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MINI-REVIEW

Role of monocarboxylate transporters in human cancers: state of the art

Céline Pinheiro · Adhemar Longatto-Filho · João Azevedo-Silva · Margarida Casal · Fernando C. Schmitt · Fátima Baltazar

Abstract

Keywords

Monocarboxylate transporter family

Monocarboxylic acids play a major role in cellular metabolism, with lactate having a key function (Halestrap & Price 1999). Transport of monocarboxylates through the plasma membrane was originally thought to be via non-ionic diffusion of the free acid, however, subsequent demonstration that lactate and pyruvate transport into human erythrocytes could be strongly inhibited after treatment with some chemicals (Halestrap & Denton 1974), a specific monocarboxylate transport mechanism was recognized.

The monocarboxylate transporter (MCT) family is presently composed by 14 members, and is encoded by the *SLC16* gene family (Halestrap & Meredith 2004), which is conserved among species, including rat, mouse and chicken.

Functional and phylogenetic relationship of MCTs

According to the Transport Classification Database (www.tcdb.org), MCTs are members of the Major Facilitator Superfamily (Saier et al. 2009), belonging to the TC# 2.A.1.13, the Monocarboxylate Porter (MCP) family. By sharing a high level of conservative amino acid sequences, the topological prediction of MCTs shows 12 transmembrane helices (TMs), an intracellular N- and C-terminus and a large cytosolic loop between TMs 6 and 7, with the most conserved regions belonging to the TMs domains, and the most variable ones matching the loops and the C-terminus (Poole et al. 1996).

The 14 human MCT homologue members are assigned as the solute carrier (SLC16A) gene series by the Human Genome Organization (HUGO) Nomenclature Committee Database (www.genenames.org). As shown in Fig. 1, the phylogenetic analysis provides valuable information regarding the functional clustering of the human MCT family. The differences in amino acid sequence reflect an evolutionary divergence associated with their functional role, since MCT1-4, known to mediate the proton-linked transport of metabolic monocarboxylic acids, appear associated in the same cluster. This cluster is further sub-divided into two

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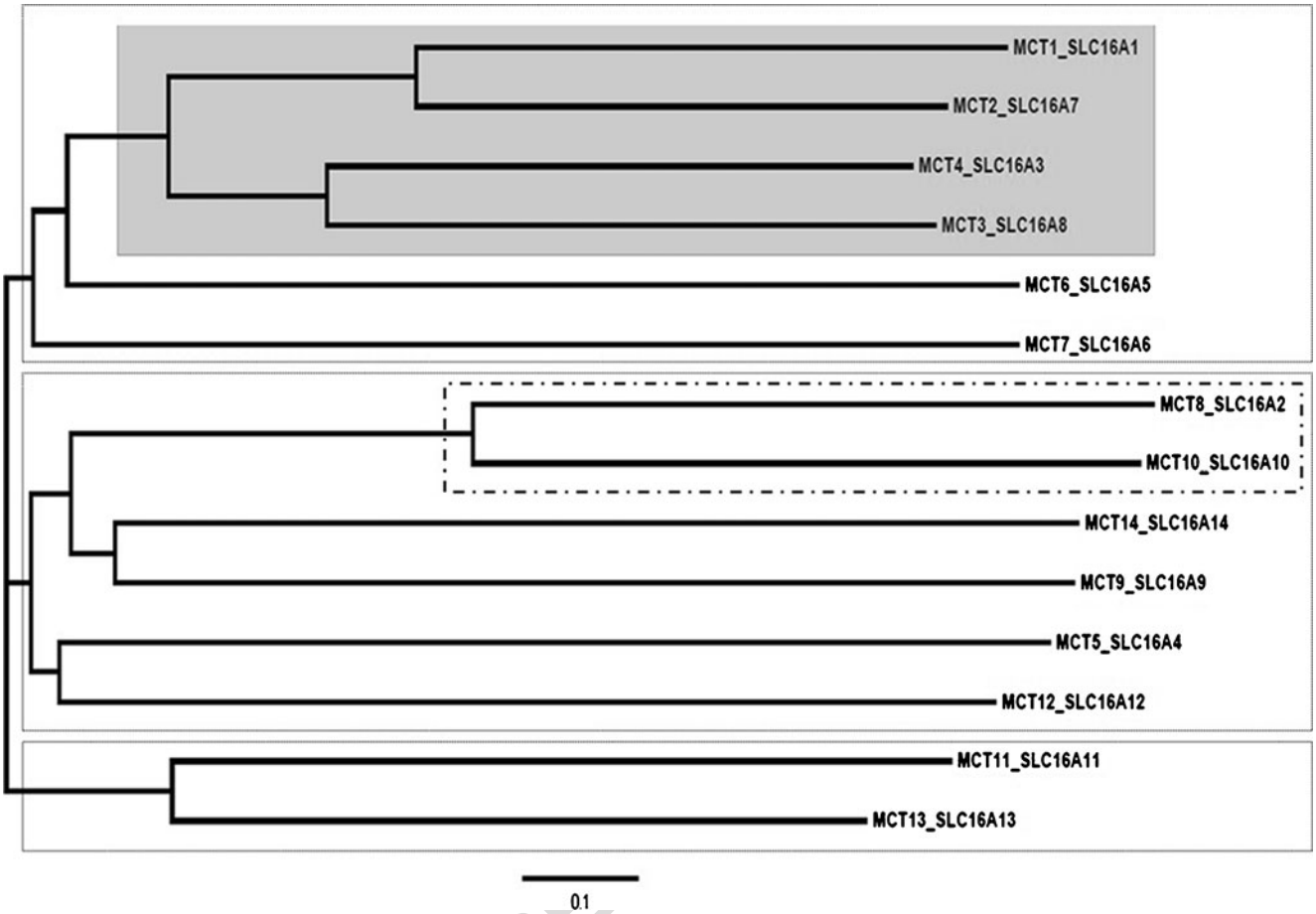

Fig. 1 Human MCT family members' phylogram, based on amino acid sequence. Boxes limited by dots represent three main clusters. In the doted-dashed box are the thyroid hormone (MCT8) and aromatic amino acids (MCT10) transporters. Solid grey box represent the proton-linked transported cluster (MCT1-4). The amino acid sequences were analyzed using CLUSTALW and tree plotting was performed using FigTree v1.3.1 (http://tree.bio.ed.ac.uk/). MCT1-14 UniProt accession numbers: P53985; O60669; O95907; O15427; O15374; O15375; O15403; P36021; Q7RTY1; Q8TF71; Q8NCK7; Q6ZSM3; Q7RTY0; Q7RTX9 Q3

shorter branches, the MCT1-2 and MCT3-4, which correlate with their range of substrate specificity and affinities found for the mammalian (human, mouse and rat) transporter isoforms (Table 1).

MCT1 has a broader distribution and transports a wider range of substrates when compared to other family members. Its kinetic parameters have been studied for the mouse isoform in tumor cells (Carpenter et al. 1996) and for the rat

Table 1 Km values (mM) of mammalian MCT isoforms for a range of monocarboxylates. (h) – human; (m) – mouse; (r) – rat; (*)- tumor cells

	MCT1 (Carpenter et al. 1996; Broer et al. 1998; Cuff et al. 2002; Kido et al. 2000; Poole et al. 1990)	MCT2 (Broer et al. 1999)	MCT4 (Dimmer et al. 2000; Manning Fox et al. 2000)
L-Lactate	$2.2^{(r)}$–$4.5^{(m*)}$	$0.7^{(r)}$	$28.0^{(h)}$–$34.0^{(r)}$
D-Lactate	$51.0^{(r)}$	–	$519.0^{(h)}$
Pyruvate	$0.6^{(r)}$–$1.0^{(r)}$	$0.08^{(r)}$	$153.0^{(h)}$
L-β-hydroxybutyrate	$8.1^{(r)}$–$11.4^{(m*)}$	n.d.	$824.0^{(h)}$
D-β-hydroxybutyrate	$8.1^{(r)}$–$10.1^{(m*)}$	$1.2^{(r)}$	$130.0^{(h)}$
Butyrate	$9.1^{(h*)}$	n.d.	n.d.
Acetoacetate	$5.5^{(r)}$	$0.8^{(r)}$	n.d.
Benzoate	$1.1^{(h)}$	n.d.	n.d.
Propionate	$1.5^{(r)}$	n.d.	n.d.
Acetate	$3.7^{(m*)}$	n.d.	n.d.

(*n.d.* not determined)

isoform expressed in *Xenopus laevis* oocytes (Broer et al. 1998). The main function of this transporter has been associated with the uptake or efflux of monocarboxylates through the plasma membrane, according to cell metabolic needs and behaving as a high affinity transporter for L-lactate, but not for D-lactate, as well as for pyruvate, acetate, propionate, D,L-β-hydroxybutyrate and acetoacetate (Halestrap & Meredith 2004). It has also been implicated in the transport of butyrate and propionate in human colonocytes (Cuff et al. 2002). Furthermore, its role in the uptake of benzoate in the human blood–brain barrier, as well as in vitro, using both immortalized and primary cultured brain capillary endothelial cells, has also been demonstrated (Kido et al. 2000).

The MCT2 rat ortholog was characterized by heterologous expression in *Xenopus laevis* oocytes (Broer et al. 1999), displaying a higher affinity for L-lactate, pyruvate, D-β-hydroxybutyrate and acetoacetate than MCT1. When expressed in the same tissue, MCT1 and MCT2 are located in distinct cells as they have been suggested to play different roles in metabolic shuttles (Garcia et al. 1995; Jackson et al. 1997).

MCT3 was first identified in chicken and displays a tissue-specific expression pattern, being only expressed in retinal pigment epithelium and choroid plexus epithelia, mediating the efflux of metabolic lactate in the retina (Philp et al. 1998; Bergersen et al. 1999). The heterologous expression of chick-MCT3 in yeast revealed a Km of 6 mM for L-lactate (Grollman et al. 2000).

The physiological role of the human MCT4 is mostly associated with the export of lactate in cells with high glycolytic rates related to hypoxic energy production (Dimmer et al. 2000). It was characterized by heterologous expression in *Xenopus laevis* oocytes (Manning Fox et al. 2000), exhibiting the highest Km values (Table 1) for most substrates and inhibitors when compared to MCT1 and MCT2.

Finally, MCT8 (rat isoform) and MCT10 (mouse isoform) mediate the transport of thyroid-hormones (Friesema et al. 2003) and aromatic amino acids (Kim et al. 2001) respectively, in a proton and sodium-independent manner. According to Fig. 1, their human orthologs share a closer phylogenetic relationship. For the remaining family members, few or no information is available about their properties and functional roles.

The role of MCTs in cell homeostasis is widely recognized and described in detail in some tissues. However, further work is needed in what concerns their role in tumor biology. Even so, if one looks at the microenvironmental scenario and molecular events occurring in carcinogenesis, it is possible to anticipate an important contribution of MCTs in the progression to malignancy.

Cancer cell metabolic adaptations

More than half a century ago, Otto Warburg demonstrated that cancer cells rapidly convert the majority of glucose into lactate, even in the presence of sufficient oxygen to support mitochondrial oxidative phosphorylation (Warburg 1956). This phenomenon is presently known as “aerobic glycolysis” or “Warburg effect”. Although Warburg’s hypothesis that impaired mitochondrial metabolism underlies the high rates of glycolysis has proven incorrect (Wang et al. 1976; Brand 1985; Moreno-Sanchez et al. 2007), the original observation of increased glycolysis in tumors has been confirmed repeatedly. In fact, this increased glucose uptake by cancer cells is the rationale behind the whole-body non-invasive ^{18}F-fluorodeoxyglucose positron emission tomography (FdG-PET) technique. This widespread clinical application is used for diagnosis, initial staging, restaging, prediction, monitoring of treatment response and surveillance in a variety of cancers (Jadvar et al. 2009).

Early carcinogenesis and development of the malignant phenotype occur in an avascular environment, and cancer cells become dependent on glucose and oxygen diffusion through blood vessels and basement membrane to fulfill their major metabolic demands (Gatenby & Gillies 2004; Gillies & Gatenby 2007). Hence, if early hyperplastic lesions develop further than a few cell layers beyond the basement membrane, regional development of hypoxia will occur, limiting cell growth. This intermittent hypoxia will promote selection for cells with anaerobic glycolysis constitutively up-regulated, allowing further cell growth (Gatenby & Gillies 2004; Gillies & Gatenby 2007; Smallbone et al. 2007). It is widely known that the major regulator of adaptation to hypoxic stress is the transcriptional factor HIF-1α, which has been widely associated with cancer progression (Semenza 1998a,b, 1999, 2000; 2001; Greijer et al. 2005). In fact, many enzymes from the glycolytic pathway like glucose transporter 1 (GLUT1) (Chen et al. 2001; Baumann et al. 2007), lactate dehydrogenase A (LDH-A) (Firth et al. 1995), among others (Greijer et al. 2005; Hu et al. 2003; Kim et al. 2006; Papandreou et al. 2006; Warnecke et al. 2004), are HIF-1α targets. Besides contributing to the constitutive glycolytic metabolism, HIF-1α also contributes to the acid-resistant phenotype, by up-regulating, at least, two important pH regulators, MCT4 (Ullah et al. 2006; de HF et al. 2009) and CAIX (Wykoff et al. 2000; Svastova et al. 2004; Chiche et al. 2009). In fact, MCT4 will not only be important for the acid-resistant phenotype, but also for the hyper-glycolytic phenotype, by exporting newly formed lactate, allowing continuous conversion of pyruvate to lactate and, therefore, continuous aerobic glycolysis.

The frequency and severity of tumor hypoxia and its association with malignant progression make the hypoxia-induced metabolic adaptations promising targets for cancer

therapy (Dang & Semenza 1999). Actually, the development of treatments that target tumor metabolism is receiving renewed attention, with several potential drugs targeting metabolic pathways currently in clinical trials (for review see (Porporato et al. 2011)). Importantly, MCT1 is included in this list of metabolic targets for cancer therapy.

The biological relevance of lactate transport in cancer

As already mentioned, the acid-resistant phenotype is an essential condition for cancer cell survival. Hence, different pH regulating systems are present in the plasma membrane of cancer cells, including MCTs, the Na^+/H^+ exchanger 1 (NHE1), carbonic anhydrase IX (CAIX) and anion exchanger 1 (AE1). Although MCTs are not the major H^+ transporters, they perform a double role in the adaptation to hypoxia: export of lactate, essential to the hyper-glycolytic phenotype, and pH regulation, important to the acid-resistant phenotype.

Besides its role as tumor acidifier, inducing mutagenesis/clastogenesis, cancer cell invasive behavior, radio- and chemoresistance (Gatenby & Gillies 2004), lactate has other properties which can contribute to the malignant behavior of cancer cells (Fig. 2). T cell activation is dependent on high rates of glycolysis and, therefore, dependent on a rapid efflux of lactate from T cells (Frauwirth & Thompson 2004). However, if the extracellular concentration of lactate is high, lactate efflux from T cells will be inhibited. This is the case of the tumor micromilieu and, as a consequence, T cell metabolism and function will be disturbed, decreasing the immune response against tumor cells (Fischer et al. 2007). Also, evidence shows that both lactate and pyruvate regulate hypoxia-inducible gene expression, independently from hypoxia, by stimulating the accumulation of HIF-1α (Lu et al. 2002). This indicates that, lactate, per se, stimulates the hyper-glycolytic phenotype, providing a positive feedback. Moreover, exogenous lactate was demonstrated to increase cellular motility (Walenta et al. 2002), vascular endothelial growth factor (VEGF), the major angiogenic factor (Spector et al. 2001; Kumar et al. 2007; Hunt et al. 2007), as well as hyaluronan and its receptor CD44, which are molecules involved in the process of cancer invasion and metastization (Stern et al. 2002; Rudrabhatla et al. 2006). Altogether, this evidence shows the various biological activities of lactate that can enhance the malignant phenotype of tumor cells, contributing to the association of high tumor lactate concentrations with incidence of metastases (Schwickert et al. 1995; Walenta et al. 1997; Walenta et al. 2000; Brizel et al. 2001), tumor recurrence, patient survival (Walenta et al. 2000; Brizel et al. 2001) and radioresistance (Quennet et al. 2006). As a result, MCTs, as the transporters responsible for lactate efflux from cancer cells, will be involved in the lactate-induced malignant behavior of cancer cells.

Besides being an end-product of different metabolic pathways, lactate may also be a substrate for oxidative phosphorylation and, as described in skeletal muscle and brain (Juel 1997; Pellerin et al. 1998), a cell-cell lactate shuttle has been proposed for cancer cells. Therefore, lactate has been recently described as the key metabolic intermediate in a metabolic symbiosis between glycolytic and oxidative cancer cells, in which the peripheral and oxygenated oxidative cells consume the lactate produced by the central and less oxygenated glycolytic cells (Fig. 2) (Sonveaux et al. 2008).

Although glucose is the major source of lactate in most solid tumors, it is important to note that other cancer pathways rather than glycolysis, like glutaminolysis and serinolysis (Mazurek et al. 2000, 2001a, b; DeBerardinis et al. 2007), can lead to lactate production. Nevertheless, lactate will always be an important metabolic end-product, either cancer cells use glycolysis or other energetic pathways for energy and biomass production.

MCT expression in human cancers

Although less explored than other proteins involved in the glycolytic phenotype or even than other pH regulators, reports on the role of MCTs in cancer are becoming more frequent with years (Table 2).

Colon

The first report on MCT expression in human tumor samples described a decrease of MCT1 expression (by Western blot) in the colonic transition from normality to malignancy (Ritzhaupt et al. 1998), which was further supported by a larger study analyzing MCT1, MCT2, and MCT4 expressions by Northern blot, Western blot and, immunohistochemistry only for MCT1, in healthy colon samples, adenomas and carcinomas. MCT1 protein decrease was confirmed, while MCT2 and MCT4 protein expression was not detected, despite mRNA expression of MCT4 (Lambert et al. 2002). However, more recent evidence showed a high expression of MCTs in colon adenocarcinoma (Pinheiro et al. 2010a), as well as significant increase of MCTs expression in cancer cells when comparing to normal colonic samples (Koukourakis et al. 2006; Pinheiro et al. 2008a). These contradictory results are probably due to antibody specificity, with special attention to the fact that the first immunohistochemical study failed to show MCT1 expression in the plasma membrane of cancer cells, which is essential for plasma membrane lactate efflux. In opposition, one of these recent studies showed a significant increase of MCT1 and MCT4 in the plasma membrane of colorectal cancer cells accompanied by a significant decrease in MCT2 at the plasma membrane. This finding is in accordance with the dependence of hyperglycolytic cancer cells in

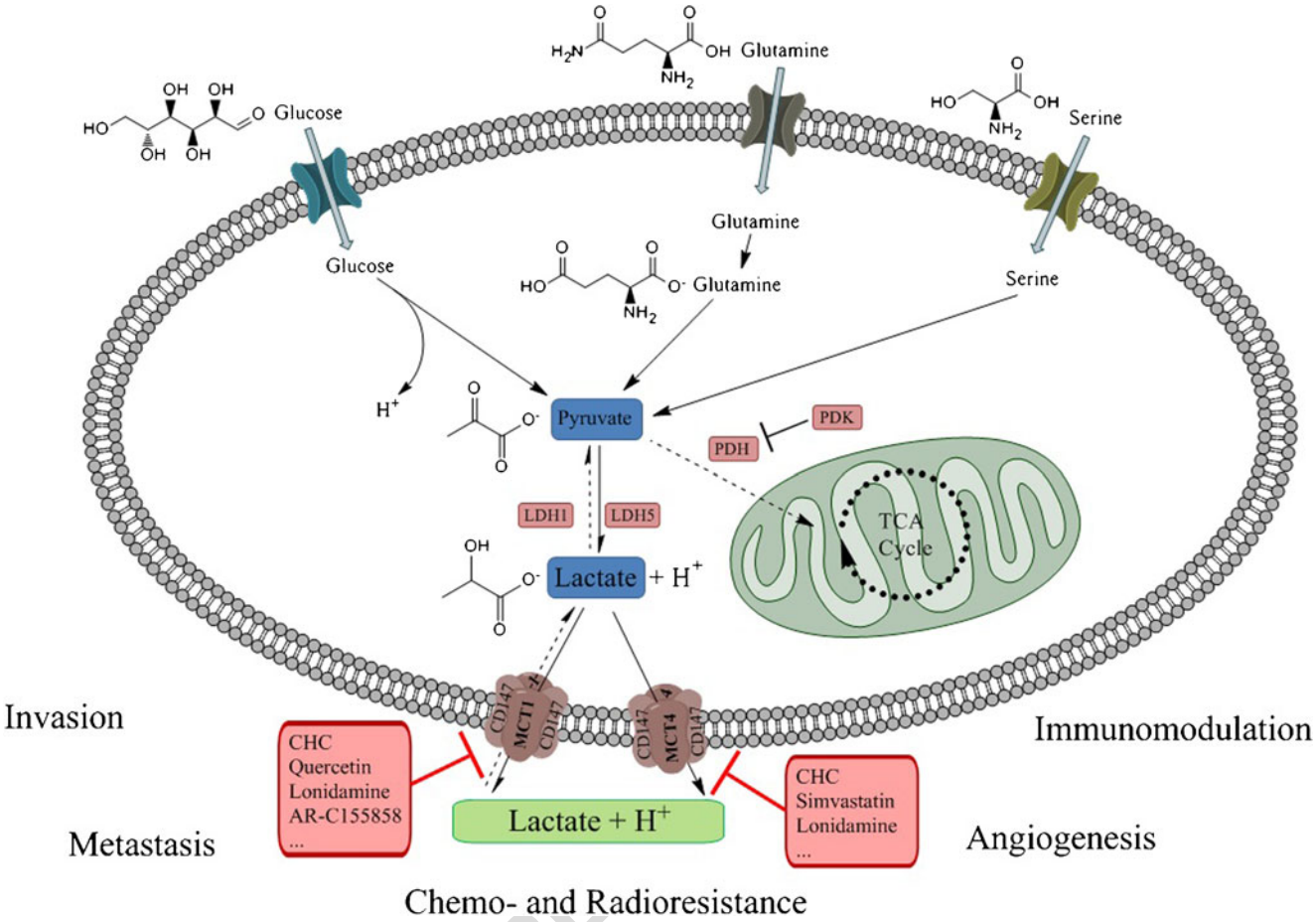

Fig. 2 Overview on the metabolic pathways leading to lactate production (continuous lines) and transport across the plasma membrane, as well as strategies of lactate transport inhibition. Discontinuous arrows represent lactate uptake and flow inside oxidative cancer cells. Abbreviations: CHC, α-cyano-4-hydroxycinnamic acid; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; PDH, pyruvate dehydrogenase; PDK1, pyruvate dehydrogenase kinase 1

exporting the accumulating lactate through MCT1 and MCT4, but not MCT2. Additionally, MCT2 and MCT4 were strongly expressed in the cytoplasm of cancer cells indicating a possible role of these isoforms in the mitochondrial uptake of pyruvate (Pinheiro et al. 2010a; Koukourakis et al. 2006; Pinheiro et al. 2008a). Importantly, analysis of MCT expression in regard to the clinic-pathological parameters showed associations of MCT1 plasma membrane expression with vascular invasion, which could be explained by the role of extracellular lactate and acidity on cancer cell invasion (Pinheiro et al. 2008a), which will need further confirmation. Koukourakis and collaborators also found MCT1 expression in tumor-associated fibroblasts, favoring absorption of the accumulating lactate from the extracellular matrix, to be used as energy source, as well as lack of endothelial MCT1, to avoid lactate absorption and vascular destruction by acidosis. Additionally, MCT2 was strongly expressed in the cytoplasm of cancer cells and tumor-associated fibroblasts, indicating a possible role of MCT2 in the mitochondrial uptake of pyruvate. Finally, MCT4 was weakly expressed in the tumor micromilieu, suggesting a minimal role in the metabolic intratumoral communication (Koukourakis et al. 2006).

Central nervous system

In neoplastic human tissues of the central nervous system, the few existing studies point to a possible important role of MCT expression, especially MCT1 (Froberg et al. 2001; Mathupala et al. 2004; Fang et al. 2006; Li et al. 2009). Strong expression of MCT1 was found in ependymomas, hemangioblastomas and high grade glial neoplasms (anaplastic astrocytomas and glioblastoma multiforme (GBM)), whereas low-grade glial neoplasms (oligodendrogliomas and astrocytomas) were either negative or showed weak MCT1 expression (Froberg et al. 2001). Additionally, Western blot analysis in total protein extracts from normal brain and primary brain tumors (GBMs) demonstrated that normal brain predominantly expressed MCT3, whereas MCT1 and MCT2 were the major isoforms present in GBM tumors. MCT4 was not detected in any of the tumor tissues

t2.1 **Table 2** Overview on MCT1, MCT2, MCT4 and CD147 expression and prognosis in different tumor types

t2.2	Tumor site	MCT1 expression	MCT2 expression	MCT4 expression	CD147 expression*
t2.3	Colon	↓ from normality to malignancy (Ritzhaupt et al. 1998; Lambert et al. 2002)	Not detected in either normal or tumor tissues (Lambert et al. 2002)	Not detected in either normal or tumor tissues (Lambert et al. 2002)	(+) in tumor cells; no significant associations with MCTs (Pinheiro et al. 2010a)
t2.4		(+) in tumor cells but (−) normal epithelium (Koukourakis et al. 2006)	+ in tumor cells cytoplasm, but not in plasma membrane (Koukourakis et al. 2006)	Cytoplasm of cancer cells (Koukourakis et al. 2006)	
t2.5		↑ in tumor cells, compared to normal epithelium/associated with vascular invasion (Pinheiro et al. 2008a)	↑ in cytoplasm expression but ↓ in tumor cells plasma membrane compared to normal epithelium (Pinheiro et al. 2008a)	↑ in tumor cells, compared to normal epithelium (Pinheiro et al. 2008a)	
t2.6		(+) in tumor cells (Pinheiro et al. 2010a)	(+) in tumor cells (Pinheiro et al. 2010a)	(+) in tumor cells (Pinheiro et al. 2010a)	
t2.7	Central nervous system	Strongest in high grade glial neoplasms, compared to low grade glial neoplasms (Froberg et al. 2001)	↑ in glioblastoma, compared to normal tissue (Mathupala et al. 2004)	(-) in glioblastoma (Mathupala et al. 2004)	
t2.8		(+) in glioblastoma and (−) in normal tissue (Mathupala et al. 2004)			
t2.9		(+) in neuroblastoma/associated with age >1 year at diagnosis, stage 4 disease, unfavorable Shimada histopathology, DNA diploid index, *n-myc* amplification and high-risk clinical group (COG criteria) (Fang et al. 2006)			
t2.10	Breast	↓ due to gene hypermethylation (Asada et al. 2003)	(+) in tumor cells and normal epithelium cytoplasm, but not in plasma membrane (Pinheiro et al. 2010a)	Tendency to be ↑ in tumor cells, compared to normal epithelium (Pinheiro et al. 2010b)	(+) in tumor cells (Pinheiro et al. 2010a; Pinheiro et al. 2010b) and normal epithelium (Pinheiro et al. 2010b); significantly associated with MCT1 (Pinheiro et al. 2010b) and MCT4 (Pinheiro et al. 2010a, b)
t2.11		↑ in tumor cells, compared to normal epithelium/associated with basal-like subtype, high histological grade, estrogen and progesterone receptors, cytokeratins 5 and 14 and vimentin (alone or co-expressed with CD147) (Pinheiro et al. 2010b)		↑ in tumor cells, compared to normal epithelium (Pinheiro et al. 2010a)	
t2.12	Lung	Cytoplasmic accumulation in alveolar soft-part sarcoma (Ladanyi et al. 2002)	(+) in tumor cells but (−) normal epithelium (Koukourakis et al. 2007)	(+) in tumor cells but (−) normal epithelium (Koukourakis et al. 2007)	(+) in tumor cells; tendency to be associated with MCT1 and significantly associated with MCT4 (Pinheiro et al. 2010a)
t2.13		(+) in tumor cells but (−) normal epithelium (Koukourakis et al. 2007)	(+) in tumor cells and normal epithelium cytoplasm, but not in plasma membrane (Pinheiro et al. 2010a)	↓ in tumor cells, compared to normal epithelium (Pinheiro et al. 2010a)	
t2.14		(+) in tumor cells and normal epithelium (Pinheiro et al. 2010a)			
t2.15	Gynecologic tract	↑ from preinvasive to invasive cervical cancer/associated with metastases in AC (when co-expressed with CD147) (Pinheiro et al. 2008b)	No progressive change from preinvasive to invasive cervical cancer/↑ ASC (Pinheiro et al. 2008b)	↑ from preinvasive to invasive cervical cancer/↑ AC (Pinheiro et al. 2008b)	↑ from preinvasive to invasive cervical cancer; significant association with MCT1 and MCT4 but not MCT2 (Pinheiro et al. 2009a)

t2.17 **Table 2** (continued)

Tumor site	MCT1 expression	MCT2 expression	MCT4 expression	CD147 expression*
t2.16	(+) in ovarian tumor cells (Pinheiro et al. 2010a; Chen et al. 2010), but (−) in normal and benign epithelium (Chen et al. 2010)/associated with low grade, high FIGO stage, residual tumor, lack of tumor relapse and presence of ascites (Chen et al. 2010)	(+) in ovarian tumor cells cytoplasm, but not in plasma membrane (Pinheiro et al. 2010a)	(+) in ovarian tumor cells (Pinheiro et al. 2010a; Chen et al. 2010), but (−) in normal and benign epithelium (Chen et al. 2010)/associated with high grade, high FIGO stage, residual tumor, tumor relapse and presence of ascites (Chen et al. 2010)	(+) in ovarian tumor cells (Pinheiro et al. 2010a; Chen et al. 2010), but (−) in normal and benign epithelium (Chen et al. 2010); tendency to be associated with MCT1 (Pinheiro et al. 2010a), significantly associated with MCT1 and MCT4 (Chen et al. 2010)
t2.17 Prostate	(+) in tumor cells but (−) normal epithelium and PIN lesions/associated with high pretreatment PSA, high Gleason score, high pathological grade and nodal involvement (Hao et al. 2010)	↑ in tumor cells, compared to normal epithelium (Pertega-Gomes et al. 2011)	(+) in tumor cells but (−) normal epithelium and PIN lesions/associated with high pretreatment PSA, high Gleason score, high pathological grade and nodal involvement (Hao et al. 2010)	(+) in tumor cells but (−) normal epithelium and PIN lesions; co-localization with MCT1 and MCT4 (Hao et al. 2010)
t2.18	↓ in tumor cells, compared to normal epithelium/associated with high PSA, absence of perineural invasion and presence of biochemical recurrence (Pertega-Gomes et al. 2011)		↑ in tumor cells, compared to normal epithelium/high PSA levels, advanced tumor stage, higher Gleason score, presence of perineural invasion, and presence of biochemical recurrence (Pertega-Gomes et al. 2011)	(+) in tumor cells and normal epithelium; significantly associated with MCT1 and MCT4, but not MCT2 (Pertega-Gomes et al. 2011)
t2.19 Gastric	(+) with no change along progression/associated with advanced gastric cancer, Lauren's intestinal type, stage III+IV and lymph-node metastases when (co-expressed with CD147) (Pinheiro et al. 2009b)		↓ from normal tissue, to primary tumor, to lymph-node metastases/associated with early gastric cancer and Lauren's intestinal type (Pinheiro et al. 2009b)	(+) with no change along progression; significantly associated with MCT1 and MCT4 (Pinheiro et al. 2009b)

(Mathupala et al. 2004). A more recent study on the sympathetic nervous system tumor neuroblastoma, showed, by mRNA quantification, that MCT1 expression is also high and is associated with age >1 year at diagnosis, stage 4 disease, unfavorable Shimada histopathology, DNA diploid index, *n-myc* amplification and high-risk clinical group (Children's Oncology Group criteria) (Fang et al. 2006). Finally, expression analysis revealed that *SLC16A1* transcript, encoding for MCT1, was elevated in 90 % of the medulloblastomas analyzed (Li et al. 2009).

Breast

Evidence for MCT down-regulation was not only observed in colon carcinoma (Ritzhaupt et al. 1998; Lambert et al. 2002). In fact, silencing of *SLC16A1* by gene promoter hypermethylation was suggested in 4 of 20 breast cases (20 %), however, the resultant decrease of mRNA and protein were not demonstrated (Asada et al. 2003). In fact, results from our group showed a significant increase of MCT1 cytoplasmic and plasma membrane expression in breast carcinoma, when comparing to normal breast epithelium (Pinheiro et al. 2010a, b). MCT2 and MCT4 were also evaluated, however, while MCT2 was only present in the cytoplasm in a similar frequency in normal and tumor samples, MCT4 only showed a significant increase in tumor samples for cytoplasm expression (Pinheiro et al. 2010a), with no differences in plasma membrane expression (Pinheiro et al. 2010a, b). Importantly, MCT1, alone or in co-expression with CD147, was associated with basal-like subtype (a more aggressive breast cancer group) and other poor prognostic variables, including tumor high grade, pointing at an important role of MCT1/CD147 in breast carcinoma aggressiveness (Pinheiro et al. 2010b).

Lung

The literature is also controversial in lung cancer. In a first study by Koukourakis and collaborators, no expression of MCTs in normal lung was found, while expression of MCT1 was found in all tumors examined and both MCT2 and MCT4 were also expressed in cancer cells. This study also

analyzed the possible metabolic cooperation between lung cancer cells and tumor-associated stroma, however, tumor-associated stroma expressed MCTs weakly (Koukourakis et al. 2007). In opposition, a recent study by our group showed that normal lung presents a high frequency of MCT expression and, in fact, MCT4 is less expressed in tumor samples than in normal epithelium. However, as this last study was performed in a small number of cases, further work is needed to confirm these results (Pinheiro et al. 2010a). MCT1, in association with its chaperone CD147, was also described in the cytoplasm of alveolar soft part sarcoma (Ladanyi et al. 2002).

Gynecologic tract

MCT expression has also been described in some gynecological tumors like cervical and ovarian cancer (Pinheiro et al. 2010a, b; Chen et al. 2010). In cervical cancer, a significant increase in overall and plasma membrane expression of MCT1 and MCT4 was observed from pre-invasive to invasive squamous lesions and from normal glandular epithelium to adenocarcinomas. For MCT2, the significant alterations in the expression along the progression to the invasive phenotype did not follow a clear increase/decrease pattern. Also, MCT2 was more frequently observed in squamous cell carcinomas, while MCT4 was more frequently observed in adenocarcinomas. Importantly, high risk HPV-positive pre-invasive cases expressed more MCT1 and MCT4 than HPV negative pre-invasive cases, and also presented more MCT1 in plasma membrane (Pinheiro et al. 2008b). Additionally, CD147 was more frequently expressed in MCT1 and MCT4 positive cases and co-expression of MCT1 and CD147 was significantly associated with lymph-node metastasis in adenocarcinomas (Pinheiro et al. 2009a). In ovarian cancer, staining for MCT1 and MCT4 as well as their chaperone CD147 was not found in normal ovarian tissues and benign ovarian tissues, while around 80 % of epithelial ovarian primary and metastatic tumors showed expression of these proteins. MCT1 was significantly associated with low grade tumors, high FIGO stage, presence of residual tumor, lack of relapse and presence of ascites; MCT4 was significantly associated with high grade tumors, high FIGO stage, presence of residual tumor, relapse and presence of ascites. Importantly, MCT expression was associated with the expression of the multidrug resistance markers MDR1 and MRP2 (Chen et al. 2010). Our group also reported expression of MCT1, MCT2 and MCT4 in ovarian carcinoma, but with a lower frequency for MCT4 (around 45 %) and around 95 % for MCT2 (Pinheiro et al. 2010a).

Prostate

Association of MCTs with MDR1 was also described in prostate cancer (Hao et al. 2010). In this study, MCT1 and MCT4 were found to be expressed in around 90 % of prostate cancer cases, with 20 % of positive cases showing a weak immunostaining, while no expression was found in normal prostate tissues, prostate intraepithelial lesions or in non-tumor regions adjacent to primary prostate cancer tissues. Importantly MCT1 and MCT4 expressions were associated with high pretreatment PSA levels, high Gleason score, high pathological stage, and nodal involvement (Hao et al. 2010). In another study evaluating the expression of MCTs in prostate cancer (Pertega-Gomes et al. 2011), MCT1 was expressed in all normal samples and significantly less frequently expressed in tumor samples, being accompanied by its chaperone CD147. Conversely, MCT2 and MCT4 were significantly more frequently expressed in the cytoplasm of tumor cells when compared to normal tissue. All MCT isoforms and CD147 were expressed, at different frequencies, in PIN lesions. In accordance with some of the findings from the first study (Hao et al. 2010), MCT1 expression was associated with higher PSA levels, absence of perineural invasion, and presence of biochemical recurrence, while MCT4 expression was associated higher PSA levels, advanced tumor stage, higher Gleason score, presence of perineural invasion, and presence of biochemical recurrence (Pertega-Gomes et al. 2011). Further studies are warranted to better elucidate the expression pattern of MCTs in prostate tissues.

Stomach

In contrast to what was found in the previous types of tumors, neither MCT1 nor MCT4 were found to be up-regulated in gastric adenocarcinomas (Pinheiro et al. 2009b). Actually, MCT4 expression was more frequently observed in normal gastric mucosa than in gastric cancer cells and even less frequently observed in lymph-node metastasis, indicating a progressive loss of this MCT isoform with disease progression. Also, MCT4 expression was associated with Lauren's classification of intestinal-type carcinoma. MCT1 was similarly expressed in normal gastric mucosa, primary tumors and lymph-node metastasis, being present in the majority of samples (around 80 %). These findings may indicate that MCT1 has a major contribution in gastric homeostasis, which is maintained along carcinogenesis (Pinheiro et al. 2009b).

Overall, the data available in the literature support the hypothesis of a major role of MCTs in the emergence of the hyper-glycolytic and acid-resistant phenotypes, as adaptations to the hypoxic microenvironment. The up-regulation of MCTs in the plasma membrane of different type of tumors is an adaptive mechanism to allow continuous high glycolytic rates, by exporting the accumulating end-product, lactate, as well as to counteract acid-induced apoptosis or necrosis. However, this may not be the case for all tumor

types, hence, further studies characterizing MCT expression in other tumors are warranted.

MCTs as therapeutic targets in cancer

Considering the major role of MCTs in cancer metabolic adaptations, MCT inhibition will have a direct effect on cell pH regulation, therefore having an important effect on cancer cell viability. Also, MCTs have a crucial role as gatekeepers of the metabolic symbiosis between cancer cells (Sonveaux et al. 2008), so, targeting these transporters will "shut-down" the advantageous symbiosis, having an important impact on tumor homeostasis. Finally, taking into account the contribution of lactate to the malignant phenotype, together with the up-regulation of MCT in some tumors, MCT inhibition may be a useful therapeutic approach in cancer. This will then counteract the effects of lactate and, therefore, increase the immune response against tumor cells and decrease migration capacity of cells, among others.

In fact, it was demonstrated that in vitro MCT1 inhibition decreases intracellular pH (Sonveaux et al. 2008; Fang et al. 2006; Wahl et al. 2002), leads to cell death (Sonveaux et al. 2008; Mathupala et al. 2004; Fang et al. 2006; Wahl et al. 2002; Colen et al. 2006) and, importantly, enhances cancer cell radiosensitivity (Colen et al. 2006). Additionally, silencing of MCT4 results in decreased cancer cell migration (Gallagher et al. 2007), by mechanisms that also involve interaction of MCT4 with β_1-integrin (Gallagher et al. 2009). In opposition, another study showed that silencing of MCT1 or MCT4 inhibited cancer cell invasion, but did not influence cell migration (Izumi et al. 2011). Importantly, promising results using in vivo models have also been reported, where administration of α-cyano-4-hydroxycinnamic acid (CHC), a classical non-specific inhibitor of MCT1 (Fig. 1), retarded tumor growth, rendered tumor cells sensitive to radiation (Sonveaux et al. 2008), induced tumor necrosis and decreased tumor invasion (Colen et al. 2011). The importance of MCTs for in vivo tumor growth was confirmed by a more specific approach, where combined silencing of MCT1 and MCT4 or silencing of CD147 significantly reduced glycolytic flux and tumor growth (Le et al. 2011). There are also other MCT inhibitors described (Fig. 1) (Le et al. 2011; Ovens et al. 2010; Wang & Morris 2007; Belt et al. 1979; Kobayashi et al. 2006; Ben-Horin et al. 1995; Ben-Yoseph et al. 1998), which are either non-isoform specific (AR-C155858 targets both MCT1 and MCT2 (Ovens et al. 2010)) or target other molecules besides MCTs (e.g., lonidamine primary target is hexokinase II (Floridi et al. 1981)). However, these compounds have been little explored as lactate transport inhibitors in the cancer context (Le et al. 2011; Wang & Morris 2007; Belt et al. 1979; Ben-Yoseph et al. 1998).

MCT regulation by chaperones

As previously mentioned, functional expression of MCTs is regulated by accessory proteins, such as CD147, that are involved in trafficking and anchoring of plasma membrane proteins.

Regulation of MCT1 and MCT4, but not MCT2, by CD147, was supported by evidence on human tissues (Pinheiro et al. 2009a, b,2010a, b), complementing the in vitro and some in vivo studies previously described (Gallagher et al. 2007; Kirk et al. 2000; Makuc et al. 2004; Philp et al. 2003; Deora et al. 2005; Wilson et al. 2005). Indeed, the prognostic value of CD147 appears to be associated with its co-expression with MCT1, as observed in breast and gastric carcinomas (Pinheiro et al. 2009b, 2010b). Therefore, targeting CD147, which will also impair MCT activity, appears to be a rational therapeutic approach against human cancer, as already described both in vitro and in vivo (Schneiderhan et al. 2009; Su et al. 2009; Baba et al. 2008). Besides the role of CD147 as chaperone for MCT1 and MCT4 plasma membrane trafficking and activity, these MCT isoforms also have been implicated in CD147 proper membrane expression (Gallagher et al. 2007; Deora et al. 2005). Thus, the contribution of MCTs to the malignant phenotype is not limited to their own function as lactate transporters and pH regulators, but may also be further enhanced by their role in regulating CD147 expression. If so, MCTs may also have indirect roles in tumor growth and angiogenesis, as well as cancer cell migration and invasion (Nabeshima et al. 2006; Yan et al. 2005; Iacono et al. 2007; Slomiany et al. 2009).

In vitro studies show that CD44 may also function as a chaperone for MCT expression (Slomiany et al. 2009). Additionally, parallel analysis of CD44 and MCTs expressions in human cancer samples, show that CD44 is associated with MCT1 in lung cancer (Pinheiro et al. 2010a) and both MCT1 and MCT4 in prostate cancer (Hao et al. 2010). As a result, MCT expression may also have a role in cell growth control, adhesion, migration, invasion, and chemoresistance (Marhaba & Zoller 2004; Toole & Slomiany 2008a, b), through interaction with CD44.

Importantly, there is a relevant number of cases with MCT plasma membrane expression lacking ~~both~~ CD147 or CD44 in the plasma membrane ~~co-expression~~, suggesting that a not yet identified chaperone may be involved in MCT trafficking to the plasma membrane.

Conclusion

Carcinogenesis has been viewed as a progressive process described as "somatic evolution" as it requires a sequence of genetic changes; however, recent models of carcinogenesis

integrate the neo-Darwinian evolution, stating that phenotypic properties are retained or lost based on their contribution to fitness for survival, with cell-environment interactions. This new concept of carcinogenesis was applied to explain the Warburg phenomenon, i.e., the preference for the glycolytic phenotype, even in the presence of oxygen. Thus, as cancer progression proceeds, mutations in tumor cells increase and traits that are found in invasive cancers, like the hyper-glycolytic and acid-resistant phenotypes, arise as adaptive mechanisms to environmental proliferative constraints, such as hypoxia.

Many players have been associated with these cellular adaptations; however, although an important role of lactate transporters could be anticipated in the context of the Warburg effect, the underlying role of MCTs in solid tumors are far from being understood. Thus, additional studies characterizing MCT expression in tumor types not yet analyzed, confirmation of the results already published as well as additional functional studies are needed to reinforce the contribution of MCTs for cancer maintenance and aggressiveness.

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