylsulfonyl fluoride (PMSF, 100 μM), cyclopiazonic (50 μM), pyrimethamine (1 μM), and bafilomycin A1 (100 nM) were incubated for 30 min before quercetin stimulation. Cellular flavonoid quantification was performed according to the method described in the section above.

**Immunofluorescence Assay.** HepG2 and hMATE1 cells were cultured on polylysine-coated glass slides for 24 h and fixed with 4% paraformaldehyde for 20 min. Mouse liver and intestine tissues from C57BL/6j mice were fixed in 4% paraformaldehyde, embedded in optimal cutting temperature (OCT) compounds, and sectioned on a cryotome at −25 °C (10–20 μM thickness, CM3050S, Leica, Wetzlar, Germany). The human tissue sections were purchased from Genetex (San Antonio, TX). For immunofluorescence staining, fixed cells and tissues were incubated with 5% bovine serum albumin (BSA) in PBS for 30 min and immunostained with a rabbit polyclonal MATE1 antibody (Abcam, Cambridge, MA), together with an antibody for a marker protein of intracellular organelles, for 1 h. Antibodies for mouse monoclonal pan-cadherin, goat polyclonal calnexin, mouse monoclonal lysosome-associated membrane protein-1 (LAMP1), goat polyclonal calnexin, goat polyclonal peroxisomal, and goat polyclonal catalase were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). As a secondary fluorescent antibody, Alexa Fluor 532 goat anti-rabbit was used and incubated for another 1 h with either Alexa Fluor 488 goat anti-mouse or Alexa Fluor 488 donkey anti-goat antibody. The coverslips were embedded in anti-fade mounting medium after staining with 4,6-diamidino-2-phenylindole. Image analysis was performed using a Zeiss LSM510 META confocal microscope and the Zeiss LSM510 version 3.2 software (Carl Zeiss, Jena, Germany). Co-localized spots were quantified using ImageJ software (version 1.47, National Institutes of Health (NIH), Bethesda, MD) and calculated using [co-localization (%) = yellow spots/total spots × 100].

**Immunoblotting.** Plasma membrane, ER, and peroxisomal fractions were isolated from mouse livers using density gradient centrifugation as described previously. The protein concentration was determined using the Bradford reagent, with BSA as a standard. Protein samples (40 μg) in Laemmli sample buffer were boiled for 5 min and separated on 8–10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels. Separated proteins were electroforetically transferred onto nitrocellulose membranes. Non-specific binding was blocked with 5% nonfat dry milk in Tris-buffered saline with Tween-20 (TBST) buffer for 1 h at room temperature (RT), and membranes were incubated with primary antibodies. After washing several times with 1% TBST wash buffer, membranes were incubated with the secondary antibody for 1 h at RT. Immunoreactive bands were detected by enhanced chemiluminescence western blot detection reagents and quantified by the ChemiDoc XRS system (Bio-Rad, Hercules, CA) using the Quantity One software (version 4.6.9, Bio-Rad). The enzyme-linked immunosorbent assay (ELISA) assay for MATE1 protein quantification was performed according to the instructions of the manufacturer (Cusabio, Wuhan, China).

**Measurement of Cellular Lipoxygenase, Peroxidation, and Glucose Uptake.** Oleic acid was dissolved in ethanol at 100 mM for use as a...
Figure 1. Cellular localization of MATE1 in cultured cells and tissues. (A) Localization of MATE1 in HepG2, hMATE1 cells, and mouse and human tissues. Antibodies against pan-cadherin [plasma membrane (PM)], prohibitin [mitochondria (MI)], LAMP-1 [lysosome (LY)], calnexin (ER), and peroxin [peroxisome (PER)] labeled cellular organelles. Blue, green, and red signals indicate nuclei, cellular organelles, and MATE1, respectively. Images were taken using a confocal microscope. (B) Immunoblotting of MATE1 with cellular organelles from the mouse liver. (C) Concentrations of MATE1 in mouse liver plasma, ER, and peroxisomal membrane determined by ELISA. (D) Localization of MATE1 (green) on peroxisome (catalase, red) in MATE1 overexpressed and knockdown HepG2 cells. (E) Co-localization of cellular quercetin (green) and catalase (red), a marker for peroxisomes in HepG2 cells. The values are the mean ± SEM. Statistical significance was analyzed using a one-way ANOVA and Student’s t test. p < 0.05 was considered statistically significant.
stock solution. To make lipid-loading media, the oleic acid stock solution was diluted to 400 \( \mu \text{M} \) in serum-free media, which included 0.5% BSA. Control and hMATE1 cells were seeded in 6-well plates at a concentration of 5 \( \times \) 10^5 cells/well and stimulated with normal media, oleic acid-containing media, or oleic acid- and flavonoid-containing media for 10 min. The cellular flavonoids were quantified every 2 min as described in the Materials and Methods. The values are the mean \( \pm \) SEM. \((\ast) p < 0.05\) versus the control. (F) Cell viability. Control (HEK293T) and hMATE1 cells were incubated in PBS buffer at pH 4–9 for 5 min. (F) Intracellular quercetin accumulation at various pH values. The values are the mean \( \pm \) SEM. The group comparison was performed using a one-way ANOVA. QG, quercetin-3-O-glucoside; KG, kaempferol-3-O-glucoside; LG, luteolin-7-O-glucoside; and AG, apigenine-7-O-glucoside.

**Figure 2.** Effect of MATE1 overexpression on cellular flavonoid accumulation. (A–D) Time-course accumulation of intracellular flavonoids. Quercetin (Q), kaempferol (K), luteolin (L), and apigenin (A) were incubated (100 \( \mu \text{M} \) each) with control HEK293T or hMATE1 cells for 10 min. The cellular flavonoids were quantified every 2 min as described in the Materials and Methods. The values are the mean \( \pm \) SEM. \((\ast) p < 0.05\) versus the control. (E) Cell viability. Control (HEK293T) and hMATE1 cells were incubated in PBS buffer at pH 4–9 for 5 min. (F) Intracellular quercetin accumulation at various pH values. The values are the mean \( \pm \) SEM. The group comparison was performed using a one-way ANOVA. QG, quercetin-3-O-glucoside; KG, kaempferol-3-O-glucoside; LG, luteolin-7-O-glucoside; and AG, apigenine-7-O-glucoside.

**Statistical Analysis.** The data are expressed as the mean \( \pm \) standard error of the mean (SEM). Differences were considered statistically significant at \( p < 0.05\) or by a one-way analysis of variation (ANOVA) test. Each experiment was performed at least in triplicate.

**RESULTS**

**Cellular Localization of MATE1 in the Liver.** The tissue distribution of MATE1 expression in mice revealed substantial
gene expression of MATE1 in the liver and several other tissues, such as the kidney, which suggests that MATE1 may play a role in the cellular accumulation of the flavonoid quercetin in the liver (see Figure S1 of the Supporting Information). Immunofluorescence analysis investigated the subcellular localization of MATE1 in mouse and human liver tissues as well as in cultured cells with antibodies for marker proteins of plasma (pan-cadherin), mitochondrial (prohibitin), lysosomal (LAMP), ER (calnexin), and peroxisomal (peroxin or catalase) membranes (Figure 1). Our results indicate that the MATE1 protein is expressed in liver tissues. The expression levels were similar in both mice and human tissues. Immunofluorescence analysis suggested that MATE1 is primarily expressed in the plasma, ER, and peroxisomal membranes in cultured hepatocytes, hMATE1 cells, and both mouse and human liver tissues (Figure 1A). These results were confirmed with isolated membrane fractions from mouse liver using immunoblotting analysis and ELISA (panels B and C of Figure 1). Analysis with intestinal tissues was confirmed (see Figure S2 of the Supporting Information). To confirm the MATE1 expression on peroxisome, HepG2 cells were transfected with MATE1 siRNA and were analyzed with immunofluorescence analysis (Figure 1D). Result confirmed the reduction of MATE1 expression in cells with MATE1 knockdown (~56% versus MATE1 overexpression; p < 0.05). Next, we performed immunofluorescent microscopy to localize the subcellular accumulation of quercetin (Figure 1E). The results suggest that a significant amount of quercetin is accumulated in the peroxisome. The accumulation of quercetin in peroxisomes may be due to uptake by MATE1 in the peroxisomal membrane. A significant amount of quercetin was also found in the nucleus.

**Effect of MATE1 on Flavonoid Transport.** Because MATE1 was highly expressed in the liver tissue, we next investigated cellular flavonoid uptake by MATE in HEK293 cells overexpressing hMATE1. Eight representative flavonoids that are abundant in common dietary plants were examined (see Figure S3 of the Supporting Information). The flavonoid concentration used in this study was chosen on the basis of preliminary assay results (see Figure S3 of the Supporting Information). Cellular flavonoid uptake occurs rapidly, and of the tested compounds, quercetin exhibited the most efficient uptake by MATE1, which was determined by comparing the uptake in hMATE1 and control cells. The quercetin concentration at 8 min was increased by 110% in hMATE1 cells when compared to the controls (17 versus 8 nmol/5 × 10^5 cell in hMATE1 and controls, respectively; p < 0.05). Kaempferol was transported by MATE1 to a lesser degree than quercetin. The intracellular accumulations of quercetin-3-O-glucoside, kaempferol-3-O-glucoside, luteolin, luteolin-7-O-glucoside, apigenin, and apigenine-7-O-glucoside were marginally but significantly altered by hMATE1 overexpression, which suggested that MATE1 might transport diverse flavonoids, including both aglycones and glycosides, with different affinities.

It is difficult to predict the substrate specificity of MATE1, as evidenced by our results. However, four factors appear to determine the efficiency of MATE1-dependent flavonoid uptake: molecular weight, the number of hydrogen bond donors and acceptors, hydrophobicity, and ionization status.
First, our results demonstrate that aglycones were superior substrates for MATE1 compared to glycosides and exhibited greater cellular uptake. The reason for this is not clear; however, it is possible that flavonoid glycosides may be too large for MATE1, because most known substrates for MATE1 have a molecular weight of less than 300 g/mol. Additionally, aglycones with a greater number of hydrogen bond donors and acceptors and a lower degree of hydrophobicity showed greater cellular uptake. Thus, quercetin showed the highest level of cellular uptake among all of the tested flavonoids because it is a small aglycone and shows the lowest hydrophobicity among tested flavonoid aglycone (see Table S1 of the Supporting Information).

Finally, the ionization of flavonoid aglycones, at a basic pH, hindered cellular uptake. Flavonoids are not ionized at a neutral pH ($pK_a = 6.74–7.76$), but hydroxyl groups progressively remove protons in accordance with increasing pH. In quercetin, cell viability was reduced slightly at various pH values compared to at pH 7, but the reductions were not statistically significant (Figure 2E). Cellular quercetin uptake was pH-dependent and greatest at pH 6, at which quercetin is not ionized. The quercetin concentration was increased in hMATE1 cells by 105% compared to controls, at this pH value ($p < 0.001$; Figure 2F). In contrast, at pH 9, the cellular quercetin accumulation was markedly reduced in comparison to at pH 6 and was only moderately higher in hMATE1 cells compared to controls. These findings demonstrate that a negative charge on flavonoids may prohibit uptake by MATE1.

Quercetin Accumulation in Cells with hMATE1 Knockdown in HEK293T Cells and MATE Inhibitor Treatment in HepG2 Cells. Transfection with hMATE1 siRNA knocked down its protein expression in HEK293T cells by $-41\%$ compared to the control (Figure 3A). Cellular flavonoid accumulation was decreased by 29% with statistical significance in siRNA hMATE1 cells compared to the control ($p < 0.05$; Figure 3A). Next, the effects of several inhibitors of membrane transporters were investigated in HepG2 cells. Pyrimethamine is a competitive and specific inhibitor of MATE1. In HepG2 cells that were stimulated with pyrimethamine, quercetin uptake was dramatically reduced by 75% at 3 min when compared to controls ($p < 0.05$ versus the control; Figure 3B). We also quantified quercetin transport in hMATE1 cells that were stimulated with pyrimethamine, quercetin uptake was dramatically reduced by 75% at 3 min when compared to controls ($p < 0.05$ versus the control; Figure 3B).

We also quantified quercetin transport in hMATE1 cells that were stimulated with PMSF, cyclopiazonic acid, and bafilomycin A1 (Figure 3C), which are inhibitors for bilitranslocase and P- and V-type ATPases, respectively. PMSF significantly blocked quercetin accumulation by up to 48%, as reported previously ($p < 0.05$). Cyclopiazonic acid and bafilomycin A1 reduced intracellular quercetin accumulation by 48 and 35%, respectively, when compared to controls ($p < 0.05$). These results suggest that multiple membrane proteins, which include MATE1 and bilitranslocase, mediate intracellular hepatic quercetin transport. Additionally, the data also suggest...
that quercetin uptake by MATE1 is mediated by secondary active transport, which requires the activity of both P- and V-type ATPases as well as a proton gradient across the membrane.

**Biological Activity of Quercetin in Lipid-Loaded hMATE1 Cultured Cells.** Quercetin ameliorates symptoms of metabolic syndrome, including dyslipidemia, insulin sensitivity, and obesity. Hepatic lipid accumulation is one of the common symptoms in metabolic syndrome; thus, we investigated the effect of hMATE1 expression on hepatic lipid accumulation. hMATE1 cells that were stimulated with quercetin had intracellular cholesterol and triglyceride concentrations that were significantly reduced by 30 and 41%, respectively, compared to control cells (p < 0.05; panels A and B of Figure 4). The antioxidative activity of quercetin (10 μM) increased in hMATE1 cells when compared to controls; thus, the levels of lipid peroxides were reduced after lipid loading (p < 0.05; Figure 4C). In addition, insulin-mediated cellular glucose uptake was improved in lipid-loaded hMATE1 cells when compared to the control (Figure 4D).

### DISCUSSION

Flavonoids are plant-specific; approximately 8000 different types have been isolated from various plants. Quercetin, which is one of the most abundant flavonoids, has been intensively studied for its various biological activities in humans, including its hypolipidemic effects and regulation of metabolic syndrome symptoms. However, considering the amount of biological activity studies with flavonoids, including quercetin, their cellular transport mechanism in mammals remains unknown.

The mechanism of flavonoid transport has been investigated intensively in plant tissues, and MATE1 has been reported to carry out the vacuolar transport of anthocyanin in *Arabidopsis*. Interestingly, human MATE1 is expressed at high levels in the kidney, liver, and intestine. Human MATE1 has 95% amino acid sequence homology compared to that expressed in *Arabidopsis*. It has been reported that the major role of human MATE1 is to transport various organic cations, however, some anionic drugs, including estrone sulfate, are substrates for MATE1. As such, quercetin demonstrated its activity cellular transporter. Kaempferol, luteolin, and apigenin uptake were moderately enhanced with MATE1 overexpression. Knocking down the expression of MATE1 with siRNA diminished the cellular quercetin accumulation. Additionally, treatment of cells with pyrimethamine, which is a MATE inhibitor, reduced cellular flavonoid accumulation by 75%, demonstrating the critical role of MATE1 in cellular flavonoid accumulation. A reduction of quercetin accumulation in cells that were incubated with V- and P-type ATPase inhibitors also confirmed the secondary active transport of human MATE1. The hypolipidemic, antioxidative, and cellular glucose uptake activities of quercetin in cultured hepatocytes were exaggerated in cells that overexpressed MATE1, which suggests that the increased bioavailability of quercetin enhanced its biological activity. An immunoblotting analysis with isolated subcellular membranous organelles revealed that MATE1 is highly expressed in both the liver and intestine, especially in peroxisomes and the ER as well as in the plasma membrane. Immunohistochemistry with mouse and human liver tissues as well as with cultured hepatocytes demonstrated a similar subcellular localization of MATE1, which was consistent with the immunoblotting analysis. Finally, the fluorescent staining analysis revealed that quercetin was co-localized in peroxisomes and the nucleolus, which suggests the potential accumulation of quercetin in the corresponding organelles, in which peroxisomal accumulation is mediated by MATE1 at least in peroxisome.

In plant tissues, MATE1 has a high affinity for glycoside forms of flavonoids, such as epicatechin-3-O-glucoside and cyanidine-3-O-glucoside, over the corresponding aglycones. However, the present data demonstrate that aglycones showed higher levels of cellular accumulation than glycosides in cells that overexpressed hMATE1, except for apigenin-7-O-glycoside. The reasons for the differences in substrate specificity between human and plant MATE1 are unclear at present. Minor differences in amino acid sequence may be associated with differential substrate specificity in human and plant MATE1. It appears that human MATE1 has broad substrate specificity. MATE1 is thought to be involved in the transport of various organic cations, including tetraethylammonium, 1-methyl-4-phenylpyridinium, and metformin, as well as some anionic compounds, including estrone sulfate, acyclovir, and ganciclovir. In this study, we demonstrate that MATE1 also transports flavonoid aglycones with high affinity.

Prediction of the common structural components of MATE1 substrates appears to be highly problematic. However, Wittwer et al. described the major physicochemical parameters of MATE1 substrates in animal cells, including molecular weight, the number of hydrogen bond donors, and degree of hydrophobicity, as being in line with our results pertaining to flavonoid transport. We demonstrated that flavonoid aglycones are superior substrates for MATE1 compared to their glycosides, suggesting that molecular weight may be the key determinant of MATE1 substrate specificity. Among the tested flavonoid aglycones, those with a higher number of hydrogen bond donors and acceptors, together with low hydrophobicity (assessed via the log P value), were superior substrates for MATE1. As such, quercetin demonstrated the highest uptake, followed by kaempferol, luteolin, and apigenin. These prerequisites for MATE1 substrate specificity were applied to explain other drug-based substrates for MATE1, such as metformin and 1-methyl-4-phenylpyridinium.

In the MATE1 inhibitor and gene knockdown experiments, the basal level of quercetin uptake was maintained, which indicated that the uptake of quercetin and potentially other flavonoids occurs in a highly complex manner through multiple cellular transporters. With regard to quercetin, we know that MATE1 and bilitranslocase mediate the cellular uptake of quercetin; however, HepG2 cells that were incubated simultaneously with two inhibitors, namely, PMSF and pyrimethamine, revealed slightly increased quercetin accumulation (data not shown), which suggested that there may be an additional low-capacity, high-affinity cellular transporter.

The subcellular localization of MATE1 revealed that MATE1 is also expressed in intracellular organelles, including the ER and peroxisomal membrane as well as the plasma membrane. To our knowledge, this study is the first to confirm the expression of MATE1 in subcellular organelles in animal cells. Previously, Otsuka et al. reported the localization of human MATE1 in the liver, which suggested the expression of MATE1 in the canalicular and apical membranes of bile ducts. The
authors reported that MATE1 may be a polyspecific exporter for various types of organic compounds into the bile canaliculi with multidrug resistant gene-1.\textsuperscript{60} Another study also claimed the expression of MATE1 in the brush border membrane of the proximal tubule of the kidney.\textsuperscript{31} However, both studies did not report the intracellular localization of MATE1 in humans, which was previously confirmed in plant tissues. The findings of the expression of MATE1 in hepatic subcellular organelles and the accumulation of cellular flavonoids in organelles add a novel function for human MATE1, in addition to the previously reported role of exporting organic cations.

In conclusion, MATE1 is a novel flavonoid transporter in mammalian cells, particularly for quercetin in the liver and intestine. MATE1 is mainly expressed in plasma, ER, and peroxisomal membranes, and increased cellular flavonoid accumulation was observed in hMATE1 cells. Cellular quercetin accumulation in mammalian MATE1 eliminates lipid and glucose disorders. Owing to the limited bioavailability and Technology Development (Project PJ010528032014) and the proximal tubule of the kidney.\textsuperscript{31} However, both studies did not the expression of MATE1 in the brush border membrane of the which was previously con

The authors declare no competing financial interest.

**ABBREVIATIONS USED**

ER, endoplasmic reticulum; LAMP, lysosome-associated membrane protein; MATE1, multidrug and toxic compound extrusion transporter-1; PMSF, phenylmethylsulfonyl fluoride; TBARS, thiobarbituric acid reactive substances; TBST, tris-buffered saline with Tween-20.

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