Multiple Biological Activities of Lactic Acid in Cancer: Influences on Tumor Growth, Angiogenesis and Metastasis

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Abstract: High rate of glycolysis is a metabolic hallmark of cancer. While anaerobic glycolysis promotes energy production under hypoxia, aerobic glycolysis, the Warburg effect, offers a proliferative advantage through redirecting carbohydrate fluxes from energy production to biosynthetic pathways. To fulfill tumor cell needs, the glycolytic switch is associated with elevated glucose uptake and lactic acid release. Altered glucose metabolism is the basis of positron emission tomography using the glucose analogue tracer [^{18}F]-fluorodeoxyglucose, a widely used clinical application for tumor diagnosis and monitoring. On the other hand, high levels of lactate have been associated with poor clinical outcome in several types of human cancers. Although lactic acid was initially considered merely as an indicator of the glycolytic flux, many evidences originally from the study of normal tissue physiology and more recently transposed to the tumor situation indicate that lactic acid, i.e. the lactate anion and protons, directly contributes to tumor growth and progression. Here, we briefly review the current knowledge pertaining to lactic acidosis and metastasis, lactate shuttles, the influence of lactate on redox homeostasis, lactate signaling and lactate-induced angiogenesis in the cancer context. The monocarboxylate transporters MCT1 and MCT4 have now been confirmed as prominent facilitators of lactate exchanges between cancer cells with different metabolic behaviors and between cancer and stromal cells. We therefore address the function and regulation of MCTs, highlighting MCT1 as a novel anticancer target. MCT1 inhibition allows to simultaneously disrupt metabolic cooperativity and angiogenesis in cancer with a same agent, opening a new path for novel anticancer therapies.

Keywords: Tumor metabolism, hypoxia, Warburg effect, lactate, lactic acidosis, monocarboxylate transporters, hypoxia-inducible factor-1, nuclear factor-κB.

INTRODUCTION

Cancers exhibit extensive heterogeneity in almost all phenotypic features, such as cellular morphology, gene expression, metabolism, as well as angiogenic, proliferative, and metastatic potentials. This heterogeneity is mainly attributed to multiple mutations in oncogenes and tumor suppressor genes. Some of them may have a genetic predisposition to microenvironmental stresses such as hypoxia, depletion of glucose and other nutrients, and acidosis.

Under normoxic conditions, quiescent cells in normal tissues generally depend on energetically efficient aerobic metabolic pathways to generate ATP. Glucose enters into the cells through glucose transporters (primarily GLUT1 to GLUT4), is sequestered intracellularly in the form of glucose-6-phosphate (G6P) after phosphorylation by hexokinases (HKs), and undergoes glycolysis to generate pyruvate. If glucose is fully metabolized through glycolysis, two molecules of pyruvate are produced per molecule of glucose consumed. Then, the fate of pyruvate depends on the oxygen condition prevailing in the tissue. Under aerobic conditions, pyruvate enters into mitochondria where it is converted to acetyl-CoA in the pyruvate dehydrogenase (PDH) reaction, and acetyl-CoA enters into the tricarboxylic acid (TCA) cycle to be metabolized to CO_2, H_2O and energy metabolites. Glycolysis coupled to oxidative phosphorylation (OXPHOS, Table **1** is a box defining specific terminology) yields up to 38 molecules of ATP per molecule of glucose. In anaerobic conditions, the limited availability of O_2 to accept electrons from the electron transport chain slows down the OXPHOS flux. A significant proportion of pyruvate is reduced into lactate by lactate dehydrogenase-5 (LDH-5), a reaction serving to perpetuate anaerobic glycolysis by oxidizing NADH into NAD^+ to aliment the glyceraldhehyde-3-phosphate dehydrogenase (GAPDH) reaction

Table 1. Terminology Box.

Term	Definition
Anaplerosis	Processes that replenish the TCA cycle with energy-containing intermediates to produce ATP in the mitochondrion.
Cataplerosis	Processes that deplete the TCA cycle through redirecting energy-containing intermediates from ATP production in the mitochondrion towards biosynthetic pathways.
Oxidative phosphorylation	An oxygen-dependent process that occurs in mitochondria where it couples the oxidation of macromolecules and electron transport with the production of ATP.
Pasteur effect	Negative feedback exerted by energy metabolites on key glycolytic enzymes.
Warburg effect	Uncoupling between glycolysis and oxidative phosphorylation commonly observed in proliferating cells including tumor cells.

Fig. (**1**). An inverse correlation between intracellular pyruvate levels and the activity of histone deacetylases in colon cancer cells further suggested that the LDH-5 reaction could prevent pyruvate-induced apoptosis [1, 2]. However, although pyruvate in these studies induced colon cancer cell death upon ectopic re-expression of the pyruvate-sodium symporter SLC5A8 [1, 2], SLC5A8 is usually silenced in cancer cells [3] and we have found that exogenous pyruvate (delivered at a 10 mM concentration in its methyl-esterified form to human cervix cancer cells) rather supports tumor cell survival [4]. Generation of lactate under hypoxia is further facilitated

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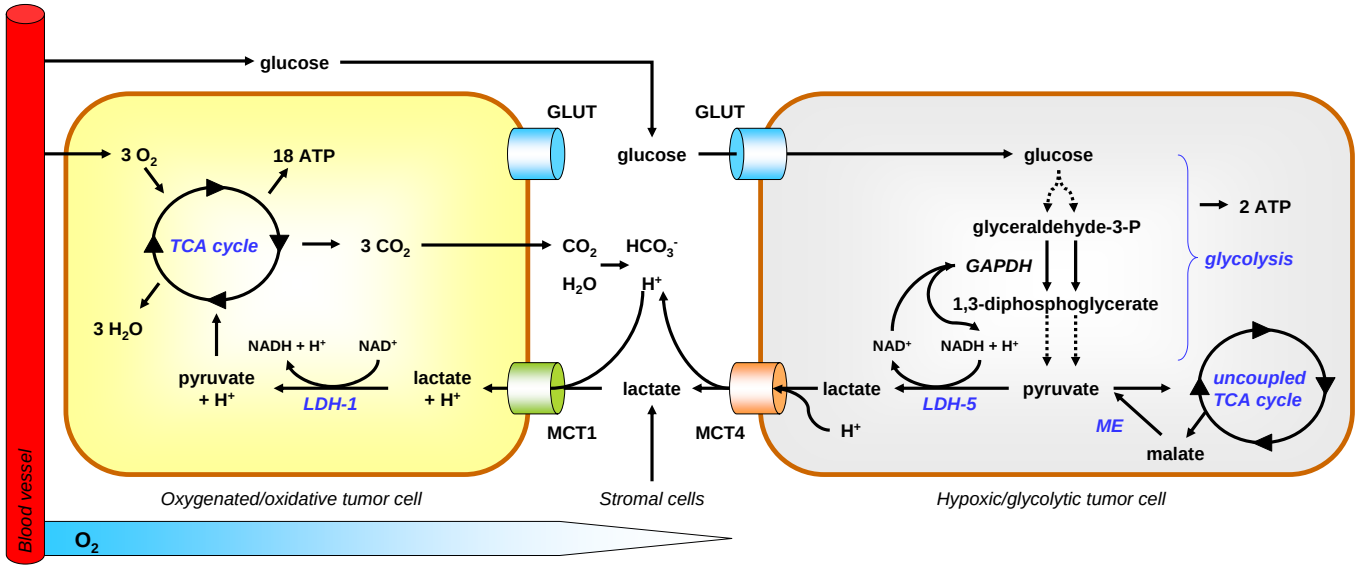

Fig. (1). Model of lactate shuttles in cancer. Solid tumors typically comprise oxygenated tumor cells close to blood vessels and hypoxic cells at distance from blood vessels. Hypoxic cells produce energy from glucose using glycolysis uncoupled from oxidative phosphorylation of the tricarboxylic acid (TCA) cycle, which requires high-rate glucose import by glucose transporters (GLUT) to produce 2 ATP per molecule of glucose. Pyruvate, produced either from glycolysis or generated by the malic enzyme (ME) from cataplerotic malate, is reduced to lactate by lactate dehydrogenase-5 (LDH-5). NADH is oxidized to NAD^+, which is as a substrate of glyceraldehyde-3-P (P = phosphate) dehydrogenase (GAPDH) and therefore maintains glycolysis at high rate. Lactate is exported together with a H^+ by monocarboxylate transporters (MCT), primarily MCT4. Once exported from glycolytic tumor cells or, as recently described [32], from stromal cells, lactic acid readily dissociates to lactate and H^+ (lactic acidosis). Oxidative tumor cells have a preference for lactate compared to glucose to fuel their oxidative metabolism [4, 125]. Lactate together with a H^+ is taken up by MCT1, oxidized to pyruvate by LDH-1, and pyruvate is incorporated into the TCA cycle to yield up to 18 ATP per molecule of lactate. CO_2 is produced and exported to the extracellular space where it generates bicarbonate and additional H^+ (carbonic acidosis). According to the model, tumors are metabolic symbionts in which lactate as a preferential oxidative fuel increases glucose bioavailability for glycolytic activities. Disrupting the symbiosis therapeutically is achievable with MCT inhibitors, especially drugs targeting MCT1 in cells close to blood vessels [30, 166].

by the increased activity of pyruvate dehydrogenase kinase 1 (PDK1) that blocks PDH activity [5, 6], and by the conversion of malate, a cataplerotic product of anaerobic mitochondrial activity, into pyruvate by the malic enzyme [7]. Lactate serves as a counter-ion for the export of protons, a process meant to avoid intracellular acidification and facilitated by the lactate-proton symporter monocarboxylate transporter 4 (MCT4) [8] Fig. (**1**). Although glycolysis uncoupled from OXPHOS only yields 2 molecules of ATP per molecule of glucose, several molecular pathways including hypoxia-inducible transcription factor-1 (HIF-1), c-Myc, AMP-activated protein kinase (AMPK) and molecular target of rapamycin (mTOR), cooperate to accelerate the glycolytic flux, as recently reviewed in reference [9]. Consequently, anaerobic glycolysis offers the possibility to match cell energy production with demand under hypoxia, at the expense of high glucose consumption and abundant lactic acid release. Lactate levels in tumors can indeed reach up to 40 mM, with an average level of 10 mM [10].

The glycolytic switch represents more than a mere adaptation to hypoxia. Indeed, some tumor cells especially in advanced cancers may perform aerobic glycolysis (i.e., high rate of glycolysis uncoupled from OXPHOS even in the presence of O_2), a metabolic phenotype known as the Warburg effect [11]. Constitutive upregulation of glycolysis under normoxic conditions allows carbohydrates to be redirected to biosynthetic pathways instead of getting cleaved to lactate, thus promoting tumor cell proliferation and tumor growth. These pathways include the pentose phosphate pathway (PPP) for DNA synthesis and NADPH production, alanine biosynthesis from pyruvate, and cataplerosis during which organic acids produced in the TCA cycle are exported from mitochondria to promote lipid synthesis and amino-acid synthesis and exchanges.

High rate of glucose uptake associated with increased glycolytic fluxes has been exploited clinically for the detection of tumors using positron emission tomography (PET) with the radiolabeled glucose analogue tracer [^{18}F]-fluorodeoxyglucose (FDG) [12]. FDG-PET has high sensitivity for metastases, making it a prevailing clinical tool for diagnosis (see references [13, 14] for comprehensive reviews). Elevated rates of FDG uptake have further shown a strong negative correlation with patient outcome in many cancers [13, 14]. Interestingly, although no clinical diagnostic application has been developed to date, studies concerning lactate accumulation in human tumors have shown that tumors with metastatic spread such as cervical tumors, head and neck cancers and rectal adenocarcinomas, exhibit a wider range and higher levels of lactate as compared to non-metastatic tumors [15-19]. Elevated levels of lactate also showed a correlation with poor patient prognosis and overall survival in cervical cancer [17], head and neck cancer [18], high grade gliomas [20-22] and non small cell lung cancer [23]. This makes lactate a suitable candidate as a diagnostic and prognostic indicator for a wide variety of tumors. While most of these studies considered lactic acid merely as a byproduct of fermentative glycolysis and consequently lactate measurements as a reflection of the glycolytic activity of tumors, lactic acid itself could be an important tumor growth-promoting factor. This review briefly summarizes the current understanding of the multiple biological activities of lactic acid in cancer.

LACTIC ACID: FROM WASTE BYPRODUCT TO TUMOR-GROWTH PROMOTING AGENT

In biological fluids, lactic acid (pK_a 3.86) is almost 99 % dissociated into lactate anions and protons [24]. For much of the 20th century, lactic acid was largely considered as a dead-end waste product of glycolysis, a major cause of muscle fatigue, and a key factor in acidosis-induced tissue damage. In 1929, Carl and Gertrude 'Gerty' Cori [25] demonstrated that the liver takes up circulating lactate from the blood to undergo hepatic gluconeogenesis, a phenomenon nowadays known as the Cori Cycle. Lactate uptake and metabolism were later studied in the muscle, leading to the emergence of the concept of lactate shuttles initially between muscle cells [26], then between different intracellular compartments [27], and more recently between different cell populations within a same organ as exemplified by the astrocyte-neuron lactate shuttle in the brain [28]. These seminal advances in our understanding of lactate activities in normal tissues have only recently been translated into new knowledge in the tumor context. Increasing evidence indicates that lactate in tumors is a fuel for the oxidative metabolism of oxygenated tumor cells [4, 29-33], a signaling agent in tumor and endothelial cells [34-36], and an important contributor to wound repair and angiogenesis [37-39]. Here, we review these biological actions of lactate in cancer first by describing the necessary lactate transporters mediating lactate exchanges. We then focus on lactate shuttling, starting with insights from normal tissue physiology to the hypothesis of a tumor metabolic symbiosis in cancer, to conclude with descriptions of extracellular (lactic acidosis at the bedside of metastasis) and intracellular influences on redox homeostasis, lactate signaling and tumor angiogenesis.

LACTATE TRANSPORTERS AND THEIR REGULATION IN CANCER

Lactate is an anion and therefore requires transporters to efficiently cross cell membranes. This function is predominantly exerted by transporters of the MCT family. MCTs are proton-coupled 12-span transmembrane proteins with both *N*-terminal and *C*-terminal tails located in the cytosolic domain. Of the 14 identified MCTs encoded by the *SLC16A* gene family, only 6 are functionally characterized to date [40]. Of these, MCT1 to MCT4 are able to carry out the proton-coupled transport of lactate across cell membranes. They behave as passive transporters driven by the gradient of protons across cell membranes and are not selective for lactate. With the highest affinities for lactate, MCT2 ($K_{m\ lactate}$ = 0.5 mM) and MCT3 ($K_{m\ lactate}$ = 5 mM) are specialized in the import of lactate but only in very specific tissues such as the liver (Cori cycle), the kidney and the brain for MCT2, and the retina for MCT3 [41-43]. MCT1 is ubiquitously expressed and has an intermediate affinity for lactate ($K_{m\ lactate}$ = 3.5-10 mM). It may function bidirectionally depending on the cellular and environmental context: for example outwardly in erythrocytes [44], proliferating lymphocytes [45] and some tumor cells [46]; and inwardly in slow-twitching muscle fibers [47], neurons [28], endothelial cells [36], and oxidative tumor cells [4, 33]. With the lowest affinity for lactate and a high turnover rate, MCT4 ($K_{m\ lactate}$ = 22 mM) is adapted for the export of lactic acid from glycolytic cells [8]. The other transporters of the MCT family have been identified to transport other endogenous and/or exogenous molecules, for instance diuretics (MCT6), thyroid hormones (MCT8) and aromatic amino acids (MCT10) [48, 49].

The transport of lactate is of critical importance for tumors with elevated glycolysis, not only to prevent cellular acidification through exporting lactic acid, but also to sustain growth through importing lactate, as detailed below. In the vast majority of tumors analyzed to date, MCT1 and MCT4 play a predominant role, but exceptions exist. For example, MCT1 and MCT2 are apparently the primary isoforms expressed in human glioblastoma multiforme and glioma-derived cell lines; targeting these MCTs with specific siRNAs in U-87 cells significantly reduced cell viability [50]. The membrane expression and function of MCT1 and MCT4 are dependent on their association with the mature glycosylated form of the chaperone protein basigin/CD147, also known as extracellular matrix metalloproteinase inducer (EMMPRIN) [48, 51, 52]. Conversely, CD147 is also affected by MCT expression: in the breast cancer cell line MDA-MB-231, MCT4 regulates the maturation and trafficking of CD147 to the plasma membrane [53], whereas in Caco-2 cells, silencing of MCT1 leads to accumulation of the immature and core-glycosylated form of CD147 [52]. Additional MCT chaperones may exist. CD44 was indeed shown to co-immunoprecipitate with and to regulate the intracellular trafficking of MCT1 and MCT4 in breast cancer cells [54]. Another report suggests the existence of an additional chaperone protein different from CD147 and CD44 in human tumor biopsies where a mismatch was observed between the plasma membrane expression of MCTs, CD44 and CD147 [46].

There have been several reports discussing changes in MCT expression in different types of cancers. In xenografts of the human cervix carcinoma cell line SiHa and human colon cancer cell line WiDr, we have, for example, reported a preferential expression of MCT1 in normoxic cells with oxidative potential [4], and others in other models have reported that MCT4 expression is predominant in glycolytic/hypoxic cells [55]. MCT localization and function could therefore be matched. Accordingly, MCT4 has been identified as a HIF-1-target gene product that facilitates the release of lactate from glycolytic cells [8, 56], and MCT1 could primarily mediate lactate uptake by oxygenated cells in tumors [4, 33]. However, little is known about the regulation of its expression. Although by using the hypoxia marker EF5 and immunohistology we have confirmed the absence of MCT1 in hypoxic areas of primary human lung cancer biopsies [4], hypoxia may not exert its influence independently of other factors: *in vitro*, hypoxia did not significantly decrease MCT1 mRNA and protein expression in HeLa cells [56]. In support of additional microenvironmental controls, *in vivo* muscle studies showed increased MCT1 protein expression in response to exercise [57], which was better characterized *in vitro* by showing that lactate promotes both MCT1 and CD147 transcription and protein expression in rat and human muscles [58]. To our knowledge, a similar regulation has not been reported in cancer cells. Interestingly, hypermethylation of a CpG island in the 5' upstream region of the *SLC16A1* gene encoding MCT1 has been demonstrated in the notably Warburg phenotype MDA-MB-231 cell line, and proposed to account for gene silencing [59]. However, this study was not supported by measurements of MCT1 protein expression.

Cancer cells like those of esophageal squamous cell carcinomas and endometrial carcinomas have been shown to have an elevated expression of CD147, which the corresponding normal tissues do not express [60-62]. Through homophilic interactions, elevated CD147 in tumor cells stimulates the production of matrix metalloproteinases (MMPs) 1, 2, 3, 9, 11, 14 and 15 by neighboring tumor cells, stromal fibroblast and endothelial cells [63], leading to a remodeling of the extracellular matrix promoting tumor growth and tumor cell mobility [64-66]. Many of these MMPs are notoriously associated with malignant cancers [67]. CD147 was also reported to stimulate vascular endothelial growth factor (VEGF) production through the PI(3)K/Akt pathway [68], potentially contributing to tumor aggressiveness by stimulating angiogenesis. To what extent its interactions with MCTs participate to the tumor growth-promoting activities of CD147 is still largely unknown.

Sodium-coupled MCTs (SMCTs) belonging to the *SLC5A* family transport substrates similar to those carried by *SLC16A* MCT family members. SMCT1, which has a very high affinity for lactate ($K_{m\ lactate}$ = 160-240 μM), regulates the uptake of short-chain fatty acids in the colon [69] and the tubular reabsorption of lactate and pyruvate in the kidney [70]. In colon cancer, rather than primarily

ensuring lactate exchanges, it behaves as a tumor suppressor by mediating the uptake of pyruvate and butyrate, two pro-apoptotic molecules acting as potent inhibitors of histone deacetylases (see reference [71] for an extensive review). Consequently, SMCT1 is silenced in many types of cancers, including colon, thyroid, stomach, brain, breast, pancreas, and kidney cancers [3].

LACTATE SHUTTLES IN CANCER: FROM NORMAL TISSUE PHYSIOLOGY TO THE HYPOTHESIS OF A METABOLIC SYMBIOSIS IN TUMORS

Muscle cells and myocytes use glucose, fatty acids and ketone bodies as energy sources to generate ATP. During intense exercise, fast-twitching glycolytic muscle fibers (white muscle) become hypoxic and produce lactic acid, which is then released to the extracellular compartment. Once exported, extracellular lactate may be taken up by slow-twitching muscle fibers (red muscle) where it is utilized as a respiratory fuel, or shuttled into the blood stream for further clearance. Lactate export and uptake is a MCT-dependent process reviewed in reference [72]. Briefly, muscles express MCT1 and MCT4 with isoform-specific pattern: tissues like heart and oxidative red muscle show higher expression of MCT1, whereas MCT4 expression is seen largely in glycolytic white muscles [47]. Lactate shuttles between glycolytic muscle fibers (where it is produced) and oxidative muscle fibers where, after oxidation to pyruvate by LDH-1, it fuels the TCA cycle. Interestingly, training increases MCT1 expression and the anaerobic threshold point at which lactate starts to accumulate in the blood [73]. Conversely, human patients deficient for MCT1 expression experience reversible muscle cramps upon intensive exercise, a condition known as cryptic exercise intolerance [74].

A similar lactate shuttle is proposed between astrocytes and neurons in the brain. Recent studies have shown that the brain tissue is a fervent consumer of lactate from blood and there is evidence that lactate is a preferred metabolic substrate for neurons even in presence of glucose [75, 76]. Nuclear magnetic resonance (NMR) studies in the brain have indeed shown that on elevating plasma lactate levels, the uptake of glucose decreases [77]. Astrocytes express both MCT1 and MCT4 and perform aerobic glycolysis, exporting a substantial amount of lactate into the extracellular space. Lactate is then taken up by the neighboring neurons through the high affinity lactate transporter MCT2 expressed on the cell surface [43, 48]. NMR studies have shown that following [^{13}C]-lactate administration, the labeled isotope was traced to TCA cycle intermediates and amino acids [78].

Both muscle and brain point towards a two compartment shuttle, wherein in one location lactate is produced glycolytically and at the other location it is utilized oxidatively. Similarly, the vast majority of solid tumors also contains populations of glycolytic tumor cells (being aerobic or not) and oxidative tumor cells. On this basis and because tumors are known usurpers of physiological functions, we [4] have studied lactate shuttling in cancer. We found that lactate is an important metabolic fuel for oxygenated tumor cells Fig. (**1**). In presence of glucose and lactate, these cells switch from glucose to lactate uptake and thus import consistently less glucose as compared to the situation when no lactate is supplied in the media. When glucose is removed, oxidative tumor cells import large amounts of lactate that is used to fuel the TCA cycle for ATP production. In this study, we therefore proposed that the existence of the hypoxic tumor cell compartment relies on a metabolic symbiosis by which glycolytic/hypoxic and oxidative/oxygenated tumor cells mutually regulate their access to energy metabolites. The symbiosis involves lactate recycling: oxidative tumor cells prefer to use lactate in the presence of glucose as an oxidative fuel, allowing glucose to diffuse farther from blood vessels where it is taken up by glycolytic tumor cells, in turn producing lactate in large quantities Fig. (**1**). In tumor cells under aerobic conditions, we proposed that lactate oxidation is more efficient than glucose as the latter claims initial energy input for phosphorylative reactions and the homeostasis of glycolytic enzymes, whereas each molecule of lactate produces up to 18 ATP. Oxidation of lactate into pyruvate by LDH-1, a necessary step for anaplerosis with lactate, further involves the continuous production of reduced NADH that could buffer oxidative stress. Several observations support the symbiont hypothesis: (i) inhibition of MCT1, which we found to be the prominent path for lactate uptake by oxidative tumor cells, induces hypoxic/glycolytic tumor cell death *in vivo* as a consequence of a metabolic switch from lactate-fueled respiration to glycolysis in the oxidative tumor cell compartment [4], (ii) MCT1 is expressed in an array of primary human tumors and preferentially in normoxic tumor cells in biopsies of lung cancer [4], and (iii) *ex vivo* measurements made at early time points during chronic MCT1 inhibition with the experimental drug α-cyano-4-hydroxycinnamate (CHC) showed significant inhibition of the Pasteur effect in FaDu and SiHa tumor xenografts [79]. The latter study, however, failed to evidence a reduction in average glucose levels and hypoxia in viable tumor areas 4 hours (FaDu) and 4 days (SiHa) after initiating CHC delivery, whereas in other models we evidenced radiosensitization after 3 days (LLc) and reduced hypoxia after 18 days of treatment (WiDr) [4]. These disparities may well be explained by our incomplete understanding of the pharmacology of CHC (including biodistribution with respect to different tumor models and/or different treatment times), which certainly warrants further investigation. Other MCT1 inhibitors with increased inhibitory activities have been identified [80, 81]. The high clinical potential of targeting tumor symbiosis with MCT1 inhibitors is currently being investigated with AZD3965, an orally administered compound now entering Phase I/II clinical trials for advanced solid tumors (http://science.cancerresearchuk.org/). An alternative could be to target lactate export through MCT4 [53], but the strategy is confronted with the difficulty of reaching the target *in vivo* (a large proportion of hypoxic/glycolytic tumor cells are located at distance from drug-supplying blood vessels), and no specific small molecule inhibitor nor blocking antibody selective for MCT4 has been identified so far. Interestingly, metabolic cooperativity in tumors has recently been shown to involve stromal cells: cancer-associated fibroblasts have been reported to be glycolytic and to fuel the oxidative metabolism of cancer cell [31, 32]. Co-culture experiments supported with analyzes of human breast cancer samples also showed increased MCT4 expression in stromal cells, whereas MCT1 expression was increased in tumor cells [33], thus revealing an additional rationale for anticancer MCT1 inhibition.

LACTIC ACID IN TUMOR INVASION AND METASTASIS

Glycolysis is nominally a faster succession of reactions than ATP production through OXPHOS [82]. Compared to normal paced OXPHOS, high rate of glycolysis coupled to biosynthetic pathways yields an elevated production of lactic acid, which must be exported to avoid intracellular acidification and death. A direct mechanism is provided by MCTs which, as passive lactate-proton symporters, couple the export of each molecule of lactate with a proton. With low affinity for lactate but hypoxia-induced expression and a high turnover rate [55, 56], MCT4 is particularly well adapted to ensure this function [8]. It does not exclude the contribution of other MCTs such as MCT1. It is particularly striking to realize that the lactate concentration detected in human tumors such as cervix cancer may range from 4 mM up to 40 mM (median 14 mM) [18], whereas the physiological concentration of lactate in normal tissues at rest is set between 1.8 mM and 2 mM [16, 24]. It represents at least an equivalently high release of protons, not taking into account lactic acid recycling by oxidative tumor cells Fig. (**1**) and the clearance of lactate by circulation. Besides MCTs, the equipment of tumor cells ensuring proton export includes carbonic anhydrases, membrane-bound vacuolar ATPase (V-ATPase), and the sodium proton exchanger NHE1 (recently reviewed in reference [9]). These activities collectively create a transmembrane gradient

of protons opposite to that in normal cells: in cancer cells, the intracellular pH (pH_i) is either neutral or even slightly alkaline ($pH \geq 7.4$), and extracellular pH (pH_e) is acidic (pH = ~6.7 - 7.1) [83, 84]. Alkaline pH_i provides a supplemental glycolytic advantage to tumor cells: the activity of LDH-5 is maximal at pH 7.5 [85], and the activity of phosphofructokinase-1 (PFK1) increases with the pH due to the repression of allosteric repressors [86].

There are two main forms of acid in tumors: carbonic acid (carbonic acidosis) and lactic acid (lactic acidosis). Glucose and lactate respiration, glutaminolysis (fueling the TCA cycle), cataplerosis (which necessitates TCA cycle activities but no O_2 consumption), malate decarboxylation (malic enzyme reaction), and overactive PPP (producing 1 CO_2 per molecule of G6P), as well as titration of bicarbonate with metabolically produced acids, are important sources of CO_2 [9, 87, 88]. It is important to stress out that glycolysis when yielding 2 molecules of lactate from 1 molecule of glucose does not contribute directly to the production of protons required for lactate exportation. On one hand, the various sources of CO_2 listed above may (OXPHOS, glutaminolysis, oxidative arm of the PPP) or may not (cataplerosis, malate decarboxylation) be coupled with oxygen consumption, and CO_2 may generate H^+ and HCO_3^- intracellularly following hydration by carbonic anhydrases (CAs) [89]. On the other hand, ATP hydrolysis and the catabolism of nitrogen may be other sources of acidity. The hypothesis that lactic acid is a major cause of tumor acidification is based on a correlation between elevated lactic acid concentration and low pH_e in tumors [90]. However, other studies have shown that tumors derived from glycolysis-deficient cells (lacking phosphoglucose isomerase activity or LDH) still produced a pH_e of 6.7 (i.e., as low as with parental tumors) despite negligible lactic acid production *in vitro* [91, 92]. The authors concluded that lactic acid production is not the only and perhaps even not a major cause of tumor acidification. Supporting these conclusions, it was observed that both pCO_2 and bicarbonate levels were higher in the interstitial fluids around glycolysis-deficient tumors as compared to those in normal tissues [87]. Others reported a high pCO_2 in rodent solid tumors (59 - 84 mm Hg *versus* 50 - 66 mm Hg for venous blood) [93], further indicating that CO_2 rather than lactic acid could be the main contributor to tumor acidification. Once produced, CO_2, being freely membrane permeable, exits the cell. Located at the cell membrane with an extracellular orientation, CAIX catalyses the hydration of CO_2 into HCO_3^- and H^+, thereby contributing to extracellular acidification of the tumor microenvironment [94].

Several studies have described the key contribution of tumor acidity to local invasion and metastasis, which has been the topic of recent reviews [95, 96]. Briefly, changes in the tumor microenvironment bestow clever proliferative advantages on the tumor cells for invading the adjacent normal tissue. Indeed, extracellular acidification leads to obliteration of normal tissues *via* caspase-mediated activation of p53-dependent apoptotic pathways [97, 98], whereas cancer cells are well equipped to export protons (reviewed in reference [9]) and/or lack p53 expression [98]. In addition, a low pH_e promotes (i) angiogenesis through acid-induced expression of VEGF and IL-8 [99, 100], (ii) extracellular matrix degradation through activating proteolytic enzymes such as cathepsin B [101, 102], and (iii) inhibition of immune functions [103]. One of the most characterized influences of acidosis to cancer progression is metastasis facilitation. Cancer cells on their metastatic route wave their way through the extracellular matrix (ECM), which requires ECM degradation and remodeling, a process facilitated by extracellular acidification [96, 104]. It has been shown that low pH_e provides a favorable microenvironment for the activation of proteases, including MMPs [105, 106], urokinase-type plasminogen activator [107], and cathepsins B [101, 102, 108], D [109], and L [110]. For example, maximal activity of human MMP-3 is seen within the optimum pH range of 5.75 to 6.25 [105]. Acidosis also contributes to MMP activation by promoting the proteolytic cascade that converts pro-MMPs to active MMPs, as is the case for MMP-9 [106].

Studies of lactic acidosis have largely neglected the contribution of the lactate anion to tumor invasion and metastasis, which has only recently gained some attention. Interestingly, Izumi *et al.* [111] showed that, in addition to MCT4 (creating extracellular acidification), MCT1 plays an important role in tumor cell invasion. MCT1 expression indeed correlated with *in vivo* invasiveness of human lung cancer cells and MCT1 inhibitors decreased both migration and invasiveness. Interestingly, in the same study [111], MCT1 deletion reduced tumor cell migration to a larger extend than CD147 silencing, suggesting a contribution of MCT1 not overlapping the well-known pro-metastatic activities of CD147 [63-66]. Whether changes in intra- or extra- cellular lactate concentration drive tumor cell migration and invasion remains an open question. On one hand, Izumi *et al.* [111] using A110L lung tumor cells expressing both MCT1 and MCT4 failed to show a significant influence of exogenous sodium lactate (5 mM) on cell migration and invasion over a 22-h time course. In the same pH-buffered conditions, there was no correlation between extracellular lactate levels and invasion activities. On the other hand, others [112] showed that sodium *L*-lactate (10 – 40 mM) but not *D*-lactate or changes in osmolarity or intracellular pH induced a time- and dose-dependent migration of human SQ20B squamous larynx carcinoma cells in Boyden chamber assays. Migration was random (as observed using videomicroscopy) and was also stimulated by pyruvate, suggesting that lactate oxidation into pyruvate could be involved in the migratory phenotype of these tumor cells [112]. Further studies are needed to confirm and further identify the underlying mechanism(s). In another set of experiments, lactate was shown to stimulate the production of hyaluronan (HA) and the expression of CD44, its main transmembrane receptor, by fibroblasts [113] and melanoma cells [114]. In fibroblasts, it is supported by the existence of a lactate-induced transcriptome, including genes encoding CD44 and hyaluronidases HYAL1 and HYAL2, but also c-fos, c-jun, c-ets, and caveolin-1 [115]. HA-induced activation of CD44 at the tumor cell surface reduces cell adherence and promotes cell mobility, as reviewed in reference [116]. Still in line with a potential involvement of lactate in metastasis, it has been shown that lactate upregulates the expression of transforming growth factor β2 (TGF-β2) in glioblastoma cells [117]. TGF-β2 is a key regulator of invasion in high-grade gliomas, inducing a mesenchymal pro-migratory phenotype and promoting ECM remodeling [118, 119]. Thus, lactate-induced TGF-β2 expression could play a role in brain metastasis.

Lactate and lactic acid are often confounded. While the contribution of acidosis to tumor progression and metastasis is well documented, the role of the lactate anion is only starting to be characterized. Based on existing data, one may envision a potential use of MCT inhibitors for the modulation of cancer metastasis, as previously suggested by others [111].

LACTATE AND INTRACELLULAR REDOX POTENTIAL

The mitochondrion is a main site of O_2 consumption in the cell and any reduction in pO_2 leads to the increased production of reactive oxygen species (ROS). If hypoxia persists, the transcription factor HIF-1 along with c-Myc stimulates adaptive mechanisms to reduce ROS and to re-establish redox homeostasis. Indeed, when PDH is inhibited by the HIF-1-target gene product PDK1 under hypoxia, the glycolytic flux is pushed towards the LDH-5 reaction for NAD^+ production, which prevents excessive ROS production [5, 6, 120]. HIF-1 activation is further promoted by ROS themselves [121].

Pyruvate represents a crucial metabolic regulatory point. It is the product of glycolysis, the product of malate decarboxylation, a main fuel for the TCA cycle, and it can be converted to lactate by LDHs in a reversible redox reaction Fig. (**1**). Due to different affini-

ties for their substrates, LDH-5, a target gene product of c-Myc and HIF-1, preferentially couples the reduction of pyruvate into lactate with the oxidation of NADH into NAD^+ [122], whereas LDH-1 preferentially catalyzes the reverse reaction and is most generally silenced in glycolytic cancer cells [2, 123]. The LDH-5 reaction serves to replenish the NAD^+ pool to make glycolysis self-sufficient. As a product of pyruvate reduction in glycolytic cells and as a substrate for pyruvate generation in oxidative tumor cells, lactate influences the NADH/NAD^+ ratio and thereby the cell redox status Fig. (**1**). Lactate as a fuel further influences cell metabolism through competing with the GAPDH reaction for NAD^+ (LDH-1 being a more efficient user of NAD^+) [4, 124], and potentially through allosterically inactivating the glycolytic enzymes HKs (as shown in skeletal muscle and liver) and phosphofructokinase (liver and kidney) [125]. Interestingly, transactivation mediated by HIF-1 and HIF-2 can be modulated by NAD^+ levels, providing a further link between the interconversion of lactate and pyruvate, cell metabolism and tumor progression: the NAD^+-dependent enzyme poly(ADPribose) polymerase 1 (PARP1) binds to HIF-1α and co-activates HIF-1-dependent gene expression [126], and the NAD^+-dependent deacetylase sirtuin 1 (SIRT1) increases transcriptional activation by HIF-2 but not by HIF-1 [127].

LACTATE SIGNALING

Lactate is not only a metabolic intermediate; it may also act as a signaling molecule. Lactate (20 mM, 6-h) was indeed shown to activate the transcription of 673 genes in L6 myocytes, including genes involved in metabolism and mitochondrial activities, transcription activation, signal transduction, transport, oxidative stress, apoptosis, cell growth, and calcium signaling (see the supplemental data of reference [58] for a complete list of genes). Only 3 genes were downregulated. Interestingly, among the regulated genes were *Jun* (a component of AP-1 transcription factor), *Ras* and *CREB* (effectors of the mitogen-activated protein kinase [MAPK] pathway), suggesting a lactate-induced activation of ROS-sensitive pathways. Lactate indeed induced a small but significant increase in ROS production and stimulated the DNA binding of NF-κB and NRF-2, as shown using electrophoretic mobility shift assays [58]. AP-1 activation was not evidenced. Increased activity of NRF-2 together with increased DNA binding of CREB suggests that lactate could be involved in mitochondrial biogenesis (as also documented with increased cyclooxygenase-IV expression in whole muscle homogenates), which is physiologically relevant for an increased clearance of lactate by oxidative muscle fibers [58].

In the context of cancer, lactate has been identified as a hypoxia-mimetic able to activate the transcription factor HIF-1 originally in normoxic glioma cells [34, 35]. The underlying pathway was shown to require lactate oxidation into pyruvate (LDH-1 reaction) in order to support a functional competition between pyruvate and 2-oxoglutarate (a by-product of the TCA cycle) for the control of HIF PHD activity [34, 35] Fig. (**2**). PHDs are Fe(II)- and 2-oxoglutarate-dependent dioxygenases known to inactivate HIF-1α through prolylhydroxylations followed by proteasomal degradation [128-131]. Oxygen being a necessary substrate of the reaction, their activities decrease under hypoxia [132, 133]. Assays with immobilized 2-oxoglutarate and radiolabeled PHDs showed that pyruvate is

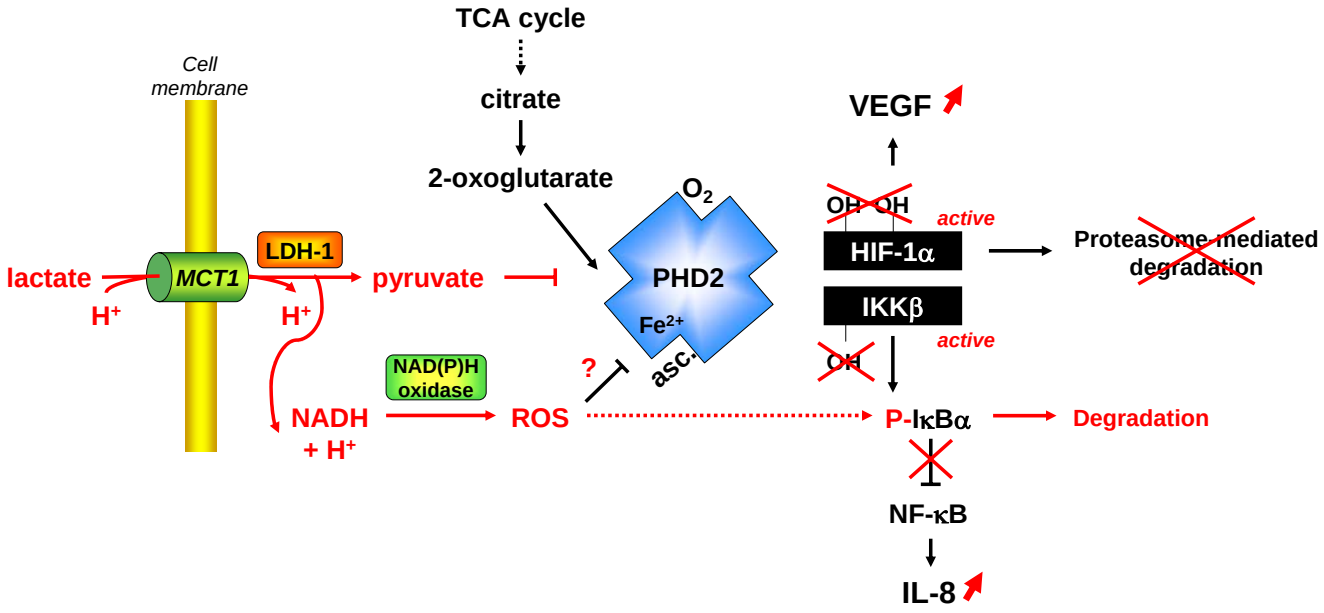

Fig. (2). Signaling pathway of lactate supporting HIF-1 and NF-κB activation. Lactate, its downstream effectors and influences are represented in red. Prolylhydroxylases (PHD) are Fe(II)- and 2-oxoglutarate-dependent oxygenases that catalyze (i) hydroxylation of hypoxia-inducible factor-1α (HIF-1α) on 2 proline residues addressing the subunit for proteasome-mediated degradation, and (ii) inactivating hydroxylation of IKKβ. Unphosphorylated IκBα represses nuclear factor-κB (NF-κB). Oxygen and 2-oxoglutarate (arising in the cytosol from citrate exported from mitochondria) are necessary substrates of the reactions. Exogenous lactate originating e.g. from glycolytic tumor cells is taken up primarily by monocarboxylate transporter 1 (MCT1) and oxidized to pyruvate by lactate dehydrogenase-1 (LDH-1). The reductive arm of the reaction yields NADH + H^+ from NAD^+. Both pyruvate and NADH mediate lactate signaling intracellularly. On one hand, pyruvate functionally competes with 2-oxoglutarate leading to PHD inactivation and, consequently, HIF-1α protein stabilization and IKKβ activation. On the other hand, NAD(P)H oxidase yields reactive oxygen species (ROS) from NADH; ROS activate a cascade leading to the phosphorylative inactivation of IκBα which, together with IKKβ activation, account for NF-κB activation. ROS may further mediate the effects of lactate through either maintaining iron in an oxidized Fe^{3+} state and/or through oxidizing ascorbate (asc.) thereby preventing Fe^{3+} reduction. While in tumor cells vascular endothelial growth factor (VEGF) was evidenced as a final effector of lactate signaling [34], IL-8 was found to be induced by lactate in endothelial cells [36]. Both pathways cooperate for lactate-induced tumor angiogenesis.

able to displace the binding of 2-oxoglutarate on PHDs independently of hypoxia [35], offering a potential rationale for exogenous lactate stimulating HIF-1 activity in normoxic cancer cells. Of note, soft ionization mass spectrometric assays in *in vitro* nondenaturing conditions failed to detect a direct interaction between pyruvate and PHD2 [134]. This study using *N*-terminally truncated PHD2 (amino acids 181- 426) also failed to show PHD2 inhibition by pyruvate, suggesting that pyruvate may not interact directly with the 2-oxoglutarate-binding site but would rather induce a conformational change interfering with the binding of 2-oxoglutarate to full length PHD2. This possibility remains to be demonstrated. The Verma study [35] and our unpublished data clearly show that pyruvate is more rapid and more effective than lactate in inducing HIF-1α protein stabilization, highlighting pyruvate as a potential intermediate supporting PHD inhibition by lactate. Pyruvate increased the transcription of several HIF-1-target genes including *VEGF*, *GLUT3*, *aldolase-A* (in U87 glioma cells); and *erythropoietin* (*EPO*) in Hep3G human hepatoma cells [34]. The inability of NADH or catalase (administered exogenously to permeabilized cells) to recapitulate the effects of lactate was originally interpreted to rule out an important contribution of redox changes to lactate-induced HIF-1 activation in glioma cells [34]. However, the observation that ascorbate (a necessary PHD cofactor allowing iron reduction for enzyme recycling) and glutathione (GSH) block the activity of pyruvate later revealed that redox changes are an integral part of the signaling pathway [35]. This particular issue obviously warrants further investigation. Lactate-induced HIF-1α stabilization was confirmed in many cancer cell lines but surprisingly not in Hep3G cells where pyruvate consistently induced *EPO* gene transcription. Pyruvate also induced HIF-1α expression in normal astrocytes and normal human prostate epithelium [34], thus suggesting that hypoxia mimicry by lactate could be a physiological phenomenon.

Interestingly, in addition to lactate and pyruvate, Lu *et al.* [35] initially reported that oxaloacetate, α-ketoisocaproate, α-keto-β-methylvalerate and α-keto-isovalerate (possessing 2-oxo groups) also induced HIF-1α expression in some cancer cell lines, an activity which was not shared by citrate, fumarate, 2-oxoglutarate, succinate, acetoacetate, β-hydroxybutyrate, pyruvaldehyde, malate and alanine in the same cancer cells. Their conclusion that molecular features mimicking those of 2-oxoglutarate are necessary for the biological activity has now been challenged by others having shown that succinate (a product of the PHD reaction), fumarate, (iso)citrate and 2-hydroxyglutarate (substituting the 2-oxo group by a 2-hydroxyl group) are also potent PHD inhibitors [134-138]. Fumarate and succinate inhibit PHD1 to 3 but not factor inhibiting HIF (FIH) [137], citrate inhibits PHD3 and FIH [137], and 2-hydroxyglutarate competitively inhibits the interaction of 2-oxoglutarate with multiple dioxygenases including PHDs, collagen hydroxylases, and histone demethylases [138]. These activities link HIF-1 activation with the aggressive phenotype of tumors harboring loss of function mutations in fumarate hydratase (*FH*) [136, 139] or succinate dehydrogenases (*SDHs*) [135, 139]; or gain of function mutations in isocitrate dehydrogenase *IDH1* and *IDH2* supporting the production of 2-hydroxy-glutarate instead of 2-oxoglutarate from isocitrate [138, 140].

In macrophages, lactate was shown to increase TLR4 signaling and NF-κB-dependent inflammatory gene expression [141]. These responses were blocked using antioxidants or the MCT inhibitor CHC, indicating that lactate oxidation into pyruvate could play a role and that targeting MCT pharmacologically could inhibit lactate signaling. This pathway has been better characterized in human umbilical vein endothelial cells (HUVECs) by Végran *et al.* who demonstrated lactate-induced NF-κB activation based on a gene signature array showing lactate-induced IL-8 transcription [36]. Both ROS and PHD inhibition were shown to be equally important in mediating lactate signaling Fig. (**2**). Indeed, on one hand, lactate-induced ROS production was evidenced to mediate the degradation of the NF-κB inhibitor IκBα through serine phosphorylations and protein degradation. On the other hand, 2-oxoglutarate dose-dependently inhibited lactate-induced NF-κB activity and IL-8 expression, and lactate signaling was further found to be inoperative in the absence of PHD2. In support of this, Cummins *et al.* [142] have shown that hypoxia may activate NF-κB through a decreased hydroxylation of IKKβ by PHDs, triggering the phosphorylation-dependent degradation of IκBα. In the study of Végran *et al.* [36], a functional link between both ROS and PHD2 pathways was provided by showing that targeting NAD(P)H oxidase with diphenylene iodonium (inhibiting ROS production from NADH) also prevented the increase in basal NF-κB activity measured after PHD2 silencing. An important *in vivo* finding was that lactate-induced IL-8 angiogenesis requires MCT4 expression in glycolytic tumor cells (for the release of lactate), and MCT1 expression in endothelial cells (for lactate uptake). Lactate shuttles in tumors, therefore, influence non-metabolic systems that could be therapeutically targeted with MCT1 inhibitors.

Some evidence indicates that lactate could affect additional pathways. For example, as already mentioned before, lactic acid was shown to induce both transcription and protein secretion of TGF-β2 in primary cultures of high-grade glioma [117]. TGF-β2, in turn, increases the membrane expression of integrin $\alpha_v\beta_3$ and MMP2 expression and activation. Conversely, silencing LDH-5 with a specific siRNA resulted in decreased TGF-β2 expression, MMP2 activity, and tumor cell migration *in vitro*. These data suggest the existence of a lactate-driven autocrine signaling pathway coupling glycolysis with increased tumor cell migration and metastasis. Others have reported lactate-induced TGF-β1 production *in vivo* [143]. Although microenvironmental acidification may certainly play a role, the molecular mechanisms regulating this pathway are still incompletely understood. Another example of potential lactate signaling has been provided by immunologists: lactic acid (10 - 20 mM) enhances the transcription of IL-23p19 (subunit p19 of pro-inflammatory and tumor-promoting IL-23) in monocytes stimulated with a TLR2/4 ligand, but low pH and sodium lactate were devoid of such activity [144]. In co-culture systems, lactic acid further enhanced IL-17A and IL-21 gene and protein expression in regulatory/memory $CD4^+$ T cells [144, 145]. Although many evidences point at an intracellular action of lactate, the underlying mechanisms of this mode of signaling have not been identified to date. A final example of lactate signaling is the well-known regulation of protein ADP-ribosylation, described in detail in the next section pertaining to lactate-induced angiogenesis.

The transcription and signaling pathways modulated by lactate have potentially wide spread effects on tumor metabolism, angiogenesis and metastasis. Although some activities of lactate have already been elucidated, major mechanistic insights are still expected to deliver suitable therapeutic targets. In cancer, MCT1 inhibition is emerging as a novel strategy with multiple action sites. It is now crucial to validate the contribution of MCT1 and perhaps of other MCTs in defined cell types with different (epi)genetic backgrounds exposed to variable microenvironmental conditions.

LACTATE IN TUMOR ANGIOGENESIS: CLUES FROM WOUND REPAIR

Despite wound transformation is an uncommon and rare event, gene expression analysis of healing skin wounds indicated a pattern which strongly resembles to that of highly malignant cancers [146]. Striking similarities between wounds and tumors have been pointed out by Harold Dvorak [147] who, in the mid 1980s, provocatively compared tumors to wounds that do not heal. Coherently with these observations, wounds, similar to tumors, are notoriously hypoxic lesions characterized by an increased glycolytic rate and lactate secretion [148, 149]. In normal physiological conditions, on average, lactate levels range from 1.8 mM to 2.0 mM, whereas in wounds and tumors it may reach 6 - 15 mM. [10, 16, 24, 37, 150].

Several efforts have been made in order to understand whether lactate accumulation in wounds was just an epiphenomenon or actually involved in the wound repair process. It is currently recognized that lactate concentrations similar to those present in wounds improve by themselves many processes essential to the regenerative process. In the next paragraphs, we review the main evidences pointing at a functional link between lactate production and angiogenesis in the context of wound healing, which would logically also exerts an influence in cancer despite few specific data are available today.

Wound healing is one of the most composite biological features, involving several signaling cascades and biological processes that have to be transiently activated and coordinated in order to lead to proper wound repair [151]. Among these processes, the onset of angiogenesis supporting the development of new blood vessels from pre-existing ones is a critical event to restore perfusion, oxygenation, and nutrient supply. Lactate has been shown to exert several pro-angiogenic activities: it stimulates VEGF secretion by macrophages [152], endothelial cell migration [38], vascular morphogenesis [153] and the recruitment of circulating vascular progenitor cells [154]. In addition, lactate has been shown to trigger tissue repair by augmenting collagen deposition, TGF-β production, and fibroblast proliferation [37, 39, 155]. Several lactate-sensitive pathways support these biological responses. In order to understand the underlying mechanisms, it is important to point out that lactate-induced angiogenesis rely on lactate oxidation by LDH-1 [153, 154], calling into play the products of the enzymatic reaction (namely pyruvate and NADH) and lactate transporters. On one hand, raising levels of pyruvate interfere with PHD activity to activate HIF-1 and NF-κB, as mechanistically detailed above and in Fig. (**2**). HIF-1 is a master regulator of angiogenesis: its activation is sufficient to trigger the expression of several growth factors required for angiogenesis, among which VEGF, basic fibroblast growth factor (bFGF) and stromal cell-derived factor-1 (SDF-1) [156, 157]. While VEGF and bFGF regulate angiogenesis through binding with their cognate receptors on endothelial cells, the combined action of VEGF and SDF-1 in response to lactate is involved in the recruitment of endothelial progenitor cells for vasculogenesis at the wounded site. Milovanova *et al.* [154] indeed showed that lactate is sufficient to trigger the recruitment and *in vivo* vascular differentiation of $CD34^+$ cells in Matrigel plugs, and both phenomenons were blocked using neutralizing antibodies against VEGF and SDF-1. They were also blocked with LDH inhibitors (oxamate and siRNAs), the NAD(P)H oxidase inhibitor apocinyn, the antioxidants *N*-acetylcysteine and dithioerythritol, and a specific siRNA against HIF-1, thus confirming lactate-induced HIF-1 activation as an important pathway for vasculogenesis. On the other hand, NADH production associated with lactate oxidation depletes the pool of NAD^+, which has consequences on NAD^+-dependent enzymes such as GAPDH (explaining in part why lactate-fueled respiration inhibits glycolysis [4, 124]) and PARPs. PARPs use NAD^+ for the synthesis of polyadenosine diphosphoribose (pADPR) used for the posttranslational modification of histones and other proteins including p53, Sp-1, and NF-κB [158, 159]. By depleting NAD^+, LDH-1 activity logically reduces pADPR synthesis and affects gene transcription [152, 160]. In the context of wound healing, Ghani *et al.* [150] reviewed the work of Thomas Hunt having shown that VEGF transcription and synthesis and collagen transcription are enhanced by the lactate-mediated downregulation of pADPR, which thereby participates in lactate-induced angiogenesis. There is further evidence that lactate can decrease the mono-ADP-ribosylation of VEGF (leading to its activation in macrophages and in endothelial cells) [152, 161, 162] and of collagen PHD, which increases its enzymatic activity and collagen maturation in fibroblasts [163].

To date, whether lactate exerts similar influences in cancer as those evidenced in wounds is largely unknown. To our knowledge, there is no report addressing the influence of lactate on ADP-ribosylation in tumors. Recently, Lu *et al.* demonstrated increased VEGF production in several cancer cell lines exposed to lactate [34], but these findings were never translated in functional assays. Perhaps the most extensive study is that of Végran *et al.* [36], linking lactate-induced NF-κB activation in endothelial cells with IL-8-mediated autocrine angiogenesis. The authors documented that this pathway drives endothelial cell migration and tube formation *in vitro*, and lactate-induced tumor angiogenesis *in vivo*. The latter experiment involved the co-injection of notably Warburg WiDr tumor cells with HUVECs in Matrigel plugs in immunodeficient mice, wherein either the use of MCT4-deficient tumor cells (unable to release lactate), the use of MCT1-deficient HUVECs (unable to take-up lactate), or an IL-8-blocking antibody all repressed tumor angiogenesis and delayed tumor growth [36]. These experiments further confirmed MCT1 as an antitumor target. While MCT1 inhibition in oxidative tumor cells exerts antimetabolic activities [4], targeting MCT1 in endothelial cells could exert potent anti-angiogenic effects [36]. Further studies are now needed to define the therapeutic dimension of the strategy.

CONCLUDING REMARKS

Although the involvement of lactate in wound healing and the existence of lactate shuttles in normal tissue physiology have been identified years if not decades ago, lactate in cancer has attracted attention only recently. Most of its tumor-growth activities still need to be discovered. In tumors, lactic acid is exported from glycolytic tumor cells (being aerobic or anaerobic) and stromal cells. However, theoretically, glycolysis uncoupled from any other metabolic pathway would yield lactate but no proton. Lactic acidosis therefore involves metabolic activities different from glycolysis itself and relies on the necessity of a pH gradient across the plasma membrane to export lactate through MCTs. There are two potential readings: (i) although many other proton transporters are overexpressed in glycolytic cancer cells, lactate serves to export protons to prevent intracellular acidosis; and/or (ii) protons serve to export lactate in order to maintain the pH buffering capacity of the LDH-5 reaction. Far to be trivial, resolving this black box could help to position MCT inhibitors with respect to inhibitors of other proton transporters, such as CAIX, NHE-1 and membrane-bound V-ATPase, most of which are currently undergoing clinical trials [9].

Once exported, lactate and protons may separately exert tumor-promoting influences, or act in a coordinated manner. Coordinated activities have been described in the context of the lactic acid induction of IL-23 in monocytes, an activity that cannot be reproduced with low pH or sodium lactate when administered separately [144]. If, as exemplified in many studies and proposed in others, the intracellular activities of lactate first require MCT1-mediated lactate uptake, protons are necessarily involved. Intracellular activities include the use of lactate as an oxidative fuel, lactate signaling supporting angiogenesis (HIF-1 and NF-κB activation) [34-36] and metastasis (induction of TGFβ, HA and CD44) [113, 114, 117], and the lactate regulation of mono/poly-ADP-ribosylation in angiogenesis [150]. A consequence of this is that targeting MCT1 with a single therapeutic agent could exert multiple anticancer influences. A first MCT1 inhibitor, AZD3965, is currently entering Phase I/II clinical trials for advanced solid tumors (http://science.cancerresearchuk.org/). Its evaluation could help to better define the role of MCT1 in cancer. Lactate may also exert MCT-independent activities in tumors, as done by protons. Over the past decades, the contribution of low pH_e to tumor progression and metastasis has been well characterized but the direct activities of lactate are still to be explored. Some could be conveyed by GPR81, a G protein-coupled receptor initially identified in adipocytes and recently revealed as a receptor for lactate [164, 165].

In summary, while many of the biological activities of lactic acid in tumors could be targeted therapeutically with MCT inhibi-

tors and other strategies have been identified to exploit tumor acidity independently of lactate, most intrinsic activities of the lactate anion remain to be identified, characterized and, potentially, tailored for therapy.

CONFLICT OF INTEREST

The authors report no potential conflict of interest.

ACKNOWLEDGMEMENTS

Works at the authors' lab are supported by the European Research Council (FP7/2007-2013 ERC Independent Researcher Starting Grant 243188 TUMETABO to P.S.), the Belgian *Fonds National de la Recherche Scientifique* (F.R.S.-FNRS), the *Communauté Française de Belgique* (ARC 09/14-020), and the *Fondation Belge Contre le Cancer* (200-2008). P.S. is a F.R.S.-FNRS Research Associate.

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Received: November 30, 2011 **Accepted: December 26, 2011**

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