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Tumor metabolism of lactate: the influence and therapeutic potential for MCT and CD147 regulation

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Abstract

Tumor metabolism consists of complex interactions between oxygenation states, metabolites, ions, the vascular network and signaling cascades. Accumulation of lactate within tumors has been correlated with poor clinical outcomes. While its production has negative implications, potentially contributing to tumor progression, the implications of the ability of tumors to utilize lactate can offer new therapeutic targets for the future. Monocarboxylate transporters (MCTs) of the *SLC16A* gene family influence substrate availability, the metabolic path of lactate and pH balance within the tumor. CD147, a chaperone to some MCT subtypes, contributes to tumor progression and metastasis. The implications and consequences of lactate utilization by tumors are currently unknown; therefore future research is needed on the intricacies of tumor metabolism. The possibility of metabolic modification of the tumor microenvironment via regulation or manipulation of MCT1 and CD147 may prove to be promising avenues of therapeutic options.

Keywords

Cancer; CD147; lactate; MCT1; MCT1 inhibitors; MCT4; tumor metabolism

Much of the past research on lactate in cancer has focused on its production. Under the influence of hypoxia, glucose is catabolized to lactate via glycolysis to yield ATP; mitochondria cannot produce ATP in oxidative phosphorylation without oxygen to aid in electron transport. This type of production is termed the 'Pasteur effect.' Cancer cells can also produce lactate under aerobic conditions. The switch from a 'normal' metabolic profile, consisting of glycolysis followed by oxidative phosphorylation, to cytosolic glycolysis in the presence of adequate oxygen, is termed the 'Warburg effect.' The discovery of aerobic glycolysis resulting in increased lactate production within tumors in the 1920–1930s by Otto Warburg led to a reevaluation of the potential role and significance of lactate in cancer studies [1–4]. Since the pioneering work of Warburg, production and accumulation of lactate has been documented to have clinical significance in a number of different cancer types. In the 1970s, lactate metabolism in malignant metastatic carcinomas and colorectal cancers was beginning to be explored with blood samples from patients that were injected or infused with radio-labeled glucose and pyruvate [5,6]. These studies indicated that lactate levels in plasma and venous

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blood were elevated in patients with metastatic cancer [6]. Nuclear magnetic resonance (NMR) studies following the fate of ^{13}C -labeled lactate are still used today.

In the 1980s, quantitative bioluminescence imaging was used to assess metabolites such as lactate and glucose from flash-frozen biopsies to study ischemia in brain tissue [7,8]. This technique was later applied to assess these same metabolites in cancer [9–11]. More recent studies investigating lactate accumulation in human tumors have found that cervical tumors, head and neck cancers and rectal adenocarcinomas, with metastatic spread, exhibited a wider range and significantly higher levels of lactate than nonmetastatic tumors [12–14]. Subsequently, it was found that elevated lactate levels correlate with poorer patient prognosis, poor disease-free or metastasis-free survival and poor overall survival in human cervical cancers [15], head and neck cancer [16], high-grade gliomas [9,17,18] and non-small-cell lung cancer [19] (Table 1). Mean lactate content can vary widely between individual tumors, even if tumors are of the same size, grade or entity [13,15]. The reason and mechanisms behind this variation are still poorly understood; further research on monocarboxylate transporters' (MCTs') functions and interactions within the tumor microenvironment could help to explain this variation. Elucidating the intricacies of lactate metabolism and MCT interaction, we may have the potential to modify the metabolism of the tumor. Coaxing tumors to utilize lactate (decreasing its accumulation) or finding ways to reduce glycolysis and lactate formation may affect tumor aggressiveness and invasiveness. In summary, lactate has been demonstrated to be a prognostic indicator across a wide variety of tumors and types of cancer; this feature makes lactate metabolism of interest for further investigation, not only as a biological marker, but also as a potential therapeutic end point or target.

Characteristics of the tumor microenvironment

Tumor microenvironment pathology is influenced by several key physiological factors: oxygen transport, vascular structure/perfusion, nutrient and metabolite transport and pH. Oncogenic and tumor suppressor genomic regulation also greatly contribute to the phenotype [20,21], but will not be emphasized in this review. Features of tumor physiology that affect oxygen transport can be summarized in 'seven points of regulation', as described by Dewhirst *et al.* [22]. Compared to normal tissues, these points are:

- The steep longitudinal oxygen gradients within the tumor;
- Increased intravascular gradients;
- Shunt flow in the vascular structure to evade the tumor tissue;
- Lower vascular density;
- Inefficient and unorganized vascular orientation and structure;
- Imbalance between oxygen consumption and delivery rates;
- Slower blood flow due to increased blood viscosity from intravascular hypoxia [22].

While these effects develop gradually, temporal oxygen fluctuations can occur in a matter of minutes [23,24].

A consequence of tumor hypoxia is the upregulation of the α -subunit of the heterodimer hypoxia-inducible transcription factor (HIF-1). Both the overlying physiological and the underlying temporal influences promote hypoxia; in turn, HIF-1 α is stabilized [25]. HIF-1 α participates in genetic regulation and signaling cascades that control angiogenesis, invasiveness, oxidative stress resistance, treatment-resistance and the glycolytic switch to anaerobic metabolism [22,26].

The physiological influences mentioned above, paired with upregulation of genetic factors that promote glycolysis, lead to altered ionic and metabolic gradients within the tumor [27]. Spatial relationships between vasculature, ATP and metabolites exist within the tumor; lactate accumulation often occurring within areas of hypoxia and/or necrosis [28]. Metabolic conditions exacerbate and induce prosurvival pathways through transcriptional or translational regulation of tumor suppressor genes and oncogenes. This can result in cancer cell proliferation due to loss of proapoptotic factors and an increase in prosurvival and growth signaling [29].

What has become more apparent in the last few decades of metabolism research is that lactate is more than merely a byproduct of glycolysis; it has the ability to influence angiogenesis, expression of surface molecules, genetic response, ATP generation and cell migration.

Lactate metabolism introduction

There are five requisite conditions for lactate oxidation:

- Lactate must enter the cell or be produced internally by the cell;
- The movement of lactate in (and out) of the cell requires a membrane-bound monocarboxylate transporter, such as MCT1;
- Lactate dehydrogenase (LDH) is required to convert lactate to pyruvate before it can enter into the tricarboxylic acid (TCA) cycle;
- Adequate concentration of oxygen is required – lactate cannot be consumed anaerobically;
- Healthy, functional mitochondria are required to carry out the TCA cycle and the electron transport chain (ETC).

Beyond this, there are other secondary influences on lactate metabolism. For example: pyruvate dehydrogenase complex (PDC) regulation by phosphorylation via pyruvate dehydrogenase kinase (PDK); PDK inhibitors/phosphatases determine if pyruvate is converted to acetyl coA for the first step of TCA cycle; and regulation of glycolysis via phosphofructokinase (PFK) and other glycolytic enzymes or intermediaries will contribute to overall cell metabolism. Other factors to mention are pH, ratios of ATP:ADP, AMP:cAMP and NAD⁺:NADH, and 5' adenosine monophosphate-activated protein kinase (AMPK) and inorganic phosphate (Pi) levels [30,31]. Lactate utilization can encompass oxidation for energetic purposes, but utilization also includes amino acid production or shuttles for anabolic purposes.

Monocarboxylate transporter family

Proton-coupled monocarboxylate transporters are 12-span transmembrane proteins with N-terminus and C-terminus in the cytosolic domain. While there are 14 members/subtypes of MCTs coded by the *SLC16A* gene family, not all of these are currently characterized [32]. MCTs account for 70–90% of muscle lactate transport [33]. While it could be said that the distinguishing common feature among members of this family was the transport of monocarboxylates (lactate, pyruvate, butyrate, acetate and propionate), it has been found that certain subtypes have the ability to transport a wider range of substrates such as ketone bodies, some aromatic amino acids, and even some pharmacological agents, such as γ -hydroxybutyrate (GHB) and statins, among others [34,35]. Other members, MCT6, MCT8 and MCT10, will transport substrates such as diuretics, thyroid hormones T3 and T4, and aromatic amino acids, respectively [32,34].

Of the characterized family members of *SLC16A*, MCT1 is reported to have the most ubiquitous tissue expression [32] (Table 2). Other subtypes demonstrate tissue-specific expression.

Expression and regulation of MCT1, MCT2, MCT3 and MCT4 have been reported in a wide variety of cancers [36–44]. For brevity and clarity, we will be addressing the subtypes of proton-coupled MCTs 1–4 best characterized in human tissue, largely focusing on MCT1 and MCT4 with only passing mention of MCT2 and MCT3 when relevant.

Sodium-coupled MCTs (SMCTs) of the *SLC5A* gene family were discovered in the early to mid 2000s, namely *SLC5A8* [45,46] and *SLC5A12* [47]. SMCTs transport similar substrates as many of the *SLC16A* MCT family members; however, they show differences in patterns of tissue distribution and their potential roles in cancer or cancer progression [34,48]. *SLC5A* members will be mentioned when relevant, but the focus for this review will be *SLC16A* MCTs.

Lactate consumption in normal tissue

Studies on direct utilization of lactate in normal cells can provide a window into the potential metabolic processes in cancer cells; therefore, we will briefly explore lactate metabolism in normal tissue. Lactate movement and metabolism as participating in a cell-to-cell shuttle system was first intimated by the work of Carl and Gerty Cori with their discovery of the Cori cycle, a cycle in which lactate picked up from the bloodstream by the liver undergoes gluconeogenesis [49]. Later, in 1984, Brooks initiated the new wave of lactate shuttle research [50]. The basic observations of lactate transport and utilization were first studied in muscle. From here, as Gladden summarizes [51], the lactate shuttle theory has grown to include the cell-to-cell lactate shuttle within muscle, intracellular lactate shuttle [52–54], the astrocyte–neuron lactate shuttle [55–58], a lactate–alanine shuttle [59,60], a peroxisomal lactate shuttle [61] and the spermatogenic lactate shuttles [62].

Lactate utilization in muscle

Muscle cells have the ability to produce and export lactate, as well as import and directly utilize lactate. During exercise, myocytes, which normally participate in oxidative phosphorylation, will begin to undergo glycolysis as the energy demand increases and oxygen supply decreases. Cells will export the accumulated intracellular lactate concentrations. Lactate will filter into the bloodstream and enter the liver, primarily, to undergo the Cori cycle for gluconeogenesis [31,49]. What is of more interest for this review is the ability of myocytes to directly consume lactate.

Myocytes are efficient in their ATP generation, utilizing glucose, lactate, fatty acids and ketone bodies as energy sources. Briefly, the muscle–lactate shuttle occurs when lactate generated and excreted by exercising muscle is taken up by other muscle cells via bloodstream delivery, or directly by nearby resting muscle. Of the lactate generated during exercise, 55–75% will be oxidized by muscle [63]. Muscle cells express both MCT1 and MCT4, although the expression pattern and regulation in tissue is isoform-specific. Expression of MCT1 ($K_m \sim 3.5$ mM for lactate) is significantly higher in heart [64] and oxidative muscle fibers compared with glycolytic muscle fibers, and strongly correlates with lactate uptake [65], while MCT4 ($K_m \sim 34$ mM for lactate) expression is seen primarily in glycolytic muscle [64]. Lactate accounts for up to 40% of oxidative substrate utilized by the heart at rest, and this portion climbs to 60% with work [66]. Experiments in rabbits demonstrate that oxidative myocytes take up and oxidize lactate at 2.5 mM, while glycolytic myocytes use lactate for gluconeogenesis and will not take it up until the concentration reaches 4 mM [67]. Other studies have demonstrated that lactate transport capacity in slow twitch muscle fibers is nearly twice that of fast twitch fibers [33].

Tumor cells may demonstrate similar heterogeneity in the ability to take up and utilize lactate; some tumor types may take up lactate as a threshold mechanism at high lactate concentrations, while others may be avid consumers, utilizing as little or as much lactate as they are exposed

to without the requirement for high concentrations to force uptake [68]. Similar to muscle, this may depend on which MCTs are expressed on the cell surface.

Lactate uptake rates

Many of the K_m values cited for MCT sub-type 1 and 4 are relatively consistent among the literature. Determination of K_m values for lactate uptake can be found in studies conducted with *Xenopus laevis* oocytes. *In vitro*, oocytes expressing MCT1 demonstrated a rate of 828 ± 79 pmol/10 min for uptake of 5 mM lactate [69]. In a separate study, MCT4-expressing oocytes took up lactate at a rate of 323 ± 25 pmol/10 min per oocyte when incubated with 1 mM lactate, which is still higher than oocytes without MCT4 expression (60 ± 3 pmol/10 min) [70]. When comparing one MCT subtype to another, many studies use their K_m or V_{max} values as a basis for comparison. As stated above, MCT1 as well as MCT2 both have a lower K_m than MCT4 for lactate, illustrating that subtype 1 and 2 have a greater affinity for lactate than MCT4 [32]; however, the affinity for lactate in MCT4 can be increased (and the K_m decreased) by exposure to lower pH values. When *X. laevis* oocytes were incubated in a pH of 7, 6 and 5 the MCT4 K_m value for lactate dropped from 34 ± 5 mM to 10 ± 2 mM to 1.4 ± 0.5 mM, respectively [70].

Lactate utilization in brain

Although glucose was thought of as the primary energy substrate of normal, healthy brain tissue, recent studies have found that brain tissue is an avid consumer of lactate, with the capability of taking up lactate from the bloodstream after exercise, as well as direct consumption by neurons from astrocytic production of lactate [71]. Neurons account for approximately 90–95% of energy consumption as compared to glial cells, and there is evidence that lactate is a preferential substrate to glucose by neurons [72]. Lactate and glucose administered in equimolar concentrations *in vitro* are utilized for neuronal oxidative metabolism, 90% (direct lactate) and 10% (glucose-derived pyruvate), respectively [57]. In a metabolic model within the brain, astrocytes, which express MCT1 and MCT4, undergo aerobic glycolysis, exporting a considerable amount of lactate into the extracellular space. The lactate is then taken up to be used as an energy source by neighboring neurons via the lactate transporter, MCT2 (K_m for lactate ~ 0.7 mM) [32] expressed on the surface [73].

Not all lactate is of equal energetic value. Studies in the early 2000s have demonstrated that neurons in culture have a kinetic preference for extracellular lactate over intracellular lactate (or pyruvate) generated via glycolysis from previously acquired glucose [74]. This supports a two-compartmental theory of lactate utilization (discussed in more detail below). To support the assertion that lactate is being used as an energy substrate and not merely shuttled back out of the cell, NMR studies in neurons have demonstrated the output of labeled TCA cycle intermediates and amino acids following administration of ^{13}C -lactate [60]. Further evidence for lactate as a preferential substrate for brain comes from *in vivo* studies with NMR, demonstrating decreased utilization of glucose after raising plasma lactate levels [75,76].

Lactate in wound healing

Induction of cell proliferation and intracellular oxidant production are seen in fibroblasts exposed to exogenous lactate during wound healing [77]. This induction of oxidants may be due to increased oxidation of lactate through oxidative phosphorylation, generating the free radicals and ATP necessary to meet the energetic demands of wound healing. During wound healing, lactate functions to stimulate production of VEGF, as well as a number of cytokines and growth-promoting factors that assist in angiogenesis and collagen synthesis [78].

The growth-promoting effects of lactate that are seen in wound healing likely occur within the tumor environment as well; lactate may act as a growth or survival signal to the surrounding stromal fibroblasts in tumors with lactate accumulation.

Lactate utilization in tumors

Although a majority of studies on lactate in relation to cancer have predominantly focused on its presence and accumulation, a number of studies have found that tumor cells have the ability to take up lactate and utilize it for energetic purposes as well as amino acid formation. Lactate uptake is not to be confused with its metabolism to generate ATP, as it is possible that the cell can take up lactate that may then participate in the lactate–alanine shuttle or another mechanism that is not related to energetic metabolism. Here we will focus on its uptake and metabolism with mention of the lactate–alanine shuttle when appropriate. Lactate uptake occurs *in vitro* in cervical cancer SiHa cells [68] and breast cancer MDA-MB-231 cells in a pH-dependent manner [79]. If lactate uptake in tumor tissue is similar to muscle, areas with increased blood flow would show greater uptake [80]. While muscle and brain tissue may be able to make use of lactate as an efficient substrate, the microenvironment of a tumor often does not have adequate oxygenation or an efficient and functional vascular network. Therefore, tumor utilization of lactate is dependent upon oxygen availability, lactate concentrations, presence of healthy mitochondria and appropriate MCT subtype expression for lactate uptake [50,81].

In 1991, metabolic studies were conducted on EMT6/Ro multicellular spheroids. A benefit of this method is that the spheroid mimics tumor micro-areas *in vivo*. Since the cells are not grown in a monolayer, they are exposed to varying degrees of oxygenation and nutrient availability based on their spatial distribution. Metabolic data on glucose and lactate levels were measured with Boehringer Mannheim enzymatic tests after spheroids were subjected to progressively increasing concentrations of lactate under conditions of 20% O₂ and 5% O₂. Cells showed lactate consumption at the highest lactate concentration (20 mM) tested in both oxygenation states [82]. Other experiments in this same study demonstrated a depressed cell growth rate when exposed to high lactate as compared to glucose, but yet the viable rim of the spheroids showed high metabolic activity and O₂ consumption in the presence of high lactate concentrations [82]. Likewise, studies conducted in the 1970s found that three strains of Ehrlich ascites tumor cells oxidized exogenous ¹⁴C-labeled lactate resulting in incorporation of ¹⁴C in amino acids, acetate and CO₂ to varying proportions among the strains [83]. Approximately a third of a series of 29 rat hepatomas were demonstrated to utilize lactate based on calculations of arteriovenous substrate differences. The production or utilization of lactate was dependent on arterial lactate concentration and not arterial glucose concentration or glucose utilization [84,85]. These results demonstrated that tumor utilization of lactate occurred when the arterial lactate concentrations rose above 2–3 mM [85]. The ‘metabolic fate’ of the lactate removed from the arterial blood was not investigated in this particular study, and the mechanisms underlying this observation were not fully understood at the time.

Later studies with *in vitro* glioma cell lines and *in vivo* rat gliomas demonstrated that exogenous lactate was found to be a major source for oxidative metabolism in spite of net lactate production from aerobic glycolysis [86,87]. *In vitro* studies using NMR with [3-¹³C]L-lactate and [1-¹³C]D-glucose in C6 glioma cells were conducted. Cells were incubated with either 5.5 mM of labeled glucose, 11 mM of labeled lactate or a combination of 5.5 mM of labeled glucose with 11 mM of unlabeled lactate for 4 h before NMR samples were collected. Accounting for the fact that [1-¹³C] D-glucose metabolizes to 1 M of unlabeled lactate and 1 M of [3-¹³C]L-lactate, they found there was a high yield of glucose conversion to lactate [86]. From the NMR spectrum, peaks corresponding to 3C alanine and 4C glutamate were also seen; the former indicating [3-¹³C]L-lactate conversion to alanine, and the latter indicating [3-¹³C]L-lactate entering the TCA cycle to produce glutamate. Comparing the results from the dishes with lone-

labeled substrates, they found a high yield of ^{13}C incorporation into glutamate when starting from $[3-^{13}\text{C}]\text{L-lactate}$ [86]. Additionally, their findings suggest that lactate was a better precursor for alanine and glutamate than glucose [86]. From these results, we can conclude that certain tumors have the ability to utilize the lactate they take up for either energetic metabolism, as indicated by the labeled glutamate metabolite, or for amino acid formation, as indicated by the labeled alanine metabolite.

Based on observations that lactate production and utilization can occur simultaneously and/or shift from net lactate production to net lactate uptake based on the external lactate concentrations [82,86], a two-compartmental theory was proposed. Briefly, it described two separate metabolic pools of both lactate and pyruvate within the cell; one pool of lactate being a result of cytosolic glycolysis that is separate from oxidative metabolism, and the other pool being the imported exogenous lactate to be used for oxidative phosphorylation [86,88]. Using NMR, results from a 2001 study in perfused rat hearts support the idea of lactate compartmentalization. Glycolytically-derived lactate efflux can occur simultaneously with exogenous labeled lactate uptake, demonstrating that these metabolic pathways function separately [89].

These studies beg the question of what effects lactate utilization will have on tumor survival, aggressiveness and clinical profile. If accumulation of lactate correlates with increased metastasis and poor disease-free and overall survival [9,12–16,18,19], does the ability of the tumor to utilize lactate contribute to its survival, furthering cancer progression? Or does the ability to utilize lactate reduce the accumulation of lactate, and, in turn, lessen the negative effect of a hostile tumor environment? Does the ability to utilize lactate indicate a less aggressive tumor that shows a more ‘normal’ metabolic ability, namely, using available oxygen as opposed to undergoing the ‘Warburg effect’? Or will the increased oxygen consumption necessary for lactate oxidation ultimately lead to a more hypoxic tumor with increased reactive oxygen species? Of course, lactate may not be utilized for oxidation or energetic purposes; it may contribute to amino acid production through transamination reactions. In this case, will it serve to promote tumor survival, providing the tumor with the building blocks of protein synthesis? These are important questions in the field of cancer metabolism – ones that strongly encourage more research on the intricacies of tumor metabolism.

Lactosis versus acidosis

Lactate and acidity are concepts that are often assumed as a singular phenomenon. Although the molecule is often referred to as ‘lactic acid,’ lactate exists primarily in ionic form within the body at physiological pH, with over 99% of it being dissociated from the proton [51]. Upon transport, the proton and lactate anion are associated as they cross the cell membrane by MCTs [80]. Although MCTs have the ability to contribute to pH regulation, there are other transporters on the cell surface that specialize in pH regulation, such as the vacuolar proton pump (V-ATPase), sodium-proton exchangers (NHE1) and bicarbonate transporters (BCT). Many of these pH-regulatory transporters undergo expression or regulatory changes with malignant transformation, contributing to enhanced growth signaling, motility and multidrug resistance [90]. Low extracellular pH (pH_e) within the tumor cannot be primarily attributed to lactate or the ionic transport of MCTs. Table 3 provides a summary of the relevance of pH regulators to the tumor microenvironment, including their basic functions, expression in tumors, influences on cell migration and downstream targets, and a brief reference to potential inhibitors.

Glycolysis-generated lactate itself is not inherently a source of acidity; acidification can occur without glycolysis. Two different studies were conducted comparing LDH-competent versus LDH-deficient cell lines [91] and glycolysis-competent versus glycolysis-impaired *ras*⁹⁴[−] and *7ras*³[−] cell lines [92] in both *in vitro* and *in vivo* systems. The non-lactate producing cells from

both studies showed acidification of the culture media and tumor. The drop in pH_e tended to be greater in the LDH-competent cells than the LDH-deficient cells although this finding was not statistically significant. However, the amount of $^{14}\text{CO}_2$ produced from either ^{14}C glucose or ^{14}C alanine was significant when comparing the cell lines; the LDH-deficient cells, showed a significantly greater fraction of their $^{14}\text{CO}_2$ production from ^{14}C alanine rather than from ^{14}C glucose [91]. *In vivo*, the pH_e of the tumor tissue was similar for both LDH-competent (7.03 ± 0.03) and LDH-deficient (7.03 ± 0.05) tumors, despite the wide disparity in lactate content (1877 ± 135 vs 488 ± 91 $\mu\text{g/g}$ wet weight, respectively) [91]. Studies with the glycolysis-impaired *ras94⁻* and *7ras3⁻* cells demonstrated similar results to the LDH-deficient cells in regards to media acidification. The $\text{H}_3\text{O}^+:\text{lactate}$ ratio was significantly higher in the glycolysis-impaired cell lines, indicating that there was a source of acidity besides lactate. *In vivo* studies with tumors of these cell lines demonstrated a mean interstitial pH that was not significantly different between the glycolysis-competent versus glycolysis-impaired. After measuring metabolites in the tumor interstitial fluid of parental and glycolysis-impaired tumors, it was found that lactate levels were 28% lower in the glycolysis-impaired tumors, although CO_2 and pH levels were comparable with the parental tumors [92].

Implicated in the increased acidification of the abovementioned studies was increased levels of CO_2 [92], as hydrated CO_2 generates bicarbonate (HCO_3^-) and H^+ [93]. Briefly, a recent review highlights three sources of CO_2 that can lead to acidification the extracellular milieu. CO_2 can be generated from the TCA cycle since one carbon atom that passes through will generate one molecule of CO_2 . The pentose phosphate shunt is another pathway that generates CO_2 . Finally, as indicated at the beginning of this paragraph, titration of bicarbonate with protons can lead to increased CO_2 [93]. This discussion is expanded and detailed in a review by Swietach *et al.* [93]. Carbonic anhydrase (CA) catalyzes this reaction; there are 14 mammalian isoforms. Certain CA isoforms, such as 9 and 12, are increased in many cancers and are HIF-1 α -regulated [94]. Bicarbonate transport has been found to be increased through CA colocalization and interaction with some bicarbonate transporters [95,96], which is partially regulated by protein kinase C (PKC) [97]. Additionally, when a sodium-bicarbonate cotransporter (NBC) was expressed together with MCT1 in *Xenopus* oocytes, the transport of lactate and H^+ by MCT1 increased almost twofold, suggesting that the buffering capability of NBC reduced H^+ intracellular accumulation, which would normally hinder MCT1 activity [98]. It is clear that no one transporter is responsible for cellular pH regulation, and not only can many different transporters fulfill the same function, they can also assist or enhance the activity of other transporters.

Although lactate accumulates within tumors via the Pasteur effect, the cellular response to hypoxia is not the same as with lactosis or acidosis. Both hypoxia and accumulated lactate show correlation to more aggressive cancers, poor patient prognosis and disease-free and overall survival [99–101]. However, in studies that carefully controlled for conditions of hypoxia, (nonlactate) acidosis, lactosis and lactic acidosis, the range of cell responses was intriguing. A strong lactic acidosis genomic response has been found to not only negatively correlate with a hypoxic response score, but additionally the lactic acidosis response predicts a favorable prognosis in a series of clinical breast cancer trials [102]. Found within the same data set, the cellular response to high lactic acidosis was upregulated genes associated with aerobic metabolism, including factors involved in the TCA cycle and electron transport, while the response for low lactic acidosis showed an upregulation in genes associated with matrix metalloproteinases (MMP) [102], enzymes implicated in many aggressive cancers that are also induced by HIF [103].

MCT1 & MCT4 expression & regulation

The characteristics of these two transporters are being covered together since they share some common traits. Regulation is summarized in Figure 1. It is known that the function of both transporters is dependent upon interactions with other proteins, such as the chaperone CD147 [32]. Many studies have demonstrated the tight association of CD147 with MCT1 and MCT4 with coimmunoprecipitation, and immunohistochemical techniques have demonstrated co-expression on the plasma and mitochondrial membranes [104–107]. MCT1, MCT3 and MCT4 depend on association with the mature, glycosylated form of CD147 in order for these MCTs to be expressed and functional on either plasma or mitochondrial membranes [107,108]. Conversely, it appears that CD147 maturation is affected by MCT expression. In MCT1, knockdown experiments in Caco-2 cells, accumulation of the immature, core-glycosylated form and disappearance of the mature glycosylated form occurred with no difference in the mRNA [107]. In other experiments, abrogation of fully glycosylated CD147 was observed and the core-glycosylated form was restricted to the endoplasmic reticulum when MCT4 siRNA was added to MDA-MB-231 cells [40].

Studies on rat and human muscle cells have demonstrated that MCT1 mRNA is upregulated in response to 10 mM lactate *in vitro* [53], or in response to the increase of local lactate in exercising muscle, *in vivo* [109]. The increase in MCT1 mRNA also corresponds to significant increases in PGC-1 α mRNA [53]; PGC-1 α signaling is important to muscle metabolism, promoting oxidation [110]. Total and mitochondrial protein levels of MCT1 and CD147 have been demonstrated to increase significantly with exposure to exogenous lactate, while MCT4 expression remains unchanged [53]. *In vivo* exercise studies found that while MCT1 and MCT4 will increase after training, the increase of MCT1 correlates with citrate synthase activity, indicating an active TCA cycle [109]. MCT4 did not show the same correlation, indicating that MCT1 regulation can be important to the ability of the muscle cell to directly consume lactate to be used in TCA cycle/oxidative phosphorylation while MCT4 regulation is more likely to correspond to lactate efflux from the more glycolytic myocyte. This begs the question of whether cancer cells also have the ability to consume lactate or if the upregulation of MCT1 is more likely an adaptation to higher concentrations of intracellular lactate generated from increased glycolysis in cancer cells.

Lactate is not the only substrate that can upregulate MCT1 expression. Butyrate, a short chain fatty acid synthesized by bacterial fermentation in the colon, has been demonstrated to stimulate MCT1 promoter activity [111]. Hormones act to regulate the expression of MCT1; both testosterone [112] and thyroid-stimulating hormone (TSH) [113] will stimulate tissue protein expression of MCT1. The metabolite milieu is affected by the expression and function of MCTs. Inhibition of MCT1 and MCT2 via siRNA showed significant decrease in malignant glioma cell viability and lactate efflux (i.e., glycolysis) *in vitro*; these effects were increased when both were inhibited simultaneously [114]. MCT1 overexpression in rat β -cells has been demonstrated to increase pyruvate oxidation, and when co-overexpressed with lactate dehydrogenase A, islet cells will additionally increase lactate oxidation [115]. A recent study in cultured astrocytes found that basal lactate uptake was decreased with MCT1 silencing, but not with MCT4 silencing. The effect was more profound than CD147 silencing, which decreased basal lactate influx and efflux and glutamate-activated lactate release. The results from this study indicate the possibility of an uncharacterized MCT subtype or an unidentified transporter functioning within astrocytes [116].

MCTs & cancer

MCT expression in cancer is summarized in Table 1. Colorectal cancer provides intriguing information regarding MCT expression in cancer. MCT1 is expressed in normal colonic

epithelium to facilitate the transport of butyrate, the primary energy source for these cells [117]. Butyrate has been demonstrated to be important in proliferation, apoptosis and differentiation, with declining levels indicative of colon cancer progression [118]. Since butyrate is imperative for differentiation of colon cells [119,120], as well as breast cells [121], it makes sense that low levels of this substrate would contribute to carcinogenic transition or progression. Butyrate unavailability may be due to microenvironmental levels as well as a decreased ability of the cells to take up the substrate. The loss or silencing of MCT1 has been demonstrated to correlate with the deregulation of butyrate-responsive genes involved in differentiation and apoptosis [43,122], transition from normalcy to malignancy in colonic epithelium [123], along with a simultaneous increase in GLUT-1 expression [37,123], supporting the hypothesis that the primary energy source for cancerous colon cells switches from butyrate to glucose. *SLC5A8*, termed SMCT1, is also responsible for mediating uptake of butyrate [48] and has been demonstrated to act as a tumor suppressor in colon epithelium, with its silencing via aberrant correlating to neoplastic transformation of colon cells [124]. An opposing finding on MCT expression in colon tissue indicates that there is an increase in MCT1, MCT2 and MCT4 in colorectal carcinoma compared with normal colonic epithelium [36]. Expression patterns of MCT1 within a separate study demonstrated that both membrane and cytoplasmic MCT1 expression was seen in both normal colonic tissue as well as in colonic tumor cells and tumor-associated fibroblasts [37]. Studies mentioned used immunohistochemical analysis to assess MCT1 abundance; no conclusion can be drawn about the discrepancy between results. Further studies are needed to elucidate MCT expression and its implications in colorectal cancer.

Differential expression of MCT1 has been seen in clinical breast samples with hypermethylation of a CpG island (CGI) in the 5' upstream region of MCT1 accounting for silencing, similar to the expression seen in the MDA-MB-231 cell line [121]. In lung cancer, MCT1, MCT2 and MCT4 expression are found among tumor cells while very weak expression was seen in stroma, and no MCT expression was found in normal lung tissue [125]. Although MCT signaling pathways are not completely known, MCT2 expression/regulation has been linked to IGF-1 [126] and PI3k/Akt or mTor pathways [127], which may also be relevant to other subtypes. Interestingly, current research on SMCT1 demonstrates a more consistent expression pattern in different cancers compared with MCT1; SMCT1 is reputedly silenced in thyroid, stomach, brain, breast, pancreas and kidney cancers [48]. Perhaps this indicates that the SMCTs may have an unambiguous expression pattern in cancers compared with proton-coupled MCTs; further research on the expression and function of SMCT and MCT subtypes in cancer is warranted to elucidate these possibilities.

Monocarboxylate transporter subtypes may be specifically expressed among tumor cell types, with MCT1 being more abundant in cells with the potential for lactate oxidation while MCT4 is restricted to the more glycolytic cells. SiHa cells, which were found to be able to take in lactate, express abundant MCT1 and little MCT4, while the reverse was found for the more glycolytic cell line WiDr [68]. Hypoxia, specifically HIF-1 α , will upregulate MCT4 mRNA and protein expression while having no effect on MCT1 expression [128]. Our laboratory has seen similar results in 0.5% oxygen conditions with MCT4 upregulation and MCT1 downregulation in A549 and HT1080 cells [Wergin M, Kennedy K, Dedeugd C, Dewhirst MW. Duke University Medical Center, NC, USA. Unpublished data]. In sections of some human tumors, an inverse spatial distribution of MCT1 and MCT4 was found when compared to the distribution of hypoxia marker, EF5: MCT4 showing a positive correlation to EF5 staining and MCT1 showing a negative correlation [Wergin M *et al.* Unpublished data]. To support the concept of an inverse spatial relationship between MCT1/aerobic tissue and MCT4/hypoxic tissue, non-small-cell lung cancer biopsies showed that MCT1 did not colocalize with EF5, a hypoxia marker drug [68].

CD147 & cancer

It is well established that cancer cells show enhanced expression of CD147 [129] (Table 1). Esophageal squamous cell carcinomas (ESCC) (stage 3 and 4) and endometrial (97.3% incidence) carcinoma show significant upregulation of tissue expression of CD147, while CD147 is not expressed in the respective normal tissues [130,131]. CD147 holds promise as a reliable biomarker in cancer progression, showing a significant expression increase in carcinomas compared with sarcomas and normal epithelial tissues, as well as positively correlating with tumor recurrence and progression and negatively correlating to survival in breast cancer patients in a retrospective study [132].

Much of the influence of CD147 on the invasiveness and metastatic potential of tumor cells is related to the induction of MMPs, which break down extracellular matrix proteins. This induction of MMPs by CD147 occurs largely in the adjacent fibroblasts found within the stroma or adjacent normal tissue [130,133]. MMP subtypes under CD147 induction include 1, 2, 3, 9, 11, 14, and 15 [134] in both fibroblasts and endothelial cells. Many subtypes are notorious for their association and correlation to malignant cancers [135–138]. A study in breast cancer cells has demonstrated that disruption of the homophilic CD147 interaction with antibody treatment inhibited MMP2 production from tumor cells and MMP2-dependent invasion *in vitro* through matrigel [139]. Invasive properties conferred to melanoma tumor cells by CD147 are a result of stimulation of MMPs [140]. CD147 has been demonstrated, in gallbladder carcinoma, to predict for poor prognosis and overall survival, especially in association with MMP-2 expression [141]. Adding to the tumorigenic effects of CD147 is its ability to stimulate VEGF via the PI3K/Akt pathway [142]. VEGF will likewise be upregulated by increased MMP production (Figure 2).

Invasiveness and multidrug resistance of hepatocellular carcinoma cells is mediated by CD147 and shows increased levels of MMP11, which are both decreased with CD147 RNAi [143]. In human breast cancer, positive CD147 correlates with high tumor grade and volume, increased mitotic index, decreased tumor-specific survival and negative estrogen receptor (ER) and progesterone receptor (PR) staining [144]. CD147 facilitates survival of suspended breast carcinoma cells MDA-MB-231s, while knockdown of CD147 decreases viability of suspended cells. CD147 inhibits anoikis, and when silenced, MDA MB 231 cells undergo apoptosis. Anoikis is due to CD147 activating the MAPK pathway with phosphorylation of Erk1/2 leading to a decreased level of Bim (pro-apoptotic) by proteosomal degradation, which confers survival. This partially explains why increased levels of CD147 in cancer correlate with metastasis [145].

Additionally, CD147 is associated with a number of accessory or regulatory proteins beyond MCTs, including β 1-integrins, cyclophilin A, caveolin-1 [135] and transporters CD98 heavy chain-LAT1 complex, ASCT2 (amino acid transporter) and EpCAM (epithelial cell adhesion molecule, a regulator of cell proliferation) [146]. CD147 will induce production hyaluronan from adjacent cells [147]. Another large complex recently explored is the CD44 variant hyaluronan-associated CD147/MCT complex that will ultimately affect lactate transport [148].

Larger cell membrane CD147-associated complexes include the MCT/CD147-CD98hc/LAT1-ASCT2-EpCAM, which likely suppresses AMPK, as indirectly supported by CD147/CD98 RNAi experiments demonstrating that AMPK activation reduces expression of the large complex [146]. AMPK affects many metabolic operations, including stimulating fatty acid oxidation and glucose uptake, which lead to increased lactate production [30,149]. Even as an indirect target of CD147-associated complexes, the activation or suppression of AMPK will impact a myriad of metabolic effects. This indicates indirect participation of molecules

contribute to complex metabolic signaling pathways, and that manipulating one target can change the metabolic phenotype in potentially unexpected ways.

MCTs & CD147 as potential targets

Rationale behind MCT inhibition is multi-factorial (Figure 3). Inhibition of MCTs will have a direct effect on monocarboxylate transport and pH. It has been demonstrated that MCT1 inhibition decreases intracellular pH, resulting in cell death [68,90,150,151]. Since MCT1 is bidirectional, inhibition of this transporter may also cause the extracellular environment to become more acidic. While this effect is usually associated with a more aggressive phenotype, the increase in acid allows for more lactate uptake [79] and may have additional aid in treatment if candidate drugs require lower extracellular pH to enhance their uptake [152].

Another hypothesis regarding potential efficacy of MCT1 inhibition as a cancer treatment is in relation to the proposed 'metabolic symbiont' model between hypoxic and aerobic cells within the tumor microenvironment. Briefly, this model states that aerobic cells, which have the ability to consume lactate, can confer a survival advantage to the hypoxic tumor cells by allowing glucose to reach the hypoxic cells farther from vessels. By inhibiting MCT1, lactate is no longer available to the aerobic cells, forcing them to take up the glucose. This starves the more treatment-resistant hypoxic cells [68]. Inhibition of MCT1 has been found to decrease tumor growth rate [68] and elicit cell death via apoptosis and necrosis when silenced in conjunction with MCT2 [114].

Finally, inhibition of MCTs may suppress cell migration. Silencing of MCT4 via siRNA has been demonstrated to reduce transwell migration of MDA-MB-231 cells by as much as 85% [40]. More recent studies by Philp's group have illustrated the coimmunoprecipitation and colocalization of MCT4 and β -1-integrin in the leading edge lamellapodia of migrating cells [153]. Both CD147 and MCT4 interact closely with β -1-integrin; these findings, paired with the dependency of CD147 expression on MCT4 expression [40], indicate that MCT4 may be exerting more of an influence on focal adhesion- and integrin-associated cell migration than previously imagined.

There are several small molecules that inhibit MCT function. The efficiency of inhibition of MCT4 function tested under conditions of 30 mM lactate for the following inhibitors has been evaluated: α -cyano-4-hydroxycinnamate (CHC or CINN) ($K_i \sim 27 \mu\text{M}$), phloretin ($K_i \sim 1.4 \mu\text{M}$), 5-nitro-2-(3-phenylpropylamino) benzoate, 3-isobutyl-1-methylxanthine and p-chloromercuribenzenesulfonate (pCMBS) ($K_i < 1.0 \mu\text{M}$), which inhibits MCT1 via reaction with CD147 as opposed to direct MCT1 interaction [154,155]. There are some variations of efficiency of functional inhibition of the different inhibitors based on the subtype. CHC is supposedly a specific MCT1 inhibitor [154]. Of these CHC, pCMBS and phloretin are well-established MCT1 inhibitors, which reduces lactate influx by up to approximately 80% in normal hepatocytes in a dose-dependent manner, starting at concentrations of 5 mM. Similar effects have been demonstrated with 4,4'-dibenzamidostilbene-2,2'-disulfonate and 4,4'-diisothiocyanostilbene-2,2'-disulfonate [156] and with the tumor cell line Ehrlich-Lettre, although with some variation [157,158]. A recent MCT1-specific inhibitor, AR-C117977, has been found to have immunosuppressive properties that will significantly prolong skin graft and heart allograft survival in mice [159]. Some of these inhibitors can be used as broad spectrum pH regulator inhibitors, and likewise will inhibit lactate and proton efflux via MCTs. More MCT-specific inhibitors hold promise in facilitating tumor metabolism manipulation, as well as potentially acting as immunosuppressors.

Inhibition studies on CD147 with RNAi (Figure 4) have demonstrated significant decreases in invasiveness [39,160–162], MMP secretion [163], multidrug resistance [143,162,164] and increased cell death, both through apoptosis and necrosis [145,165]. Inhibition by a mouse

monoclonal antibody led to specific cancer cell death while sparing normal fibroblast; this same antibody also disrupted the CD147–MCT1 association [165]. Since some MCT subtypes rely on CD147 for expression and regulate pH via the monocarboxylate symport, CD147 inhibition helps to kill cells by indirectly inducing a drop in intracellular pH, as in the case of pCMBS. CD147 regulation has been demonstrated to effect lactate influx/efflux [116].

When considering MCTs as a target for therapy, it is imperative to bear in mind and evaluate toxicity to normal tissue. These molecules have the potential for altering metabolism, inflammatory response, intracellular pH, and angiogenic response on a broad spectrum. Local delivery may be required to prevent deleterious whole-body effects. Systemic delivery of an MCT inhibitor, and specifically MCT1, could affect almost every organ of the body, with the most drastic effects on cardiac and skeletal muscle. Possible side effects in skeletal muscle include muscle fatigue and inability to tolerate moderate- to high-intensity exercise due to the build-up of intracellular lactate as well as hydrogen ions [166]. In the worst-case scenario, the side effects of MCT inhibition in the heart may lead to exercise intolerance or other more severe cardiac toxicities [155,166]. Since MCT1 is one of the transporters that will take up butyrate, it is possible that with MCT1 inhibition there may be potential problems with proper differentiation or cell proliferation may be reduced in the colon. Inhibition of CD147 systemically may severely interfere with a number of the body's systems and cell processes. For perspective, CD147-null female mice will have severely impaired embryonic implantation, and CD147-null male mice are sterile [135]. It is obvious that for these molecules to have prospects for treatment, local delivery to the tumor may be preferable.

Future perspective

While some MCT inhibitors demonstrate potential for clinical usage, there are none that are currently in clinical trials. Local delivery of MCT inhibitors is an important first step towards clinical use. Once that hurdle is cleared, future prospects could include tumor-targeted combined therapies of CD147 silencing and/or MCT subtype-specific inhibition by small molecules. With further research on gene therapy, manipulation of CD147, MCT or SMCT expression may be possible for treatment of cancer for future generations. Manipulation of CD147 may drastically reduce tumor cell invasion, ECM breakdown, metastases and multi-drug resistance. Therapeutic change in MCT expression could reduce cell migration, increase cell death through intracellular acidification or through hypoxic cell starvation. Reintroducing SMCT expression in cases of loss of expression through hypermethylation may re-establish the tumor-suppressing effects of SMCT1. While ultimately it is unknown what future place MCT-, SMCT- or CD147-related therapies will have in the clinic, the manipulation of these molecules have and will continue to grant us great insight into connections between tumor metabolism, microenvironment and metastatic propensity.

Executive summary

Physiologic tumor microenvironment

- Unorganized vascular structure and shunt flow.
- Inefficient red blood cell flux and oxygen transport can lead to hypoxia and increased HIF-1 α levels.
- Lactate accumulation:
 - Anaerobic glycolysis (Pasteur effect).
 - Aerobic glycolysis (Warburg effect).

Requirements for lactate utilization & sites of regulation

- Membrane transporter (MCTs) – regulated by metabolites, chaperones and transcription factors.
- Lactate dehydrogenase: lactate ↔ pyruvate.
- Adequate oxygen – regulated by consumption and delivery.
- Healthy, functional mitochondria.
- Pyruvate dehydrogenase complex: pyruvate can be converted into acetyl CoA, pyruvate dehydrogenase complex is regulated by PDK1.

Lactate in cancer

- Accumulation correlates with poor prognosis, metastases-free and overall survival in many cancer types.
- Lactate has been demonstrated to spatially correlate to hypoxic regions in tumor sections in some tumors.
- Mean lactate content has a wide variation between tumor samples and within individual tumors.
- Certain cancer cell lines have the ability to take up lactate.
- Hepatomas and gliomas have been documented to utilize lactate *in vivo* according to nuclear magnetic resonance studies.
- Strong lactic acidosis genomic response predicted for favorable prognosis of clinical breast cancer trials.

MCTs & CD147 in cancer

- MCTs demonstrate variable regulation by several stimuli.
- MCT regulation profiles differ among cancer types.
- CD147 has been found to be upregulated in most cancers.
- CD147 stimulates matrix metalloproteinases that degrade extracellular matrix components and facilitate invasiveness.
- CD147 stimulates VEGF production, potentially increasing angiogenesis.
- MCTs and CD147 are involved in several large membrane complexes that can be under regulation of hyaluronan or 5' adenosine monophosphate-activated protein kinase.

Targeting MCTs & CD147

- MCT1 inhibition:
 - Decreases pHi leading to cell death.
 - Blocks aerobic tumor cell lactate utilization, starving hypoxic cells of glucose.
- MCT4 siRNA decreases tumor cell migration.
- CD147 siRNA decreases MMP secretion, multidrug resistance and tumor cell invasiveness.
- CD147 siRNA increases cell death by apoptosis and necrosis.

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Figure 1. Monocarboxylate transporter 1 and 4 (MCT1 and MCT4) regulation

Blue boxes indicate upregulation of the specific MCT subtype while green boxes indicate a downregulation. There is no distinction between transcriptional versus translational regulation. HIF: Hypoxia-inducible transcription factor; MCT: Monocarboxylate transporter; NO: Nitric oxide.

Figure 2. CD147 signaling and interactions

Black arrows indicate stimulation/activation. Gray arrows indicate associated molecules and their additional signaling or augmentation of effect. MCT: Monocarboxylate transporter; MMP: Matrix metalloproteinase.

Figure 3. Potential metabolic pathways between tumor cells (see left)

Cells close to vessels will have the advantage of high/adequate concentrations of oxygen and nutrients, such as glucose. Cells farther from vessels will experience varying degrees of hypoxia and starvation. **(A)** The metabolic pathways possible between cancer cells without MCT1 inhibition when the aerobic cells are able to consume/utilize lactate. Shown here is the Pasteur effect (anaerobic glycolysis) in the hypoxic cells farther from vessels. The well-oxygenated cells close to vessels may undergo healthy oxidative phosphorylation, possibly the Warburg effect (aerobic glycolysis), or lactate utilization. **(B)** Illustrates the consequences of MCT1 inhibition on cell-to-cell metabolism and intracellular pH. MCT4, having a high K_m , is unlikely to take up lactate unless there is a very high extracellular concentration of lactate. Excluded from this diagram are pH regulators other than MCTs, such as Na^+/H^+ exchanger (NHE1), which would serve to remove some of the H^+ from the cell. MCT1 inhibition can lead to cell death by two different means: the hypoxic cells are starved since cells close to vessels are forced to only take up glucose since the ability of lactate consumption is blocked, as seen by comparing **(A)** to **(B)** or the decrease in intracellular pH leads to toxicity, indicated by the protons represented in the diagram.

Figure 4. Influence of CD147 silencing on MCT expression, tumor cell lactate metabolism and MMP regulation in neighboring fibroblasts

Above tumor cell (left) has upregulated CD147 expression on the surface and adequate membrane expression of MCT subtypes 1 and 4, which allows various metabolic options for the cell (see Figure 3). Overexpression of CD147 can lead to increased matrix metalloproteinase production by neighboring fibroblasts (right). Bottom tumor cell shows the consequences of CD147 silencing with either CD147 siRNA or monocarboxylate transporter 4 (MCT4) siRNA. The gold bubble indicates the difference between these two. With MCT4 siRNA, CD147 is present in the cell, but in the low glycosylated form and therefore restricted to the cytosol. With CD147 siRNA, CD147 expression is ablated. Both scenarios lead to decreased MCT1 and MCT4 membrane expression, which will prevent monocarboxylate transport in and out of the cell. Lack of mature CD147 expression on the cell surface will also reduce MMP production by fibroblasts in comparison with a tumor cell with high CD147 expression.

MCT: Monocarboxylate transporter; MMP: Matrix metalloproteinase.

Table 1

MCT1, MCT4, CD147 expression and lactate levels within tumors.

Type of cancer	MCT1 expression	MCT4 expression	CD147 expression	Lactate levels
Cervical	↑ From preinvasive to invasive [44]	↑ From preinvasive to invasive [44]		↑ With metastases [12], poor DFS, OS [15]
Head and neck	(+) [Wergin M, Kennedy K, Dedeugd C, Dewhirst MW. Duke University Medical Center, NC, USA. Unpublished data]	(+) [Wergin M <i>et al.</i> Unpublished data]		↑ With metastases [13], poor DFS, OS [16]. HMRS studies: lactate SI did not correlate with tumor pO ₂ , treatment response or locoregional response [167]
Colorectal	(+) In tumor cells in samples tested [37], ↑ in tumor cells compared to normal epithelium [36] (–) Or loss from normalcy to malignancy [123]	Weak (+) in tumor cells [37], ↑ in tumor cells compared to normal epithelium [36]	(+) Increased cell death with silencing [165]	↑ With metastases [14]
Liver			Hepatocellular carcinoma: (+), correlated with ↑ invasiveness [168], multidrug resistance [143] and ↑ MMP (?)	Utilization in rat hepatomas [84,85]
Melanoma	(+) [161]	(+) [161]	↑ Correlated with ↑ invasiveness and ↑ MMP production [140], linked to metastasis [160], increased cell death with silencing [165] and decreased VEGF with silencing [161]	
Breast	↓ In MDA-MB-231 and some clinical samples by hypermethylation of CGI upstream [121]	↑ In MDA-MB-231 and metastatic breast cancer [40]	↑ With multidrug resistance, <i>in vitro</i> invasiveness [164], histopathological risk factors [144] and resistance to anoikis [145]	
Prostate			↑ Correlates with ↑ invasiveness [163] +/↑ correlative to TNM stage, histological subtype and poor prognosis, independent prognostic factor [169, 170]	
Brain	(+) In gliomas, silencing leads to cell death [114]			↑ In gliomas [7, 9]. Utilization in rat glioma [57,86,88]
Esophageal, ESSC			↑ With ↑ MMP2 resulted in poor	

Type of cancer	MCT1 expression	MCT4 expression	CD147 expression	Lactate levels
			prognosis in ESCC [130]	
Lung	(+) In tumor cells, (-) in normal cells [125], cytoplasmic accumulation in alveolar soft-part sarcoma [171]	(+) In tumor cells, (-) in normal [125]	Cytoplasmic accumulation in alveolar soft part sarcoma [171]	[Lactate] significantly ↑ in patients with recurrence, and correlates with poorer survival in men [19]
Gallbladder carcinoma			↑ Levels predict for poor prognosis and overall survival [141]	
Bladder			(+)/↑ Correlates with TNM stage, histological subtype and poor prognosis, Independent prognostic factor [170]	
Renal			+ / ↑ Correlates with TNM stage, histological subtype and poor prognosis, Independent prognostic factor [170]	
OSSC			↑ In OSCC compared to normal, linked with ↑ multidrug resistance [172]	
Pancreatic	(+) [39]	(+) [39]	↑ Compared to normal tissue, Expression in tumor and stromal cells [173] (+), linked to invasiveness and tumorigenic potential [39]	
Ovarian			(+)/↑ Confers invasiveness [162]	
Neuroendocrine	↑ In neuroblastoma [150]			

The (+) sign indicates that the protein has been shown experimentally to be expressed (positive for expression), but information regarding whether it is upregulated as compared to normal tissue was not found. Likewise, the (-) sign indicates that there was found to be no expression (negative for expression), as opposed to downregulation. ↑: Increase; ↓: Decrease.

CGI: CpG island; DFS: Disease-free survival; ESCC: Esophageal squamous cell carcinoma; MMP: Matrix metalloproteinase; OS: Overall survival; OSSC: Oral squamous cell carcinoma; TNM: Tumor node metastasis.

Table 2

MCT1 and MCT4 expression tissue distribution.

	Tissue expression/distribution	High expression
MCT1	Ubiquitous	Heart, oxidative skeletal muscle
MCT4	Skeletal muscle, chondrocytes, leukocytes, testis, lung, heart, placenta, skin, retina, brain	Glycolytic skeletal muscle, astrocytes, leukocytes

Table 3

pH regulators relevant to tumor progression.

pH regulator*	Function/mechanism	Expression in tumors	Cell migration	Associated/regulated molecules	Inhibitors
Na ⁺ /H ⁺ exchanger: NHEs (1)	Regulates pH _e and pH Expels H ⁺ from cytosol, promotes cell polarity [174] necessary for cell proliferation and stable cell volume [90]	Antiapoptotic activity [90]	↑ Invasiveness of breast carcinoma cells [174,175], required for migration, accumulates on leading edge of lamellipodium [174,176] → tumor cell pseudopodia	MMP activity dependent on NHE1 activity [174] Ras-mediated ERK, G protein-coupled receptors, PKC, RhoA and integrin receptors regulate NHE1 activity [90]	Amiloride and derivatives Guamiline and derivatives
Bicarbonate transporters: BCTs : <i>SLC4</i> = Na ⁺ -HCO ₃ ⁻ cotransporters (NBCs 1-4), Cl ⁻ /HCO ₃ ⁻ exchangers (NBCE) and anion exchangers (AE1 —4) <i>SLC26</i>	Regulates pH _i and cell volume ↑ in NHE1-deficient cells [174,177]		NBC has less influence on cell migration compared with NHE1, but will contribute to migration during acute intracellular acid load [177]	Coexpression with CA9 for AE1-3 increases AE-mediated bicarbonate transport [178]	
Carbonic anhydrases: CAs (9 and 12)	Extracellular production of HCO ₃ ⁻ is transported into cytosol H ⁺ ions remain at cell surface, ↓ pH _e [174]	CA9 ↑ in solid tumors, ↑ tumor cell growth [174]		CA9 regulated by HIF-1 [174]	
Monocarboxylate transporters: <i>SLC16A</i> = MCTs (1 and 4)	Facilitated passive H ⁺ transport with monocarboxylates	Refer to Table 1	MCT4 colocalized with β1 integrin at focal adhesions, expressed on leading edge of migrating cells [153,174]	CD147 chaperone MCT4 regulated by HIF-1	Aromatic monocarboxylates Anion transport inhibitors Bioflavonoids Others [90]
V-ATPases	H ⁺ pump, cytoplasmic pH homeostasis Endocytosis, intracellular transport [90]	↑ In multidrug resistant cells [90]	C subunit interacts with β1 integrin [90]	E subunit interacts with mSos-1 → Rac-1 signaling [90]	

* Names of pH regulators are given in normal text, bold typeface denotes abbreviations, italicizing denotes a gene family, and relevant isoforms or subtypes are given in parentheses.

↑: Increased; ↓: Decreased; MCT: Monocarboxylate transporter; MMP: Matrix metalloproteinase; NBC: Sodium-bicarbonate cotransporter; NHE1: Na⁺/H⁺ exchanger; PKC: Protein kinase C.