Human Monocarboxylate Transporter 2 (MCT2) Is a High Affinity Pyruvate Transporter*

The transport of pyruvate and lactate across cellular membranes is an essential process in mammalian cells and is mediated by the H⁺/monocarboxylate transporters (MCTs). We have molecularly cloned and characterized a novel human monocarboxylate transporter, MCT2. The cDNA is 1,907 base pairs long and encodes a polypeptide of 478 amino acids with 12 predicted transmembrane domains. Human MCT2 is the product of a single gene that mapped to chromosome 12q13 by fluorescence in situ hybridization. The kinetic properties of human MCT2 fulfill the criteria to establish it as a H⁺/monocarboxylate transporter; however, the unique biochemical feature of human MCT2 is its high affinity for the transport of pyruvate (apparent \( K_m \) of 25 µM), implying that it is a primary pyruvate transporter in man. Comparison of human MCT1 and MCT2 with regard to tissue distribution and RNA transcript variants disclosed substantial differences. Human MCT2 mRNA expression was restricted in normal human tissues but widely expressed in cancer cell lines, suggesting that MCT2 may be pre-translationally regulated in neoplasia. We found co-expression of human MCT1 and MCT2 at the mRNA level in human cancer cell lines, including the hematopoietic lineages HL60, K562, MOLT-4, and Burkitt’s lymphoma Raji, and solid tumor cells such as SW480, A549, and G361. These findings suggest that the two monocarboxylate transporters, MCT1 and MCT2, have distinct biological roles.

The transport of pyruvate and lactate across cellular membranes is an essential process in mammalian cells (1–3). In liver and kidney, pyruvate and lactate produced by peripheral tissues are converted back to glucose through the gluconeogenic pathway (4). On the other hand, tissues with few or no mitochondria, such as erythrocytes and tumor cells, depend largely on glycolysis to generate ATP. The major end products of this metabolic pathway, pyruvate and lactate, are therefore produced in quantity and must be eliminated from the cells to enable continued glycolytic flux and prevent toxic effects in the cells (5). Similarly, under conditions of hypoxia (6, 7), the rate of glycolysis increases prominently in muscle in an effort to generate ATP to drive contraction, and it is critical for the cell to efflux the lactate to avoid intracellular acidosis. It is now widely accepted that the transport of lactate and pyruvate is mediated by a family of H⁺/monocarboxylate transporters, MCTs,1 named for their characteristic substrate specificity for short chain monocarboxylates (1). The MCTs share substrate specificity, have a characteristic stereoselectivity for the L isomer of lactate, and are inhibited by c-cyanocinnamate derivatives and by less specific anion transporter blockers such as stilbene disulfonates and phloretin. Pyruvate and lactate transport are accelerated at acid pH, implying a mechanism of H⁺/monocarboxylate cotransporter (reviewed in Ref. 1).

Several isoforms of MCTs have been molecularly characterized and their tissue distribution described. Accumulating evidence has led to the notion that each isoform of the transporter may have slightly different kinetic and pharmacological properties related to the unique metabolic requirements of the tissues where it is localized (1, 8). The first monocarboxylate transporter (designated MCT1) was cloned from Chinese hamster ovary cells by Garcia et al. (9). MCT1 is a membrane-bound protein with 12 predicted transmembrane regions (9). The motif of the 12 transmembrane regions is shared by many other transporters, including p-glycoprotein (10, 11), erythrocyte band 3 anion exchanger (12), and glucose transporters (GLUT) (13). MCT1 was not categorized as a member of the ABC (ATP Binding Cassette) super family of membrane transporter proteins (14) because of its lack of ATP-binding domains. MCT1 homologues were later cloned and sequenced from human, rat, and mouse and found to be highly conserved between species (15–18). A second isoform, named MCT2, was isolated from a Syrian hamster liver library that has ~60% amino acid sequence identity with MCT1 (19). A rat MCT2 homologue was also cloned and sequenced (20). Unlike MCT1, MCT2 appears to be less highly conserved between species (20). Northern and Western blotting have shown that MCT1 is expressed in the majority of tissues examined in rodents (15, 19). MCT2, in contrast, has substantial tissue restriction (19). Recently, a third isoform of the MCT family, designated MCT3, was isolated from chicken retinal pigment epithelium (21). Its tissue distribution remains to be determined.

Although much of our understanding of these transporters was derived from the studies of hamster MCTs, relatively little is known about the human monocarboxylate transporters at both biochemical and molecular levels. Human MCT1 cDNA has been isolated and its gene (gene symbol SLC16A1) mapped to chromosome bands 1p13.2-p12 (15). Detailed functional characterization and tissue distribution of human MCT1, however, has not yet been reported. In the present study, we report the cloning of the human monocarboxylate transporter MCT2 and provide a comparative characterization of the human

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AF049608.

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1 The abbreviations used are: MCT, monocarboxylate transporter; kb, kilobase(s); PCR, polymerase chain reaction; FISH, fluorescence in situ hybridization; DAPI, 4,6-diamidino-2-phenylindole.
MCT1 and MCT2 transporters expressed in Xenopus laevis oocytes. Because of its degree of sequence identity to hamster MCT2, human MCT2 may be presumed to have similar biological properties as its hamster homologue. Human MCT2, however, is novel because it has a high affinity for the transport of pyruvate (apparent \( K_m \) of 25 \( \mu \)M), suggesting that it is a primary pyruvate transporter in man. Also, multiple mRNA transcripts of human MCT2 are detected (whereas there is only one mRNA species for hamster MCT2), implying a mechanism of alternative splicing or multiple promoters to generate the human MCT2 RNA variants. We also found differential expression of the human MCT2 mRNA between normal tissues and cancer cell lines, whereas no difference was seen among the transcripts of human MCT1, implying that human MCT2 may be pretranslationally regulated in neoplasia. Finally, we found co-expression of human MCT1 and MCT2 at the mRNA level in neoplastic hematopoietic lineage lines including HL60, K562, MOLT-4, and Burkitt’s lymphoma Raji and in solid tumor cell lines such as SW480, A549, and G361. The presence of multiple MCTs is likely physiologically important in cancer cell lines.

**EXPERIMENTAL PROCEDURES**

**Materials**—Sodium [2-\( ^{14} \)C]pyruvic acid (6.25 mCi/mM) and sodium \( \alpha \)-[\( \text{\textit{L}} \)-2\( ^{3} \)H]lactic acid (20 Ci/mM) were obtained from American Radiolabeled Chemicals (St. Louis, MO). All other chemicals were purchased from Sigma.

**Cloning of Human MCT2**—Complementary DNA encoding hamster MCT2 (GenBankTM/EBI Data Bank accession number L31957) was digested with NdeI, and the resulting two fragments (corresponding to nucleotides 220–925 and 925–1908) were used as templates to generate uniformly random primed, \( ^{32} \)P-labeled probes. These probes were then used to screen a human liver 5'-stretch (oligod(T)- and random primed) cDNA library in bacteriophage \( \lambda gt10 \) (CLONTECH, Palo Alto, CA) (22). Over one million plaques were transferred to replicate filters and probed at 60 °C with 2 \( \times \) 10\(^{-5} \) cm/\( \mu l \) of the probes using ExpressHyb hybridization solution (CLONTECH). The filters were washed under low stringency conditions, once in 2 \( \times \) SSC and 0.05% SDS at room temperature for 15 min and twice in 1 \( \times \) SSC and 0.1% SDS at room temperature for 30 min. Positive recombinant phage plaques were plaque purified, and cDNAs were subcloned in the plasmid pGEMZ (Promega) and pCAV3 (Intragen) for further studies.

**DNA Sequence Analysis**—cDNAs were sequenced using an Applied Biosystem 373A automated DNA sequencer and ABI 377 from Applied Biosystems. PCR extension was performed on a Perkin-Elmer 9600 using AmpliTaq DNA polymerase. The final sequence was confirmed from both strands using vector-specific or internal primers. Sequence comparison and base searches were performed using the Navigator (DNASTAR Inc., Madison, WI) and the BLAST program (23) provided by the NCBI server at the National Library of Medicine/National Institutes of Health.

**Reverse Transcriptase-Polymerase Chain Reaction**—500 ng of poly(A) RNA from human liver (CLONTECH) was reverse transcribed using a Life Technologies, Inc. SuperScript\textsuperscript{TM} premplification system for first strand cDNA synthesis. 1/10 of the product was subjected to PCR using vent polymerase. The 5'-oligonucleotide (5'-CTGAGGATCCCACTAGAGGAGGAAAGT-3') introduced a 5'-\( \text{ BamHI } \) restriction site and 20 downstream nucleotides that match nucleotides 145–164 in the human MCT2 cDNA sequence. The 3'-oligonucleotide (5'-ATGGCTCGAGCATCAGGAGGCGGAT-3') introduced a 3'-\( \text{XhoI} \) restriction site. The last 20 nucleotides of the 3'-oligonucleotide complement nucleotides 1598–1617 of the human MCT2 cDNA. The PCR conditions used were 35 cycles of 94 °C for 30 s, 57 °C for 1 min, followed by 74 °C for 1 min. Amplified cDNAs were digested with \( \text{BamHI} \) and \( \text{XhoI} \) and cloned into the vector pCDNA3, which was cleaved with the same restriction endonucleases. The identity of the amplified MCT2 cDNA fragment was confirmed by sequencing.

**cDNA Synthesis**—The plasmid cDNA containing human MCT1 was obtained from the American Type Culture Collection (Manassas, VA) and linearized with \( \text{BamHI} \), followed by \textit{in vitro} transcription using T7 RNA polymerase. The plasmid cDNA containing human MCT2 was linearized with \( \text{XhoI} \) digestion and transcribed \textit{in vitro} using T7 RNA polymerase. All the \textit{in vitro} transcription reactions were done in the presence of RNaase inhibitors and the cap analog m\( ^{7} \)Gppp\( ^{5} \)G (Ambion, Austin, TX). The resulting cRNA was phenol-chloroform extracted and isopropyl alcohol precipitated. RNA concentration was determined by UV spectrophotometry, and RNA integrity was verified by denaturation and formaldehyde-agarose gel electrophoresis.

**Functional Expression in X. laevis Oocytes**—X. laevis oocytes (stages V-VI) were microinjected with 50 ng of cDNA in \( \text{H}_{2} \text{O} \) or MCT1 or MCT2 or water and maintained in Barth's solution at 18 °C for 3 days (24). For transport studies, groups of three oocytes were incubated in the OR2 reaction mixture (15 mM Hepes, 82.5 mM NaCl, 2.5 mM KCl, 1 mM \( \text{Na}_{2} \)HPO\(_{4} \), and 1 mM MgCl\(_2 \)) containing \( ^{14} \)Cpyruvate at room temperature. The initial velocities of pyruvate transport were determined at 5 min for various pyruvate concentrations (0.5–15 mM for MCT1 and 1–100 \( \mu \)M for MCT2). For substrate specificity and inhibitor studies, inhibitors of compounds (10 \( \mu \)M for MCT1 and MCT2 studies, respectively) were included in the reaction mixture simultaneously. Nonspecific transport was determined in parallel experiments with water-injected oocytes. After incubation, transport was stopped by washing oocytes three times with 4 ml of ice-cold phosphate-buffered saline. Each oocyte was then lysed individually with 0.5% SDS. The incorporated radioactivity was determined by liquid scintillation counting.

**Northern Analysis**—Multiple tissue RNA blots containing size-fractionated human poly(A)+ RNAs (2 \( \mu g/lane \)) were obtained from CLONTECH. These blots were hybridized with \( ^{32} \)P-labeled cDNA inserts corresponding to nucleotides 611–1490 of human MCT1 and nucleotides 1–1229 of human MCT2, respectively. Hybridization was carried out at high stringency hybridization conditions (65 °C for 1 h with ExpressHyb hybridization solution. The blots were washed three times at 50 °C in 0.1 \( \times \) SSC and 0.1% SDS. The blots were then exposed to XAR-5 film at −70 °C with two intensifying screens. Signals were visualized by autoradiography.

**Chromosomal Localization**—The 1.9-kb full-length human MCT2 cDNA was used for chromosomal localization of the gene encoding MCT2. Localization was done by somatic cell hybrid analysis using a \( ^{32} \)P-labeled cDNA probe and by fluorescence \textit{in situ} hybridization (FISH) using biotin-labeled cDNA. Mapping panel 2 (Coriell Cell Repository) consisting of monochromosomal hybrid cell lines obtained from the NIGMS, National Institutes of Health, was used for somatic cell hybrid analysis following conventional methods. FISH was employed for subregional localization following biotin-labeling of the MCT2 cDNA. Metaphase chromosome preparation, \textit{in situ} hybridization, and detection of the labeled probe were performed as described previously (25). Separate images of hybridization signal and DAPI-stained chromosomes were captured using a cooled charge-coupled device (CCD) camera (Photometrics, Tucson, AZ) and analyzed using the Smartcapture image analysis system (Vysis, Downers Grove, IL).

**RESULTS**

**Cloning and Sequence Analyses of cDNAs Encoding Human MCT2**—To isolate the cDNA for human MCT2, a human liver cDNA library in bacteriophage \( \lambda gt10 \) was screened with a \( ^{32} \)P-labeled cDNA probe corresponding to the entire coding region of hamster MCT2 under low stringency hybridization conditions. Over one million plaques were screened, and three weakly positive clones were purified and sequenced and found to contain overlapping cDNA sequences that were 75% identical to hamster MCT2. The longest cDNA was 1.9-kb in size and was chosen for functional expression. This clone was subsequently named human monocarboxylate transporter 2 (MCT2). The nucleotide and the derived amino acid sequences of human MCT2 are presented in Fig. 1A.

The isolated cDNA of human MCT2 is 1907 base pairs long, with a predicted open reading frame of 1,437 nucleotides, a 5'-untranslated region of 161 nucleotides, and a 3'-untranslated region of 309 nucleotides. The putative initiation codon ATG (nucleotides 162–164) lies within a consensus translation start site (26). Although the exact size of the 5'-untranslated region is unknown, the occurrence of an in-frame stop codon at nucleotides 138–140 precludes the possible utilization of a distal, upstream initiator ATG. Neither a poly(A) tail nor a polyadenylation signal was evident in the sequence, and therefore the length and complete sequence of the 3'-untranslated region remain to be determined.

The open reading frame in Fig. 1A reveals that human MCT2...
comprises 478 amino acids and has a calculated $M_r$ of 55,247. The protein is rich in hydrophobic amino acid residues (Ala, Ile, Leu, Phe, Trp, and Val), which account for 41% of the residues in the predicted sequence and may explain the migration pattern of the in vitro translated product of human MCT2 (apparent $M_r$ of 40,000) upon electrophoresis in SDS-polyacrylamide gel.2 As shown in Fig. 1B, Kyte-Doolittle hydropathy analysis of the primary amino acid sequence of human MCT2 revealed the presence of 12 putative transmembrane domains with a lengthy hydrophilic segment between transmembrane domains 6 and 7. This pattern of hydropathy was nearly superimposable with MCT1 (15–20). The amino acid sequence of human MCT2 exhibited 77% sequence identity to the hamster and 72% identity to the rat homologue (Fig. 2A) (19, 20). Human MCT2 also showed 49% sequence identity to human MCT1 (Fig. 2B). Sequence identity between human MCT1 and MCT2 exists throughout the coding region. Most sequence conservation lies within the 12 predicted transmembrane regions. Most sequence divergence is found at the amino- and carboxyl-termini and in the hydrophilic loop between the predicted transmembrane domains 6 and 7.

Comparison of Pyruvate Transport Activity, Substrate, and Inhibitor Selectivity of Human MCT1 and MCT2 Expressing X. laevis Oocytes—To access the functional properties of human MCT2 and compare them to MCT1, $[^{14}C]$pyruvate uptake was measured in X. laevis oocytes that express limited endogenous transport activity. Oocytes injected with cRNA derived from either human MCT1 or MCT2 exhibited a 5–10-fold higher $[^{14}C]$pyruvate uptake than water-injected oocytes (data not shown). The transport of pyruvate was time-dependent and approximately linear in the first 20 min for both MCT1 and MCT2 (Fig. 3A). A 5-min incubation time was used in subsequent experiments to estimate the initial velocities. Uptake of $[^{14}C]$pyruvate by either MCT1 or MCT2 was saturable and conformed to simple Michaelis-Menten kinetics. Surprisingly, pyruvate transport in MCT1- or MCT2-expressing oocytes revealed strikingly different $K_m$ values. An apparent $K_m$ value of 2.5 mM for pyruvate was observed in human MCT1-expressing oocytes (Fig. 3B, left panel), a value that is in agreement with previously published data regarding hamster MCT1 (19). The calculated $K_m$ for human MCT2-expressing oocytes was 25 mM as determined by double reciprocal plot (Fig. 3B, right panel). In contrast, the hamster MCT2 has a reported $K_m$ for pyruvate transport of 0.8 mM (19). Apparent $K_m$ values of 6.0 and 6.5 mM were determined for transport of L-lactate in oocytes expressing human MCT1 and MCT2, respectively (data not shown), indicating that neither of the human transporters handles lactate at high affinity.

Uptake of pyruvate by MCT1 or MCT2 was stimulated by decreasing the extracellular pH from 8.5 to 6.0 (Fig. 3C). The rate of pyruvate uptake in MCT1 cRNA-injected oocytes increased by more than 1.5-fold when the extracellular pH was lowered from 8.5 to 6.0, whereas there was only a minor increase in the rate of pyruvate transport in water-injected oo-

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2 R. Y. Lin and D. W. Golde, unpublished observations.
The rate of pyruvate uptake in MCT2 cRNA injected oocytes increased by 35 and 65%, respectively, when the pH was lowered from 8.5 to 7.5 and to 6.0. Together, these data suggest that both MCTs function as H\[^+\]/monocarboxylate cotransporters.

To investigate substrate specificity, we examined inhibition of MCT1- or MCT2-mediated \[^{14}\text{C}\]pyruvate uptake by various unlabeled monocarboxylates (Fig. 3D). At an extracellular concentration of 1 mM pyruvate, MCT1-mediated pyruvate transport was inhibited by 10-fold excess L-lactate (100% inhibition) and to a lesser extent by \[^{\beta}\]-hydroxybutyrate (85%), DL-\[^{a}\]-hydroxybutyrate (78%) and DL-\[^{b}\]-hydroxybutyrate (65%). Similarly, at an extracellular concentration of 15 mM pyruvate, MCT2-mediated pyruvate transport was strongly inhibited by a 60-fold excess of L-lactate (100% inhibition), DL-\[^{\beta}\]-hydroxybutyrate (78%) and DL-\[^{a}\]-hydroxybutyrate (65%). These results provided further evidence that both MCTs exhibit broad substrate specificity for unbranched aliphatic monocarboxylates. Human MCT1 and MCT2 also showed stereoselectivity for L- over D-lactate (Fig. 3D). This stereospecific transport of the L-isomer of lactate is in good agreement with that of other MCTs as reported previously (1, 27, 28). Deoxyglucose was used here as a negative control because it is not transported by MCTs (Fig. 3D). Classical inhibitors of monocarboxylate transport such as \[^{a}\]-cyanocinnamates decrease pyruvate uptake mediated by MCT1 and MCT2. Of the two \[^{a}\]-cyanocinnamate compounds that we examined, \[^{a}\]-cyano-3-hydroxycinnamate demonstrated more potent inhibitory effects than did \[^{a}\]-cyano-4-hydroxycinnamate in MCT1-expressing oocytes, whereas \[^{a}\]-cyano-4-hydroxycinnamate was found to be able to block more transport activity mediated by MCT2.

**Fig. 2.** Alignment of human MCT2 with hamster and rat homologs (A) and human MCT1 (B). Identical amino acid residues are boxed, and the amino acids are numbered on the left.

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tissue (Fig. 4A). Other tissues found to express MCT1 included spinal cord, skeletal muscle, testis, ovary, placenta, small intestine, colon, prostate, thymus, spleen, liver, brain, stomach, thyroid, lymph node, trachea, adrenal gland and bone marrow.

No hybridization signals were obtained with mRNA isolated from kidney, pancreas, lung, and leukocytes (Fig. 4A). In contrast to the widely distributed MCT1 transcript, MCT2 mRNA showed substantial tissue restriction and appears to be more complicated. Three mRNAs of MCT2 from testis (ranging from 1.2- to 2.8-kilobases) yielded intense hybridization signals (Fig. 4A). Moderate to low levels of longer transcripts (3.6 and 11 kb) were observed in RNA samples derived from spleen, heart, kidney, pancreas, skeletal muscle, brain, and leukocytes (Fig. 4A). The lack of detection of MCT2 transcripts in liver, the tissue from which we cloned MCT2, is probably partially because of low abundance of MCT2 message and also related to limited quantities of RNA on the blot with respect to the hybridization signals for β-actin (Fig. 4A). Liver has a relatively low abundance of MCT2 message compared with pancreas which also has low β-actin hybridization signals, but a strong human MCT2 hybridization signal. Using sequence-specific primers, we amplified the full-length MCT2 by reverse transcriptase-PCR from liver (Fig. 4B). Of the tissues containing the two MCT2 transcripts (3.6- and 11.0-kb, respectively), the 11-kb mRNA is more abundant than the smaller 3.6-kb transcript.

Southern blot analysis of human genomic DNA hybridized with 32P-labeled probe corresponding to nucleotides 634–1096 in the MCT2 cDNA sequence yielded a simple pattern (data not shown), suggesting that human MCT2 is a single copy gene in the human genome. The Northern analysis therefore suggests that human MCT2 RNA transcripts undergo alternative splicing, or possibly multiple promoters drive transcription of the human MCT2 gene.

Expression of MCT1 and MCT2 mRNAs in Human Cancer Cell Lines—Northern blot analysis of human cancer cell lines revealed that MCT1 mRNA was abundantly expressed in hematopoietic lineage lines HL60, K562, MOLT-4, and Burkitt’s lymphoma Raji, representing myeloid, erythroid, and T- and B-lymphoid differentiation, respectively, and in epithelial tumor cell lines SW480, A549, and G361. MCT2 mRNA was also expressed in all the cell lines. In contrast to the human tissues where the longer, 11-kb transcript is the predominant species in tissues expressing it (except testis, Fig. 4A), the smaller, 3.6-kb transcript appeared to be more abundant in the human cancer cells. Longer exposure of the same blot revealed the presence of a weak but clear 11-kb mRNA transcript in all the cell lines. The abundance of the two MCT2 transcripts is therefore differentially displayed between normal human tissues and cancer cell lines. It is worth noting that HL60 leukemia cells have an extra MCT2 transcript of 4.4 kb. Its function remains to be determined.

Chromosomal Localization of the Human MCT2 Gene to 12q13—Southern blotting of the cDNA probe to the monochromosomal somatic cell hybrid panel revealed a strong band in the lane containing human chromosome 12 without cross-hybridization in any other lane (data not shown). The result unambiguously assigned the human MCT2 gene to chromosome 12. For subregional assignment, normal human metaphase spreads were evaluated, following FISH of the biotin-labeled probe. In ten metaphases, twin hybridization spots were detected on both chromosome 12 homologues at band 12q13, thereby assigning the gene to this band (Fig. 5)

**DISCUSSION**

Accumulating evidence has led to the notion that mammals express multiple isoforms of MCT. We isolated the human MCT2 cDNA by low stringency screening of a liver cDNA library. Western blot and immunohistochemical studies revealed the presence of MCT2 in hamster liver (19), and Jackson et al. (20) reported the expression of an abundant 2.4-kb message of MCT2 in hamster and mouse liver by Northern blot analysis. We found that human liver, however, contains low abundance MCT2 message that can only be detected by reverse transcriptase-PCR. Contrasting results have also been reported concerning expression of MCT2 in the brain. Neither MCT1 nor MCT2 were detected in hamster brain by Western blot analysis (19). In contrast, Jackson et al. (20) reported the expression of MCT2 mRNA in hamster brain but not in rat or mouse brain by Northern blot analysis (20). Recently, Pellerin et al. (29) reported the presence of three transcripts for MCT2...
in mouse brain. We found expression of MCT2 at the mRNA level in human heart, brain, kidney, spleen, pancreas, skeletal muscle, testis, and leukocytes. The tissue distribution of MCT2 therefore appears to be species-dependent. We also found three transcripts for MCT2 in human testis and two larger transcripts for MCT2 in other tissues. Human testis abundantly expresses both MCT1 and MCT2. The physiological significance of the unique MCT2 transcripts in testis is unknown. Previous immunohistochemical studies showed a cell-type-specific expression of MCT1 and MCT2 in the hamster male reproductive system. Hamster MCT1 was present on sperm heads in the testis and proximal epididymis and on the microvillar surface of the epithelium in the distal epididymis. In contrast, MCT2 was present on the tails of sperm throughout the testis and epididymis (19). Although it is not clear why sperm express one isoform of MCT in the head and another in the tail, it is postulated that MCT2 is coupled to LDH-X, the isoform of lactate dehydrogenase that is expressed exclusively
in sperm tail (30). Because the relative abundance of MCT2 transcripts varies from one tissue to another, MCT2 is presumably posttranscriptionally regulated, and the roles of each of the transcripts may be tissue-specific. Although the genomic clone for MCT2 is not yet available, our Southern blot analysis yielded a simple hybridization pattern, suggesting that MCT2 is a single copy gene in the human genome. The multiple transcripts of MCT2 may arise from alternative splicing or are generated by different promoters and/or have different polyadenylation sites.

We studied the uptake of pyruvate in oocytes expressing human MCT1 and MCT2 because it is difficult to interpret data on MCTs expressed in mammalian cell lines because of the presence of high endogenous monocarboxylate transport activity. X. laevis oocytes, proved to be a reliable system for investigating the transport properties of MCTs using radiolabeled substrates (31). A preliminary analysis of human MCT1 and MCT2 substrate selectivity demonstrated that the two MCTs had similar but distinguishable substrate preferences. The major difference observed between human MCT2 and MCT1 was an almost 2-orders of magnitude difference in the Km value for the transport of pyruvate. The high affinity for pyruvate transport of the human MCT2 points to this isoform as the primary transporter of pyruvate in man. Hamster MCT2 only has a slightly higher, less than 4-fold, affinity for pyruvate as compared with hamster MCT1. The differential kinetic properties of MCT1 and MCT2 among the two species may relate to the fact that the amino acid sequence identity is only 49% between human MCT1 and MCT2 as compared with the 60% identity between hamster MCTs. Recombinant chimeric transporter studies will be helpful to identify regions within the MCT1 and MCT2 transporters that dictate their unique patterns of substrate specificity. DL-β-Hydroxybutyrate and DL-α-hydroxybutyrate were found to be inhibitors of pyruvate transport in MCT1- or MCT2-expressing oocytes. Furthermore, pyruvate transport was strongly inhibited by α-cyanoacetamides. Our studies show that α-cyano-3-0H-cinnamate exerted a more potent inhibitory effect than did α-cyano-4-0H-cinnamate in MCT1-expressing oocytes, whereas α-cyano-4-0H-cinnamate was found to block more transport activity mediated by MCT2. When decreasing the extracellular pH from 8 to 6.0, the uptake of pyruvate by MCT1 or MCT2 was stimulated, suggesting both MCTs function as H⁺/monocarboxylate cotransporters. Our data indicate that the two human MCTs are likely to have distinct biological roles.

The physiological significance of the presence of both MCTs in the same tissue is unknown. Previously, Bröer et al. (31) proposed a mechanism of lactate shuttle between neurons and astroglial cells based on the molecular evidence of the existence of MCT1 mRNA in astroglia-rich culture and the expression of MCT2 mRNA in neuron-rich primary culture (31). Direct evidence of a transfer of lactate between Müller glial cells and photoreceptors in mammalian retina has also been provided (32). Using in situ hybridization, Pellerin et al. (29) provided an extensive characterization of the regional distribution of both MCT1 and MCT2 mRNA during development in the mouse brain. Their results showed that both transporter mRNAs are highly expressed in the cortex, the hippocampus, and the cerebellum. A peak in the expression of both transporters was observed at about postnatal day 15 and declined rapidly by 30 days, suggesting a mechanism of developmental regulation.

We found wide distribution of both MCT1 and MCT2 in human tumor cell lines. Both transporters were expressed in the neoplastic hematopoietic lines, and the smaller 3.6-kb MCT2 transcript was favored. Our findings suggest that human MCT2 functions primarily as a pyruvate carrier. Further, human MCT2 may play a special role in the metabolism of cancer cells, perhaps facilitating anaerobic glycolysis.

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