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Monocarboxylate transporters in the brain and in cancer☆

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ABSTRACT

Monocarboxylate transporters (MCTs) constitute a family of 14 members among which MCT1–4 facilitate the passive transport of monocarboxylates such as lactate, pyruvate and ketone bodies together with protons across cell membranes. Their anchorage and activity at the plasma membrane requires interaction with chaperon protein such as basigin/CD147 and embigin/gp70. MCT1–4 are expressed in different tissues where they play important roles in physiological and pathological processes. This review focuses on the brain and on cancer. In the brain, MCTs control the delivery of lactate, produced by astrocytes, to neurons, where it is used as an oxidative fuel. Consequently, MCT dysfunctions are associated with pathologies of the central nervous system encompassing neurodegeneration and cognitive defects, epilepsy and metabolic disorders. In tumors, MCTs control the exchange of lactate and other monocarboxylates between glycolytic and oxidative cancer cells, between stromal and cancer cells and between glycolytic cells and endothelial cells. Lactate is not only a metabolic waste for glycolytic cells and a metabolic fuel for oxidative cells, but it also behaves as a signaling agent that promotes angiogenesis and as an immunosuppressive metabolite. Because MCTs gate the activities of lactate, drugs targeting these transporters have been developed that could constitute new anticancer treatments. This article is part of a Special Issue entitled: Mitochondrial Channels edited by Jean-Claude Martinou.

1. Introduction

Monocarboxylate transporters (MCTs) constitute a family of 14 transmembrane proteins encoded by the *SLC16A* family of genes. According to the transporter classification system of Milton Saier (http://www.tcdb.org), MCTs belong to the monocarboxylate porter (MCP) family (2.A.1.13), itself a member of the major facilitator superfamily (MFS). MCTs have been identified in all eukaryotic organisms of which genomes have been sequenced to date. They can transport a wide variety of substrates (Table 1). Four members of the MCT family, MCT1, MCT2, MCT3 and MCT4 are monocarboxylate transporters responsible for the proton-linked transport of several monocarboxylate metabolites, such as pyruvate, L-lactate and ketone bodies (acetoacetate and *D*-β-hydroxybutyrate) across the plasma membrane [1,2]. Other best characterized MCTs are MCT8 that has a high affinity for thyroid hormones T3 and T4, and MCT10/TAT1, a transporter of aromatic amino acids [2,3]. MCT6 has been reported to facilitate the proton-linked transport of bumetanide, but its natural substrate is unknown [4]. MCT7 has been implicated in the export of ketone bodies by hepatocytes fueled with circulating fatty acids upon fasting [5]. Accordingly, loss of MCT7 expression resulted in hepatic steatosis in Zebrafishes, which was prevented by the introduction of human MCT7. MCT9 has been clearly identified as a sodium- and pH-independent carnitine efflux transporter when it was expressed in Xenopus oocytes injected with [3H]-carnitine [6]. The substrates and functions of the five other MCT family members are currently unknown.

MCTs have numerous physiological functions as they are expressed in a wide range of tissues (such as brain, skeletal muscle, heart, bowel and liver) where they control, among others, the central metabolism of glucose, gluconeogenesis, activation of T-lymphocytes, spermatogenesis,

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AMPK, AMP-activated protein kinase; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; CA, carbonic anhydrase; CAF, cancer-associated fibroblast; CHC, α-cyano-4-hydroxycinnamate; CN, calcineurin; CTL, cytolytic T lymphocyte; DBDS, 4,4′-dibenzamidostilbene-2,2′-disulphonate; DC, dendritic cell; DIDS, 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid; EAAT1, excitatory amino acid transporter 1; glpT, glycerol phosphate transporter; IGF1, insulin-like growth factor 1; IkBα, inhibitor of NF-kB α; IκκβIκκβ, inhibitor of NF-κB kinase β; LDH, lactate dehydrogenase; MCP, monocarboxylate porter; MCT, monocarboxylate transporter; MDSC, myeloid-derived suppressor cell; MFS, major facilitator superfamily; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NFAT, nuclear factor of activated T cells; NF-kB, nuclear factor-kB; NK, natural killer (cell); NMDA, *N*-methyl-*D*-aspartate; NSCLC, non-small cell lung cancer; OXPHOS, oxidative phosphorylation; pCMBS, p-chloromercuribenzene sulphonate; PHD, prolylhydroxylase; ROS, reactive oxygen species; TM, transmembrane (domain); VEGF, vascular endothelial growth factor.

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Table 1
The MCT family of transporters.[§]

Human gene name	Protein name	Sequence accession ID	Main substrates	Tissue distribution
SLC16A1	MCT1	NM_003051	Lactate, pyruvate, ketone bodies	Ubiquitous except β cells of the endocrine pancreas
SLC16A2	MCT8	NM_006517	T2, rT3, T3, T4	Ubiquitous
SLC16A3	MCT4	NM_004207	Lactate, ketone bodies	Skeletal muscle, chondrocytes, leucocytes, testis, lung, brain, ovary, placenta, heart
SLC16A4	MCT5	NM_004696	–	Brain, muscle, liver, kidney, lung, ovary, placenta, heart
SLC16A5	MCT6	NM_004695	Bumetanide probenecid nateglinide	Kidney, muscle, brain, heart, pancreas, prostate, lung, placenta
SLC16A6	MCT7	NM_004694	Ketone bodies	Liver, brain, pancreas, muscle, prostate
SLC16A7	MCT2	NM_004731	Pyruvate, lactate, ketone bodies	High expression in testis, moderate to low in spleen, heart, kidney, pancreas, skeletal muscle, brain and leucocytes
SLC16A8	MCT3	NM_013356	Lactate	Retinal pigment epithelium, choroid plexus
SLC16A9	MCT9	NM_194298	Carnitine	Endometrium, testis, ovary, breast, brain, kidney, spleen, retina
SLC16A10	MCT10/TAT1	NM_018593	Aromatic amino acids, T3,T4	Kidney (basolateral), intestine, muscle, placenta, heart
SLC16A11	MCT11	NM_153357	–	Skin, lung, ovary, breast, lung, pancreas, retinal pigment epithelium, choroid plexus
SLC16A12	MCT12	NM_213606	–	Kidney, retina, lung testis
SLC16A13	MCT13	NM_201566	–	Breast, bone marrow stem cells
SLC16A14	MCT14	NM_152257	–	Brain, heart, muscle, ovary, prostate, breast, lung, pancreas liver, spleen, thymus

[§] Adapted from references [5,6,10].

pancreatic β cell activity, thyroid hormone metabolism and drug transport. In addition to their implication in various physiological processes, they play significant roles in pathological situations as can be exemplified by the case of tumors. This review will summarize the current knowledge pertaining to their functions in the brain, where their physiological roles are starting to emerge in greater details, and in cancer, which represents a pathology in which MCTs are clearly occupying an important position.

2. General description of monocarboxylate transporters

2.1. The MCT family: structural and functional characteristics

2.1.1. Structure

Theoretical predictions and biochemical assays with MCT1 as a model protein indicate that all the members of the MCT family share a common topology. Hydropathy plots predict the presence of 12-transmembrane (TM) helices with intracellular C- and N-termini, a large cytosolic loop between TM helices 6 and 7, and two highly conserved sequences in TM1 and TM5 [7]. A common feature shared with MFS members is a better sequence conservation in TM regions compared to loops and C-termini. To date, no crystallographic characterization of MCTs has been reported, but Halestrap et al. [7–9] have proposed 3D models based on molecular modeling, the structure of the *E. coli* glycerol phosphate transporter GlpT, site-directed mutagenesis and the binding sites for 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS), a MCT1 inhibitor. These models suggest that the structure of MCT1 at the plasma membrane may swing between two states: a closed conformation where the substrate-binding site is cytosolic and an open conformation where this site is extracellular (for a graphical representation, see Fig. 3 in reference [7]).

2.1.2. Mechanism of activity

The predicted open and closed conformations of MCTs and kinetic analyses of proton-linked transport of lactate into erythrocytes are the basis for the proposed translocation mechanism of lactic acid transport by human MCT1 through the plasma membrane. MCT1 preferentially facilitates the uptake of lactic acid and operates in an ordered process that starts when a proton binds to K38 at the extracellular surface of MCT1, providing a positive charge to the lysine [8,9,11]. Proton binding is followed by the binding of one molecule of lactate to form an ionic pair, which promotes a conformational change from closed to open state. It follows that the proton is transferred to D302 and lactate to R306 (both residues are localized at the inner surface of the channel), thus deprotonating K38, which induces the return to the closed conformation and exposure of the D302/R306 site to the cytosol. The pair H^+/lac^- is released into the cytoplasm. Another essential residue for MCT1 activity is F360, protruding into the channel of the transporter where it controls substrate selectivity by steric hindrance. According to this mechanism, the transport of lactic acid by MCT1 is passive and bidirectional: import and export depend on the intra- and extracellular concentrations of lactate and protons [2,7]. This molecular model highlights the importance of three residues, which are conserved in the four members of the MCT family that transport monocarboxylates (MCT1, MCT2, MCT3 and MCT4) and in MCT7, where there is a conservative substitution of D302 by E302.

2.1.3. Substrates

MCT1, MCT2, MCT3 and MCT4 are responsible for the bidirectional proton-linked transport of monocarboxylates across the plasma membrane, and will be the focus of this review. These MCT isoforms show preference for short chain monocarboxylates, including those substituted on positions two and three, such as pyruvate, L-lactate, D-β-hydroxybutyrate and acetoacetate. Quantitatively, lactate is one of the most important metabolites for these transporters, with a stereoselectivity for L- over D-lactate [7] consistent with the fundamental role of the L stereoisomer in eukaryotic cell metabolism. With a Km of 22–28 mM, MCT4 has the lowest affinity for lactic acid [12] (Table 2). However, it has a high turnover rate [13], making it particularly well adapted for the export of lactate by glycolytic cells where it helps to control intracellular pH homeostasis [12,14]. Comparatively, MCT1 has an intermediate affinity for lactate ($Km_{lactate}$ = 3.5–10 mM) and is widely expressed in healthy and cancer tissues [2,15]. MCT2 ($Km_{lactate}$ = 0.5–0.75 mM) and MCT3 ($Km_{lactate}$ = 5–6 mM) show the highest affinity for lactate, but their expression is restricted to very specific tissues (see Section 2.1.5) [16–18]. Differences in the Km of the transporters for pyruvate are more pronounced, with $Km_{pyruvate}$ values of 1.0, 0.1 and 153 mM for MCT1, MCT2 and MCT4, respectively [7]. MCT2 has the highest affinity for ketone bodies, with $Km_{D\text{-}\beta\text{-}hydroxybutyrate}$ = 1.2 mM and $Km_{acetoacetate}$ = 0.8 mM determined in Xenopus oocytes [19]. For MCT1, these values are $Km_{D\text{-}\beta\text{-}hydroxybutyrate}$ = 12.5 mM and $Km_{acetoacetate}$ = 5.5 mM [20]. The transport of ketone bodies by MCT3 has not been evaluated, and is not likely to occur through MCT4 due to the very low affinity of the transport for these substrates [7]. Beyond natural substrates, recent evidence also shows that MCT1 is controlling the uptake of 3-bromopyruvate [21] (an alkylating agent developed for cancer therapy) and of dichloroacetate [22] (a pyruvate dehydrogenase kinase inhibitor that restores the mitochondrial metabolism of pyruvate

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