

Review

Monocarboxylate transporters as targets and mediators in cancer therapy response

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Summary. Monocarboxylate transporters (MCTs) belong to a family of transporters, encoded by the *SLC16* gene family, which is presently composed by 14 members, but only MCT1 to 4 have been biochemically characterized. They have important functions in healthy tissues, being involved in the transmembrane transport of lactic acid and other monocarboxylic acids in human cells.

One of the recently recognized hallmarks of cancer is altered metabolism, with high rates of glucose consumption and consequent lactate production. To maintain this metabolic phenotype, cancer cells upregulate a series of plasma membrane proteins, including MCTs. MCT1 and MCT4, in particular, play a dual role in the maintenance of the metabolic phenotype of tumour cells. On one hand, they facilitate the efflux of lactate and, on the other hand, they contribute to the preservation of the intracellular pH, by co-transporting a proton. Thus, MCTs are attractive targets in cancer therapy, especially in cancers with a hyper-glycolytic and acid-resistant phenotype.

Recent evidence demonstrates that MCTs are involved in cancer cell uptake of chemotherapeutic agents, including 3-bromopyruvate. In this way MCTs can act as “Trojan horses”, as their elevated expression in cancer cells can mediate the entry of this chemotherapeutic agent into the cells and selectively kill cancer cells. As a result, MCTs will be mediators of chemotherapeutic response, and their expression can be

used as a molecular marker to predict response to chemotherapy.

Key words: Monocarboxylate transporters, Cancer therapy, Glycolysis, Molecular targets

Introduction

Cancer is one of the leading causes of death worldwide. Estimates from 2012 indicate that there are annually about 14.1 million new cases of cancer and 8.2 million deaths due to the disease (Globocan, 2012). Despite the progress in therapeutic optimization, there is still much to be done in the cancer field and, thus, understanding cancer cell physiological behaviour is crucial for the discovery of new molecular targets and novel drugs.

One of the emerging hallmarks of cancer is altered metabolism, in which cancer cells exhibit high rates of glucose consumption with concomitant lactate production (Warburg, 1956; Hanahan and Weinberg, 2011). To maintain this metabolic phenotype, cancer cells upregulate a series of proteins, including glycolytic enzymes and pH regulators, including monocarboxylate transporters (MCTs) that will facilitate the efflux of lactate, co-transported with a proton.

MCTs have been described to be involved in the transport of lactic acid and other monocarboxylic acids in human cells, with important functions in healthy tissues (Halestrap and Price, 1999; Halestrap, 2012; Halestrap and Wilson, 2012). There is also evidence for their role in cancer, given the increased lactic acid

production by cancer cells (Froberg et al., 2001; Pinheiro et al., 2012). Additionally, they have been described as the transporters for chemotherapeutic agents like 3-bromopyruvate (3-BP) (Cardaci et al., 2012; Queiros et al., 2012; Matsumoto et al., 2013). Therefore, exploiting MCTs either as specific molecular targets or drug transporters in cancer cells can be of great clinical value, opening new perspectives in cancer therapy.

The monocarboxylate transporter family

Monocarboxylate transporters (MCTs) belong to a family of transporters, encoded by the *SLC16* gene family, which is presently composed by 14 members (Pao et al., 1998; Halestrap and Meredith, 2004). MCT1-4 have been demonstrated to facilitate the transmembrane proton-linked transport of metabolically important monocarboxylates such as lactate, pyruvate, branched-chain oxoacids, and the ketone bodies acetoacetate and β -hydroxybutyrate (Deuticke, 1982; Yoon et al., 1997; Lin et al., 1998; Halestrap and Price, 1999; Dimmer et al., 2000). MCT1 has a ubiquitous distribution in human tissues, with an important expression in heart and muscle (Lin et al., 1998; Price et al., 1998; Fishbein et al., 2002). This isoform has an intermediate affinity for its substrates and is involved in both uptake and efflux of monocarboxylates from cells. MCT2 is considered a high affinity transporter, being adapted to the uptake of monocarboxylates into cells (Lin et al., 1998; Broer et al., 1999). This isoform shows a more restricted tissue distribution (Lin et al., 1998; Fishbein et al., 2002), being mostly found in tissues that use lactate as a respiratory fuel, like brain (Fishbein et al., 2002; Pierre et al., 2002) or cardiac and red skeletal muscles (Juel, 1997; Halestrap, 2013), as well as kidney and liver, where lactate is the major gluconeogenic substrate (Garcia et al., 1995; Fishbein et al., 2002). MCT3 has an even more restricted distribution, it has been identified in the retinal pigment and choroid plexus epithelia, and implicated in the efflux of lactate in the retina (Philp et al., 1998; Bergersen et al., 1999). On the other hand, MCT4 is known as a low affinity transporter and has been observed particularly in highly glycolytic tissues, such as white skeletal muscle fibers, astrocytes, and white blood cells (Price et al., 1998; Wilson et al., 1998; Dimmer et al., 2000), being associated with lactate efflux (Wilson et al., 1998; Halestrap and Price, 1999). Although isoforms 1-4 are the best characterized isoforms over the years, the function of other MCT isoforms is already known. For example, *SLC16A10* gene encodes for an aromatic amino-acid transporter (T-type amino-acid transporter 1, named TAT1) (Kim et al., 2001) and MCT8 transports thyroid hormones (Friesema et al., 2003, 2008), while MCT6 transports bumetanide, but not L-lactic acid (Murakami et al., 2008). Although the substrate for MCT12 is still unknown, recent studies suggest that it may be involved in the establishment and/or maintenance of homeostasis in the eye lens and probably also in the kidney (Kloeckener-Gruissem et al.,

2008; Zuercher et al., 2010). The substrates of the remaining members of the family (MCT5, MCT7, MCT9, MCT11, MCT13 and MCT14) are still unknown.

Although the regulatory mechanisms of MCT expression are not completely elucidated, evidence indicates that MCTs are regulated at both transcriptional and post-transcriptional levels. Importantly, hypoxia is known to be a major regulator of MCT expression. While there is some controversy around MCT1, evidence for MCT4 upregulation by hypoxia is more consistent (McClelland and Brooks, 2002; Ord et al., 2005; Zhang et al., 2005; Ullah et al., 2006; Kay et al., 2007; Sonveaux et al., 2008; Perez de Heredia et al., 2010). Actually, MCT4 was described to be regulated by the hypoxia inducible factor 1 α (HIF-1 α), a transcription factor with a major role in the adaptation to hypoxia (Ullah et al., 2006).

The association with ancillary proteins also appears to be crucial for the functional regulation of MCTs, as they are involved in trafficking and anchoring of membrane proteins to specific cellular locations. In this context, CD147 emerges as the major and best-studied regulator of the expression of MCT isoforms 1, 3 and 4. CD147, also known as basigin or EMMPRIN, belongs to the immunoglobulin superfamily and is a broadly distributed plasma membrane protein (Biswas et al., 1995). CD147 was shown to specifically interact with MCT1 and MCT4, but not MCT2, at the plasma membrane (Kirk et al., 2000; Zhao et al., 2001; Ladanyi et al., 2002; Wilson et al., 2002). Besides regulating MCT membrane location (Makuc et al., 2004; Deora et al., 2005; Gallagher et al., 2007; Baba et al., 2008; Su et al., 2009), CD147 also regulates the expression of MCT1, 3 and 4 (Philp et al., 2003; Gallagher et al., 2007; Schneiderhan et al., 2009; Su et al., 2009), as well as their activity as lactate transporters (Makuc et al., 2004; Wilson et al., 2005). In turn, MCT1 and MCT4 were also demonstrated to regulate CD147 maturation and trafficking to the plasma membrane (Deora et al., 2005; Gallagher et al., 2007). In opposition, MCT2 was found to preferentially interact with another member of the immunoglobulin superfamily, gp70 (Huang et al., 1990; Wilson et al., 2005). More recently, the glycoprotein CD44 and its main ligand, hyaluronan, were also identified to associate to the MCT1/MCT4/CD147 complex in the context of drug resistance in cancer therapy (Slomiany et al., 2009). Being also a glycoprotein, one can hypothesize that CD44 could also behave as a chaperone of these MCT isoforms.

The role of monocarboxylate transporters in cancer

Although the role of MCTs in physiological homeostasis is well known in some tissues, less is known about their role and clinical relevance in the cancer context.

Almost a century ago, Otto Warburg demonstrated that cancer cells have a preference to use glycolysis for energy production, independently of the levels of oxygen

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(Warburg, 1956), a phenomenon currently known as “aerobic glycolysis” or “Warburg effect”. Despite the flaws in Warburg’s original hypothesis (Wang et al., 1976; Moreno-Sanchez et al., 2007), the observation of increased glycolysis in cancer has been confirmed repeatedly, being the rationale behind the whole-body non-invasive ¹⁸F-fluorodeoxyglucose positron emission tomography (FDG-PET) imaging technique. Although much less energetically efficient than oxidative phosphorylation, glycolysis provides several advantages to cancer cells, such as allowing survival under intermittent hypoxia (Gatenby and Gillies, 2004; Gillies and Gatenby, 2007), being a source of anabolic substrates essential for biosynthetic pathways (Gatenby and Gillies, 2007; DeBerardinis et al., 2008) and providing DNA protection from damage by oxygen radicals produced during oxidative phosphorylation (Kondoh et al., 2007). Additionally, the resulting production of lactate gives rise to an acidic microenvironment, providing a further competitive advantage over normal cells (Gatenby et al., 2007; Gillies and Gatenby, 2007), also facilitating cancer cell invasion (Martinez-Zaguilan et al., 1996; Smallbone et al., 2005; Rofstad et al., 2006). To avoid necrosis or apoptosis induced by acid overproduction (Park et al., 1999; Williams et al., 1999), cancer cells undergo a series of adaptations, including upregulation of membrane pH regulators (Gatenby and Gillies, 2004), like the Na⁺/H⁺ exchanger 1 (NHE1), CAIX (carbonic anhydrase 9), AE1 (anion exchanger 1) and MCTs, MCT1 and MCT4 in particular. Here, MCTs play a dual role; on one hand, they facilitate the efflux of lactate, allowing the maintenance of the glycolytic phenotype and, on the other hand, by co-transporting a proton along with lactate, they contribute to the preservation of the intracellular pH. Thus, MCTs appear as attractive targets in cancer therapy, especially in cancers with the hyperglycolytic and acid-resistant phenotype.

Expression of MCTs in human tumours

Studies on the role of MCTs in cancer are becoming more frequent over the years. The expression of MCTs in human tumours has been recently reviewed by Pinheiro and collaborators (Pinheiro et al., 2012). Despite some controversies, MCTs appear to be differentially expressed among solid tumours, a fact most likely related to the different metabolic profiles of tumours.

In colorectal carcinoma, there is controversy in the literature around MCT expression. The first two reports on colon carcinoma described downregulation of MCT1 expression (Ritzhaupt et al., 1998; Lambert et al., 2002), but more recent evidence describes upregulation of both MCT1 and MCT4 isoforms at the plasma membrane of colorectal cancer, compared to the non-neoplastic counterparts. Importantly, MCT2 and MCT4 were strongly expressed in the cytoplasm of cancer cells indicating a possible role in intracellular organelles such

as mitochondria (Koukourakis et al., 2006, Pinheiro et al., 2008a). Also, plasma membrane MCT1 expression was found to be associated with vascular invasion (Pinheiro et al., 2008a). Although this association is easy to understand, assuming the possible role of MCT1 in lactate efflux, it needs further investigation.

Another tumour type with controversies on MCT expression is breast cancer. A first report described that *SLC16A1* gene is silenced in 20% of breast cancer cases due to promoter hypermethylation although the results were not supported by mRNA or protein expression analyses (Asada et al., 2003). In contrast, a more recent study, comprising around 250 cases of breast carcinoma cases, showed an overall increase of MCT1 expression. MCT1 and its chaperone CD147 were associated with the basal-like subtype of breast cancer and other poor prognostic variables, such as high histological grade, estrogen and progesterone receptor negativity and high proliferation index, pointing at a role of MCT1/CD147 in breast carcinoma aggressiveness (Pinheiro et al., 2010).

In tumours of the central nervous system, MCT1 expression was described to be much higher in ependymomas, hemangioblastomas and high grade glial neoplasms (anaplastic astrocytomas and glioblastoma multiforme (GBM)), than in low-grade glial neoplasms (oligodendrogliomas and astrocytomas) (Froberg, 2001). In another study, MCT3 was predominantly found in normal brain, whereas MCT1 and MCT2 were the major isoforms present in GBM tumours. MCT4 was not detected in any of the tumour tissues (Mathupala et al., 2004). MCT1 expression in neuroblastoma was also high and was associated with poor prognostic factors, such as advanced disease, DNA diploid index, n-myc amplification and high-risk clinical group (Fang et al., 2006). In a more recent study, MCT1, MCT4, and CD147 were described to be overexpressed at the plasma membrane of glioblastoma cells, compared with diffuse astrocytomas and non-neoplastic brain tissue (Miranda-Goncalves et al., 2013).

In uterine cervix there is only one report showing a significant increase in MCT1 and MCT4 expression from pre-invasive to invasive squamous lesions and from normal glandular epithelium to adenocarcinomas, in a comprehensive series of squamous cell carcinomas and adenocarcinomas (Pinheiro et al., 2008b).

MCT1, MCT2 and MCT4 were reported to be highly expressed in gastro-intestinal stromal tumours (GISTs), with some important associations with clinicopathological data. CD147 expression was associated with mutated KIT as well as a progressive increase in Fletcher’s Risk of Malignancy, while co-expression of MCT1 with CD147 was associated with low patient overall survival. These findings suggest that the pair MCT1/CD147 is involved in GIST aggressiveness, contributing to cancer cell metabolic adaptations in GIST development and/or progression (de Oliveira et al., 2012).

Finally, MCT downregulation was found in both

gastric and prostate cancers (Pineiro et al., 2009; Pertega-Gomes et al., 2011). In gastric cancer, while there were no differences in MCT1 and CD147 expressions between non-neoplastic mucosa and either primary tumour or lymph-node metastasis, there was a significant downregulation of MCT4 in the progression to malignancy. However, the pair MCT1/CD147 was associated with poor prognosis factors, such as advanced gastric carcinoma, Lauren's intestinal type, TNM staging and lymph-node metastasis (Pineiro et al., 2009). In prostate cancer, the first report on MCT expression described both MCT1 and MCT4 upregulation in prostate cancer tissues. The authors also suggested that overexpression of CD147 and MCT4 is associated with prostate cancer progression (Hao et al., 2010). A second report described a significant increase in the cytoplasmic expression of both MCT2 and MCT4, and a significant decrease in both MCT1 and CD147 expression in prostate tumour cells, when compared to normal tissue (Pertega-Gomes et al., 2011). Associations were found between MCT1, MCT4 and CD147 expressions and poor prognostic markers. Importantly, MCT4 and CD147 were associated with high PSA levels, Gleason score and pT stage, as well as perineural invasion and biochemical recurrence. More recently, the value of MCT2 expression in the diagnosis of prostate cancer was also explored. In this context, the pattern of expression of MCT2 in normal prostate, precursor lesions and prostate cancer was compared with that of alpha-methylacyl-CoA racemase (AMACR), currently used in prostate cancer diagnosis. In combination with AMACR (positive marker) and p63 or 34 β E12 (as negative markers), MCT2 (positive marker) helped to improve the diagnosis of prostate carcinoma, increasing the sensitivity and specificity to detect prostate cancer (Pertega-Gomes et al., 2013).

Besides playing an important role in cancer cells, there have been increasing reports of MCT expression in the tumour stroma. Actually, it appears that there is a metabolic symbiosis between tumour and stroma cells, where tumour cells produce lactate that will be used as an energy substrate by stromal cells. The first evidence was given by the group of Koukourakis and collaborators, which demonstrated this metabolic complementarity in colon cancer (Koukourakis et al., 2006). The authors showed that cancer cells exhibit high glucose transporter 1 (GLUT1), lactate dehydrogenase 5 (LDH5) and MCT1 expression, consistent with a glycolytic phenotype, while cancer associated fibroblasts express LDH1 and MCT1/MCT2 and low GLUT1, consistent with a more oxidative phenotype. A similar metabolic cooperation between lung cancer cells and tumour-associated stroma was later described by the same group, where MCTs were overexpressed in cancer cells, with a weak expression of MCTs in the tumour-associated stroma, while no expression was found in normal lung (Koukourakis et al., 2007). More recently, Lisanti and collaborators proposed a theory known as "reverse Warburg effect", in which the fibroblastic

tumour stroma feeds the epithelial cancer cells, in a host-parasite relationship (Pavlides et al., 2009). Here, the epithelial tumour cells would manipulate the normal stroma to produce energy-rich metabolites to feed on (Pavlides et al., 2009).

MCTs as therapeutic targets in cancer

From the above described, it is easy to conjecture that inhibition of MCT activity and/or expression will certainly disturb cancer cell homeostasis, by interfering with monocarboxylate transport and pH regulation. Taking into account that there is up-regulation of MCTs in several tumours, inhibition of these molecules can be a useful strategy to explore in cancer treatment.

There are several known classical MCT inhibitors, which can be divided into three major categories: a) bulky or aromatic monocarboxylates like α -cyano-4-hydroxycinnamate (CHC); b) amphiphilic compounds including bioflavonoids like quercetin and phloretin; and c) stilbene-derived compounds such as 4,4-O-diisothiocyanostilbene-2,20-disulphonate (DIDS) and 4,4-O-dibenzamidostilbene-2,20-disulphonate (DBDS) (Halestrap and Price, 1999). The sensitivities to the different inhibitors vary among the MCT isoforms, according to each isoform affinity for the substrates. MCT2 is more sensitive to inhibition by CHC, DBDS and DIDS than MCT1, but is insensitive to p-chloromercuribenzenesulphonate (pCMBS) (Garcia et al., 1995; Bonen et al., 2000). This difference in pCMBS sensitivity results from the different accessory proteins required for MCT1 and MCT2 activities, as the inhibitor is now known to target CD147 (Wilson et al., 2005). However, in opposition to MCT1, MCT3 is insensitive to inhibition by CHC, pCMBS and phloretin (Grollman et al., 2000). MCT4 exhibits a much lower sensitivity for a wider range of inhibitors than MCT1 (Manning Fox et al., 2000).

However, these inhibitors are not specific and may target other molecules. For example, the well-known MCT inhibitor CHC, frequently used as an MCT1 inhibitor, also inhibits the mitochondrial pyruvate transporter, as well as the anion exchanger isoform 1 (AE1) (Halestrap and Price, 1999). Quercetin and phloretin also inhibit AE1 (Halestrap and Price, 1999), efflux transporters including P-glycoprotein, multidrug resistance protein 1/2 and breast cancer resistance protein (Wang and Morris, 2007; Chen et al., 2010), as well as some intracellular pathways including phosphatidylinositol 3-kinase (PI3K)/Akt and extracellular regulated kinase (ERK).

In addition, other drugs have also been described as inhibitors of lactate transport. For example, MCT4 activity is inhibited by a range of statins, which are antihyperlipidemic drugs (Kobayashi et al., 2006). Lonidamine was firstly described as hexokinase II inhibitor (Floridi et al., 1981) but was also demonstrated as an inhibitor of lactate transport (Ben-Yoseph et al., 1998; Ben-Horin et al., 1995).

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Since the above compounds are not MCT specific, the functional role of MCTs in cancer should be studied using specific inhibitors. Thus AstraZeneca developed a series of small molecule compounds like AR-C155858, which promise to be MCT1 isoform specific and are now under clinical trials (Porporato et al., 2011). However, competition studies demonstrated that AR-C155858 inhibits both MCT1 and MCT2, but not MCT4, when expressed in oocytes (Ovens et al., 2010).

Over the last years, several authors have been demonstrating the effect of MCT inhibition in cancer, using both *in vitro* and *in vivo* models, which are summarized below.

In vitro studies with cancer derived cells

The classical inhibitor CHC is described as disturbing cancer cell homeostasis inducing cell death, decreasing cell proliferation, invasion and other important features in cancer cells through MCT inhibition. In a study with glioma cells, treatment with CHC prior to low-dose radiation exposure led to rapid morphological changes, followed by apoptotic cell death (Colen et al., 2006). CHC inhibition also induced cell death in colorectal and cervix carcinoma cells, as well as a decrease in intracellular pH (Sonveaux et al., 2008). Colen and collaborators used CHC in two glioma cell lines and in an *ex vivo* brain slice culture and demonstrated that lactate efflux inhibition clearly impaired glioma cell invasion (Colen et al., 2011).

CHC and DBDS were used in another study with melanoma cells and *ex vivo* DB-1 human melanoma xenografts, in which a great potential to selectively disturb melanoma cell viability, with a demonstrated decrease in intracellular pH, which improved the effectiveness of these chemotherapeutic drugs. These findings point at MCTs as major pH regulators in melanoma cells (Wahl et al., 2002).

More recent studies showed a decrease in lactate production, accompanied by a decrease in cell proliferation, migration and invasion capacity upon lactate efflux inhibition with CHC in glioma (Miranda-Goncalves et al., 2013) and breast cancer cells (Morais-Santos et al., 2014). Importantly, CHC was able to enhance the effect of temozolamide, a gold standard drug currently used in the treatment of gliomas (Miranda-Goncalves et al., 2013).

Inhibition of MCT1/2 with a more specific inhibitor (AR-C155858) in RAS-transformed fibroblasts inhibited lactate export, glycolysis rates and tumour growth. Interestingly, when MCT4 was expressed, cells became resistant to MCT1/2 inhibition and tumourigenicity was restored (Le Floch et al., 2011).

The drug lonidamine was used in MCF-7 human breast cancer cells, promoting a decrease in initial glucose uptake and lactate formation (Ben-Horin et al., 1995). However, other authors used the same drug in glioma cultured tumors and concluded that lonidamine action also involves inhibition of lactate efflux and

intracellular acidification, suggesting its exploitation for sensitizing gliomas to radiation, chemotherapy or hyperthermia (Ben-Yoseph et al., 1998). Additionally, in neuroblastoma cells, MCT1 inhibition by lonidamine also promoted intracellular acidification, and consequent decrease in cell viability, causing cell death by apoptosis, similarly to CHC (Fang et al., 2006). A more recent study demonstrated that lonidamine and quercetin were able to decrease lactate production, cell proliferation, migration and invasion in breast cancer cells, also similarly to CHC (Morais-Santos et al., 2014).

Since specificity of MCT inhibition can be limited when using small molecule compounds, some studies started to use RNA interference (RNAi) or other technologies to more accurately study the role of MCTs in cancer.

In a glioma cell line, lactate efflux was reduced by 30% for individual silencing of either MCT1 or MCT2, and by 85% for combined silencing, decreasing intracellular pH, cell viability and increasing both apoptosis and necrosis (Mathupala et al., 2004). In another study, silencing of MCT1 in glycolytic glioma cells reduced lactate efflux, migration and sensitivity to CHC (Miranda-Goncalves et al., 2013).

Gallagher and collaborators, by silencing MCT expression by RNAi, described an interaction between MCT4 and β 1-integrin and investigated the role of MCT4 knockdown in migration. They found that MCT4 silencing slowed migration and increased focal adhesion size in epithelial and breast cancer cell lines (Gallagher et al., 2007, 2009), probably mediated by MCT/CD147 complex disruption, while MCT1 silencing did not alter these features (Gallagher et al., 2009).

In a human colon adenocarcinoma cell line, silencing of either MCT1 plus MCT4 or CD147 reduced cell glycolytic flux as well as tumour growth (Le Floch et al., 2011). Furthermore, specific inhibition of MCT1 with siRNA corroborated the results obtained with CHC, lonidamine and quercetin inhibition, decreasing *in vitro* cancer cell aggressiveness in breast cancer cells, by decreasing proliferation, migration and invasion (Morais-Santos et al., 2014).

Studies using in vivo models

The well-known MCT inhibitor CHC has also been used in *in vivo* models, namely mouse xenografts and chick chorioallantoic membrane (CAM) models.

In induced intracranial glioma tumours, CHC caused tumour necrosis and, importantly, control animals did not demonstrate any adverse neurologic effects during its administration (Colen et al., 2011). Other authors reported a reduction in tumour growth and cell sensitization to radiation, after MCT1 inhibition with CHC using colorectal and cervical cells injected intramuscularly in the leg of mice (Sonveaux et al., 2008). In experiments using human colorectal and breast cancer cells silenced for MCT expression, injected subcutaneously in nude mice, MCT4 was pointed out as

an important link between tumour metabolism and angiogenesis, mediated by the released lactate, which stimulates the NF-KB/IL-8 pathway (Vegran et al., 2011). A recent study using nude mice for prostate tumour induction, where carbohydrate restriction was combined with lactate transport inhibition by CHC, demonstrated that MCT1 inhibition did not have a significant effect on tumour volume, but was associated with an increased necrotic fraction (Kim et al., 2012). Additionally, subcutaneous cervix tumours were treated with CHC and, unexpectedly, lactate concentration, necrosis and hypoxic areas were not affected. However, CHC treatment induced inhibition of glycolysis and activation of OXPHOS (Pasteur effect), showing that CHC is able to influence intratumoural distribution of glucose between hypoxic and non-hypoxic tumour areas (Busk et al., 2011).

In the chick chorioallantoic membrane (CAM) *in vivo* model, treatment with CHC was able to decrease glioma tumour size, proliferation and the number of tumour vessels associated with the tumour (Miranda-Goncalves et al., 2013).

MCTs as transporters of anticancer compounds

The above described shows the important activity of MCTs, namely of MCT1 and MCT4, in the proliferation and aggressiveness of tumour cells. MCTs are thus important targets in cancer therapy, leading to tumour regression when inhibited. This is due, mainly, to the important role of lactic acid for tumour characteristics; indeed, high levels of lactic acid are associated with poor prognosis, contributing directly to tumour progression (Dhup et al., 2012).

However, other monocarboxylates or monocarboxylate derivatives, which are also MCTs substrates,

can have an opposite effect in cancer cells. Actually, some haloderivatives of monocarboxylic acids like 3-bromopyruvate (3-BP), dichloroacetate (DCA) and iodoacetate (IAA), are described as antiglycolytic agents, and their inhibitory effect in tumours is described either in *in vivo* or *in vitro* systems (Fahim et al., 2003; Ko et al., 2004; Bonnet et al., 2007).

From this standpoint, overexpression of monocarboxylic acid transporters can be seen as a molecular target for cancer therapy, not only due to its direct effect on tumour growth, but also, in an opposite way, due to their capacity to mediate the transport of monocarboxylic acid-related compounds with anticancer properties.

A major challenge in developing an anticancer drug is to specifically target cancer cells, avoiding the toxic side effects in normal tissues. Different compounds targeting the glycolytic metabolism or signalling pathways that regulate cellular metabolism have emerged in the last years. Several of them showed a promising, selective and significant cytotoxicity to cancer cells, and pre-clinical and clinical trials to validate their therapeutic anticancer effect were already established or are ongoing. Table 1 summarizes some of these anticancer agents that inhibit the cancer glycolytic phenotype, their putative molecular targets, mechanism of action and the current clinical status.

From the antiglycolytic agents listed in Table 1, 3-BP, DCA and IAA are haloderivatives of monocarboxylic acids. All these molecules display, at physiological pH, a hydrophilic nature, requiring a plasma membrane transporter to reach their intracellular molecular target(s). There are several reports pointing at the involvement of some MCT or SMCT family members in the uptake of these compounds by mammalian cells (Fishbein et al., 1988; Babu et al.,

Table 1. Therapeutic agents specifically targeting glycolytic metabolism in tumours. Compiled from (Kroemer and Pouyssegur, 2008; Rodriguez-Enriquez et al., 2009; Tennant et al., 2010; Porporato et al., 2011; Vander Heiden, 2011; Granchi and Minutolo, 2012; Jones and Schulze, 2012).

Agent	Molecular target(s)	Effect	Current clinical status
Phloretin	GLUTs	Inhibition of glucose uptake	Preclinical
Sylibin	GLUTs	Inhibition of glucose uptake	Phase I/ II
2-DG	GLUTs HK	Inhibition of glucose uptake, Blocks glycolytic flux; ATP depletion	Phase I/ II
Lodamine	HKII MCTs	Blocks glycolytic flux Inhibition of lactate transport	Phase II/ III
3-BP	HKII GAPDH	Alkylating agent Blocks glycolytic flux Dissociates HK II from the mitochondria	Preclinical Translational study reported
3PO (and derivatives)	PFKFB3 GAPDH	Blocks glycolytic flux	Preclinical
Iodoacetate	GAPDH	Alkylating agent Blocks glycolytic flux	Preclinical
TLN232	PKM2	Peptidic inhibitor	Phase II
FX11	LDH	Blocks glycolytic flux	Preclinical
DCA	PDK	PDK inhibitor Reactivates PDH and redirects OXPHOS glucose metabolism from glycolysis towards	Phase I/ II
CHC	MCT	Inhibition of lactate transport	
AZD3965	MCT1	Inhibition of lactate transport	Phase I/ II

2-DG, 2-deoxyglucose; 3-BP, 3-bromopyruvate; 3PO, 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one; CHC, α -cyano-4-hydroxycinnamate; DCA, dichloroacetate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLUT, glucose transporter; HK, hexokinase; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; PFKFB3, bifunctional enzyme phosphofruktokinase2/fructose-2,6-bisphosphatase; PKM2, pyruvate kinase M2.

2011; Queiros et al., 2012; Birsoy et al., 2013).

3-bromopyruvate

3-BP is a pyruvate haloderivative that alkylates proteins, generally in the -SH group of cysteine residues, leading to the corresponding loss of functionality. Several studies have been published, reporting 3-BP toxic effect on cancer cells, both *in vitro* and *in vivo* (Ko et al., 2001, 2004; Kim et al., 2008; Davidescu et al., 2012; El Sayed et al., 2012; Icard et al., 2012; Nakano et al., 2012; Tang et al., 2012), without relevant side effects. Very recently, a translational study demonstrated that 3-BP is a powerful and specific anticancer agent in humans (Ko et al., 2012). 3-BP is known for the ability to inhibit cancer cell energy metabolism, depleting cellular ATP and leading to cell death, which could implicate autophagy (Davidescu et al., 2012), apoptosis (El Sayed et al., 2012) or necrosis (Bhardwaj et al., 2010), depending on the tumour type.

One of the main molecular targets of 3-BP is the glycolytic enzyme hexokinase II (HKII). This HK isoform is highly expressed in several tumours and is, similarly to MCTs, upregulated by HIF-1 α , favouring the “Warburg effect” (Wolf et al., 2011). Overexpression of HKII is related to poor prognosis, as glycolysis is the primary energy source used by cancer cells to sustain their uncontrolled cell growth. 3-BP induces a covalent modification of HKII, likely in cysteine residues, and dissociates it from the mitochondria, promoting the release of the apoptosis inducing factor (AIF) and triggering apoptosis (Chen et al., 2009). However, 3-BP is a highly reactive alkylating agent and is toxic in concentrations that are too low to inhibit HKII, indicating that it may have other targets in cancer cells (Pereira da Silva et al., 2009). At the mitochondrial level, it seems to affect not only the energy production coming from glycolysis, but also mitochondrial respiration, inducing a whole cell factory ATP depletion (Ko et al., 2001, 2004). Different reports also point to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the prime target for 3-BP (Ganapathy-Kanniappan et al., 2010, 2013; Tang et al., 2012). GAPDH is a glycolytic enzyme that catalyses the production of 1,3-bisphosphoglycerate from glyceraldehyde 3-phosphate and Pi, with simultaneous reduction of NAD⁺ to NADH. Although widely used as housekeeping control in several assays, since it is constitutively expressed in all tissues, GAPDH was found to be upregulated in different types of cancer, in agreement with its function in glycolysis (Guo et al., 2013). Like other proteins involved in the Warburg effect, its expression is induced by hypoxic conditions, in a process dependent of HIF-1 α transcription factor (Higashimura et al., 2011). The ability of 3-BP to inhibit Histone Deacetylase (HDAC) isoforms 1 and 3 leading to apoptosis in breast cancer cell line MCF-7, has also been shown (Thangaraju et al., 2009).

The selective uptake of 3-BP by cancer cells could

be the reason for such a specific effect. A first report revealed the involvement of SMCT1 on 3-BP uptake (Thangaraju et al., 2009). However, SMCT1 is a tumour suppressor protein found downregulated in most cancer types (Ganapathy et al., 2008). In opposition, MCT members, namely MCT1 and MCT4, contribute to the malignant phenotype of cancer cells and, as already mentioned, they are upregulated in several tumours (Pinheiro et al., 2012). Several reports attribute to MCTs the role of 3-BP transport and it is believed that they play a crucial role in the specific tumour-induced death effect of 3-BP. Queirós and collaborators demonstrated that butyrate pre-treatment of breast cancer cells upregulates plasma membrane expression of both MCT1 and MCT4 and simultaneously potentiates 3-BP antitumour activity, clearly indicating an association between MCT expression and 3-BP effect (Queiros et al., 2012). More recently, it has been reported that glutamine starvation also enhanced 3-BP cytotoxicity, through an increase in MCT1 stability (Cardaci et al., 2012). Also supporting the role of MCT1 in 3-BP transport is the inhibitory effect of CHC, which promoted a decrease in the cytotoxic effect of 3-BP in a squamous cell carcinoma bearing mouse model (Matsumoto et al., 2013). Further evidence for 3-BP as a substrate of MCT1 was given in a genome wide analysis of a mutated cells library exposed to 3-BP. Massive parallel sequencing identified MCT1 and its chaperone CD147 as the two most frequently inactivated genes in 3-BP resistant clones (Birsoy et al., 2013). In the same work, MCT1 stable expression in a cancer cell line devoid of MCT1 sensitized it to 3-BP treatment. Overall, these results indicate a clear role of MCTs in 3-BP cytotoxic effect, most probably by mediating its entrance into cancer cells.

Acetate derivatives: Dichloroacetate and Iodoacetate

Besides pyruvate and its derivatives, MCTs can mediate the transport of acetate too (Rae et al., 2012). DCA and IAA are antiglycolytic agents with antitumour activity that are acetate haloderivatives, being reasonable to expect that they could also be MCT substrates.

DCA is a compound already in clinical use to treat patients with mitochondrial deficiencies that develop lactic acidosis (Stacpoole et al., 1988). The target of DCA is pyruvate dehydrogenase kinase (PDK), a regulatory enzyme that phosphorylates and inhibits pyruvate dehydrogenase (PDH), a component of the pyruvate dehydrogenase complex (PDC) that catalyses the decarboxylation of pyruvate to acetyl-CoA, CO₂ and NADH. DCA mimics pyruvate and binds to its binding site in the N-terminal domain of PDK, inactivating it (Knoechel et al., 2006), which disrupts the Warburg effect by switching the glycolytic metabolism towards mitochondrial oxidative phosphorylation. DCA treatment reactivates PDH, leading to an increased entrance of acetyl-CoA in TCA. The aerobic glucose oxidation in cancer cells increases toxic mitochondrial

reactive oxygen species (ROS), damaging the mitochondrial complex I, and depolarizing the mitochondrial membrane, one of the earliest events in apoptosis (Heshe et al., 2011). DCA showed relevant cytotoxic effects in different types of cancers (Granchi and Minutolo, 2012), and its effect was even more pronounced *in vivo* than *in vitro* (Papandreou et al., 2011). Its safety in clinical cases has been demonstrated in patients with congenital or acquired lactic acidosis treated with DCA.

It has been reported that DCA must be used in a high concentration to be effective in cancer treatment

(Stockwin et al., 2010), much higher than that needed to inactivate PDK (Cooper et al., 1974, Whitehouse et al., 1974). Babu and collaborators (Babu et al., 2011) hypothesised that this could be due to the absence of a transport system, as DCA is ionized and, so, unable to cross the plasma membrane by simple diffusion. Indeed, mitoplatin, a dual anticancer agent constituted by one molecule of cisplatin with two molecules of DCA, is considerably more cytotoxic than DCA alone, even in cisplatin resistant cells. The DCA component induces mitochondrial dysfunction, overcoming cisplatin resistance, and the increased lipophilicity of the

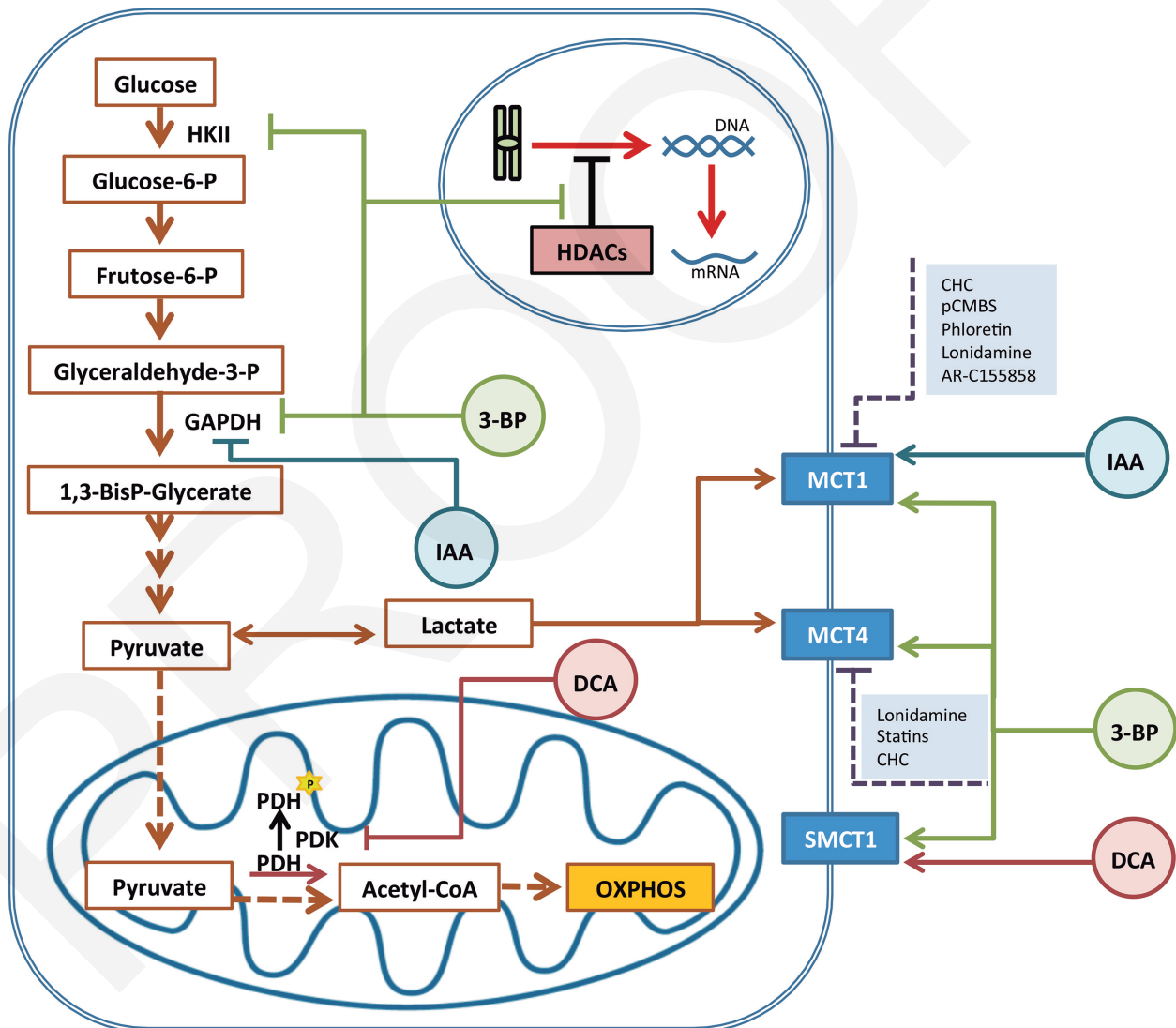


Fig. 1. Schematic representation of the pivotal role of MCTs in cancer: as lactate exporters to support the metabolic demands of tumours or as targets for cancer therapy using inhibitory compounds (blue boxes) or mediating the uptake of monocarboxylate analogues with anticancer properties. 3-BP: 3-Bromopyruvate; CHC: α -cyano-4-hydroxycinnamic acid; DBDS: 4,4-dibenzamidostilbene-2,2-disulfonate; DCA: dichloroacetate; DIDS: 4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; HDACs: histone deacetylases; HK: hexokinase; IAA: iodoacetate; MCT: monocarboxylate transporter; OXPHOS, oxidative phosphorylation; SMCT: sodium monocarboxylate transporter; PDH: pyruvate dehydrogenase; PDK: pyruvate dehydrogenase kinase.

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molecule allows DCA entrance in the cell (Dhar and Lippard, 2009). SMCT1 has been identified as responsible for DCA uptake, with high affinity (Babu et al., 2011) but it is epigenetically silenced in most tumour cells (Ganapathy et al., 2005), which could explain why high DCA concentrations should be used to achieve cytotoxicity in cancer cells. However, the MCT family members that are expressed in cancer cells display low affinity for DCA (Jackson and Halestrap, 1996). To our knowledge, no further report evidenced MCTs as being involved in DCA transport in cancer cells.

Few studies have been done concerning the effect of the antiglycolytic agent IAA in cancer, at least using *in vivo* assays, and even fewer concerning its uptake by cells. Like 3-BP, IAA is an alkylating agent that reacts with the -SH group of Cys149 from the active site of GAPDH (Harris and Walter, 1976; Granchi and Minutolo, 2012) and it has been shown to target key enzymes of the pentose phosphate pathway (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase), inhibiting the synthesis of cellular components essential for tumour growth and survival (Granchi and Minutolo, 2012). Although the anticancer action mechanism of IAA has not yet been entirely investigated, the different studies where IAA has been used showed its potential use as an anticancer agent (Bhardwaj et al., 2010; Sanchez-Arago and Cuezva, 2011). IAA is an acetate derivative and, like 3-BP or DCA, it may be transported by MCTs and/or SMCT1. However, in contrast to the other two compounds, no reports are published showing these transporters' influence on IAA activity. The only evidence that (S)MCTs can be involved in IAA transport is given in a report from 1988, where the addition of IAA promoted the efflux of lactate in human erythrocyte cells (Fishbein et al., 1988). Further investigation of IAA action in cancer cells and its transport through the plasma membrane may be important to develop novel anticancer therapies.

Final remarks

In conclusion, these data show that MCTs, especially MCT1 and MCT4 can be of great clinical value for cancer therapy, as they can be explored either as specific molecular targets or as drug transporters, opening new perspectives in cancer therapy (Fig. 1).

On one hand, the efflux of lactate and protons through monocarboxylic acids transporters allows the maintenance of cancer cell hyperglycolytic and acid-resistant phenotype, also creating an environment which promotes tumour cell invasion and metastization (Pinheiro et al., 2012). The upregulation of MCT isoforms 1 and 4, is, therefore, important for cancer survival, being important potential targets for cancer therapy. Lactate efflux via MCTs can be blocked using specific inhibitors or siRNA, leading to tumour regression. On the other hand, MCTs can behave as Trojan horses, mediating the transport of anticancer

drugs, resulting in cell death. From the candidate compounds currently known, 3-BP is the only confirmed MCT substrate so far, although we cannot exclude the possibility of MCTs being also involved in DCA and IAA uptake into cancer cells. Considering the upregulation of MCTs (the gate) and glycolytic enzymes/regulators (the targets) in several cancers, antiglycolytic compounds that are MCT substrates can be exploited as anticancer agents in the treatment of a wide range of cancers.

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