

Role of Human Monocarboxylate Transporter 1 (hMCT1) and 4 (hMCT4) in Tumor Cells and the Tumor Microenvironment

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Abstract: In recent years, the abnormal glucose metabolism of tumor cells has attracted increasing attention. Abnormal glucose metabolism is closely related to the occurrence and development of tumors. Monocarboxylate transporters (MCTs) transport the sugar metabolites lactic acid and pyruvate, which affect glucose metabolism and tumor progression in a variety of ways. Thus, research has recently focused on MCTs and their potential functions in cancer. The MCT superfamily consists of 14 members. MCT1 and MCT4 play a crucial role in the maintenance of intracellular pH in tumor cells by transporting monocarboxylic acids (such as lactate, pyruvate and butyrate). MCT1 and MCT4 are highly expressed in a variety of tumor cells and are involved the proliferation, invasion and migration of tumor cells, which are closely related to the prognosis of cancer. Because of their important functions in tumor cells, MCT1 and MCT4 have become potential targets for cancer treatment. In this review, we focus on the structure, function and regulation of MCT1 and MCT4 and discuss the developed inhibitors of MCT1 and MCT4 to provide more comprehensive information that might aid in the development of strategies targeting MCTs in cancer.

Keywords: monocarboxylate transporter, tumor, tumor microenvironment, function, regulation

Introduction

The monocarboxylate transporter (MCT) superfamily contains 14 members and is encoded by the solute carrier family 16 (*SLC16A*) gene family. The MCT proteins show sequence homology. Thus far, only the functions of MCT1–4 have been elucidated. These proteins mediate the bidirectional transport of monocarboxylic acids (such as lactate, pyruvate, ketone bodies and gamma-hydroxybutyrate) across the plasma membrane.¹ A recent report also indicated that MCTs mediate succinate export in the retina.² The concentration of H⁺ and monocarboxylic acid on both sides of the cell membrane determines the direction of transport.³ Some evidence has shown that MCTs facilitate the plasma membrane transport of certain drugs, such as salicylates and valproic acid.⁴ The transmembrane movement of monocarboxylic acids is essential for cells that rely on glycolysis for energy.^{5,6} Under physiological conditions, MCT1 and MCT4 cooperate to form a lactate shuttle system that maintains lactate homeostasis between glycolytic and oxidative cell.⁷ Lactate is an important metabolite in human health and disease.⁸

MCT1 (which is encoded by *SLC16A1*) is found in most tissues. MCT4 (*SLC16A3*) is strongly overexpressed in highly glycolytic and anaerobic tissues.⁹ MCT1 and MCT2 have very high affinity for pyruvate ($K_m \approx 0.1\text{--}0.74$ mmol/L) and stereoselective L-lactate ($K_m \approx 1\text{--}3.5$ mmol/L), and MCT4 has low affinity for pyruvate ($K_m \approx 153$ mmol/L) and lactate ($K_m \approx 28$ mmol/L).^{3,10–12} Consistent with their high affinity for pyruvate and lactate, MCT1 and MCT2 are mainly expressed in red skeletal muscle, heart and neurons, where they transport lactate into cells to provide fuel for oxidative phosphorylation.¹³ MCT3 is expressed only on the basolateral membrane of the choroid plexus and retinal pigment

epithelium, with a moderate affinity for lactate ($K_m \approx 6$ mmol/L), and MCT4 is mainly expressed in highly glycolytic cells, such as white skeletal muscle fibers and astrocytes.¹⁴

Metabolic abnormalities are one of most important hallmarks of cancer. In cancer cells, glycolysis is amplified to meet the energy demands of rapid proliferation.¹⁵ Lactic acid produced by glycolysis must be transported to the extracellular environment; therefore, cancer cells require high levels of MCTs to maintain this metabolic phenotype.^{16,17} MCT1 and MCT4 play important roles in cancer metabolism and promote cancer development through multiple mechanisms.¹⁸ Some cancer cells still undergo glycolysis under aerobic conditions to produce energy to meet the high metabolic demand. This metabolic mode of aerobic glycolysis in cancer cells is called the “Warburg effect”.¹⁹ Lactic acid and other products generated by glycolysis require MCT-mediated transport to the outside of the cell to maintain intracellular pH homeostasis. The transported lactate also leads to a decrease in the pH of the microenvironment. In tumor tissue, the acidification of extracellular microenvironment caused by co-transporting protons is conducive to angiogenesis, tumor cell proliferation, invasion and metastasis.¹⁹ Therefore, targeting MCTs may represent a possible treatment strategy for cancer.

Structure and Function of MCT1 and MCT4

Structure

The crystal structures of MCTs have not been fully described. However, topological prediction indicates that all family members have 12 transmembrane helices (TMs), a C-terminus and N-terminus within the cell, and a large cytoplasmic loop between TM6 and TM7. In the MCT superfamily members, the TM regions are more conserved than the loop and C-terminus.¹¹ Lysine 38, aspartic acid 302, and arginine 306 are particularly important for substrate binding and transporter activity, as shown by human MCT1 modeling, and MCT1 and MCT 4 have been confirmed as proton-linked MCTs.¹¹ In the structure of hMCT1 and hMCT4, seven key residues are conserved and involved in the translocation cycle of L-lactate.²⁰ Conserved helix 5 is essential for transport function and is involved in stereoselectivity.²¹ The loop between TM6 and TM7 contributes to the substrate affinity of hMCT.²² In MCT1, TM6 binds to basigin (an immune protein) mainly through hydrophobic interaction.⁷ An arginine residue in TM8 is conserved in most SLC16 family members due to its involvement in carboxyl binding of the carboxyl group of monocarboxylate esters.^{23,24} D302 and R306 on the inner surface of TM8 accept protons and lactate, respectively.²⁵ F360 in helix 10 and R306 in TM8 play a key role in substrate selectivity by acting as steric hindrance protrusion channels, and residues around R306 are not always conserved, suggesting that these residues may be involved in substrate recognition.^{24,26} R278 in TM8 of hMCT4 is also a key residue for substrate recognition (Figure 1).²⁷

After synthesis, MCTs need to be transported to the plasma membrane to function. The correct localization and function of MCT1 and MCT4 require the assistance of glycosylation chaperones.

CD147/basigin, a member of the immunoglobulin superfamily, is involved in immune response and immunosuppression.²⁹ CD147/basigin is located on the cell surface and consists of an extracellular Ig-like domain, a single transmembrane fragment and a short intracellular cytoplasmic tail; it is widely distributed in vivo and promotes tumor migration by inducing matrix metalloproteinases (MMPs).^{30,31} CD147/basigin also regulates the expression of vascular endothelial growth factor (VEGF) and metalloproteinases in xenograft tumors and stimulates tumor angiogenic potential and growth rate.²⁹

There are four isoforms of basigin (BSG) proteins, of which the BSG1 isoform is located in the retina and closely interacts with MCT3.^{32,33} BSG2 (hereafter, BSG), the most prevalent and studied isoform, contains two immunoglobulin-like domains that are chaperones of MCT1 and MCT4 and help them to fold properly. BSG thus maintains their stability, promotes their cellular expression and mediates proper localization on the cell membrane surface.^{31,34} Le Floch et al found that the expression of MCT1 and MCT4 was significantly reduced in cells under normoxia and hypoxia after knockdown of the CD147/BSG gene in colon adenocarcinoma cells.³⁵ Thus, the interaction of MCT1 and MCT4 with BSG in the plasma membrane is important for their activity.^{36,37} In patients with bladder urothelial carcinoma, high expression of MCT1 and CD147 is associated with poor overall survival, while high expression of MCT4 is associated with poor recurrence-free survival; high expression of MCT1 and MCT4 is an independent prognostic indicator for poor overall survival and recurrence-free survival, respectively.³⁸ One study showed that CD147 is upregulated in bladder

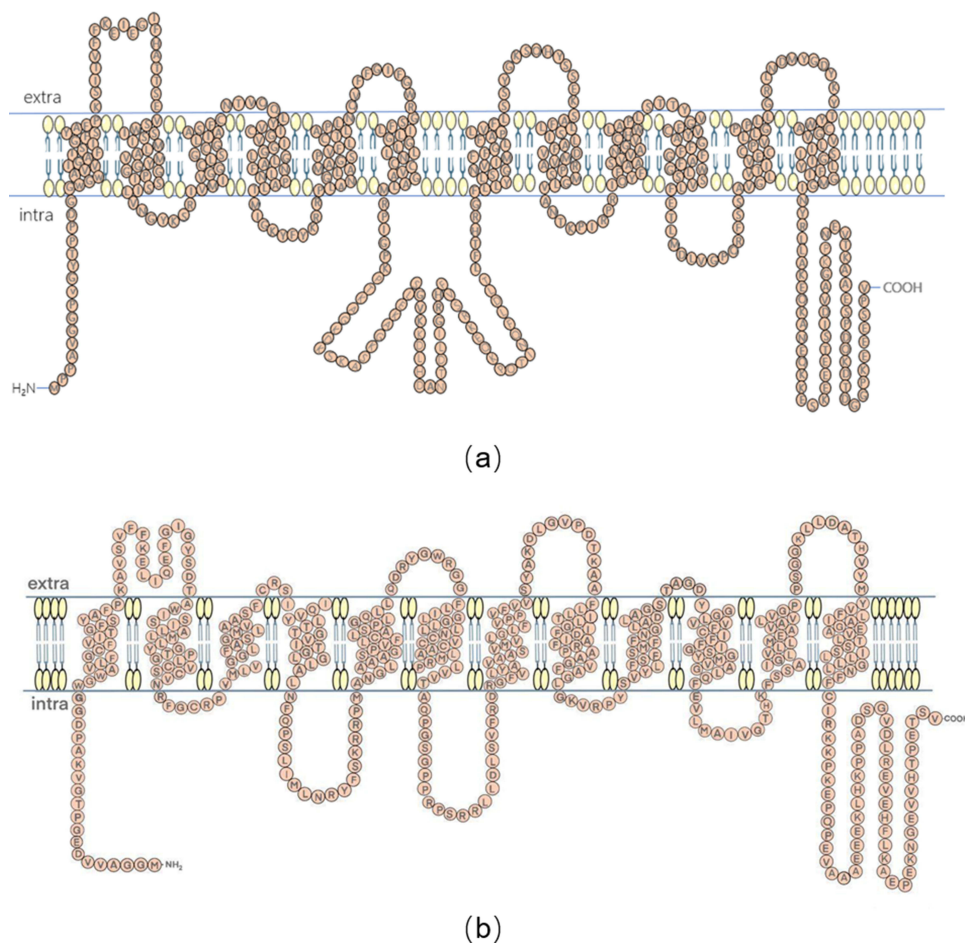


Figure 1 Prediction model of hMCT1 (a) and hMCT4 (b).

Notes: Adapted from Sasaki S, Kobayashi M, Futagi Y, et al. Crucial residue involved in L-lactate recognition by human monocarboxylate transporter 4 (hMCT4). *PLoS One*. 2013;8(7):e67690. Creative Commons.²⁸

tumor tissues and is significantly associated with tumor invasiveness and poor prognosis.³⁹ Several studies have identified CD147 expression as an independent prognostic factor in bladder urothelial carcinoma.^{40,41} CD147 and MCT1 have also been associated with drug resistance of tumor cells. Afonso et al found that CD147 and MCT1 were associated with bladder cancer progression and resistance to cisplatin-based chemotherapy, revealing that targeting CD147 and MCT1 is helpful in treating cisplatin-resistant bladder cancer.³⁹

Function

Lactic acid is mainly produced from glucose through a reaction catalyzed by a series of glycolytic enzymes. After glucose uptake by cells via glucose transporters, pyruvate is generated in the cytoplasm by the following steps: 1) glucose, catalyzed by hexokinase (HK), consumes one molecule of ATP to produce one molecule of glucose 6-phosphate (G-6-P); 2) G-6-P is converted to fructose-6-phosphate (F-6-P) catalyzed by phosphate hexose isomerase (PGI); 3) F-6-P, catalyzed by 6-phosphofruktokinase 1 (PFK1), consumes one molecule of ATP to produce fructose-1,6-diphosphate (F-1,6-BP); 4) F-1,6-BP, catalyzed by aldolase (ALDO), produces glyceraldehyde 3-phosphate (G-3-P) and dihydroxyacetone phosphate (DHAP), which are isomers of each other; 5) G-3-P dehydrogenation is catalyzed by glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to 1,3-diphosphoglycerate (1,3-PGA); 6) 1,3-PGA is catalyzed by 3-phosphoglycerate kinase (PGK) to produce 3-phosphoglycerate (3-PG) and a molecule of ATP is produced; 7) 3-PG is converted to 2-phosphoglycerate (2-PG) under the catalysis of enolase, yielding phosphoenolpyruvate (PEP); and 8) PEP forms pyruvate under the catalysis of pyruvate kinase (PKM). Through this series of reactions, one molecule of glucose is

converted to two molecules of pyruvate. In the case of sufficient oxygen supply, pyruvate enters the mitochondria to produce a large amount of energy through the tricarboxylic acid cycle (TCA). In the case of insufficient oxygen, pyruvate generates lactate under the catalysis of lactate dehydrogenase A. In normal tissue cells, such as red skeletal muscle, heart and neurons, MCT1 transports lactate into cells, where it is converted by lactate dehydrogenase B (LDHB) to pyruvate, which is supplied by oxidation through the TCA. In white skeletal muscle fibers and astrocytes, MCT4 transports lactate production out of cells and maintain intracellular acid-base balance (Figure 2).

Malignant tumors are characterized by heterogeneity; they contain both aerobic regions close to blood vessels and hypoxic regions far from blood vessels. Cancer cells in hypoxic areas obtain energy through glycolysis, which produces less energy; thus, cancer cells in hypoxic areas need more glucose to produce energy, which is called “glucose starvation”.⁴² Under hypoxic conditions, hypoxia-inducible factor-1 (HIF-1) is overexpressed, and HIF-1 induces the expression of glucose transporter 1 (GLUT1). GLUT1 is responsible for glucose uptake, and hypoxic cancer cells take up a large amount of glucose through overexpressed GLUT1. Glucose generates a large amount of energy to meet the needs of cell metabolism through the glycolytic pathway and simultaneously produce pyruvate and H⁺. Pyruvate is converted to lactate by LDHA, which leads to a decrease in intracellular pH.⁴³ MCTs transport the produced lactate and pyruvate out of the cell and play an important role in the lactate shuttle between hypoxic and aerobic cancer cells.⁴⁴ Similarly, oxidized cancer cells obtain an additional supply of lactate through MCT1/4-mediated lactate shuttle by promoting aerobic glycolysis in adjacent stromal fibroblasts.^{45,46} MCT4, which has low affinity for lactic acid, transports lactic acid produced by hypoxic cancer cells through glycolysis outside of cells. MCT1, which has a high affinity for lactic acid, can transport lactic acid to normoxic cancer cells. Lactate entering cancer cells is converted to pyruvate by lactate dehydrogenase catalyzed B (LDHB). After then, pyruvate enters the TCA as the substrate for cancer cell oxidation power (Figure 3). If lactate is the only energy source for tumor cells, inhibition of MCT1 leads to cell death.^{42,47} For aerobic cells, oxidative lactate metabolism may be more favorable than aerobic glycolysis because it produces ATP at most 7.5-fold.¹⁸ At the same time, aerobic cancer cells use lactate for energy, so more glucose can enter hypoxic cancer

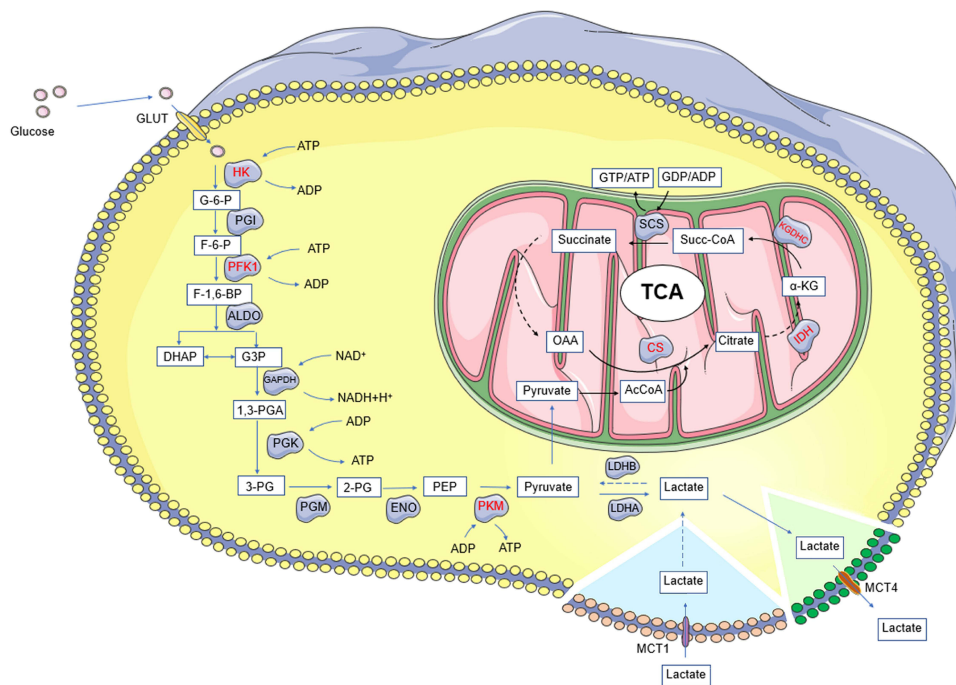


Figure 2 Glucose metabolic process. Glucose enters cells via glucose transporters (GLUT). Many enzymes are involved in the process of anaerobic glycolysis. The production of pyruvate from glucose is catalyzed by three key enzymes: hexokinase (HK), 6-phosphofruktokinase 1 (PFK1) and pyruvate kinase (PKM). Pyruvate is catalyzed by lactate dehydrogenase A (LDHA) to lactate. The tricarboxylic acid cycle (TCA) process is catalyzed by several key enzymes: citrate synthase (CS), isocitrate dehydrogenase (IDH) and ketoglutarate dehydrogenase complex (KGDHC). Blue areas (eg, red skeletal muscle, heart and neurons) and green areas (eg, white skeletal muscle fibers and astrocytes) indicate lactate transport in normal cells.

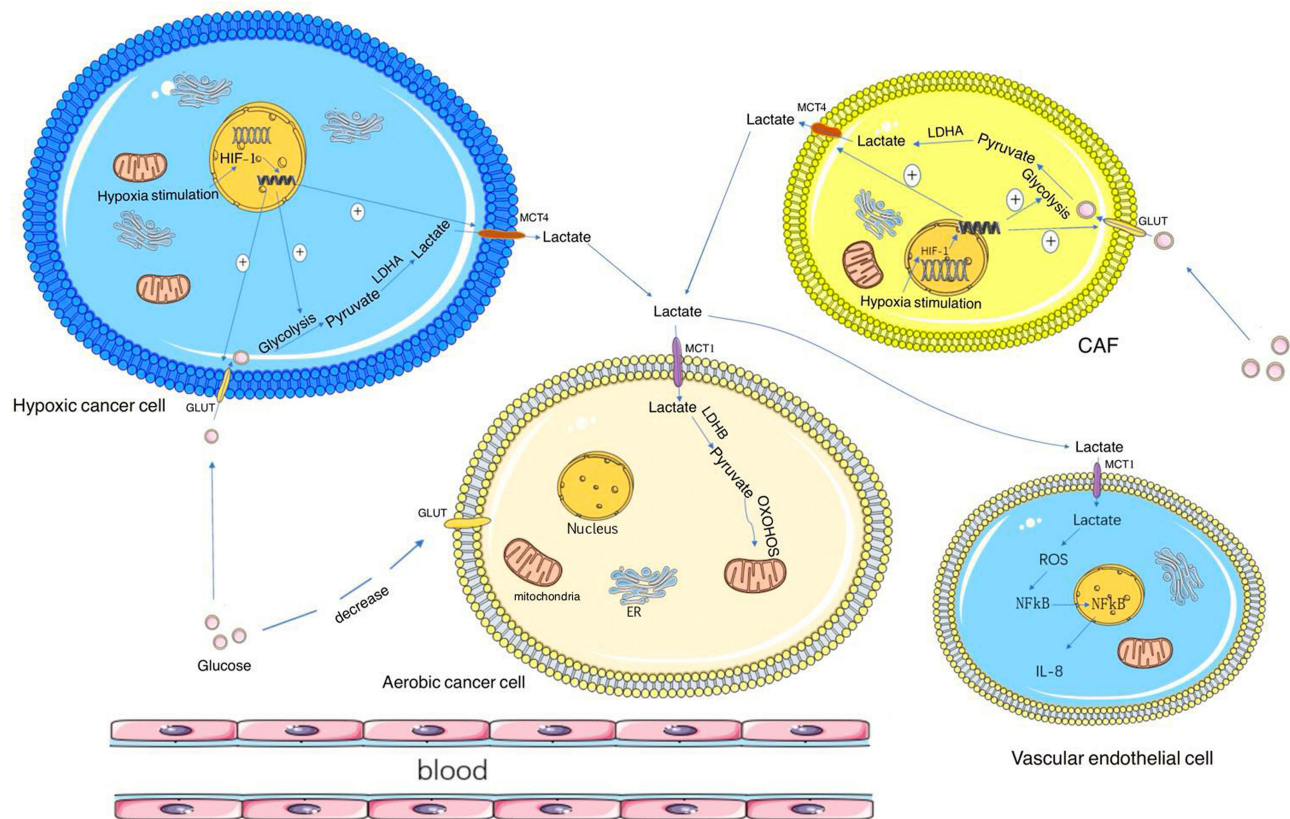


Figure 3 Schematic of lactate shuttling from cell to cell through the MCTs. Hypoxic cancer cells and cancer-associated fibroblasts (CAFs) use glucose to produce lactate through glycolysis, and lactate is transported out of the cell by MCT4. Aerobic cancer cells transport lactate through MCT1. Vascular endothelial cells transport lactic acid into cells through MCT1 and promote angiogenesis through NF- κ B/IL8 pathway (\oplus : activate).

cells to produce energy to meet proliferation and metabolism needs. Studies have shown that glucose transporters and downstream glycolytic enzymes are overexpressed in more than 70% of cancers.^{48,49}

Stromal cells in contact with tumor cells undergo metabolic reprogramming and transform into cancer-associated fibroblasts (CAFs). The lactic acid produced by CAFs through aerobic glycolysis can also be transported to the extracellular through MCT4, and lactic acid is taken up by aerobic cancer cells for oxidation energy.⁵⁰ Additionally, the lactate shuttle between glycolytic cancer cells and vascular endothelial cells is also through MCTs.⁵¹ Lactate produced by tumor cells through high glycolysis is exported through MCT4, and vascular endothelial cells transport lactate into cells through MCT1.^{52,53} Therefore, MCTs play a crucial role in lactate transport between tumor cells, between tumor cells and stromal cells as well as between tumor cells and vascular endothelial cells (Figure 3). Within the same tumor cell, MCT1 and MCT4 are expressed in different amounts. Sonveaux et al found that aerobic cancer cells in human neck and colon xenograft tissues expressed high levels of MCT1 compared with hypoxic regions that showed almost no detectable MCT1 expression, but this phenomenon was not observed in other tumor types.⁴⁷ MCT4 expression is increased in hypoxic and poorly angiogenic tumor regions.^{54,55} Another study found that MCT4 is associated with the sarcolemma of fast twitch fibers in muscle.⁵⁶ In addition to the ability of MCT1 to transport lactate into cells, MCT1 also releases lactate into the medium.⁵⁷ Mathupala et al showed that targeted inhibition of MCT1 and MCT2 expression significantly reduced lactate efflux from glioblastoma multiform cells.⁵⁸ Thus, MCT1 can transport lactate in both directions. MCT1 and 4 also have a certain ability to transport ketone bodies, and the affinity of MCT1 for ketone bodies is higher than that of MCT4. Therefore, MCT1 participates in the development of tumors by mediating the transport of ketone bodies.¹ These suggest that MCTs may be a potential target for anti-cancer therapy by knocking down MCTs (by inhibitors or siRNA) or by combining MCT knockdown with chemotherapy or radiotherapy for anti-tumor treatment.⁵⁹

Lactic acid produced by tumor cells is not only taken up by cells for oxidative energy but it also inhibits the killing effect of immune cells on tumor cells, giving cancer cells immune tolerance.^{60,61} Lactate produced by tumors is internalized by cytotoxic T cells (CTLs) and inhibits cytotoxic T cell proliferation and anticancer function by inhibiting p38 and JNK/c-Jun activation.⁶² Lactate polarizes macrophages to promote the M2 phenotype of tumors and exerts an immunosuppressive function by activating G protein-coupled receptor 132 (GPR132). Lactate also inhibits the antitumor immune response of dendritic cells (DCs) by inhibiting the differentiation of monocytes into DCs.⁶² One study showed that the elevated lactate level in the tumor microenvironment is a by-product of glycolysis in breast cancer that both weakens the anticancer immune response in a concentration-dependent manner and plays a key role in the regulation of the tumor immune microenvironment.⁶³ Another study reported that the down-regulation of MCT4 promoted the cytotoxicity of NK cells in breast cancer, indicating that MCT4 is involved in the suppression of the tumor immune microenvironment.⁶⁴ Sun et al found that MCT4 may act as an immune suppressor by reducing macrophage maturation or perturbing T cell metabolism.⁶⁵

Role of MCTs in the Proliferation, Migration and Progression of Tumor Cells

MCTs play an important role in tumor cell proliferation, invasion and migration and tumor progression. MCT4, together with proton cotransporter, transports lactate produced by cancer cells through glycolysis out of the cell and transports lactate into vascular endothelial cells through MCT1. Lactate and pyruvate stimulate the expression of HIF-1.^{66,67} HIF-1 further promotes the expression of vascular endothelial growth factor (VEGF), which promotes the generation of blood vessels, providing more nutrients for tumor cells.⁶⁸ Gloire et al found that lactic acid promotes the phosphorylation and degradation of I κ B α in endothelial cells, and then promotes the activation and transfer of free NF- κ B to the nucleus for transcriptional regulation to promote angiogenesis.⁶⁹ Other studies have shown that lactate promotes the secretion of IL-8 in endothelial cells, which in turn promotes the expression of NF- κ B and angiogenesis. Inhibition of MCT1 inhibits the formation of the tumor microvascular network and tumor proliferation.⁷⁰ Zhao et al found that MCT1 regulates tumor growth and metastasis by regulating the NF- κ B pathway, and inhibition of MCT1 reduced the growth and metastasis of osteosarcoma and improved the effect of chemotherapy in osteosarcoma.⁷¹ Additionally, lactate exported by MCT4 is the fuel for cancer cell proliferation, and cancer cells transport lactate into cells through overexpression of MCT1.⁴⁷ Hong et al showed that inhibition of MCT1 prevents breast cancer cell proliferation by preventing pyruvate transport to the outside of cells; however, it did not increase cell apoptosis.⁷² A previous study showed that silencing of MCT1 and MCT2 inhibits tumor growth and leads to apoptosis and necrosis of tumor cells.⁵⁸ Pertega et al reported that both MCT1 and MCT4 expression were increased and positively correlated with prostate cancer progression.⁷³

CAFs are important components affecting cancer cell invasion and metastasis. Giannoni et al found that CAFs trigger a pro-oxidative environment in cancer cells, profoundly affecting tumor progression and metastatic spread.⁷⁴ In another study by the same group, CAFs were found to promote epithelial-mesenchymal transition in human prostate cancer cells and promote tumor growth, increase the development of stem cell markers and induce spontaneous metastasis.⁷⁵ Studies have shown that MCT4 is involved in the metabolic reprogramming process of prostate cancer cells and CAFs, and a higher expression of MCT4 in prostate cancer cells corresponds with a worse clinical outcome.⁷⁶ Other studies reported that CAFs interact with bladder cancer cells to promote tumor progression, and the expression of MCT1 and MCT4 is up-regulated in fibroblasts.⁷⁷ Therefore, MCTs may affect cancer invasion and metastasis by affecting the metabolic reprogramming process of cancer cells and CAFs. MCT4 also discharges lactate produced by CAFs from cells and transports it into cancer cells through MCT1 to provide energy for cancer cell proliferation (Figure 3). Zhang et al found that inhibition of MCT1 reduced bladder cancer cell proliferation, migration and invasion.⁷⁸ Another study found that inhibition of MCT4 by siRNA significantly inhibited the trans-pore migration of MDA-MB-231 cells by 85%.⁷⁹ This suggests that MCTs can enhance the ability of cancer cells to migrate and invade not only by affecting stromal cells in the tumor microenvironment but also by directly influencing the tumor cells.

Under hypoxic conditions, tumor cells secrete a variety of stimuli to recruit macrophages to the hypoxic site.⁸⁰ A large amount of lactic acid produced by glycolysis of tumor cells is absorbed into cells by macrophages through MCT1. Some reports showed that lactate entering macrophages activates GPR132 to induce M2 polarization of macrophages by increasing the expression of Arg1 and Mrc1. M2 macrophages can enhance the adhesion, migration and invasion of cancer cells.^{81,82}

Regulation of MCT1 and MCT4

The expression levels of MCTs vary among different tissues. MCT levels are regulated at both pre- and post-transcriptional levels (Figure 4).⁸³

HIF-1

Tumor cells are exposed to hypoxia, which induces the expression of hypoxia-inducible factor (HIF-1), a transcription factor that initiates a series of responses, including angiogenesis and various pro-survival mechanisms.⁸⁴ HIF-1 is stably expressed in a hypoxic environment and upregulates the activity of GLUTs, glycolysis-related enzymes and other hypoxia-related genes, reducing the dependence of cells on the oxidative pathway and transferring cell metabolism to the glycolytic pathway.⁸⁵ Elevated HIF-1 in rapidly growing cells, such as embryos and tumors, not only stimulates glycolysis but also reduces pyruvate flow to the TCA by inhibiting LDH in mitochondria.^{86,87} This HIF-1-mediated inhibition of LDH reprogramming in glucose metabolism is the main basis of the “Warburg effect”.¹³

In the hypoxic environment, HIF-1 binds to two hypoxia response elements upstream of the transcription start site to up-regulate the expression of MCT4. The MCT1 promoter lacks the hypoxia response elements that are bound by HIF-1, so HIF-1 does not induce the increase of MCT1 expression.⁸⁸ Previous studies have shown that HIF-1 and specific protein 1 (SP1) jointly mediate the expression of CD147 in the hypoxic microenvironment of epithelial solid tumors.⁸⁹ This up-regulation of CD147 expression indirectly plays a role in the regulation of MCT. Additionally, several investigators have shown that hypoxia does not lead to upregulation of MCT1 and MCT4 in MCF-7 cells, but it does lead to upregulation of MCT1-associated carbonic anhydrase IX (CAIX), which promotes the rapid secretion of lactate and H⁺ from breast cancer cells in uncatalyzed reactions.⁹⁰

p53

The p53 tumor suppressor has been the subject of intensive investigation. The occurrence of cancer is closely related to mutation of the p53 gene, and approximately 50% of human cancers lose p53 function because of gene mutation.⁹¹ p53

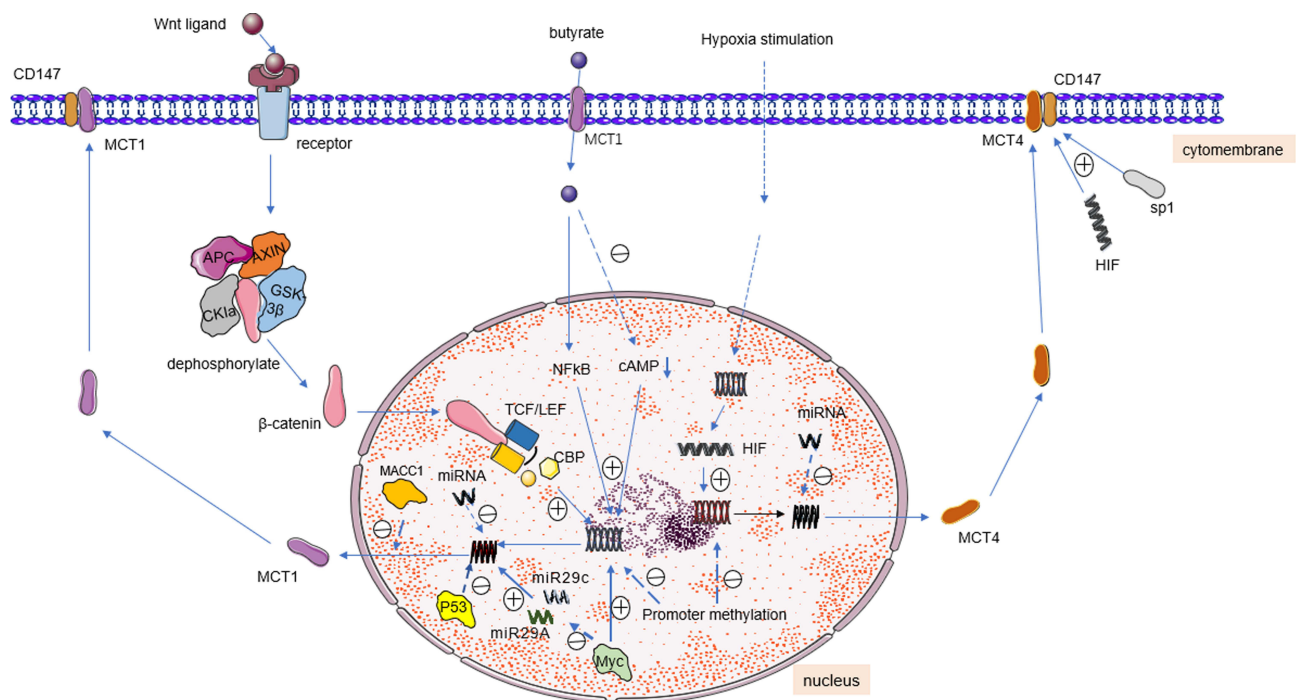


Figure 4 MCTs partial adjustment mode diagram.

Notes: ⊕: Activate, ⊖: suppress.

Abbreviations: TCF/LEF, T-cell factor/lymphoenhancer factor; CBP, CREB-binding protein; MACC1, metastasis-associated in colon cancer-I.

regulates the expression of genes related to cell cycle progression, apoptosis, cell differentiation and senescence.⁹² p53 inhibits cell proliferation and mediates homeostatic regulation of immunity and inflammation, homeostatic regulation of cell competition and homeostatic regulation of stem cell self-renewal and differentiation.⁹¹ During stress, this activates p53 transcriptional activity and promotes senescence or apoptosis of damaged cells, contributing to the protection of tissue integrity under severe stress conditions.⁹¹ p53 is also involved in the regulation of metabolic pathways in tumor cells, and loss of p53 stimulates glycolysis and impairs the mitochondrial respiratory chain, thereby promoting the switch of ATP production from oxidative phosphorylation to glycolysis.⁹³ Boidot et al found that p53 deficiency promoted the expression of MCT1 under hypoxic conditions, thereby promoting lactate release from elevated glycolytic flux.⁹⁴ Under hypoxic conditions, loss of p53 is associated with the stabilization of MCT1 mRNA, and p53 inhibits MCT1 expression. NF- κ B plays a crucial role in the hypoxia-induced increase of MCT1 expression in p53-deficient cells.

Myc

The Myc oncogene is activated in many malignant tumors and closely associated with tumorigenesis. Approximately 100,000 cancer-related deaths each year are associated with dysregulated Myc expression.⁹⁵ Upregulation of Myc expression is observed in 50% to 60% of tumors and directly contributes to malignant transformation through its pathogenic role in tumorigenesis, development and maintenance.⁹⁶ Myc transforms normal cells into tumor cells by increasing the transcription level of high-affinity target genes and even saturating them. Myc also up- and down-regulates low-affinity target genes.⁹⁷ Myc oncoproteins belong to a family of so-called “hypertranscription factors” that may regulate at least 15% of genetic transcription across the genome, including multiple targets involved in cell growth, metabolism and division.^{98,99} Prominent targets of Myc regulation include genes that encode enzymes that drive aerobic glycolysis, a hallmark of most tumors.^{100,101}

The MCT1 promoter region has a typical Myc binding site, and Myc regulates the expression of MCT1 through this site.¹⁰² MCT1 expression is increased in MCF10 breast epithelial cells expressing Myc and in certain tumors.¹⁰³ Other studies have shown that c-Myc regulates MCT1 expression by regulating the activity of the MCT1 promoter.⁹⁵ Myc also transcriptionally represses miR29A and miR29c, resulting in enhanced expression of MCT1.¹⁰⁴

Butyrate

Butyrate, a short-chain fatty acid (SCFA), is produced by bacterial fermentation of undigested carbohydrates in the colon and is the main fuel for colon cells. In the gut, SCFAs are transported across the cell membrane by pH-dependent, H⁺-coupled MCT and sodium-coupled monocyclic acid transporter (SMCT).¹⁰⁵ The main consequence of reduced intracellular SCFA oxidation is metabolic starvation and mucosal atrophy.¹⁰⁶ After entering cells, butyrate is metabolized to provide energy for cells and also has a regulatory effect on gene expression. Butyrate regulates gene expression at several levels, including through transcription, mRNA stability and elongation mechanisms.¹⁰⁷

One study showed that butyrate upregulates MCT1 expression and activity through transcriptional and post-transcriptional mechanisms.¹⁰⁸ Borthakur et al demonstrated that butyrate upregulates MCT1 promoter activity, thereby promoting the expression of MCT1, and the authors showed that this regulatory process involves the NF- κ B pathway.¹⁰⁹ In another study by the same group, butyrate and niacin were shown to reduce intracellular cAMP levels, which in turn promoted MCT1 expression.¹¹⁰

G-Protein-Coupled Receptors (GPRs)

G protein-coupled receptor 81 (GPR81), also known as hydroxycarboxylate receptor 1 (HCAR1), is a lactate receptor that is highly expressed in adipocytes and present at low levels in a variety of normal cells.^{111–113} GPR81 is also expressed in a variety of cancer cells, such as colon, breast, lung, hepatocellular carcinoma, cervical cancer and salivary gland cancer cells.¹¹⁴ GPR81 has multiple functions in vivo, including in cell development and survival, lipid metabolism, lactate transport, promotion of tumor angiogenesis, tumor growth and metastasis.^{114–117} Roland et al showed that GPR81 regulates lactate uptake and MCT1 gene expression, and silencing GPR81 reduces tumor cell activity and tumor cell proliferation when lactate is the only available energy source.¹¹⁴

GPR109A, a nicotinic acid receptor, is highly expressed in neutrophils, macrophages, monocytes, dendritic cells, skin, liver cells, retinal cells, bone and adipocytes.^{118,119} GPR109A is activated by monomethyl fumarate (MMF) and

niacin (niacin or vitamin B3).¹²⁰ Promoter methylation inhibits GPR109A expression in human colon cancer cells, and IFN- γ reverses the DNA methylation-mediated silencing of GPR109A.¹²¹ Butyrate and niacin activate the expression of GPR109A and promote the expression of MCT1 in colon cancer cells. In cells silenced for GPR1089A with siRNA, butyrate showed no effect on the expression of MCT1.¹¹⁰

Metastasis-Associated in Colon Cancer-I (MACC1)

MACC1 is an oncoprotein that regulates the hepatocyte growth factor/methionine kinase receptor epidermal growth factor (HGF/c-Met) pathway, which promotes carcinogenesis and tumor progression by promoting the migration and invasion of cancer cells and inhibiting the apoptosis of cancer cells.¹²² MACC1 plays a key role and functions as a biomarker for the progression and metastasis of a variety of solid tumors, such as gastrointestinal tumors and gynecological tumors. MACC1 has been demonstrated as a clinically useful prognostic and predictive biomarker in multiple types of solid tumors.¹²³ MACC1 plays an important role in tumor cells and tumor microenvironment to promote glucose metabolism of tumor cells and provide energy for cells. MACC1 regulates the sensitivity of tumor cells to chemotherapeutic drugs.

Wang et al found that MACC1 regulates the expression of MCT1 in gastric cancer. The overexpression of MACC1 increased MCT1 protein expression in the gastric cancer cell line MKN45, while its silencing decreased MCT1 expression.¹²⁴ Furthermore, increasing MCT1 expression in gastric cancer cells enhanced chemosensitivity to 5-FU and cisplatin. However, which pathway MACC1 regulates MCT1 expression is still unclear.

Phorbol 12-Myristate 13-Acetate (PMA) and Luminal Leptin

PMA, a protein kinase C agonist, significantly increased apical butyrate uptake and MCT1 protein expression in Caco-2 cells.¹²⁵ Intestinal MCT1 gene expression is regulated by PMA at the transcriptional level, which is dependent on PKC activation, and the up-regulation of MCT1 gene expression by PMA involves atypical PKC- ζ isoforms and the activator protein 2 (AP2) transcription factor.¹²⁶

Leptin is a hormone with multiple functions that is synthesized in different peripheral tissues, such as stomach, salivary glands, placenta and kidney.¹²⁷ Leptin is associated with food intake and energy expenditure.¹²⁸ Studies have shown that luminal leptin can regulate intestinal butyrate uptake by increasing cellular MCT1 mRNA expression.¹²⁹ The pathway by which leptin regulates MCT1 expression requires further investigation.

Promoter Methylation

DNA methylation is a key epigenetic process that involves the addition of methyl groups to a cytosine adjacent to a guanidine, a CpG dinucleotide.¹³⁰ Alterations in DNA methylation status within genes can lead to differences in gene transcription and mRNA expression levels without changing the nucleotide sequence. While hypermethylation is associated with gene silencing, hypomethylation is associated with activating gene expression.¹³¹ Many MCTs have been reported to contain CpG islands in their promoter sequences. In renal clear cell carcinoma, MCT4 is regulated by promoter methylation; the degree of promoter methylation is decreased and MCT4 mRNA expression is significantly increased in renal clear cell carcinoma tissues compared with adjacent non-tumor tissues.¹³² Asada et al found that the MDA-MB-231 human breast cancer cell line treated with 5-aza-2'-deoxycytidine, a demethylating agent, restored MCT1 mRNA expression, suggesting that MCT1 is similarly regulated by promoter methylation.¹³³

Post-Transcriptional Regulation via miRNAs

MCTs are regulated not only before transcription, but also after transcription. Increasing studies have shown that MCTs are post-transcriptionally regulated by miRNAs, which bind to MCT mRNAs, leading to the degradation of mRNA. Ng et al found that miR-124 bound to the 3'-untranslated region of MCT1 mRNA in medulloblastoma, resulting in decreased MCT1 expression, suggesting that miR-124 is a negative regulator of MCT1.¹³⁴ Liang et al demonstrated that miR-495 binds to the 3' untranslated region of human MCT1 and down-regulates MCT1 mRNA and protein expression in HeLa cells.¹³⁵ Additionally, miR-342-3p directly targets MCT1 in triple-negative breast cancer, and loss of miR-342-3p leads to MCT1 overexpression and metabolic reprogramming.¹³⁶

While several studies have evaluated the regulation of MCT1 by miRNA, few reports are available on miRNA-mediated regulation of MCT4. Xu et al found that miR-1 mimics and inhibitors down-regulate the mRNA and protein expression of MCT4 and this miRNA may indirectly regulate the expression of MCT4 by regulating HIF-1.¹³⁷ In hepatocellular carcinoma, miR-145 inhibited lactate efflux by targeting MCT4, thereby reducing the intracellular pH of tumor cells; the therapeutic effect of miR-148 was demonstrated in a HepG2 tumor-bearing mouse model.¹³⁸ In diabetic vascular complications, miR-425-5p down-regulates the expression of MCT4 in vascular endothelial cells, which becomes a target for the treatment of diabetic complications.¹³⁹

In addition to the mechanisms described above, there are a number of other regulatory modalities. Fan et al found that autophagy promotes MCT1 expression and induces glycolysis in liver cancer cells by activating Wnt/ β -catenin signaling to promote transcription.¹⁴⁰ In addition, and Sprowl-Tanio et al found that MCT1 is also a direct Wnt target gene in colon cancer cell lines.¹⁴¹ Transcription factors upstream stimulators 1 and 2 (urf1/2) suppress MCT1 expression in the human gut by inhibiting the transcription of MCT1.¹⁴² In colon cancer cells and stromal cells, the antioxidant transcription factor nuclear factor E2-related factor-2 (Nrf2) promotes metabolic symbiosis between colorectal cancer cells and stromal cells by regulating MCT1 and MCT4.¹⁴³ In L6 cells, Hashimoto et al found that lactic acid stimulates the generation of reactive oxygen species, which activates the NF- κ B and Nrf2 pathways and in turn leads to MCT1 expression.¹⁴⁴ The authors also showed through microarray analysis that AP-1 may be involved in the regulation of MCT1. Increasing carbonic anhydrase affects MCT activity on the plasma membrane and regulates MCT. Carbonic anhydrase II (CAII) enhances the transport activity of MCT1.¹⁴⁵ The activities of MCT1 and MCT4 were enhanced by carbonic anhydrases IV (CAIV) and IX (CAIX).^{146,147} Aspatwar et al found that co-expression of catalytically inactive carbonic anhydrase-related proteins VIII, X and XI in *Xenopus* oocytes enhanced the transporter activity of MCT1.¹⁴⁸ Some studies have indicated the regulation of MCT4 by AMPK, PKC, FBI-1 and interleukin 1 β .^{149–153}

The adjustment of MCTs is summarized in [Table 1](#).

Inhibitors of MCTs

MCTs transport monocarboxylic acids, especially lactic acid, provide energy for tumor cells, promote the proliferation, invasion and migration of tumor cells and are closely related to the degree of malignancy in a variety of tumors. In solid tumor cells, such as in non-small cell lung cancer, increased MCT expression indicates poor prognosis and high expression of MCT4 predicts poor prognosis.¹⁵⁴ MCT1 is an independent prognostic marker for survival in non-small cell lung cancer.¹⁵⁵ Drug resistance of tumor cells is also related to the up-regulation of MCT expression. Therefore, inhibition of MCT may be a therapeutic strategy for a variety of tumors. For example, inhibition of MCT1 has an antimetabolic effect on oxidized tumor cells, while targeting MCT1 in endothelial cells has a strong antiangiogenic effect.⁵¹ Aerobic cancer cells mainly transport lactate produced by hypoxic cancer cells into cells through MCT1 to generate energy for cell metabolism. After MCT1 inhibition, aerobic cancer cells switch from lactate metabolism to glucose metabolism to generate energy, forcing aerobic cells to take up more glucose from nearby blood vessels. Because of the high glucose consumption of aerobic cells, hypoxic cells are deprived of glucose, which is the only energy source for these cells, leading to glucose starvation and subsequent apoptosis induction in hypoxic cells, indirectly inhibiting hypoxic cancer cell survival.⁴² After MCT4 inhibition, hypoxic cancer cells are unable to transport lactate out of the cell, leading to cytosolic acidification, which in turn leads to cell death. Therefore, MCT inhibitors may play a crucial role in the treatment of a variety of cancer cells. Compared with inhibitors against MCT1, MCT4 inhibitors are relatively rare.

AZD3965 and AR-C155858

AZD3965, an analogue of AR-C155858, is a pyrrolidine derivative that is a first-rate potent inhibitor of MCT1 with high affinity for MCT1. AZD3965 has been shown to have promising tumor inhibitory effects in a variety of preclinical xenograft tumor models.^{156–159} Through *in vitro* cell experiments, AZD3965 was demonstrated to inhibit cell proliferation.¹⁶⁰ Upon co-expression of MCT1 and MCT4, tumor cells showed relative tolerance to AZD3965 single-agent activity, suggesting that MCT4 may continue to drive lactate transport even in the presence of AZD3965.¹⁰⁴ Clinical evaluation of AZD3965 is also ongoing.¹⁶¹ AZD3965 not only targets MCT1-mediated lactate transport, but it has also been reported to be involved in enhancing pyruvate/mitochondrial metabolism.^{72,162} Moreover, mitochondrial

Table I Summary of MCTs Regulation

MCTs	Regulating Modes	Ref
MCT1/4	Nrf2	[143]
	CAIV/IX	[146,147]
	Promoter methylation	[132,133]
MCT1	P53	[94]
	Myc	[95,102–104]
	Butyrate	[108–110]
	Wnt / β -catenin	[140,141]
	GPR81/109A	[110,114]
	MACC1	[124]
	PMA	[125,126]
	Luminal leptin	[129]
	API	[144]
	Urf1/2	[142]
	NF- κ B	[109,144]
	CAII	[145]
	MiR-124	[134]
miR-495	[135]	
MCT4	miR-342-3p	[136]
	HIF-1	[88]
	AMPK	[150,151]
	PKC	[151,152]
	FBI-1	[149]
	IL-1 β	[153]
	miR-1	[137]
	miR-145	[138]
miR-148	[138]	
miR-425-5p	[139]	

complex I inhibitor, metformin and mitochondrial pyruvate carrier inhibitor UK5099 increased the sensitivity to AZD3965.¹⁶²

AR-C155858, a potent MCT1 inhibitor, does not inhibit MCT4. AR-C155858 exerts its inhibitory effect by binding to the TM7–10 of MCT1.¹⁶³ AR-C155858 selectively blocks MCT1 and MCT2 activities in activated T lymphocytes and prevents lactate efflux.⁵⁷ AR-C155858 inhibits the lactate efflux of HL60 cells, a highly glycolytic leukemia cell line expressing MCT1 but not MCT4.¹⁶⁴ Furthermore, similar to AZD3965, AR-C155858 shows limited activity in tumor cells with MCT4 expression; in tumor cells expressing both MCT1 and MCT4, MCT4 was able to promote lactate efflux when MCT1 was blocked.³⁵

Coumarin Carboxylic Acids

Coumarin is an important structural unit found in many important drug molecules with favorable pharmaceutical properties.¹⁶⁵ A variety of derivatives of coumarin have MCT inhibitory effects. Draoui et al reported that the 7-alkyl amino substituent on the 3-carboxycoumarin scaffold was required for significant inhibition of MCT.¹⁶⁶ Tateishi et al showed that 7-amino-carboxycoumarin derivatives bind MCT1 in astrocytes, and targeted imaging of MCT1 by labeled 7-amino-carboxycoumarin derivatives may help further study the pathological mechanism of central nervous system diseases.¹⁶⁷ Furthermore, 7-aminocarboxycoumarin derivatives caused significant tumor growth retardation by inhibiting lactate influx in cervical cancer SiHa xenografts and HCT-116 cell transplantation models.¹⁶⁴

Bay-8002

BAY-8002 with a 5-(phenylsulfonyl benzamide) benzoic acid scaffold is an effective and specific oral hMCT1 inhibitor. BAY-8002 inhibits the proliferation of lymphoma cells and has no inhibitory effect in MCT4-expressing oocytes. BAY-8002 and AZD3965 have the same or overlapping binding sites on MCT1.¹⁶⁸

Inhibitors of MCT4

So far, only two MCT4-specific inhibitors, VB124 and Bindarit, have been reported. VB124 selectively inhibits MCT4 and has a very low inhibitory effect on MCT1. Cluntun et al found that VB124 specifically inhibits MCT4 and inhibits lactate efflux.¹⁶⁹ VB124 inhibits MCT4 in mouse H9c2 cells to prevent and reverse cardiac hypertrophy. In an in vitro HCC model, VB124 inhibited MCT4, resulting in less lactate output, which enhanced the killing effect of immune cells on HCC cells.¹⁷⁰

Bindarit, an anti-inflammatory agent that inhibits the production of inflammatory cytokines, is a highly selective and non-competitive hMCT4 inhibitor that selectively inhibits hMCT4 more than hMCT1 in *Xenopus laevis* oocytes.^{171,172}

Nonselective Inhibitors of MCTs

α -Cyano-4-Hydroxycinnamate and Analogs

The MCT1 inhibitor α -cyano-4-hydroxycinnamate (CHC), a cyanoacetic acid, has been extensively studied for its inhibitory effect on MCT1 and MCT4. In vitro cell experiments and xenograft models showed that CHC inhibited MCT1 to reduce tumor size, sensitize hypoxic tumor regions to radiotherapy and induce cell death when lactate was the only energy source.⁴⁷ Colen et al used CHC to inhibit lactate efflux in glioblastoma tumors, affecting tumor invasion and proliferation capacity.¹⁷³ CHC also inhibits MCT in the mitochondrial membrane, which reduces the flow of pyruvate to mitochondria and inhibits cell proliferation.^{174,175} Other studies have shown that CHC affects DNA repair by inhibiting MCT to limit lactate flux.¹⁷⁶

α -Cyano-4-hydroxy-3-methoxycinnamic acid (ACCA) is a derivative of CHC that inhibits MCTs and has a high affinity for MCT1.¹¹ Hamdan et al demonstrated that ACCA selectively inhibited the growth of breast cancer cells in vitro by inhibiting MCT1.¹⁷⁷

Jonnalagadda et al recently reported that N,N-dialkyl cyanocinnamic acids not only showed high potency against MCT1 but also showed excellent MCT4 inhibition.¹⁷⁸ In vivo xenograft model studies revealed that N,N-dialkyl cyanocinnamic acids exhibit excellent efficacy and selectivity in MCT1-expressing tumors.¹⁶⁵

Isobutyrate Derivatives

Fibrate lipid-lowering agents, such as bezafibrate, fenofibrate anion (the active metabolite of fenofibrate) and clinofibrate, contain butyrate, which has an isobutyrate moiety, and exhibit inhibitory effects on both MCT1 and MCT4.^{171,179} The inhibitory potency of clinofibrate on hMCT4 was similar to that of bezafibrate and fenofibrate anions; its inhibitory potency on hMCT1 was slightly higher than that of other fibrates.¹⁷¹

A previous study showed that 1-benzyl-1-hindazole-3-carboxylate, a derivative of benzylindazole and dechlorinated form of clonamide, slightly inhibits hMCT1 and hMCT4. Bendazak, a non-steroidal anti-inflammatory drug, showed a slight inhibitory effect on hMCT1 and hMCT4.¹⁸⁰

Other Inhibitors

Some early MCT inhibitors, such as phloretin, quercetin and 4,4'-di-iso-thiocyanostilbene-2,2'-disulfonate (DIDS), showed low affinity and poor specificity.⁷ Nancolas et al found that the antitumor drug ionidamine inhibited the ability of MCTs to transport lactate in *Xenopus* oocytes, but it showed nonselective inhibitory activity against transporters.¹⁸¹ Other inhibitors include the MCT1 inhibitor indolecyanoacrylate, pteridine dione and trione and the MCT4 inhibitor acriflavine (ACF).^{42,182,183} ACF disrupted the interaction of MCT4 with CD147 to inhibit MCT4 function in glioblastoma stem cells; ACF also significantly reduced angiogenesis and tumor progression under hypoxic conditions.¹⁸⁴

Some inhibitors of MCTs are summarized in [Table 2](#).

Table 2 Summary of Inhibitors

MCTs	Inhibitors	Ki or EC50*	Ref
MCT1	AZD3965	1.6nM	[156–161]
	AR-C155858	2.3nM	[57,163,164]
	7-Aminocarboxycoumarin Derivatives	11nM	[164,167]
	Indole cyanoacrylate	–	[42]
	Pteridine Dione and Trione	–	[183]
MCT4	BAY-8002	7.9nM; 10nM	[168]
	VBI24	8.6nM	[169,170]
	Bindarit	30.2uM	[171,172]
MCTs	ACF	4.6mM	[184]
	CHC	0.99uM	[47,173–176]
	ACCA	1mM	[11,177]
	N,N-dialkyl cyanocinnamic acids	–	[165,178]
	Isobutyrate derivatives	–	[171,179]
	BIC	40uM	[180]
	Bendazak	36uM	[180]

Notes: *Ki: The concentration of the corresponding free inhibitor when 50% of the substrate is bound by the inhibitor. EC50: The concentration of the inhibitor corresponding to 50% of the maximum biological effect can be achieved after a specific exposure time.

Conclusion

MCT1 and MCT4 are highly expressed in a variety of tumor cells; they play key roles in transporting monocarboxylate in the intracellular and extracellular environment and connect the lactate shuttle between tumor cells, tumor cells and non-tumor cells. MCT1 and MCT4 affect the glucose metabolism of tumor cells and play a crucial role in tumor cell proliferation, migration and invasion. MCT1 expression is closely related to the prognosis and drug resistance of tumors. Therefore, targeting MCTs has become a possible strategy for the treatment of cancer. In drug-resistant tumor cells, the combination of targeted MCT and chemotherapeutic drugs reversed the drug resistance of tumor cells. These findings suggest a potential new strategy for cancer treatment.

In recent years, increasing studies have been performed on drugs that inhibit MCTs, most of which are non-specific inhibitors. Considering the role of MCTs in tumor progression, research on specific MCT inhibitors is becoming important. Nano-drug delivery carriers have been recently developed and are widely used in pre-clinical trials because of their good biocompatibility and low toxicity. Nano-drug delivery vectors can carry not only genes but also drugs. A nano-drug delivery vector loaded with gene targeting MCTs may specifically and effectively inhibit MCTs, making this approach a potential method to inhibit MCT for cancer treatment.

The role of MCT1 and MCT4 in tumor cells goes far beyond these described above, and more studies are needed to explore other roles of MCTs in tumor cells. Inhibitors for MCTs also need to be continuously developed and explored in clinical trials.

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Disclosure

The authors report no conflicts of interest in this work.

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