"Glucose deprivation stimulates monocarboxylate transporter 1 (MCT1) expression and MCT1-dependent tumor cell migration"

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ABSTRACT

The glycolytic end-product lactate is a pleiotropic tumor growth-promoting factor. Its activities primarily depend on its uptake, a process facilitated by the lactate-proton symporter monocarboxylate transporter 1 (MCT1). Therefore, targeting the transporter or its chaperon protein CD147/basigin, itself involved in the aggressive malignant phenotype, is an attractive therapeutic option for cancer, but basic information is still lacking regarding the regulation of the expression, interaction and activities of both proteins. In this study, we found that glucose withdrawal dose-dependently upregulates MCT1 and CD147 protein expression and their interaction in oxidative tumor cells. While this transcription-independent induction could be recapitulated using glycolysis inhibition, hypoxia, the mitochondrial uncoupler rotenone or hydrogen peroxide, it was blocked with alternative oxidative substrates and specific antioxidants, pointing out at a mitochondrial control. Indeed, we found that ...

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Glucose deprivation increases monocarboxylate transporter 1 (MCT1) expression and MCT1-dependent tumor cell migration

The glycolytic end-product lactate is a pleiotropic tumor growth-promoting factor. Its activities primarily depend on its uptake, a process facilitated by the lactate-proton symporter monocarboxylate transporter 1 (MCT1). Therefore, targeting the transporter or its chaperon protein CD147/basigin, itself involved in the aggressive malignant phenotype, is an attractive therapeutic option for cancer, but basic information is still lacking regarding the regulation of the expression, interaction and activities of both proteins. In this study, we found that glucose deprivation dose-dependently upregulates MCT1 and CD147 protein expression and their interaction in oxidative tumor cells. While this posttranslational induction could be recapitulated using glycolysis inhibition, hypoxia, oxidative phosphorylation (OXPHOS) inhibitor rotenone or hydrogen peroxide, it was blocked with alternative oxidative substrates and specific antioxidants, pointing out at a mitochondrial control. Indeed, we found that the stabilization of MCT1 and CD147 proteins upon glucose removal depends on mitochondrial impairment and the associated generation of reactive oxygen species. When glucose was a limited resource (a situation occurring naturally or during the treatment of many tumors), MCT1-CD147 hetero-complexes accumulated, including in cell protrusions of the plasma membrane. It endowed oxidative tumor cells with increased migratory capacities towards glucose. Migration increased in cells overexpressing MCT1 and CD147, but it was inhibited in glucose-starved cells provided with an alternative oxidative fuel, treated with an antioxidant, lacking MCT1 expression, or submitted to pharmacological MCT1 inhibition. While our study identifies the mitochondrion as a glucose sensor promoting tumor cell migration, MCT1 is also revealed as a transducer of this response, providing a new rationale for the use of MCT1 inhibitors in cancer.

Keywords: monocarboxylate transporter 1 (MCT1); basigin; glucose metabolism; mitochondrial dysfunction; reactive oxygen species (ROS); tumor cell migration

INTRODUCTION
Cancers evolve glycolysis as an aggressive metabolic phenotype allowing sustained energy production under hypoxia and unbridled cell proliferation when oxygen is present.1 From a metabolic standpoint, it is obvious that the optimal benefits of this glycolytic switch are reached in tumor areas where glucose is abundant and from which lactic acid, the end-product of glycolysis, is efficiently cleared. Otherwise, if glucose supply and/or lactic acid removal fall below a minimum threshold, tumor cells (TCs) at distance from the vascular supply eventually die, which occurs naturally or during the course of therapy, for example, with vascular-disrupting or anti-angiogenic agents. Three strategies allow TCs to face inappropriate conditions for glycolysis: (i) to switch back to an oxidative metabolism (at the expense of proliferation and as long as enough oxygen and oxidative substrates are present), (ii) to migrate towards more favorable soils, or (iii) to manage to improve glucose delivery and lactate removal. The latter strategy largely depends on the paracrine use of lactate.2

Lactate shuttles first account for a metabolic symbiosis in tumors wherein oxygenated TCs import lactate to fuel oxidative phosphorylation (OXPHOS) through the tricarboxylic acid (TCA) cycle.3 The preferential use of lactate by these cells allows glucose to diffuse farther from blood vessels at a maximal theoretical rate of 1 glucose molecule spared for 2 molecules of lactate used. It primarily depends on the expression and activity of monocarboxylate transporter 1 (MCT1), a passive lactate-proton symporter facilitating lactic acid uptake by oxidative TCs (whereas MCT4 channels lactic acid out of glycolytic TCs).4,5 Since lactate shunting is coupled with one proton, the use of MCT1 to fuel OXPHOS with lactate opposes extracellular acidification. A second contribution of lactate to the metabolic optimization of the tumor microenvironment is referred to as ‘lactate signalling’. In oxidative TCs and in endothelial cells, it shares with the metabolic lactate pathway the dependency on MCT1 and the need to oxidize lactate into pyruvate, but requires only a low rate of lactate uptake.6–8 While TCs increase their production of vascular endothelial growth factor in response to lactate,6,9 endothelial cells are triggered to upregulate the expression of vascular endothelial growth factor receptor 2, basic fibroblast growth factor, and interleukin-8,6–8 thus providing several paracrine and autocrine pathways through which lactate induces angiogenesis. Additional activities of lactate in tumors have been reviewed recently.9 Collectively, the use of lactate as a fuel and as a signaling agent offers an aggressive advantage to tumors, which is clinically supported by the positive correlation observed between high levels of lactate and tumor progression in several types of human tumors.10 Conversely, MCT1 inhibition has been well documented preclinically to exert...
antimetabolic and anti-angiogenic effects leading to hypoxic/glycolytic TC death by virtue of glucose starvation.\textsuperscript{3,6–8} A first orally-available MCT1 inhibitor having demonstrated preclinical safety is currently entering into clinical trials.\textsuperscript{1}

Despite the fact that MCT1 has emerged as a new anticancer target, there is only limited general knowledge about the molecular mechanisms governing its expression and activity. In the exercising muscle, transcriptional induction is exerted through calcium and AMP, as recently reviewed by Halestrap and Wilson.\textsuperscript{11} In cancer cells, gene expression may further be regulated by promoter methylation/inactivation.\textsuperscript{12} At the posttranslational level, the membrane expression and transport activities of MCT1 require its direct interaction with the scaffold protein CD147.\textsuperscript{13–15} The assembly of the complex exerts a stabilizing effect, which is necessary for the trafficking, cell surface localization and function of both proteins. In this study, we focused on tumor parameters and report that glucose deprivation posttranslationally stabilizes both MCT1 and CD147. This response molecularly involves reactive oxygen species (ROS) produced as a result of mitochondrial dysfunction. The consequent formation of functional MCT1-CD147 complexes confers to TCs the ability to evade a glucose-depleted environment. This study collectively provides an unprecedented pathway linking mitochondrial ROS production to the stimulation of MCT1-dependent TC migration towards glucose.

RESULTS
Glucose deprivation stabilizes MCT1 and CD147 protein expression in tumor cells
Limited glucose bioavailability is a key feature of the tumor microenvironment\textsuperscript{16,17} that we tested as a potential modulator of MCT1 and CD147 expression. It was experimentally induced by depriving cells of glucose in a medium containing no glutamine, pyruvate, lactate or serum, and an equal amount of viable TCs were systematically compared. As models, we used SiHa cells that were previously documented to rely on OXPHOS for energy production\textsuperscript{3,8} and HeLa cells known for their ability to switch from a glucose to a glutamine- (i.e., OXPHOS-dependent) fueled metabolism.\textsuperscript{18} In a first series of experiments, we observed a dose-dependent increase in MCT1 and CD147 protein expression in SiHa cells cultured during 48 h with decreasing glucose concentrations (Figure 1a). This inverse relationship was confirmed statistically by showing that glucose starvation caused a
significant increase in the expression of both proteins in SiHa (Figure 1b) and in HeLa (Supplementary Figure 1A) cells. In these experimental settings with SiHa, the glucose transporter and hexokinase competitor 2-deoxyglucose (2-DG, 5 mM, 24 h) did not recapitulate the response to glucose starvation but it significantly increased MCT1 expression in the absence of glucose (Figure 1b). We then focused on the mechanism. When compared to measurements in the presence of glucose, glucose withdrawal with 2-DG did not significantly modulate SLC16A1/MCT1 and CD147 mRNA expression over time (Figure 1c), ruling out a transcriptional effect. It rather stabilized both proteins posttranslationally. Indeed, the addition of cycloheximide (24 h) caused a decrease in MCT1 protein expression in the presence of glucose but not under glucose starvation where higher MCT1 levels were systematically detected (Figure 1d). Induction of CD147 was similarly preserved (Figure 1d) although cycloheximide also prolonged the expression of the protein in the presence of glucose, indicating that CD147 has a longer half-life than MCT1 in SiHa cells. Increased MCT1 and CD147 protein expression was observed when culturing the cells with the irreversible glycer-aldehyde-3-phosphate dehydrogenase (GAPDH) inhibitor 2-ioma-cetate (200 μM, 24 h, Figure 1e). A similar response was observed in HeLa cells (Supplementary Figure 1B). These data collectively indicate that disruption of glycolysis increases MCT1 and CD147 protein stability in oxidative TCs.

In the absence of exogenous glutamine, pyruvate, lactate and serum, glucose removal logically caused time-dependent SiHa cell death (Supplementary Figure 2A). This and the well-known property of glucose deprivation to promote mutations in TCs prompted us to test whether clonal selection could account for changes in MCT1 and/or CD147 expression. It was ruled out by showing i) that the reintroduction of glucose after a starvation period of 24 h (resulting in increased MCT1 and CD147 protein expression) restored basal protein expression levels (Supplementary Figure 2B), and ii) that the glucose starvation of 2 independent clones isolated from the total SiHa population caused a significant increase in the expression of both proteins (Supplementary Figures 2C-D).

Mitochondrial activity controls MCT1 and CD147 expression in tumor cells

Lack of glucose or inhibition of glycolysis reduces mitochondrial anaplerosis. A potential involvement of mitochondria in the control of MCT1 and CD147 expression was first addressed by showing that hypoxia (1% O2, 24 h) triggers the expression of both proteins in SiHa (Figure 2A) and in HeLa (Supplementary Figure 1B) cells. Glucose deprivation, glycolysis inhibition and hypoxia share the common property of impairing OXPHOS. We therefore tested whether direct OXPHOS inhibition could impact MCT1 and CD147 protein expression by treating SiHa cells with the respiratory chain complex I inhibitor rotenone (1 μg/ml). Rotenone induced a ~2-fold and a ~1.4-fold increase in MCT1 and CD147 expression, respectively (Figure 2b). Similar data were obtained with HeLa cells (Supplementary Figure 1B). To test whether restoring OXPHOS could repress the increase in MCT1 and CD147 protein expression, we took advantage of our glucose starvation model. SiHa cell death upon glucose removal was rescued with lactate (Supplementary Figure 2A), a substrate known to efficiently replace glucose as an oxidative fuel for these cells. Western blots further showed that lactate totally suppressed the induction of MCT1 and CD147 expression that was otherwise detected 48 h after glucose removal (Figure 3a). It was confirmed using the two SiHa clones (Supplementary Figure 2E) and HeLa cells (Supplementary Figure 2F). Conversely, lactate in the presence of glucose did not change the expression of both proteins (Supplementary Figures 3A, B showing that increasing doses of lactate did not modulate MCT1 expression in the presence of glucose). As with lactate, the delivery of the oxidative substrate glutamine to glucose-starved SiHa cells blunted MCT1 and CD147 protein upregulation, whereas glutamine did not exert such influence in the presence of glucose (Figure 3b). In fact, refueling mitochondria prevented the response to glucose starvation irrespectively of the molecular nature of the fuel. This was further demonstrated using the cell-permeable esterified forms of the TCA cycle substrates pyruvate and succinate: in the absence of glucose, either methyl-pyruvate or dimethyl-succinate successfully blocked the induction of MCT1 and CD147 protein expression in SiHa (Figure 3c) and in HeLa (Supplementary Figure 2G) cells. In the presence of glucose, these compounds did not modulate the basal expression of both proteins (Supplementary Figure 3C).

Mitochondrial ROS mediate the induction of MCT1 and CD147 protein expression upon glucose starvation

Dysfunctional mitochondria are a main source of ROS, among which superoxide is the initial species. It can be measured in intact cells using flow cytometry with MitoSOX, a superoxide-specific fluorogenic probe with a high tropism for mitochondria. Glucose starvation (Figures 4a and b), glycolysis inhibition with iodoacetate (Figure 4c) and rotenone (Figure 4d) at the doses used in our previous experiments all independently triggered superoxide production in SiHa cells. More particularly, we documented a ~3-fold increase in the mitochondrial superoxide production of
Glucose starvation promotes the formation of MCT1-CD147 heterocomplexes at the plasma membrane of tumor cells. As MCT1 and CD147 underwent a concerted regulation in all experimental conditions, it was important to address the formation of functional heterocomplexes. Information was provided using immunocytochemistry: glucose deprivation dose-dependently caused an increase in the expression of MCT1 and CD147 at the plasma membrane of SiHa cells where both proteins colocalized (Supplementary Figure 5A). Direct protein-protein interaction was imaged using proximity ligation assay. SiHa cells incubated in a glucose-deprived medium during 24 h showed a ~2-fold increase in MCT1-CD147 interactomes compared with cells incubated in glucose-containing medium (Figure 5a). In both treatment conditions, heterocomplexes were located in the cytosol and at the plasma membrane, including in F-actin-positive protrusions, and their abundance increased at all locations in the absence of glucose (Figure 5b and Supplementary Figure 5B).

MCT1 facilitates tumor cell migration towards serum and glucose. Filopodia are sensory organelles used by invasive cancer cells to probe their microenvironment during migration.25 These cell protrusions are characterized by the presence of actin filaments. Upon glucose starvation, the apparent increase in MCT1-CD147 heterocomplexes in such structures among other locations (Figure 5b and Supplementary Figure 5B) prompted us to test the contribution of MCT1 to TC migration. In transwell assays, we first documented a pro-migratory role of MCT1 towards serum: in our experimental settings, targeting MCT1 with two different specific shRNAs impaired SiHa and HeLa cell migration by ~2- to 4-fold compared to the cells infected with a control shRNA (Figure 6a, target extinction is shown in Supplementary Figure 5A). Based on this, we functionally assayed the metabolic control of MCT1 and CD147 expression and interaction by studying TC migration towards glucose. In transwells, shCTR-expressing SiHa (Figure 6b) or HeLa (Figure 6c) cells starved from glucose in the upper well migrated towards glucose present in the lower well. In the absence of MCT1, the specific migration of both cell lines was absent in both wells of the assay compared to the situation where glucose was present in both, and lactate did not impact the migration of glucose-fueled SiHa cells (Figure 6d). It is of note that, contrary to glucose, neither lactate nor glutamine acted as a chemoattractant for SiHa cells in our experimental conditions (Supplementary Figure 5B).

The involvement of ROS in the migratory phenotype was probed with NAC (5 mM), which in transwells inhibited the specific but not the basal migration of SiHa and HeLa cells towards glucose-starved compared to glucose-fueled SiHa cells (Figures 4a and b). Lactate in the absence of glucose prevented the increase in superoxide production (Figure 4a) as also did the more direct TCA cycle intermediates methyl-pyruvate and dimethyl-succinate (Figure 4b), correlating with the MCT1 and CD147 protein data shown in Figures 3a and c, respectively.

That ROS can stimulate MCT1 and CD147 protein expression was established by showing a ~2-fold increase in the expression of both proteins 24 h after treatment with 0.8 mM H$_2$O$_2$ (Figure 4e). In line with what was observed upon glucose removal (Figure 1c), we did not detect significant changes in the abundance of the corresponding transcripts (Figure 4f). Furthermore, as with glucose starvation (Figure 1d), the induction of both proteins was preserved upon treatment with cycloheximide (Figure 4g), revealing that glucose withdrawal and ROS share the ability to stabilize MCT1 and CD147 proteins posttranslationally. We therefore sought to document a direct link. The general antioxidant and glutathione analogue N-acetyl-L-cysteine (NAC, 5 mM) blocked MCT1 and CD147 overexpression in glucose-starved SiHa cells (Figure 4h). A similar effect was observed with the mitochondria-selective superoxide scavenger MitoTEMPO (50 µM) (Figure 4i), thus confirming that ROS produced by mitochondria when they are deprived from glycolytic fuels trigger the expression of both the lactate transporter and its chaperone. ROS were also key contributors to the response to hypoxia: NAC or the H$_2$O$_2$ scavenger ebselen (20 µM) blocked hypoxia-induced MCT1 expression (Supplementary Figure 4A). None of the antioxidants influenced MCT1 and CD147 protein expression in glucose-fueled, normoxic TCs (Supplementary Figures 4A–C).
Figure 4. Mitochondrial ROS mediate the upregulation of MCT1 and CD147 in glucose-starved tumor cells. (a–d) Flow cytometry and the fluorogenic probe MitoSOX²⁵ were used to detect mitochondrial superoxide in SiHa cells. Histograms show the mean fluorescence intensity of independent measurements with 50,000 cells (%). Cells were cultured during 24 h with, where indicated, (a) glucose and sodium i-lactate (ns, P > 0.05, ***P < 0.005 compared to medium containing glucose but no lactate; **P < 0.01 compared to glucose- and lactate-deprived medium), (b) glucose, methyl-pyruvate and/or dimethyl-succinate (***P < 0.005 compared to medium supplied only with glucose; **P < 0.01, **P < 0.005 compared to glucose-deprived basal medium; n = 4), (c) 2-iodoacetate (IA, 200 μM) (**P < 0.005, n = 4–5), or (d) rotenone (1 μg/ml) (**P < 0.005, n = 4–5). Glucose (25 mM) was systematically present in (c) and (d). (e) Immunoblots and the bar graph represent MCT1 (left) and CD147 (right) protein expression in glucose-supplied SiHa cells cultured during 24 h with or without H₂O₂. β-actin was used as a loading control. (P < 0.05, n = 6). (f) Same treatments as in (e) but detecting SC16A1/MCT1 (left) and CD147 (right) mRNA expression (ns, not significant, n = 3). (g–l) Immunoblots are representative of 3 independent experiments and show MCT1, CD147 and β-actin expression in SiHa cells after 48 h of culture with, where indicated, (g) H₂O₂ and cycloheximide always in the presence of glucose (25 mM), (h) glucose and N-acetyl-l-cysteine (NAC), or (i) glucose and the mitochondrial-selective superoxide scavenger MitoTEMPO.²⁶ In (h and i), low exposition of the film was used to show the antioxidant effect on MCT1; MCT1 expression with glucose alone was detected with longer exposure times (not shown).

DISCUSSION

While the monocarboxylate transporter MCT1 has emerged as a new anticancer target and a first MCT1 inhibitor is currently entering clinical trials, little is known about its regulation by typical parameters of the tumor microenvironment.¹³,¹²,¹⁴ This basic information, however, is essential for the prediction of treatment sensitivity and resistance as well as for the rational design of combination therapies. In this study, we report that mitochondria deprived from glycolytic fuels stabilize the expression of MCT1 and of its chaperone protein CD147/basigin in human TCs, thus providing a functional complex known to be involved in the transport of monocarboxylic acids (through MCT1) and in the activation of matrix metalloproteinases (through CD147).¹³,¹⁴,¹⁵ Upon glucose starvation, the complex promoted TC migration towards serum and glucose. Our study identifies a functional link between impaired mitochondrial activity and TC migration wherein the mitochondrion acts as a glucose sensor and signals via ROS MCT1-dependent TC escape to a more favorable metabolic environment.

A disruption of the functional coupling between the TCA cycle and OXPHOS is a fundamental metabolic characteristic of cancer.¹² It is primarily achieved when TCs outgrow their oxygen and nutrient supply, marking the switch to a glycolytic metabolism. It is also typical of TCs constitutively addicted to glycolysis (the Warburg Effect). Here, using well-characterized oxidative human TCs³,⁸ we observed that both glucose starvation and hypoxia stimulate MCT1 and CD147 expression in vitro (Figures 1 and 2). These conditions are typical of the tumor microenvironment. While hypoxia has been extensively characterized to be naturally associated with tumor progression, fewer yet convincing data are currently available reporting natural glucose depletion in tumors. In experimental settings, Walenta et al. used bioluminescence to evidence a progressive decline of glucose towards the core of rat mammary tumors. Others using capillary electrophoresis coupled to mass spectrometry reported levels of glucose as low as 123 ± 43 mM/g and 424 ± 131 mM/g in patient biopsies of colon and stomach cancer, respectively.¹⁷
These concentrations were 45-times (colon) and 13-times (stomach) lower than that in the corresponding normal tissues, supporting the existence of a steep natural gradient of glucose deprivation in clinical tumors. Glucose and oxygen deprivation is further relevant to cancer therapy and the primary aim of anti-angiogenic and antivascular treatments. According to our in vitro results (Figure 1 and Supplementary Figure 5), limited glucose availability can stabilize MCT1-CD147 complexes in a dose-dependent manner and, consequently, promotes MCT1-dependent TC migration (Figure 6). The effect is posttranslational (Figure 1d) and as such may involve a reduced turnover rate of both proteins known to be subject to endosome-dependent recycling. These responses to low glucose further explain the metastatic stimulation that can be associated with the use of anti-angiogenic drugs and the higher expression of MCT1 in metastatic lesions compared to primary tumors in patients.

Conversely, increased MCT1 expression promotes the anticancer activity of 3-bromopyruvate, an alkylating agent strictly dependent on MCT1 for its uptake, thus offering a new rationale for combination therapy with anti-angiogenic or antivascular drugs.

In the absence of other nutrients, limited glucose availability deprives mitochondria of essential fuels and promotes oxidative stress. Cell survival then relies on autophagy and the ability to counter excess ROS production. Here, we show that these survivor cells overexpress MCT1 and CD147 independently of clonal selection. It may therefore be viewed as an integral part of cell adaptation to low glucose. The molecular response was mimicked by inhibiting glycolysis in otherwise oxidative TCs (Figure 1e and Supplementary Figure 6A) or by inducing OXPHOS inhibition (Figure 2). Others have recently shown that glutamine deprivation and the higher expression of MCT1 in metastatic lesions compared to primary tumors in patients.

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scavenger mitoTEMPO\textsuperscript{23} and of the glutathione analogue NAC further revealed mitochondria-derived H\textsubscript{2}O\textsubscript{2} as an important mediator of the phenotype. Inhibition was not complete though, suggesting the existence of additional pathways impacting MCT1-CD147 stability (potentially including those active upon glutamine deprivation)\textsuperscript{23} that would nevertheless exert a minor contribution under glucose deprivation as in this case NAC almost totally blocked the complete migration (Figure 6e). In other circumstances, ROS could be bypassed. Indeed, overexpressing MCT1-CD147 experimentally was sufficient to promote TC migration in a ROS-independent manner (Figure 6f). As CD147 beyond MCT1 associates with a number of partners (including MCT4) to form supercomplexes,\textsuperscript{39} additional and perhaps more indirect influences cannot be excluded. Furthermore, additional controls would be involved under hypoxia,\textsuperscript{30—42} explaining why hypoxia can fail to induce or can even repress MCT1 expression in vitro,\textsuperscript{43} whereas it stimulates MCT1 expression in vitro\textsuperscript{14} (Figure 2a), and why we detected increased MCT1 expression at early time points (Figure 2a, 24 h) whereas others did not find MCT1 induction upon a longer exposure to hypoxia (48 h).\textsuperscript{5} Modulating ROS can indeed impact the response of MCT1 to hypoxia (Supplementary Figure 4A).

Interestingly, heterocomplexes of CD147 with MCT4 (primarily involved in monocarboxylate export)\textsuperscript{6,5} have been previously reported to colocalize with \(\beta1\)-integrin at the leading edge lamellipodia of migrating cells,\textsuperscript{45} supporting a role of MCTs in migration. Here, we documented that MCT1-CD147 complexes that accumulate in glucose-deprived cells are present among other locations in such protrusions (Figure 5 and Supplementary Figure 5) and regulate the migratory phenotype (Figure 6). Two main modes of action can be envisioned. On the one hand, within a gradient of glucose but no exogenous lactate, MCT1-expressing cells migrated towards serum (Figure 2a), and why we detected increased MCT1 expression at early time points (Figure 2a, 24 h) whereas others did not find MCT1 induction upon a longer exposure to hypoxia (48 h).\textsuperscript{5} Modulating ROS can indeed impact the response of MCT1 to hypoxia (Supplementary Figure 4A).

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TCS will tend to increase their intracellular pH while decreasing the extracellular pH in the subcellular locations where MCT1 is expressed. This in turn may facilitate the activity of pro-migratory and pro-invasive enzymes and transporters, including matrix metalloproteinases.46,47 On the other hand, the MCT1-CD147 complex is known to behave as a scaffold for other proteins involved in cell migration and invasion.48 The composition of these supercomplexes is cell-type dependent and could logically be influenced by the cell environment and MCT1 expression and activity (conformation change).

In conclusion, our study shows that TCS in low glucose accumulate stabilized and functional MCT1-CD147 complexes that facilitate TC migration towards glucose. This paradigm offers a rationale for clinical data associating high MCT1 and CD147 expression and metastatic phenotype promoted by therapies limiting glucose availability in tumors.11 We propose MCT1 inhibition as a pharmacological option to counter these effects.

MATERIALS AND METHODS

Cells and reagents
SiHa human squamous cell carcinoma and HeLa human epithelial cervix cancer cells (ATCC, Manassas, VA, USA) were routinely cultured in DMEM containing D-glucose (4.5 g/l), FBS (10%) and penicillin-streptomycin (1%). SiHa clones A and B were isolated from the wild-type cell population in situ and for the metastatic phenotype promoted by therapies limiting glucose availability in tumors.11 We propose MCT1 inhibition as a pharmacological option to counter these effects.

Western blotting and immunostainings
For immunoblotting, the lysates of an equal number of viable cells were separated on SDS-PAGE and transferred onto PVDF membranes with a distinct expression pattern. Briefly, upper wells were loaded with 50 000 cells in 50 μl of medium containing no serum while the lower well always contained 0.1–0.3% of FBS in the same medium standardized for each batch according to basal migration activities. Where indicated, upper and/or lower wells were further supplemented with glucose, lactate or glutamine. After overnight incubation, migrated cells on the polycarbonate membrane interface were fixed with methanol and stained with crystal violet. They were counted under a microscope. Statistical analysis
Data are expressed as mean ± s.e.m. (error bars are sometimes smaller than symbols). Student’s t test, 1-way ANOVA (Bonferroni post-hoc test) or 2-way ANOVA (Bonferroni post-hoc test) were used where appropriate. P < 0.05 was considered statistically significant.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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