Identification of Dicarboxylate Carrier Slc25a10 as Malate Transporter in de Novo Fatty Acid Synthesis

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Mitochondrial solute carrier family 25 member 10 (Slc25a10) transports dicarboxylates such as malate or succinate across the mitochondrial inner membrane. Although fatty acid synthesis in adipose tissue or the liver is initiated by citrate transport in exchange for malate across the mitochondrial membrane, the transporter responsible for supplying malate during citrate transport has not been identified. In the present study, we clarified the role of Slc25a10 in supplying malate for citrate transport and examined the effect of Slc25a10 suppression on the lipogenic pathway and lipid accumulation. We have reported an Slc25a10 increase in white adipose tissue in obese mouse models and a decrease in a fasted mouse model using expression profiles. Next, we examined the effect of Slc25a10 suppression by small interfering RNA on citrate transport in the lipogenic cell lines HepG2 and 3T3-L1. We observed that inhibition of malate transport by Slc25a10 suppression significantly reduced the citrate transport from the mitochondria to the cytosol. We also found that suppression of Slc25a10 down-regulated the lipogenic pathway, indicated by decreases in ACC1 expression and malonyl-CoA level. Furthermore, suppression of Slc25a10 decreased triglyceride lipid accumulation in adipose-differentiated 3T3-L1 cells. These results suggested that Slc25a10 plays an important role in supplying malate for citrate transport required for fatty acid synthesis and indicated that inhibition of Slc25a10 might effectively reduce lipid accumulation in adipose tissues.

De novo fatty acid synthesis occurs in lipogenic tissues such as white adipose tissue (WAT) and the liver. A series of fatty acid synthesis reactions begins with the export of mitochondrial citrate to the cytosol using three obese mouse models and a fasted mouse model to focus on pathways or genes involved in the metabolic syndrome (11–13). For example, analysis of the livers from hyperinsulinemic rats revealed the overexpression of sterol regulatory element-binding protein-1C (SREBP-1C) (11). We also showed with the microarray study that monocyte chemoattractant protein-1 (MCP-1) is overexpressed in WAT of obese mice, indicating a relation between obesity and atherosclerosis (13). Further microarray studies of lipogenic tissues combining several obese and lean animal models may reveal more important pathways or genes in the development of increased fat mass and resultant metabolic syndrome.

However, the mechanism involving the supply of cytosolic malate for the citrate transporter, which antiports the cytosolic malate and mitochondrial citrate, remains to be elucidated.

Mitochondrial solute carrier family 25 member 10 (Slc25a10) transports a dicarboxylate, such as malate, across the mitochondrial inner membrane. The carrier is composed of six transmembrane regions with three structurally cognate repeats and is localized to the inner mitochondrial membrane (6). Mouse Slc25a10 is expressed in lipogenic tissues, with predominant expression in WAT and secondary and related expression in the liver (6, 7). Carrier expression is also induced when cells differentiate into adipocytes in vitro (6). Malate, the substrate of Slc25a10, is abundant in lipogenic tissues and antiport for citrate (8, 9). Overexpression of Slc25a10 in cultured cells results in hyperpolarization of mitochondria via an unknown mechanism, which produces reactive oxygen species (10); however, the potential involvement of Slc25a10 in fatty acid synthesis has not been examined.

DNA microarray methods have been utilized to identify important pathways or genes involved in the metabolic syndrome (11–13). For example, analysis of the livers from hyperinsulinemic rats revealed the overexpression of sterol regulatory element-binding protein-1C (SREBP-1C) (11). We also showed with the microarray study that monocyte chemoattractant protein-1 (MCP-1) is overexpressed in WAT of obese mice, indicating a relation between obesity and atherosclerosis (13). Further microarray studies of lipogenic tissues combining several obese and lean animal models may reveal more important pathways or genes in the development of increased fat mass and resultant metabolic syndrome.

The proteins involved in the pathway of de novo fatty acid and triglyceride synthesis have attracted attention as novel anti-obesity targets because suppression of the expression or function of these proteins reduces fat mass and improves obese parameters in several animal models (14, 15). A genome-wide screening of RNA interference suppression in Caenorhabditis elegans showed that inhibiting the expression of acyl-CoA synthetase reduced fat levels in wild type and mutants with increased fat content (15). Therefore, identification of new genes in this pathway may identify new anti-obesity targets.

In the present study, we performed a microarray analysis of WAT using three obese mouse models and a fasted mouse model to focus on changes in the lipogenic pathway. Slc25a10 was up-regulated in all three obese mouse models and down-regulated in the lean mouse model. We then examined the effect of Slc25a10 suppression on fatty acid synthesis. We found that Slc25a10 plays a crucial role in supplying malate for the citrate transport required for fatty acid synthesis. We also found that the suppression of Slc25a10 down-regulates fatty acid synthesis, resulting in reduced lipid accumulation in vitro.

**EXPERIMENTAL PROCEDURES**

Obese and Lean Model Mice—C57BL/6N mice (6 weeks old, CLEA Japan, Tokyo, Japan) were housed in individual cages. Mice were maintained under conditions of controlled temperature (23 ± 2 °C) and light...
Role of Slc25a10 in de Novo Fatty Acid Synthesis

Common signature genes with WAT in DIO, d-Trp34NPY-treated, and fasted mice

Table One

| Probe ID | Symbol | Gene name | DIO | d-Trp34NPY-FF | d-Trp34NPY-PF | Fasting |
|----------|--------|-----------|-----|--------------|--------------|---------|
| 102736_at | MCP-1  | Monocyte chemoattractant protein-1 | 7.2 | 6.3 | 4.9 | −3.0 |
| 92607_at  | Mest   | Mesoderm specific transcript | 3.7 | 4.2 | 2.1 | −3.0 |
| 99168_at  | Pgs    | Phosphatidylinositol glycan, class S | 2.9 | 4.4 | 1.8 | −3.7 |
| 98443_at  | Lep    | Leptin | 2.3 | 3.0 | 1.8 | −3.6 |
| 99112_at  | Slc25a10 | Solute carrier family 25 (dicarboxylate transporter), member 10 | 2.0 | 3.0 | 1.9 | −5.0 |
| 100342_i_at | Tubal | Tubulin, α 1 | 1.8 | 3.4 | 2.0 | −6.2 |

This table lists genes with more than 1.8-fold change in all the obese model mice and less than −3.0-fold change in fasted mice are listed. FF, free-fed; PF, pair-fed.

FIGURE 1. mRNA expression of Slc25a10 is increased in WAT of obese mouse models in vivo and differentiated 3T3-L1 adipocyte cells in vitro. The mRNA expression of Slc25a10 was quantified by real-time RT-PCR. The expression was analyzed in WAT of DIO mice (A), free-fed (FF) or pair-fed (PF) mice treated with appetite-stimulating peptide d-Trp34NPY (B), fasted mice (C), and pre- or postdifferentiated 3T3-L1 culture cells (D). PBS, phosphate-buffered saline; Pre-diff, predifferentiated 3T3-L1; Post-diff, postdifferentiated 3T3-L1. n = 3; *, p < 0.05; **, p < 0.01.

for differentiation. Ten days after the onset of differentiation, they were assayed.

Mitochondrial Transport Study—Freshly isolated mitochondria from HepG2 were loaded with malate by incubating in 10 ml of a transport buffer containing 100 mM KCl, 20 mM Hepes, and 1 mM EGTA at pH 7.0, in the presence of 2 mg/ml rotenone and 0.75 mM malate. After 5 min, 5 mM phenylsuccinate and 1,2,3-benzene tricarboxylate were added to inhibit the oxoglutarate carrier and citrate carrier, respectively. The malate-loaded mitochondria with the transport buffer were centrifuged at 20,000 × g for 5 min at 4 °C. The mitochondria were washed once and resuspended in 2.0 ml of the transport buffer. The kinetics of [14C]malate/malate exchange were determined using the inhibitor stop method (8, 17, 18). Malate-loaded mitochondria were incubated in 1 ml of reaction buffer containing 100 mM KCl, 20 mM Hepes, 1 mM EGTA, and 2 mg of rotenone at pH 7.0. Exchange transport was initiated by adding [14C]malate and terminated, at the indicated times, by the addition of 12.5 mM n-butylmalonate. The mitochondria were then immediately resolated by centrifugation, washed once, and acidified with 20% HClO4. The supernatant obtained after centrifugation was diluted, and radioactivity was measured using a liquid scintillation counter.

Quantification of Malate, Citrate, and Malonyl-CoA—For quantification of malate in whole cells, malate was extracted from 10⁶ cells and

(07:00–19:00). Water and food (CA-1, CLEA Japan) were available ad libitum unless noted otherwise. Models of DIO and d-Trp34NPY-treated obese mice (free-fed and pair-fed models) have been described previously (13). In the fasted model, 17-week-old male mice were fasted for 21 h, and food intake was restricted to a 3-h period daily for 7 days. All procedures involving animals complied with National Institutes of Health guidelines and were approved by the Banyu Animal Care and Usage Committee.

Microarray Study in WAT—Total RNA was extracted from WAT using TRIzol reagents (Invitrogen) and repurified with an RNase-free purification kit (Qiagen, Hilden, Germany). To determine changes in expression, 10 μg of RNA was utilized for microarray analysis (MG-U74U74A chip, Affymetrix, Santa Clara, CA) of ∼12,000 genes. For analysis of microarray data, GeneChip software (Affymetrix) was utilized. Appropriate control mice were used to provide baseline values for the experimental groups, and genes displaying values of p < 0.05 were identified using the Mann-Whitney test.

Quantitative Real-time RT-PCR—Total RNA was extracted from WAT and cultured cells as described in the previous paragraph. For cultured cells, the first purification with TRIzol reagent was omitted. Reverse transcription was performed for 500 ng of total RNA, and cDNA obtained was applied to TaqMan PCR for quantification of mRNA expression. Data were collected and analyzed using an ABI PRISM 7700 sequence detector system (Applied Biosystems, Warrington, UK). The relative mRNA expression data were normalized to β-actin or transferrin receptor. Probes and primers used for quantitative RT-PCR are listed in Supplemental Data 1. Pre-Developed TaqMan assay reagents (Applied Biosystems) were used for TaqMan probe and primers for human β-actin.

siRNA Transfection—For siRNA transfection of HepG2 cells, the cells were seeded at a confluency of 10% one day before transfection with siRNA for human Slc25a10 using Oligofectamine (Invitrogen) as follows.

Reverse transcription was performed for 500 ng of total RNA, and cDNA obtained was applied to TaqMan PCR for quantification of mRNA expression. Data were collected and analyzed using an ABI PRISM 7700 sequence detector system (Applied Biosystems, Warrington, UK). The relative mRNA expression data were normalized to β-actin or transferrin receptor. Probes and primers used for quantitative RT-PCR are listed in Supplemental Data 1. Pre-Developed TaqMan assay reagents (Applied Biosystems) were used for TaqMan probe and primers for human β-actin.

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Role of Slc25a10 in de Novo Fatty Acid Synthesis

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Quantification of Malate, Citrate, and Malonyl-CoA—For quantification of malate in whole cells, malate was extracted from 10⁶ cells and
measured using a standard enzymatic assay (19). Briefly, after collection
and washing with Hanks’ balanced salt solution, cells were dissolved in
70% EtOH. The cell lysate in EtOH was homogenized with sonication
followed by the addition of formic acid (4%). After removing cell debris
by centrifugation, the supernatants were evaporated. Dried samples
were dissolved in hydrazine solution (200 mM, pH 9.3). The solution
containing malate was reacted with malate dehydrogenase and NAD
to produce oxaloacetate and NADH. The production of NADH was
measured by spectrometry at 340 nm. For quantification of citrate, dried
samples were prepared using the same procedure used for malate. Dried
samples were dissolved in citrate detection buffer (50 mM Tris-HCl at
pH 7.4, 10 mM MgSO4, 5 mM EDTA). The citrate solution was reacted
with citrate lyase, malate dehydrogenase, and NADH to form malate
and NADH. The formation of NADH was measured by spectrometry at
340 nm. For quantification of malate and citrate in mitochondria, the
mitochondria were fractionated before measurement (20). For quanti-
fication of malonyl-CoA in cells, pellets containing 10^8 cells were dissolved in 10% trichloroacetic acid. After cen-
trifugation, the supernatant was applied to a reverse phase chromatog-
rphy column (Sep-Pak Vac C18 cartridge, Waters, Milford MA).
Extracts containing malonyl-CoA were reacted with [14C]acetyl-CoA
and purified FAS to produce palmitoyl-CoA. The radiolabeled palmi-
toy-CoA was extracted with hexane, and radioactivity was measured
using a liquid scintillation counter.

**Lipid Biosynthesis Assay**—Lipid biosynthesis was measured by the
6-h incorporation of [14C]acetate into extractable lipids added to scin-
tillation fluids (22).

**Oil Red O Staining**—Ten days after differentiation of 3T3-L1 cells
into adipocytes, the cells were washed with phosphate-buffered saline
and fixed for 2 h in 10% formaldehyde. A 0.35% solution of Oil Red O in
isopropyl alcohol was diluted with an equal volume of water, filtered,
and added to the fixed cells for 2 h. After washing with PBS, stained
triglyceride droplets were visualized and photographed. For quantifica-
tion of Oil Red O, stained cells were treated with 100% isopropyl alcohol
for 10 min to extract Oil Red O, and colorimetric intensity was meas-
ured by spectrometry at 510 nm.

**Western Blot**—3T3-L1 cells were collected in 0.5 ml of cold M-PER
buffer (Pierce). After cell debris was removed by centrifugation at
14,000 x g for 15 min at 4 °C, 30 μg of protein was separated on 8%
SDS-PAGE and electroblotted onto polyvinylidene difluoride mem-
branes (Amersham Biosciences). Membranes were blocked for 1 h at

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**FIGURE 2.** Suppression of Slc25a10 reduces malate transport activity in isolated mitochondria. A, suppression of Slc25a10-mRNA expression by siRNA in HepG2 and 3T3-L1 cells. Relative mRNA expression was measured by quantitative real-time RT-PCR analysis. B, malate transport activity across the mitochondrial inner membrane was measured in mitochondria isolated from control or Slc25a10-siRNA-treated HepG2 or 3T3-L1 cells. C, mRNA expression analysis of citrate carrier, oxoglutarate carrier, malate/Asp carrier, and H^+ carrier by RT-PCR. SCR, scrambled control. **, p < 0.01.
room temperature in Tris-buffered saline with 0.1% Tween 20 and 5% nonfat dry milk. For the detection of ACC, the membrane was incubated with anti-ACC and anti-Ser79-phosphorylated ACC antibody (Cell Signaling, Beverly, MA) followed by incubation with horseradish peroxidase-conjugated secondary antibodies. Immunoreactive bands were visualized by ECL Plus reagents (Amersham Biosciences).

**RESULTS**

Microarray Analysis of WAT in Obese and Lean Mouse Models—To understand the molecular mechanism underlying an accelerated lipogenic pathway in obesity, we performed microarray analysis in WAT of obese and fasted mouse models. The obese mouse models included DIO and D-Trp34NPY-treated mice (free-fed and pair-fed models), and the obese and fasted mouse models. The obese mouse models included DIO and D-Trp34NPY-treated mice (free-fed and pair-fed models), and the fasted mouse model was a caloric-restricted model. D-Trp34NPY is involved in the initial steps of fatty acid synthesis.

Evaluation of Data and Statistical Analysis—All data were expressed as mean ± S.D. Statistics were performed using a two-tailed unpaired Student’s t test.

**Role of Slc25a10 in de Novo Fatty Acid Synthesis**

Next, we confirmed the increased expression of Slc25a10 in obese mouse models by real-time RT-PCR (Fig. 1, A–C). Approximately 2.0-, 2.5-, and 2.0-fold increases were observed in DIO, D-Trp34NPY-FF, and D-Trp34NPY-PF mice, respectively. We also confirmed a 40-fold increase in Slc25a10 in adipose-differentiated 3T3-L1 (Fig. 1D).

Suppression of Slc25a10 Reduces Malate Transport Activity in Isolated Mitochondria—To analyze the function of Slc25a10 in lipogenesis and examine the effect of inhibition on de novo fatty acid synthesis, we designed siRNA for human and mouse Slc25a10. In lipogenic cells (HepG2 derived from liver and mouse adipocyte 3T3-L1), siRNA for Slc25a10 was transiently transfected. By selecting optimal siRNA sequences, we observed 75 and 90% of mRNA suppression in HepG2 and 3T3-L1, respectively (Fig. 2A). Next, we measured the malate-transporting activity of Slc25a10 in mitochondria isolated from the siRNA-treated cells. After suppression of Slc25a10 expression by siRNA, mitochondria were isolated from HepG2 and 3T3-L1, and the malate exchange rate was measured. Malate-transporting ability was reduced to 60 and 45% in siRNA-treated cells of HepG2 and differentiated 3T3-L1, respectively (Fig. 2B), indicating functional repression of Slc25a10.

Specificity of siRNA for Slc25a10 was confirmed by quantifying the mRNA expression of other solute carrier proteins (oxoglutarate carrier, malate/Asp shuttle carrier, citrate carrier, and H+ carrier), the sequences of which are similar to Slc25a10 (Fig. 2C). Expression was not affected by Slc25a10-siRNA treatment. The citrate carrier transport study in isolated mitochondria was also performed as a control. The citrate/citrate exchange activity of the citrate carrier was not affected by Slc25a10 suppression (Supplemental Data 2).

Suppression of Slc25a10 Reduces Cytosol Malate and Citrate Levels—We examined the effect of Slc25a10 suppression on malate and citrate levels in whole cells. After siRNA treatment of Slc25a10 in HepG2 cells, malate levels in the cells were measured. Malate level in the cells was decreased by 38% when compared with control cells (Fig. 3A). Since Slc25a10 transports malate across the inner membrane of mitochondria, mito-

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**FIGURE 3.** Suppression of Slc25a10 reduces malate and citrate in cytosol. HepG2 cells were treated with Slc25a10-siRNA or control RNA. Malate levels in whole cell fractions (A) and mitochondrial fractions (B) were measured in control or siRNA-treated cells. Citrate was simultaneously measured in whole cell fractions (C) and in mitochondrial fractions (D). SCR, scrambled control. *, p < 0.05; **, p < 0.01.
chondrial malate was also measured to determine whether the difference in whole cell malate level was derived from the mitochondria or cytosol. After isolating mitochondria, malate concentrations were measured. No difference in malate concentration between control and siRNA-treated mitochondria was observed (Fig. 3B), indicating that malate was decreased in the cytosol of siRNA-treated cells by the inhibition of malate transport from the mitochondria to the cytosol. Since citrate carrier transports cytosol malate in exchange for mitochondrial citrate, we speculated that the reduced cytosolic malate caused by Slc25a10 suppression might decrease citrate/malate exchange of citrate carrier. Thus, citrate concentration was measured. In whole cells, the citrate concentration of siRNA-treated samples was decreased by 31% when compared with the control (Fig. 3C), although no change was detected in mitochondrial concentration (Fig. 3D).

**Suppression of Slc25a10 Decreases ACC1 Expression**—ACC1 produces malonyl-CoA from acetyl-CoA, which is derived from citrate. Citrate functions as an allosteric regulator of ACC1. To examine the effect of reduced cytosol citrate on ACC1, we measured the expression of ACC1 in Slc25a10-suppressed cells. Fig. 4A shows the time course suppression of Slc25a10 in 3T3-L1 cells by siRNA. Although Slc25a10 increased 30-fold in control 3T3-L1 cells after differentiation, ~90% of Slc25a10 suppression was achieved in cells treated with siRNA for Slc25a10 after 10 days of differentiation. ACC1 induction was reduced to 50% of control in cells treated with siRNA for Slc25a10 after 10 days of differentiation (Fig. 4B). We also measured the expression of lipoprotein lipase and citrate carrier as
FIGURE 5. Protein expression of ACC in Slc25a10-suppressed cells. A, total and inactivated forms of phosphorylated ACC1 and ACC2 (Phospho-ACC1, 2) expression in HepG2 cells treated with Slc25a10-siRNA or control RNA. B, the time course protein expression of total and phosphorylated ACC1 during differentiation (Diff.) of 3T3-L1 cells treated with Slc25a10-siRNA or control RNA. SCR, scrambled control.

FIGURE 6. Suppression of Slc25a10 decreases malonyl-CoA production. After treating with siRNA, cells were collected, and malonyl-CoA, an intermediate product of fatty acid, was measured in HepG2 cells (A) and differentiated 3T3-L1 cells (B). SCR, scrambled control. *, p < 0.05; **, p < 0.01.

differentiation markers, which were increased ~100- and 25-fold, respectively, both in control and in siRNA-treated cells (Fig. 4, C and D). The expression levels of other lipogenic genes were also quantified (ATP citrate lyase and Fas) in 3T3-L1 cells. Expression was not affected by Slc25a10-siRNA (Fig. 4, E and F). In another lipogenic cell line, HepG2, reduced ACC1 expression was also observed when cells were treated with siRNA for Slc25a10 (Fig. 4G), whereas citrate carrier expression was not changed when compared with control (Fig. 4H). The decreased expression of ACC1 was confirmed with other siRNA sequences for Slc25a10 both in 3T3-L1 and in HepG2. In addition, when Slc25a10 was overexpressed in HEK293 cells with low endogenous Slc25a10 expression, ACC1 expression increased significantly in comparison with vector-transfected control cells, whereas citrate carrier expression was unchanged when compared with control (data not shown).

The decreased expression of protein ACC in Slc25a10-siRNA-treated cells was confirmed by immunoblot in HepG2 and 3T3-L1 cells, which was consistent with the decreased mRNA expression of ACC (Fig. 5A). In contrast, the inactivated form of phosphorylated ACC increased in Slc25a10-siRNA-treated cells when compared with control cells (Fig. 5B). Because citrate is known to inhibit ACC phosphorylation (3), decreased cytosolic citrate by Slc25a10 suppression might increase the phosphorylated form of ACC when compared with control cells.

Malonyl-CoA Production Is Decreased in siRNA-treated Cells—To measure ACC1 activity in Slc25a10-suppressed cells, we quantified malonyl-CoA levels in control and Slc25a10-siRNA-treated cells. Briefly, malonyl-CoA extracted from cells was reacted with labeled acetyl-CoA and FAS to produce labeled fatty acid (palmitoyl-CoA) in vitro. The amount of labeled fatty acid represents the amount of malonyl-CoA. In siRNA-treated HepG2 and 3T3-L1 cells, reductions of 30 and 50%, respectively, in malonyl-CoA level were observed when compared with control cells (Fig. 6, A and B). Since malonyl-CoA is used in fatty acid synthesis reactions catalyzed by FAS or long chain elongases, these results indicated that reduced malonyl-CoA decreases the rate of fatty acid synthesis.

Suppression of Slc25a10 Decreases Lipid Accumulation in Adipocytes—In Slc25a10-suppressed cells, factors related to fatty acid synthesis, such as citrate level, ACC1 expression, dephosphorylated ACC1, and malonyl-CoA level, were decreased. To analyze the effect of Slc25a10 suppression on the rate of fatty acid synthesis, the rate of acetate incorporation into lipid in Slc25a10-suppressed 3T3-L1 cells was measured (Fig. 7A). After acetate labeled with $^{14}$C was incubated in the siRNA-treated or control cells, the radioactivity of the lipid fraction of the cells was quantified. The rate of lipid synthesis was reduced by 40% in Slc25a10-siRNA-treated cells when compared with control. To further investigate the effect of Slc25a10 suppression on lipid accumulation, we measured the amount of triglyceride in differentiated 3T3-L1 cells treated with siRNA for Slc25a10 by staining accumulated lipid with Oil Red O (Fig. 7B). Strong staining was observed in differentiated control cells when compared with predifferentiated cells. In siRNA-treated cells, only weak staining was observed, and the size of the lipid drops was remarkably small. We quantified lipid accumulation by extracting stained Oil Red O and measuring the colorimetric intensity (Fig. 7C). In accordance with photographic data, a ~20-fold increase in lipid accumulation was observed in differentiated control cells when compared with predifferentiated cells. In contrast, lipid accumulation was reduced by 57% in Slc25a10-suppressed cells.

DISCUSSION

We clarified the role of Slc25a10 in the initial steps of fatty acid synthesis. Although the involvement of Slc25a10 in lipogenesis has been hypothesized (6, 25), this is the first report demonstrating that Slc25a10 supplies malate, which is necessary for citrate transport across the mitochondrial inner membrane. Furthermore, we showed that the inhibition of Slc25a10 down-regulated lipogenesis, leading to reduced lipid accumulation.
Inhibition of Slc25a10 decreased ACC1 mRNA expression and enzymatic activity. However, the mechanism of ACC1 regulation by Slc25a10 remains to be elucidated. We speculated that the reduced cytosolic citrate level caused by Slc25a10 suppression inactivates ACC1 activity because citrate is an allosteric effector for ACC1, leading to polymerization of ACC1 (the activated form of the enzyme) (3). As a result of decreased ACC1 activity, fatty acid synthesis was down-regulated, resulting in decreased lipid accumulation. Subsequently, reduced end or intermediate products might reduce ACC1 expression through a feedback mechanism. For example, SREBP-1, which transactivates ACC1 expression, is regulated by fatty acids (26). The decreased SREBP-1 activity caused by disturbed fatty acid synthesis could reduce ACC1 expression in Slc25a10-suppressed cells.

We demonstrated that citrate in the cytosol was reduced in Slc25a10-suppressed cells, indicating the important role of the carrier in cytosolic transport for fatty acid synthesis. The mechanism is probably due to reduced exchange of citrate and malate across the mitochondrial membrane. The citrate carrier itself excretes citrate into the cytosol for fatty acid synthesis from the mitochondrial matrix in exchange for cytosol malate (1, 27). We speculated that Slc25a10 supplies cytosol malate with the citrate carrier for fatty acid synthesis by transporting out mitochondrial malate into the cytosol. It is conceivable that reduced cytosolic malate caused by Slc25a10 suppression could decrease the malate/citrate exchange rate of citrate carrier in the initial step of fatty acid synthesis, resulting in reduced lipid accumulation. This is also supported by tissue distribution (6) or studies that showed that malate analogues have inhibitory effects on lipogenesis (25, 28). However, an additional effect of decreased malate transport on the pyruvate/malate cycle might contribute to reduced cytosol citrate. The pyruvate/malate cycle contributes to acetyl-CoA transport from the mitochondrial matrix to the cytosol. In the cycle, malate is produced from citrate by the continual reaction of citrate lyase and malate dehydrogenase. The malate is converted to pyruvate by the malic enzyme, and pyruvate is transported into mitochondria for subsequent reaction to citrate. In obese WAT, the increased cytosolic malate concentration by Slc25a10 might supply the pyruvate/malate cycle with malate, enhancing the export of citrate to the cytosol out of the mitochondria.

In Drosophila melanogaster and C. elegans, di- and tricarboxylate transporters lengthen life span and reduce fat content without affecting fertility or physical activity (29–31), although their localization to the plasma membrane differs from Slc25a10. When the Drosophila dicarboxylate transporter, Indy (I’m Not Dead Yet), was disrupted by P-element, life span was doubled (29). Drosophila Indy was also expressed mainly in fat body and midgut cells (30). For C. elegans, suppression of a Na⁺-coupled dicarboxylate transporter (NAC-2) by RNA interference led to a significant increase in life span and decreases in fat content and body size (31). The mechanism is thought to involve suppression of the transporters, which caused reduced production of metabolic energy mimicking the state of caloric restriction (29). In addition, reduced fat mass is related to the decrease in citrate supply for fatty acid synthesis caused by the transporter disruption (31). Together with our data, these reports suggest that suppression of di- or tricarboxylate carriers, which reduces the supply of citrate for fatty acid synthesis, could be an effective way to reduce fat content.

Reduced lipid accumulation by Slc25a10 suppression indicates that Slc25a10 might be a candidate for an anti-obesity drug target. In addition, specific inhibition of Slc25a10 in WAT is likely to produce minimal side effects since the carrier is expressed predominantly in adipose tissue in contrast to other lipogenic proteins expressed in a variety of tissues (32). Furthermore, Slc25a10 has been mapped near an obesity quantitative trait loci (C10bw3: Chr 11, 75 cM), indicating the need to clarify the relation between obesity and Slc25a10.

In summary, we demonstrated that Slc25a10 plays an important role in supplying malate for the citrate transport required for fatty acid synthesis and showed that inhibition of Slc25a10 down-regulates fatty acid synthesis, leading to decreased lipid accumulation. Further characterization of the function of Slc25a10 in vivo to elucidate its anti-obese effect of gene suppression is needed.

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Identification of Dicarboxylate Carrier Slc25a10 as Malate Transporter in \emph{de Novo} Fatty Acid Synthesis

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