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Regulation of Acetate Utilization by Monocarboxylate Transporter 1 (MCT1) in Hepatocellular Carcinoma (HCC)

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Altered energy metabolism is a biochemical fingerprint of cancer cells. Hepatocellular carcinoma (HCC) shows reciprocal [^{18}F]fluorodeoxyglucose (FDG) and [^{11}C]acetate uptake, as revealed by positron emission tomography/computed tomography (PET/CT). Previous studies have focused on the role of FDG uptake in cancer cells. In this study, we evaluated the mechanism and roles of [^{11}C]acetate uptake in human HCCs and cell lines. The expression of monocarboxylate transporters (MCTs) was assessed to determine the transporters of [^{11}C]acetate uptake in HCC cell lines and human HCCs with different [^{11}C]acetate uptake. Using two representative cell lines with widely different [^{11}C]acetate uptake (HepG2 for high uptake and Hep3B for low uptake), changes in [^{11}C]acetate uptake were measured after treatment with an MCT1 inhibitor or MCT1-targeted siRNA. To verify the roles of MCT1 in cells, oxygen consumption rate and the amount of lipid synthesis were measured. HepG2 cells with high [^{11}C]acetate uptake showed higher MCT1 expression than other HCC cell lines with low [^{11}C]acetate uptake. MCT1 expression was elevated in human HCCs with high [^{11}C]acetate uptake compared to those with low [^{11}C]acetate uptake. After blocking MCT1 with AR-C155858 or MCT1 knockdown, [^{11}C]acetate uptake in HepG2 cells was significantly reduced. Additionally, inhibition of MCT1 suppressed mitochondrial oxidative phosphorylation, lipid synthesis, and cellular proliferation in HCC cells with high [^{11}C]acetate uptake. MCT1 may be a new therapeutic target for acetate-dependent HCCs with high [^{11}C]acetate uptake, which can be selected by [^{11}C]acetate PET/CT imaging in clinical practice.

Key words: [^{11}C]Acetate uptake; Hepatocellular carcinoma (HCC); Monocarboxylate transporter (MCT); Positron emission tomography/computed tomography (PET/CT)

INTRODUCTION

Hepatocellular carcinoma (HCC) is an aggressive cancer that is typically treated with surgical resection, intra-arterial chemoembolization, radiotherapy, systemic chemotherapy, or molecular targeted therapy[1,2]. However, the high recurrence rate in early HCC after surgical resection and chemotherapeutic resistance in advanced HCCs results in poor patient survival outcomes. Thus, new therapeutic targets are needed to improve these outcomes. The use of carbon sources is important for the synthesis of cytoplasmic components such as nucleotides, amino acids, and membrane materials. Carbon sources also regulate the activity of enzymes and expression of genes by controlling the acetylation of non-histone proteins as well as histones[3]. Aerobic glycolysis involving glucose consumption and lactate production to produce energy and building blocks has been widely examined[4–6]. The glycolytic phenomenon is considered to be typical of aggressive cancers and has been proposed as a target for cancer therapy[7]. Glucose transporters facilitate the incorporation of glucose into cells and are overexpressed in various malignant tumors. Indeed, many studies have attempted to regulate glucose uptake through genetic and chemical methods for cancer treatment[8].

Increased glycolysis, glucose-dependent biomass synthesis, and decreased dependence on mitochondrial respiration are well-known metabolic alterations in glycolytic cancer cells. Regardless, some cancer cells use alternative carbon sources such as fatty acids, amino acids, ketone bodies, and acetate, among others. Cancers lacking increased glycolysis show a high false-negative detection rate on [^{18}F]fluorodeoxyglucose (FDG) positron emission tomography/computed tomography (PET/CT), but can be detected on [^{11}C]acetate PET/CT[9,10]. Exogenous acetate

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is converted to acetyl-CoA by cytosolic acetyl-CoA synthetase for lipid synthesis in acetate-dependent tumors[11]. In contrast, acetyl-CoA by mitochondrial acetyl-CoA synthetase is used to make energy in the tricarboxylic acid (TCA) cycle of tumors.

Despite increased interest in [^{11}C]acetate uptake in non-glycolytic human tumors, the incorporation mechanism and relevant acetate transporters have not been determined. In this study, we evaluated the role of monocarboxylate transporter (MCT) in [^{11}C]acetate transport across cell membranes in human HCCs and HCC cell lines. Additionally, we examined the effect of MCT inhibition on cellular function in HCC cell lines.

MATERIALS AND METHODS

Patients

Between January and December 2016, 37 patients who underwent [^{11}C]acetate and [^{18}F]FDG PET/CT, and surgical resection without any neoadjuvant treatment were enrolled in this retrospective study. The Institutional Review Board of our hospital approved this study and waived the requirement to obtain informed consent. Based on the results of [^{18}F]FDG and [^{11}C]acetate PET/CT, nine cases of HCCs with low [^{18}F]FDG and high [^{11}C] acetate uptake and nine cases of HCCs with high [^{18}F] FDG and low [^{11}C]acetate uptake were selected for further tissue analysis. The use of tissue samples was approved by the Yonsei University Health System Institutional Review Board at Severance Hospital, and this study was performed according to ethics guidelines (IRB No. 4-2015-0904).

Cell Culture

Human HCC cell lines (HepG2, Hep3B, Huh7, and SK-hep1) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA) containing penicillin–streptomycin (100 U/ml; Gibco) and 10% fetal bovine serum (Gibco) in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Western Blotting

Cells were washed with phosphate-buffered saline (PBS) and lysed with sodium dodecyl sulfate (SDS) lysis buffer [60 mM Tris-HCl, pH 6.8, 1 % SDS in distilled water (DW)] containing protease inhibitor cocktail (Roche, Mannheim, Germany). Equal amounts of protein from each sample were separated by SDS-polyacrylamide gel electrophoresis on 8%–12% gels and transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Membranes were then incubated with primary antibodies against monocarboyxlate transporters: MCT1 (AB3538P; Millipore), MCT2 and MCT4 (SC50322 and SC50329; Santa Cruz Biotechnology, Santa Cruz, CA, USA), acetyl Co-A synthase 2 (SC85258; Santa Cruz Biotechnology), fatty acid synthase (3189; Cell Signaling Technology, Danvers, MA, USA), and actin (A1978; Sigma-Aldrich, St. Louis, MO, USA). Membranes were washed in PBS and incubated with goat anti-rabbit (sc2004) or anti-mouse (sc2005) IgG horseradish peroxidase (Santa Cruz Biotechnology) as the secondary antibody. Labeled, specific protein bands were visualized using the ECL Kit (Thermo Scientific, Waltham, MA, USA).

[^{11}C]Acetate and [^{18}F]FDG Uptake

Cells were seeded into six-well plates (1.5×10^5 cells per well) and incubated overnight. Cultured medium was changed to glucose-free DMEM (Gibco). Approximately 0.037 MBq of [^{18}F]FDG and 0.37 MBq of [^{11}C]acetate were added to the cells and incubated for 20 min. Cells were washed with PBS, and lysis buffer (60 mM Tris-HCl, pH 6.8, 1% SDS in DW) was added to each well. The cell lysates were harvested, and the amount of radioactivity incorporated into cells was measured using a gamma counter (PerkinElmer, Waltham, MA, USA). The measured radioactivity was normalized to the protein amount in cell lysates determined using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Immunohistochemistry

Immunohistochemical staining was performed according to a standard protocol. Primary monoclonal antibodies against MCT1 were diluted 1:200 in PBS. Briefly, sections were deparaffinized and rehydrated with ethanol, and then antigen retrieval was performed in 10 mM citrate buffer, pH 6.0, for 10 min using a pressure cooker. The sections were incubated with 3% goat serum for 30 min, followed by incubation with the appropriate primary antibody overnight at 4°C. Sections were then washed with PBS three times and incubated with a biotinylated species-specific secondary antibody, followed by incubation with a streptavidin–horseradish peroxidase conjugate (Dako, Santa Clara, CA, USA). Immunoreactivity was revealed with 3,3′-diaminobenzidine (Dako). Mayer's hematoxylin was used as a counterstain in all tissue sections.

MCT1 Overexpression and Knockdown

To overexpress the *MCT1* gene in Hep3B cells, Hep3B cells were seeded into 60-mm culture plates (1×10^4 cells per well) and incubated overnight. hMCT1 cDNA plasmids were purchased from R&D Systems (Minneapolis, MN, USA). Two micrograms of plasmid was transfected into cultured Hep3B cells using 0.2 μl/well Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according

to the manufacturer's protocols. *MCT1*-overexpressing cells were then selected in a culture medium containing 300 μg/ml genectin (Invitrogen).

To knock down the *MCT1* gene in HCC cell lines, HepG2 and Hep3B cells were seeded into six-well culture plates (1×10^5 cells) and incubated overnight. Negative control siRNA (SN1003) or siRNA targeting MCT1 (1138115; Bioneer Corporation, Daejeon, South Korea) was transfected into cells, using 20 μl/well Lipofectamine 2000 according to the manufacturer's protocol.

Measurement of Cell Proliferation

HCC cells were cultured for 3 days in 96-well plates (5×10^3 cells per well). Cells were then subjected to an MTT assay. Briefly, adherent HCC cells were treated with 0.5 mg/ml thiazolyl blue tetrazolium bromide (MTT; Sigma-Aldrich) for 4 h and then washed with PBS. Afterward, the remaining MTT reagent was solubilized with DMSO. The optical density of each well was determined at 570 nm.

Cell Staining

HepG2 and Hep3B cells were seeded onto glass-bottomed cell culture dishes (NEST Biotechnology, Shanghai, P.R. China) and incubated for 24 h. Cells were fixed in 4% paraformaldehyde for 30 min and washed three times with PBS. The cells were then incubated in PBS containing 0.25% Triton X-100 for 10 min and blocked with blocking solution (3% bovine serum albumin, 0.25% Triton X-100 in PBS). Cells were treated for 30 min followed by incubation overnight at 4°C with a diluted primary antibody against mitochondria (M22426; Invitrogen) in blocking solution. After washing the cells in PBS, Hoechst 33258 was used to stain the nuclei. Cells were visualized using a Carl Zeiss LMS710 confocal microscope (Gottingen, Germany).

For Oil red O staining, HepG2 and Hep3B cells were cultured in glucose-free DMEM supplemented with acetate (500 μM final concentration; Sigma-Aldrich) with or without AR-C155858 (20 μM final concentration; Tocris Bioscience, Bristol, UK) for 2 days. Cells were fixed in 4% paraformaldehyde for 30 min followed by incubation with Oil red O solution (0.5% w/v Oil red O in isopropyl alcohol) for 20 min and counterstained with hematoxylin.

Extracellular Flux (XF) Analysis for Oxygen Consumption Rate (OCR)

Analysis of bioenergetics parameters was performed in HepG2 and Hep3B cells using the XF24 analyzer from Seahorse Bioscience (Billerica). OCR was measured using a Seahorse XF Cell Mito Stress Test kit (Seahorse Bioscience) in real time under culture conditions containing acetate (500 μM final concentration; Sigma-Aldrich) with or without AR-C155858 (100 μM final concentration; Tocris Bioscience) following the manufacturer's protocol (1 μg/ml oligomycin, 0.5 μM final concentration of FCCP, 1 μM final concentration of antimycin A and rotenone).

Statistical Analysis

Each experiment was carried out in triplicate, and quantitative data were expressed as the mean ± standard deviation (SD). Statistical analysis was conducted using the SigmaPlot analysis program (Systat Software, Inc., La Jolla, CA, USA) and GraphPad Prism Software (San Diego, CA, USA).

RESULTS

Acetate Uptake and Expression of Acetate Transporters in Human HCC Cell Lines

Using four different HCC cell lines (Hep3B, SK-HEP1, HepG2, and Huh7), we screened for acetate uptake with [11C]acetate and glucose uptake with [18F]FDG (Fig. 1A). HepG2 cells showed higher [11C]acetate uptake but lower [18F]FDG uptake compared to the other HCC cell lines. To determine which acetate transporter was responsible for the uptake, Western blot analysis was performed to evaluate the expression of MCT1, MCT2, and MCT4 in HCC cell lines (Fig. 1B). HepG2 cells with high [11C]acetate uptake showed higher endogenous MCT1 levels compared to the other cell lines. The expression of MCT2 or MCT4 was not relevant to [11C]acetate uptake.

MCT1 Expression in Patients With HCC

The primary tumors of patients were classified into two groups: HCCs with low [11C]acetate uptake and high [18F]FDG uptake, and HCCs with high [11C]acetate uptake and low [18F]FDG uptake (data not shown). Western blotting analysis confirmed that MCT1 protein levels were elevated in HCCs with high [11C]acetate and low [18F]FDG uptake compared to HCCs with low [11C]acetate uptake and high [18F]FDG uptake. In contrast, MCT4 was highly expressed in HCCs with low [11C]acetate uptake and high [18F]FDG uptake (Fig. 2A). Concordantly, membrane MCT1 levels were significantly elevated in HCCs with high [11C]acetate and low [18F]FDG uptake, as revealed by immunohistochemistry ($p=0.0002$) (Fig. 2B and C).

MCT1 as an Acetate Transporter in HCC

To confirm whether MCT1 contributed to acetate incorporation, we measured [11C]acetate uptake after modulating MCT1 expression in HCC cell lines. Based on the degrees of [11C]acetate uptake, HepG2 and Hep3B were used for further in vitro analysis. MCT1 expression was silenced by siRNA-mediated gene knockdown, and then [11C]acetate uptake was examined (Fig. 3A). MCT1

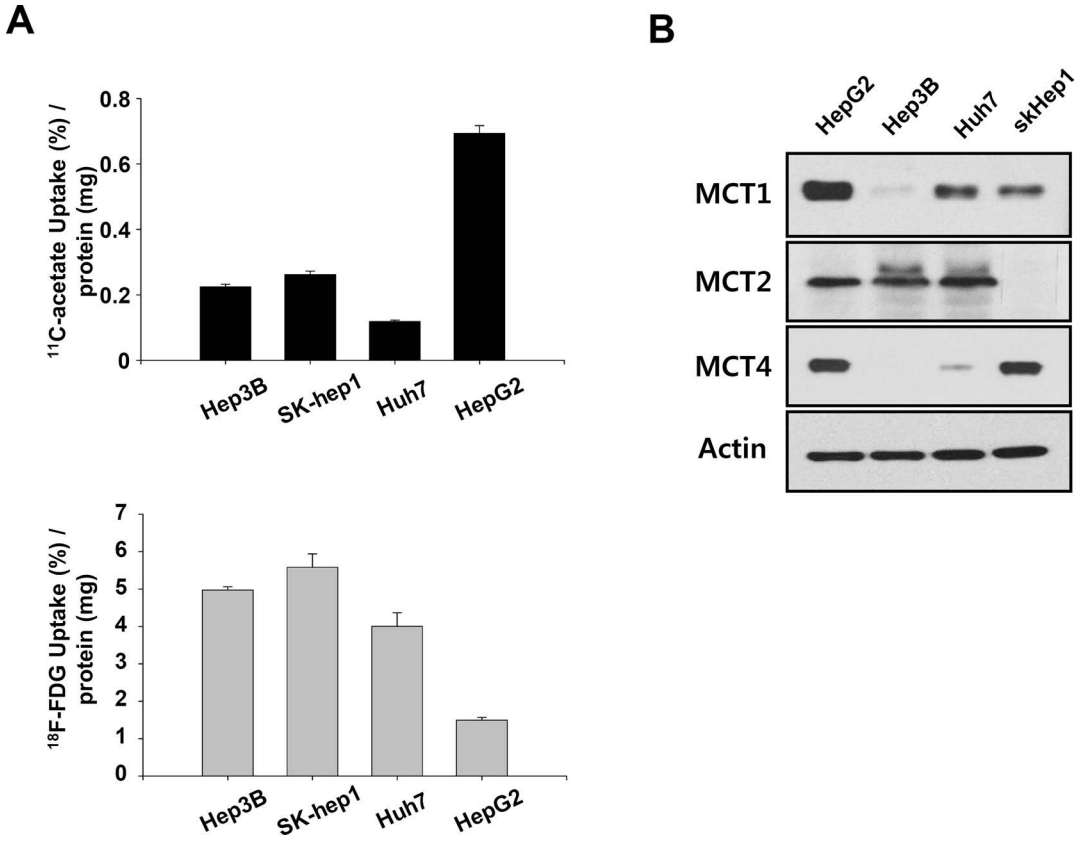

Figure 1. Patterns of metabolic radiotracer uptake in hepatocellular carcinoma (HCC) cells. (A) Radiotracer uptake ([^{18}F]FDG and [^{11}C]acetate) was measured using a gamma counter in each cell line (Hep3B, HepG2, Huh7, and SK-hep1). HCC cell lines showed high [^{18}F]FDG uptake except for HepG2 cells, in which [^{11}C]acetate uptake was dominant. Data are shown as the mean±standard deviation (SD). (B) Monocarboxylate transporter (MCT1, 2, and 4) expression in HCC cell lines determined by Western blot analysis. HepG2 cells with low [^{18}F]FDG uptake also showed high MCT1 expression compared to other cell lines.

knockdown significantly reduced [^{11}C]acetate uptake in HepG2 cells, but not in Hep3B cells. In contrast, MCT1 overexpression in Hep3B cells significantly increased [^{11}C]acetate uptake (Fig. 3B).

Next, the highly selective MCT1 inhibitor AR-C155858 was treated to further evaluate the role of MCT1 in [^{11}C] acetate uptake. Treatment with 100 μM AR-C155858 significantly reduced [^{11}C]acetate uptake in HepG2 cells (Fig. 3C). There were no remarkable changes in [^{11}C]acetate uptake after AR-C155858 treatment in Hep3B cells. This result supported the importance of MCT1 in transporting [^{11}C]acetate in HCC cells with high [^{11}C]acetate uptake.

Regulation of Oxidative Phosphorylation by Inhibiting MCT1 in HCC Cells

Acetate is a carbon source for oxidative phosphorylation (OXPHOS) in the mitochondria of acetate-dependent tumors[12]. First, the number of mitochondria in HepG2 and Hep3B cells was assessed by immunofluorescence microscopy to compare their oxidative capacity, which was significantly increased in HepG2 compared to that in Hep3B cells (Fig. 4).

Because the change in cellular OCR in the presence of pharmacological modulators is the most suitable measure of OXPHOS, real-time OCR was measured with acetate or without carbon sources in the medium. In HepG2 cells, basal OCR was 79 pmol/min without a carbon source, which was increased to 121 pmol/min after the addition of acetate (Fig. 5A). Maximum OCR showed a similar pattern to basal OCR, recovering from 58 pmol/min without a carbon source to 99 pmol/min with acetate. However, Hep3B cells showed no significant differences in basal or maximal OCRs between no carbon source and acetate in the medium (Fig. 5B). OCR analysis demonstrated that HCC cells with high [^{11}C] acetate uptake could use acetate for oxidative respiration.

In addition, HepG2 and Hep3B cells were treated with an MCT1 inhibitor, and OCR was measured. The addition of acetate in HepG2 cells increased OCR from 43 to

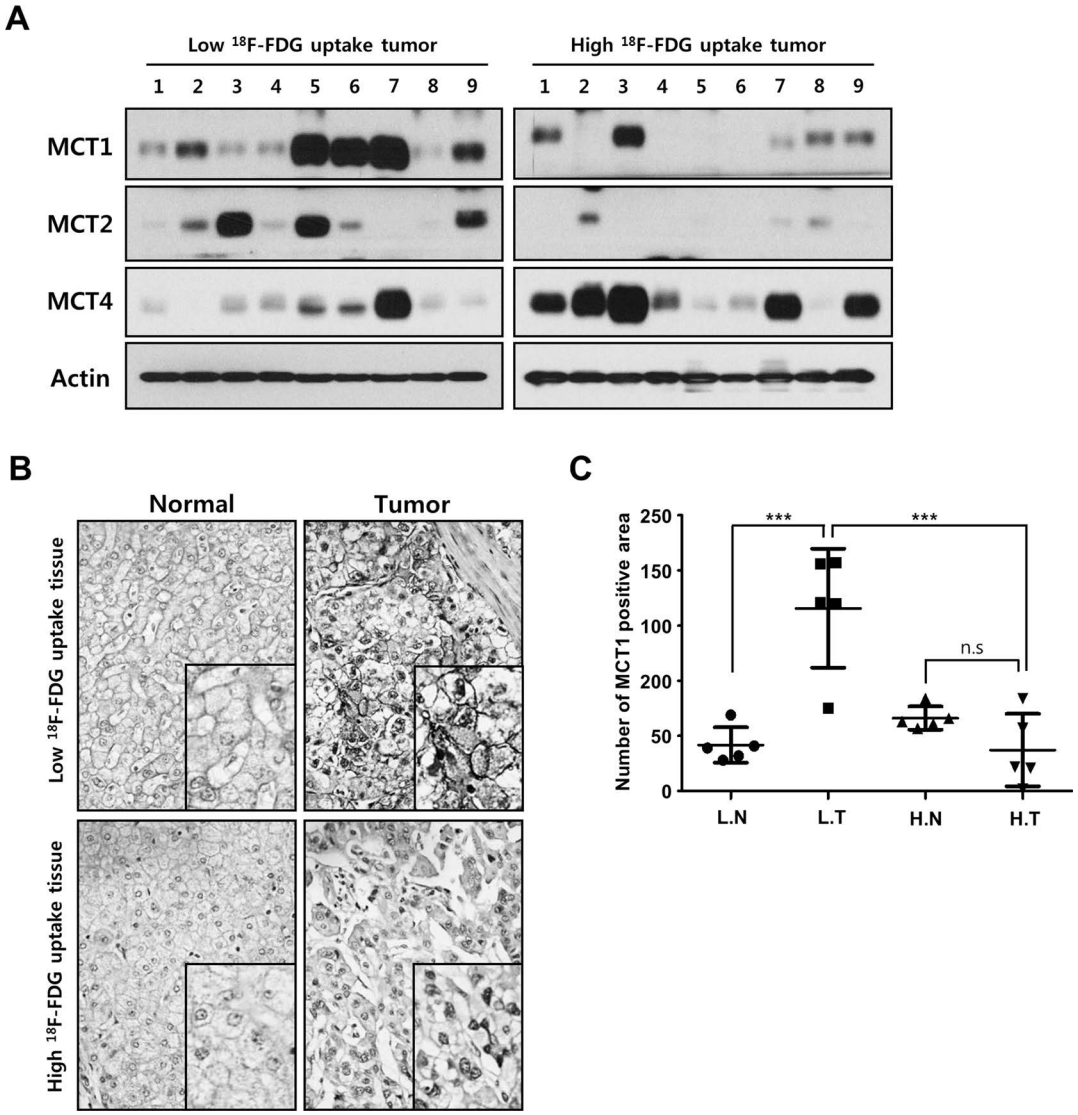

Figure 2. Monocarboxylate transporter (MCT) expression in HCC tissues. (A) Western blot analysis was performed in HCC patients. (B) MCT1 expression pattern and location were observed by immunohistochemistry analysis in HCC tissues. (C) MCT expression was quantified with the ImageJ program. Data are shown as the mean ± SD. MCT1 expression was higher in low [18F]FDG uptake tumors, but showed decreased expression in high [18F]FDG uptake tumors. Data are shown as the mean ± SD. ***$p < 0.001$.

67 pmol/min, which was then decreased to 55 pmol/min by the MCT1 inhibitor. These changes in OCR were not detected in Hep3B cells (Fig. 5C). As a result, HCC cells with high [11C]acetate uptake showed abundant mitochondria and OXPHOS capacity, using acetate taken up by MCT1.

Regulation of Lipid Synthesis and Viability by Inhibiting MCT1 Expression

Oil red O staining was performed to measure lipid synthesis in HepG2 and Hep3B cells under different conditions. Lipid staining in HepG2 cells was low without a carbon source in the medium but increased after the addition of acetate (Fig. 6A). However, Hep3B cells synthesized lipids at low levels despite acetate supplementation. Upon cotreatment with acetate and a chemical MCT1 inhibitor, the increased Oil red O staining by acetate was significantly reduced in HepG2 cells, but not in Hep3B cells.

In addition to staining, the expression of key enzymes that control lipid synthesis was evaluated. The expression of acyl-CoA synthetase 2 and fatty acid synthase was elevated in the presence of acetate in HepG2 cells but reduced by the addition of MCT1 inhibitor (Fig. 6B).

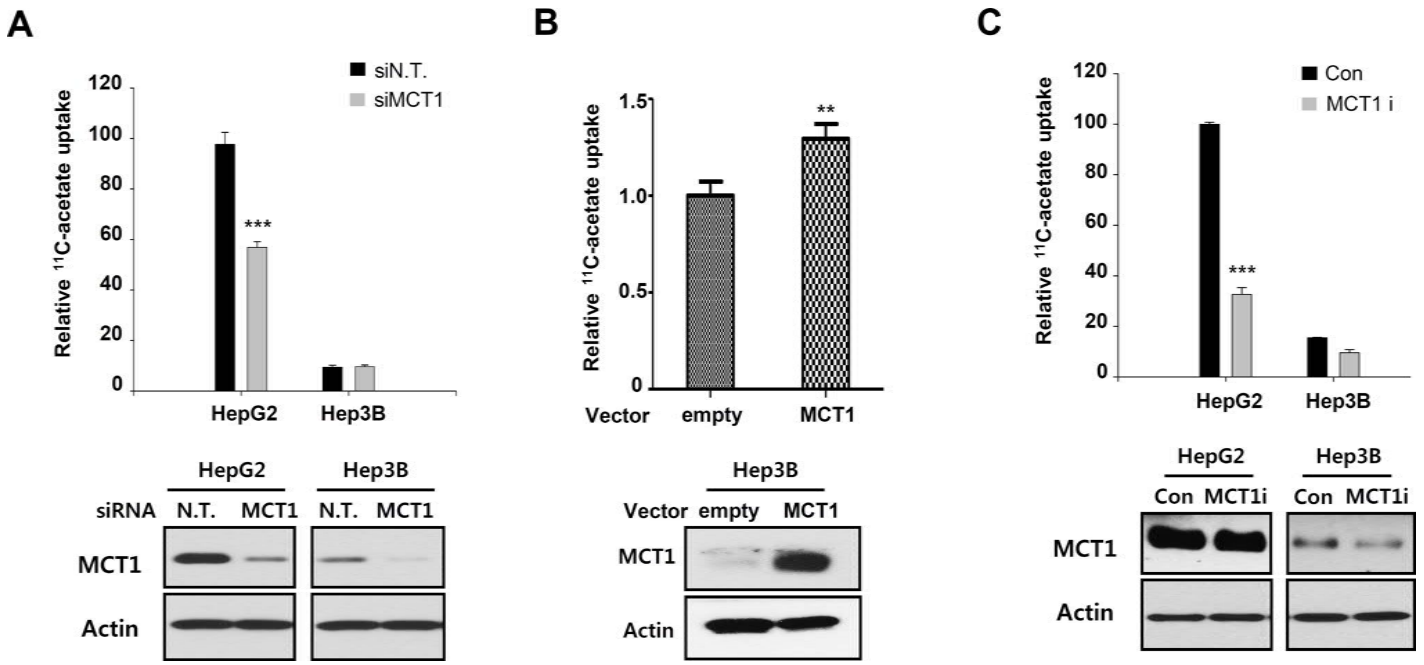

Figure 3. MCT1 inhibition and acetate uptake. [^{11}C]Acetate uptake was measured using a gamma counter in HCC cell lines under the indicated culture conditions. (A) HepG2 and Hep3B cells were transfected with nontargeted siRNA (N.T.RNA) or MCT1-targeting siRNA. Downregulation of MCT1 was confirmed by Western blot analysis and remarkably suppressed acetate uptake in HepG2 cells. Data are shown as the mean±SD. ***$p<0.001$ versus MCT1 siRNA. (B) Hep3B cells were transfected with empty vector or MCT1 gene-bearing vectors (empty or MCT1). MCT1 expression was confirmed by Western blot analysis. Despite the fact that Hep3B cells are highly glucose consuming, overexpression of MCT1 induced the uptake of [^{11}C]acetate. Data are shown as the mean±SD. **$p<0.01$ versus MCT1. (C) HepG2 and Hep3B cells were treated with the MCT1 inhibitor AR-C155858 (100 μM). [^{11}C]Acetate uptake was then measured using a gamma counter. Data are shown as the mean±SD. ***$p<0.001$ versus MCT1.

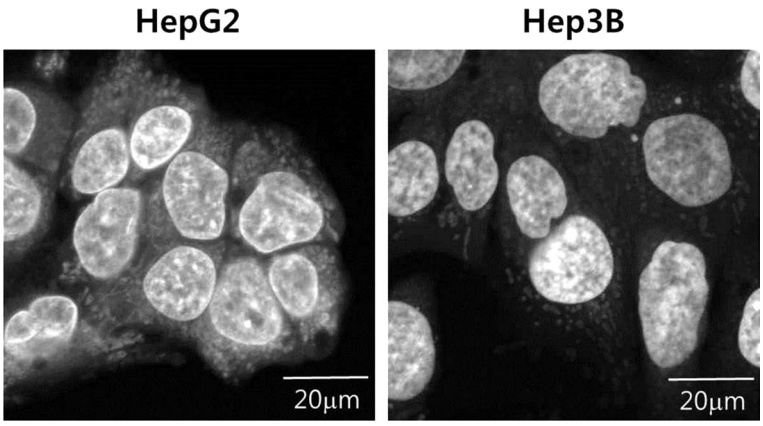

Figure 4. The number of mitochondrial in HCC cells. The number of mitochondria in HepG2 and Hep3B cells was determined by immunocytochemical analysis. The number of mitochondria was higher in HepG2 cells than in Hep3B cells.

Measurement of Cell Proliferation Upon MCT1 Inhibition

HepG2 and Hep3B cells were treated with an MCT1 inhibitor to determine the effect of MCT1 on cell viability. MCT1 inhibitor treatment resulted in a 40% reduction in cell viability in HepG2 cells, while Hep3B cells were not affected as strongly at the same drug concentration (Fig. 7). These results suggest that the viability of HCC cells with high [11C]acetate uptake was at least in part dependent on MCT1 expression.

DISCUSSION

[18F]FDG combined with PET/CT is the most commonly used radiotracer. Recently, [11C]acetate accumulation was observed in various malignancies, and acetate utilization in tumors emerged as an alternative nutrient under conditions of low cellular glucose uptake[13,14] In this study, we first found that MCT1 was overexpressed in human HCCs with high [11C]acetate uptake compared to those with low [11C]acetate uptake. The importance of MCT1 in transporting [11C]acetate into HCC cells was confirmed in in vitro uptake assays. In addition, the inhibition of MCT1 decreased OXPHOS, lipid synthesis and, subsequently, cell viability in HCC cells with [11C]acetate uptake. MCT1 may be a new potential therapeutic target in tumors with high acetate uptake, which can be detected on [11C]acetate PET/CT.

MCTs are associated with the proton-linked transport of monocarboxylates such as L-lactate, pyruvate, ketone bodies, short-chain fatty acids including acetate, and a range of α-keto monocarboxylates across the plasma membrane[15]. There are 14 MCT isoforms in the MCT family or solute carrier family[16]. Transport mediated by MCTs is considered to be facilitated diffusion rather than active transport because it depends on the concentration gradients of the monocarboxylate and proton across the membrane without further energy input. Among the MCTs, MCT1 is a bidirectional transporter that exports monocarboxylates into the extracellular space to regulate intracellular pH or imports them for reuse. Hypoxia-mediated upregulation of MCT4 is well characterized in exporting glycolytically derived lactate into the extracellular space. Similarly, MCT1 upregulation can play a critical role in monocarboxylate export in some glycolytic cancers[16–18].

In contrast to the role of MCT1 in monocarboxylate export, we found that MCT1 was important in importing acetate in human HCCs and HCC cell lines. MCT1 was highly expressed in HepG2 cells with high [11C]acetate uptake compared to that in other cells with low [11C]acetate uptake. MCT1 blocking by siRNA-mediated gene knockdown or using a highly selective MCT1 inhibitor significantly reduced [11C]acetate uptake in HepG2 cells. In human HCC specimens, membranous MCT1 levels were elevated in HCCs with high [11C]acetate and low [18F]FDG uptake, whereas MCT4 was highly expressed in HCCs with low [11C]acetate uptake and high [18F]FDG uptake. Based on these results, the import of [11C]acetate appeared to be associated with the expression of MCT1 in human HCCs and HCC cell lines. In HCCs with low [11C]acetate uptake and high [18F]FDG uptake, the high expression of MCT4 was likely attributed to glycolysis and the resulting lactate export.

In physiological states, the net direction of transport depends on the concentration gradients of protons and monocarboxylate across the plasma membrane, as all MCTs can be bidirectional[15,19]. The regulation of the

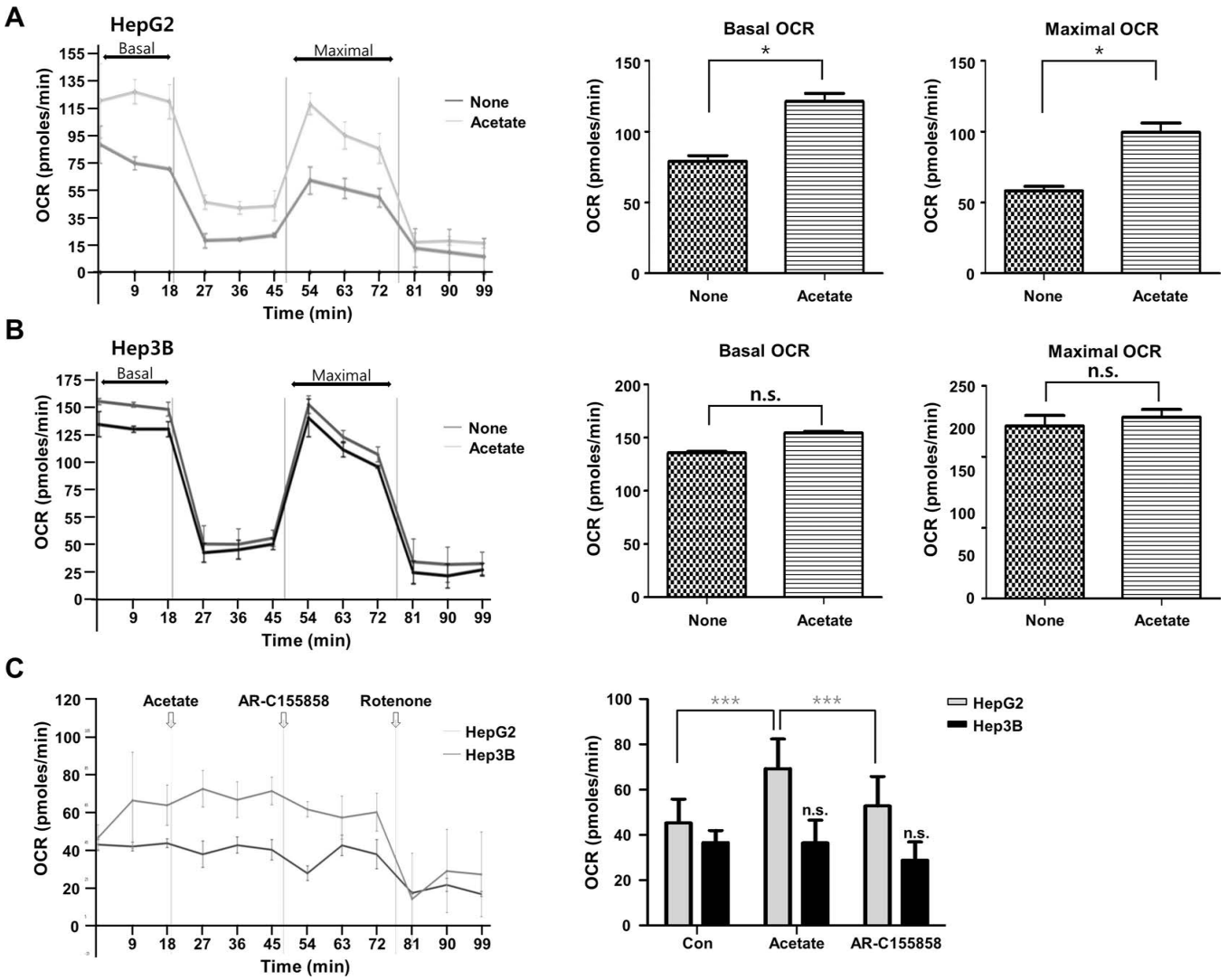

Figure 5. Oxidative phosphorylation activity upon changes in available nutrients. Oxygen consumption rate (OCR) was measured under the indicated culture conditions in real time. HepG2 and Hep3B cells were cultured in carbon source-deficient medium, with or without acetate (500 μM). Basal and maximal OCR levels were quantified in HepG2 cells (A) and Hep3B cells (B). Data are shown as the mean ± SD. *$p < 0.05$ versus acetate. n.s., nonsignificant. Basal and maximal OCR was sensitively changed with carbon source. OCR level was recovered by supplying acetate in HepG2 cells, but acetate addition did not affect Hep3B cells. (C) OCR was measured in a time-dependent manner to observe the role of MCT1 as an acetate transporter under each culture condition in HepG2 and Hep3B cells. Data are shown as the mean ± SD. ***$p < 0.001$. Acetate, carbon source; MCT1 inhibitor, AR-C155858 (100 μM); Rotenone, inhibitor of mitochondria electron transport.

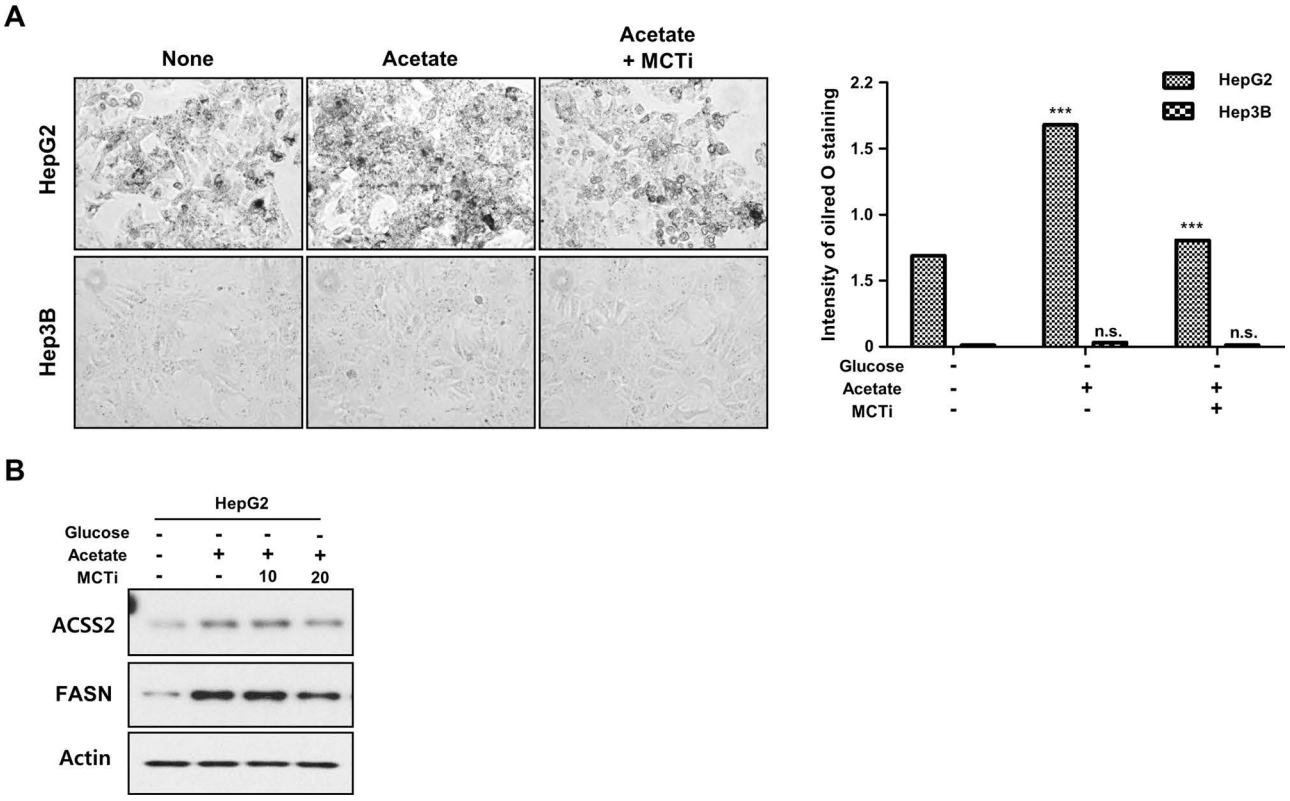

Figure 6. Acetate utilization and lipid synthesis. HepG2 and Hep3B cells were cultured for 24 h under the indicated culture conditions. (A) Lipid levels were measured and quantified using Oil red O staining for each condition. Data are shown as the means ± SD. ***$p < 0.001$. n.s., nonsignificant. Active lipid synthesis was noticeably increased in HepG2 cells by acetate addition (500 μM), and this effect was inhibited by treatment with an MCT1 inhibitor. (B) Lipid metabolism-associated proteins were measured by Western blot analysis. Expression of fatty acid synthase and ACSS2, lipid synthesis markers, was suppressed under glucose starvation but elevated by supplying acetate (500 μM) that was inhibited by MCT1 inhibitor (20 μM). MCTi, MCT1 inhibitor (AR-C155858).

direction of the MCT1 transport in cancer cells remains unknown. While glycolytic cancer cells can use MCT1 to export monocarboxylate, the import of [11C]acetate by MCT1 seemed related to OXPHOS metabolism in low-glycolytic cancer cells. In a previous study, Sonveaux et al. reported lactate uptake by MCT1 for oxidative metabolism in aerobic cancer cells[20]. In this study, we found that MCT1 imported [11C]acetate in low glycolytic human HCCs and HCC cell lines. In fact, the number of mitochondria in HepG2 cells was increased compared to that in Hep3B cells, further reflecting the maintenance of oxidative capacity. Moreover, HepG2 showed increased basal and maximal OCR after adding acetate and decreased OCR after inhibiting acetate uptake. Further studies are needed to determine whether the direction of monocarboxylate transport is associated with oxygen tension and cellular energy metabolism.

Lipid metabolism is essential for tumor cell growth and maintenance. Cancer cells mainly use glucose for lipid synthesis, but some low glycolytic cancer cells have shown the use of acetate to synthesize lipids[21]. In this

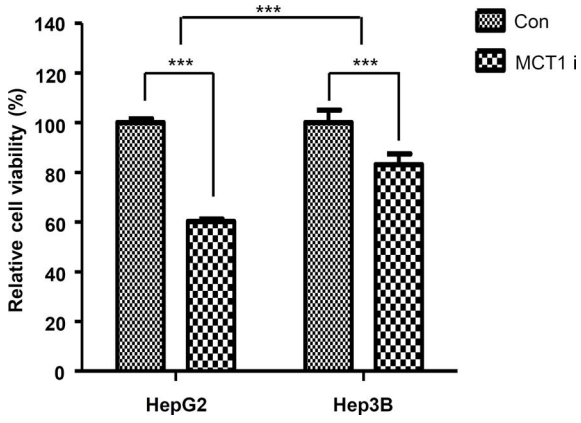

Figure 7. Effect of acetate uptake on cell viability in HCC cells. HepG2 and Hep3B cells were cultured for 24 h before treatment with the MCT1 inhibitor AR-C155858 (100 μM) for 48 h. HCC cell viability was measured by MTT assays. Data are shown as the mean ± SD. ***$p < 0.001$.

study, HepG2 cells with high [^{11}C]acetate uptake showed the ability to utilize acetate for lipid synthesis, which was reduced upon MCT1 inhibition by siRNA-mediated gene knockdown or a highly selective MCT1 inhibitor. However, acetate did not contribute to lipid synthesis in highly glycolytic HCC cancer cells. Acetate uptake by MCT1 appears to be important for maintaining lipogenesis in HCCs with high acetate uptake, but not in HCCs with low acetate uptake.

Acetate is a source of acetyl-CoA, which plays an essential role in regulating the expression of genes and activity of proteins involved in intracellular biomass, lipogenesis, and acetylation for cell survival[11,12]. In this study, the inhibition of acetate uptake by an MCT1 inhibitor significantly reduced the survival of HepG2 cells, whereas Hep3B cells with low acetate uptake were less sensitive to MCT1 inhibition; this might have been due to the differences in expression of MCTs and the ability to utilize acetate.

In the MCT family, MCT2 is expressed in the normal liver, but its expression in cancer cells is not well known. Because MCT2 is another bidirectional transport system, it may also contribute to acetate uptake. In this study, MCT1 expression was more relevant to [^{11}C]acetate uptake on PET/CT than MCT2 expression. In addition, MCT2 expression levels were not correlated to [^{11}C]acetate uptake in the cell lines. Therefore, this study focused on the role of MCT1 rather than MCT2. However, future studies of the effect of MCT2 on acetate uptake and cell survival are necessary. Another potential mechanism for acetate uptake is free diffusion across phospholipid bilayers, although MCT may significantly increase the transport rate[12,22]. Given that free diffusion can occur in any cell type, it may not contribute greatly to the increased [^{11}C]acetate uptake in cancer cells. Regardless, the effect of free diffusion in maintaining cell viability should be evaluated in future studies. Although we found the importance of acetate uptake by MCT1 in oxidative phosphorylation, lipogenesis, and cell viability, specific enzymatic pathways for acetate utilization after the import were beyond the scope of this study. Further studies using metabolomic approaches will lead to new prospects in finding therapeutic targets related to cancer-specific metabolic enzymes.

MCT1 is a novel import system of acetate in non-glycolytic HCC tumors. Acetate uptake by MCT1 is involved in oxidative phosphorylation and lipid metabolism. Thus, MCT1 may be a new potential therapeutic target in tumors with high [^{11}C]acetate uptake, which can be selected by [^{11}C]acetate PET/CT.

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