Lactate and Acidity in the Cancer Microenvironment

Scott K. Parks,1 Wolfgang Mueller-Klieser,2 and Jacques Pouysségur1,3

1Department of Medical Biology, Centre Scientifique de Monaco (CSM), 98000 Monaco
2Institute of Pathophysiology, University Medical Center, Johannes Gutenberg University Mainz, 55128 Mainz, Germany
3Institute for Research on Cancer and Aging, Nice (IRCAN), CNRS UMR 7284, INSERM U1081, Centre A. Lacassagne, University Côte d’Azur, 06189 Nice, France; email: Jacques.Pouyssegur@unice.fr

Abstract
Fermentative glycolysis, an ancient evolved metabolic pathway, is exploited by rapidly growing tissues and tumors but also occurs in response to the nutritional and energetic demands of differentiated tissues. The lactic acid it produces is transported across cell membranes through reversible H+ /lactate− symporters (MCT1 and MCT4) and is recycled in organs as a major metabolic precursor of gluconeogenesis and an energy source. Concentrations of lactate in the tumor environment, investigated utilizing an induced metabolic bioluminescence imaging (imBI) technique, appear to be dominant biomarkers of tumor response to irradiation and resistance to treatment. Suppression of lactic acid formation by genetic disruption of lactate dehydrogenases A and B in aggressive tumors reactivated OXPHOS (oxidative phosphorylation) to maintain xenograft tumor growth at a halved rate. In contrast, disruption of the lactic acid transporters MCT1/4 suppressed glycolysis, mTORC1, and tumor growth as a result of intracellular acidosis. Furthermore, the global reduction of tumor acidity contributes to activation of the antitumor immune responses, offering hope for future clinical applications.

Keywords
Warburg effect, fermentative glycolysis, lactate, LDHA, LDHB, MCT1, MCT4, glycogen, cancer, immune evasion
1. INTRODUCTION

Nearly a century has passed since Otto Warburg discovered that animal tumors produced large amounts of lactate. His experiments were performed in vitro with excised pieces of tumors and showed that fermentation (anaerobic glucose breakdown to lactate) was preferred for growth rather than respiration (oxidative glucose breakdown) present in normal cells (Warburg 1923, Warburg et al. 1927). Carl and Gerty Cori then confirmed that the preference for fermentation among tumors also occurred in living animals (Cori & Cori 1925). Furthermore, it was shown that tumor cells in a glucose-free medium survived by respiration, whereas in the absence of oxygen, glucose fermentation supported growth and survival. Interestingly, Okamoto and Warburg (Warburg et al. 1927) reported that a few hours of suppression of oxygen and glucose was sufficient to kill tumor cells. These pioneering observations a century ago illuminated the two complementary bioenergetics pathways of respiration and fermentation.

The metabolic pathways responsible for fermentation and respiration were deciphered in the mid-twentieth century, and glycolysis in particular was presumed to play a key role in the early emergence of anaerobic life, as well as in the emergence of bacteria, yeast, and animal cells in oxygenated environments. The glycolytic pathway (also known as the Embden-Meyerhof-Parnas pathway, in acknowledgment of its discoverers) converts glucose into pyruvate by a sequence of ten enzymatic reactions. The phylogenetic distribution of these enzymes has shown that they comprise an ancient metabolic pathway expressed in all organisms including eubacteria (trunk pathway only) and archaea (Fothergill-Gilmore & Michels 1993). The final and perhaps most important step of the fermentation pathway is the reduction of pyruvate into lactate with the regeneration of the coenzyme NADH into NAD$^+$ and H$^+$.  

L-Lactic acid, CH$_3$CH(OH)COOH, produced by fermentation of carbohydrates, was first isolated from milk in 1780 by Carl Wilhelm Scheele. This acid with an acid dissociation constant ($pK_a$) of 3.86 exists at neutral pH as a 99% dissociated base, L-lactate$^-$. In 1856, lactate was rediscovered by Louis Pasteur from the Gram-positive, facultative anaerobe Lactobacillus.

2. FERMENTATIVE GLYCOLYSIS AND CANCER: AN APPARENT PARADOX

For many years, the preference that rapidly growing tumors have for glucose fermentation, a low-ATP-producing pathway, in contrast to respiration, has been paradoxical, and yet this metabolic choice is almost universal for rapid proliferation as long as nutrients and glucose are provided (Vander Heiden et al. 2009). Glucose fermentation appears to also be the rule for exponential growth of microbes such as bacteria and yeast. Instead of secreting lactate, yeast reduces pyruvate into ethanol with a family of glycolytic enzymes well conserved among the Saccharomyces genus (Boonekamp et al. 2018). The extreme efficiency of this pathway, even in the presence of oxygen (termed the Crabtree effect (De Deken 1966)), is reflected by an amazing concentration of glycolytic enzymes able to represent about 30% of the total amount of soluble proteins (Fraenkel 2003). In excess of glucose, inhibition of glucose oxidation is associated with an overflow of metabolism, growth, and ethanol production, which might have emerged as a strategy to inhibit and compete with other microbes (Hagman & Piskur 2015). Most of the human tumor cell lines grown in vitro, under high-glucose and -oxygen conditions, ferment the excess glucose into lactic acid but shift to glucose/glutamine/fatty acid oxidation when glucose is scarce (below 1 mM) (Birsoy et al. 2014). However, as Otto Warburg previously reported, only a small number of tumor cell lines bear mutations that impair the mitochondrial respiratory chain, in contrast to all cancers (Birsoy et al. 2014, Warburg 1956).
3. FERMENTATIVE GLYCOLYSIS OUTSIDE CANCER: ALMOST A RULE!

In differentiated nondividing cells, metabolism is optimized to provide ATP via oxidative phosphorylation (OXPHOS). In contrast, rapidly proliferating cells, whether they are cancerous or normal cells (embryonic, immune cells, regenerating tissues, etc.), require both ATP and, above all, anabolic building blocks for increasing biomass and replenishing nutrients (Palm & Thompson 2017, Vander Heiden et al. 2009). If fermentative glycolysis is the best fit for rapid production of ATP and metabolite precursors, two branching oxidative anabolic pathways, the pentose phosphate pathway (PPP) and the serine/glycine synthetic pathway (SSP), complement the generation of anabolic precursors for lipid and nucleotide synthesis (DeNicola et al. 2015, Mitsuishi et al. 2012). In addition, the concomitant generation of NADPH by the PPP and SSP pathways contributes to the maintenance of reduced glutathione, the major cellular antioxidant (Semenza 2017) (Figure 1).

The muscle during exercise is perhaps the oldest and best example of fermentative glycolysis in a well-differentiated organ. Lactate production was long considered to be a consequence of oxygen depletion during skeletal muscle contractions. However, further quantitative biochemical studies using radioactive tracers first demonstrated that lactate production is not only confined to hypoxic environments but also takes place in well-oxygenated muscle (Brooks 1985) (for a comprehensive and rich historical review, see Brooks 2009). Additionally, the now-established idea of lactate not as a waste product but as a mobile and recycled energetic metabolite emerged initially with the Cori Cycle, a breakthrough in 1929 that led Carl and Gerty Cori to share the Nobel Prize in Physiology or Medicine in 1947 (Figures 1 and 2). Later, lactate was reported
to be recycled and stored as glycogen in the liver, muscle, kidney, and brain and became central in the understanding of carbohydrate metabolism (Brooks 2009, 2018; Cali et al. 2019; Gladden 2004; Sonveaux et al. 2008). In some organs, such as heart and liver, lactate can serve as a significant source of energy. For example, during physical exercise, 60% of the energy turnover rate in heart muscle is recruited from lactate oxidation (Jafri et al. 2001). Moreover, in C2C12 myotubes, hepatocytes, fibroblasts, and several tumor cell lines exposed to hypoxia in culture were capable of synthesizing and accumulating glycogen through gene induction by hypoxia-inducible factors (HIFs) (Pelletier et al. 2012, Pescador et al. 2010). It is remarkable that the glycolytic enzymes and the key enzymatic steps for gluconeogenesis are both induced by HIFs, ensuring glycogen replenishment in response to a hypoxic signal. Furthermore, experiments with food restriction in rats or mice demonstrated increased lactate production from glucose under conditions of restricted pyruvate oxidation through inhibition of the mitochondrial pyruvate dehydrogenase complex (Jeoung et al. 2006, Thacker et al. 1987). Finally, a recent discovery demonstrated that FOXK1 and FOXK2, two related fasting/starvation transcription factors, induced fermentative glycolysis and lactate production in muscle and adipose tissue of starved mice (Sukonina et al. 2019). Interestingly, lactate production is facilitated by concomitant inhibition of the mitochondrial pyruvate dehydrogenase complex, implicating increased activity of pyruvate dehydrogenase kinases 1 and 4 (Jeoung et al. 2006, Sukonina et al. 2019). This mechanism is reminiscent of the HIF1-induction of pyruvate dehydrogenase kinase 1, a key step in promoting fermentative glycolysis and lactic acid production in hypoxic environments (Kim et al. 2006, Papandreou et al. 2006, Pouysségur et al. 2006). Although the interplay between HIF1 and FOXK1/FOXK2 transcription factors is unknown, it is remarkable that both types activate fermentative glycolysis in response to nutrient deprivations (Figure 1).

In conclusion, fermentative glycolysis and lactate, viewed as a key glycogen precursor, are fundamental in carbohydrate metabolism and bioenergetics. This ancient evolved metabolic pathway is exploited by rapidly growing tissues and tumors but also occurs in response to the physiological nutritional and energetic demands of differentiated organs and tissues. Recently, quantitative analysis using $^{13}$C-glucose versus $^{13}$C-lactate in vivo revealed that during fasting, the contribution of glucose through the tricarboxylic acid (TCA) cycle is primarily indirect, via circulating lactate.
Figure 3
Key players in tumor cell lactate transport and pH regulation. Represented are direct movement of H\(^+\) and lactate (Lac\(^-\)) via NHE1 and MCT4, H\(^+\) shuttling and CO\(_2\) conversion via CAs at the tumor cell membrane, and extra- and transcellular mechanisms of facilitated movement/venting of both tumor acidity and lactate toward the distant vasculature. Abbreviations: CA, carbonic anhydrase; MCT4, monocarboxylate transporter 4; NHE1, Na\(^+\)/H\(^+\) exchanger 1; OXPHOS, oxidative phosphorylation; pH\(_e\), extracellular pH; pH\(_i\), intracellular pH.

(Hui et al. 2017). As reported in the next section, lactic acid is transported in and out of tumor cells via reversible monocarboxylate transporters (MCTs), which are expressed in virtually all cells (Halestrap 2012), and plays a major role in tumor progression.

4. LACTATE TRANSPORT AND TUMOR ACID-BASE REGULATION

As introduced above, a glycolytic phenotype has been a defining feature of aggressive tumor cells for nearly a century of research. Effective regulation of the lactate and H\(^+\) produced during glycolysis (Figure 3) is thus required to prevent these metabolites from becoming rate limiting in tumor cells. Lactic acid secretion into the poorly perfused extracellular space of hypoxic tumors has been established as a primary source of tumor acidity; however, CO\(_2\) production from oxidative metabolism can equally contribute to tumor acidity (see the discussion in Parks et al. 2017 and Figure 3). Therefore, it is important to interpret the literature correctly with respect to whether a given cellular effect is attributed to the influence of lactic acid versus lactate or tumor acidity in general. "Lactic acid" is often used synonymously for "lactate"; however, this can be misleading, as important areas of tumor biology such as the immune cell response can be heavily influenced by acidity but not lactate per se (Brand et al. 2016). Despite extracellular pH environments (pH\(_e\)) that can regularly approach pH 6.5 in vivo, it has been well documented that tumor cells efficiently
maintain a relatively alkaline intracellular pH ($pH_i$) to effectively maintain metabolic enzyme activity for key players such as mTORC1 (for extensive reviews see Flinck et al. 2018 and Parks et al. 2013a). Here we focus on how lactate transport is achieved in this challenging environment by focusing primarily on the current consensus for lactate transport in tumors via MCTs, their interactions with direct pH regulators [i.e., NHE1 (Na$^+$/H$^+$ exchanger 1)], and the association with carbonic anhydrases (CAs).

4.1. Monocarboxylate Transporter Regulation of Tumor Lactate Homeostasis and Tumor Growth

The $pK_a$ of lactic acid dictates that it is dissociated almost exclusively into lactate ($\text{lac}^-$) and $H^+$ within the physiological pH range. Lactate is a relatively cell-impermeant molecule, necessitating the presence of facilitated transporters of which the MCT family has been shown to provide the bulk of cellular lactate transport. Four members (MCT1–4) of the SLC16 gene family have been shown to link $H^+$ transport to lactate via electroneutral $H^+/\text{lac}^-$ symporters (Halestrap 2012, 2013). MCT1 and MCT4 in particular have been intensively studied in the context of cancer. Although MCT1, induced by c-Myc, is primarily used for lactate import or export in most tissues, HIF1 induced MCT4 (Ullah et al. 2006) is the dominant isoform found in chronic-glycolytic tissues such as tumors. This expression pattern is functionally linked to the difference in the Michaelis constant ($K_m$) for pyruvate (150 mM MCT4 versus 1 mM MCT1), which ensures continued conversion of pyruvate to lactate and thus the regeneration of cellular NAD$^+$ to enable continued glycolytic flux (Halestrap 2013). Additionally, hypoxia induction of PDK1 (pyruvate dehydrogenase kinase 1), an inhibitor of pyruvate oxidation (Kim et al. 2006, Papandreou et al. 2006), further highlighted the importance of MCT4 in channeling tumor glycolysis toward fermentation (Le Floch et al. 2011). Unsurprisingly, MCT4 is progressively becoming a prominent marker of poor prognosis in clinical literature assessments of multiple aggressive cancer types (Bovenzi et al. 2015, Doyen et al. 2014).

During the past decade, our group and others have systematically investigated disrupting lactate export to prevent glycolysis via the genetic knockout of MCT1/4 and their common chaperone, CD147. Collapse of lactate export via MCT1/4 was either genetically or pharmacologically successful in arresting glycolysis and compromising tumor cell growth under certain conditions (Chiche et al. 2012, Doherty et al. 2014, Le Floch et al. 2011). From a therapeutic development standpoint, the chaperone CD147 was an attractive target, as it controls membrane expression of both MCT1 and MCT4. Indeed, CD147 disruption was equally effective in comparison to direct MCT inhibition in the blockade of glycolytic metabolism (Granja et al. 2015, Marchiq et al. 2015). Results observed with the disruption of lactate transport were mimicked when LDHA/B knockout was achieved and consequently lactate production was eliminated (Zdralevic et al. 2018a). The potential therapeutic benefit of these approaches is discussed below in the final section. However, this large series of investigations also clearly illustrated that in the absence of glycolysis (i.e., either short-term inhibition or permanent removal), tumor cells are highly capable of utilizing OXPHOS to survive and eventually proliferate. Thus, it has become clear that any future targeted therapy against lactate transport may effectively result in temporary growth arrest; however, it must be considered in combination with short-term additional inhibition of OXPHOS, if any reduction in tumor size is to be achieved.

4.2. pH-Regulating Proteins’ Influence on Lactate Transport

Maintenance of physiological pH is essential to retain virtually all cytoplasmic protein activity, with change in pH, resulting in impairment of key cellular proteins including mTORC1 (Balgi et al. 2011, Chambard & Pouysségur 1986, Flinck et al. 2018). Consequently, cells possess highly
efficient buffering mechanisms to absorb H\textsuperscript{+} in addition to membrane transporters that remove excessive H\textsuperscript{+} to the extracellular space. The ubiquitously expressed NHE1 (Sardet et al. 1989) responds immediately to extrude cellular H\textsuperscript{+} in exchange for Na\textsuperscript{+} (reviewed in Counillon et al. 2016 and Pedersen & Counillon 2019). Other pH-regulating proteins including CAs and bicarbonate transporters are further implicated in pH\textsubscript{i} regulation and buffering of metabolic acid production (Parks et al. 2013a). Thus, the efficient mechanisms of pH\textsubscript{i} regulation in tumor cells could compete for free H\textsuperscript{+} within the cytoplasm and consequently decrease lactate transport efficiency via MCTs (Figure 3). However, we have demonstrated that a collapse of MCTs rapidly acidifies pH\textsubscript{i} (Marchiq et al. 2015) and that forced expression of MCT4 in tumor xenografts increased both pH\textsubscript{i} and glycolytic rates (Chiche et al. 2012), indicating that MCTs are required to complement other pH\textsubscript{i}-regulating proteins during heightened metabolic activity. The importance of MCTs in maintaining a more alkaline pH\textsubscript{i} in the face of glycolytic metabolism had also been shown using more broad-reaching MCT inhibitors (Zhou et al. 2001). Recently, it has been emphasized that MCTs are not capable of regulating pH\textsubscript{i} to a set point but simply act to equilibrate imbalanced gradients produced by metabolic activity (Swietach 2019). Nonetheless, the observation of either MCT inhibition or genetic removal resulting in a significant decline in pH\textsubscript{i} indicates that MCTs contribute to the tumor cell pH\textsubscript{i} value that is permissive for tumor cell survival and elevated proliferation.

Considering the potential for H\textsuperscript{+} competition between transporters such as NHE1 and MCTs, it has been of great interest to understand how MCTs obtain the H\textsuperscript{+} required for lactate transport. The fact that MCTs strip H\textsuperscript{+} from the cytoplasm at a much greater rate (∼15 times for MCT1) than would be predicted for H\textsuperscript{+} diffusion and buffering models led researchers to assume that a cooperative mechanism exists (Adelroth & Brzezinski 2004, Branden et al. 2006, Martinez et al. 2010). CAs function to catalyze hydration of CO\textsubscript{2} to HCO\textsubscript{3}− and H\textsuperscript{+} and have thus received extensive attention in the context of tumor cell metabolism due to their ability to regulate both pH\textsubscript{e} and pH\textsubscript{i} (for a recent and extensive review on CAs, see Mboge et al. 2018). As with MCT4, the extracellular-facing CAIX is one of the proteins most prominently induced by hypoxia (Wykoff et al. 2000); however, it is nearly unexpressed in most normal tissues under physiological conditions, resulting in excitement for drug development of these nearly tumor-exclusive proteins (for progress in clinical developments readers are referred to McDonald et al. 2018). CA catalytic activity is of definite importance in overall tumor bioenergetics; however, for enhancement of lactate mobility, the noncatalytic activity of CAs has come to the forefront in a large series of investigations.

Becker and Deitmer were the first to report that CA interactions with MCTs could enhance transport activity using the Xenopus oocyte heterologous expression system (Becker et al. 2005). Intriguingly, enhancement of MCT1/4 transport activity was still maintained when coexpressed with a catalytically inactive form of CAII (Becker & Deitmer 2008, Becker et al. 2010), indicating that functional coupling was not due to CA activity. Importantly, it was verified that (both intracellular- and extracellular-facing) CAs enhanced MCT transport in cancer cells, beyond just Xenopus expression systems (Jamali et al. 2015, Noor et al. 2018). Thus a concept has emerged whereby the intramolecular H\textsuperscript{+} shuttle within the CA protein structure is utilized as an H\textsuperscript{+}-collecting antenna to provide a continuous stream of H\textsuperscript{+} required for MCT cotransport of lactate (Becker et al. 2011, Noor et al. 2018) (Figure 3). Recognition that CA-MCT coexpression increases MCT activity by only twofold (Noor et al. 2018), however, necessitates further translational investigations to determine potential clinical relevance.

### 4.3. Metabolite Movement Through Cell-Cell Junctions

Acidity in the extracellular tumor space places a thermodynamic constraint on MCT-directed lactate export and glycolysis, which can be rapidly observed in vitro while monitoring metabolic flux...
Investigation of this concept within the tumor-stromal three-dimensional environment demonstrated that stromal cells could absorb metabolic acid produced by tumor cells to act as an acid conduit toward the vasculature via stromal cell-cell junctions (Hulikova et al. 2016). This work was extended to support direct tumor cell-cell junction transport whereby normoxic cells could help to stabilize the pH of hypoxic cells via movement of HCO$_3^-$ buffering units (Dovmark et al. 2018). As either tumor-stromal or tumor-tumor cell-cell interactions would alter the pH dynamics within the tumor environment, they would indirectly act on lactate transport via MCTs due to alterations in the thermodynamic status of the tumor (Figure 3). Interestingly, a role for Connexin-43 channels was revealed for the dissipation of lactate away from highly glycolytic pancreatic ductal adenocarcinoma cells (Dovmark et al. 2017) as a mechanism that could maintain elevated metabolic rates (for a review see Swietach & Monterisi 2019). An absence of therapeutic interest in tumor cell junctions has been linked to early descriptions of tumor cells lacking electrical coupling and multiple reports suggesting that the expression of connexins has tumor-suppressor properties (reviewed extensively by Aasen et al. 2016). However, it appears that in certain tumors, and perhaps more specifically at different stages of oncogenesis, this form of cell-cell metabolite movement may be considered as an effective mechanism to successfully maintain tumor bioenergetics.

5. LACTATE: AN INTEGRATIVE MIRROR OF CANCER METABOLISM WITH CLINICAL IMPLICATIONS

Steady-state concentrations of metabolites can mirror the metabolic status of live tissues. Unlike most healthy organs, malignant tumors are extremely heterogeneous with regard to the spatial arrangement of vasculature, various cellular subpopulations, and localized concentrations of metabolites (Aly et al. 2015, Jeng et al. 2015). There is evidence that this characteristic tumor heterogeneity is one major cause of therapeutic failure in medical oncology (Walther et al. 2015). Consequently, imaging metabolites in cancerous tissue in a biologically and clinically significant manner requires the quantitative detection of metabolic substances within microscopic dimensions in association with the histological tissue structure.

Metabolic analyses based on tumor biopsies are routinely performed in the clinic for pathohistological diagnosis. Spare material from this procedure is often available for scientific purposes under ethical considerations and patient consent. We have shown that appropriate removal and rapid liquid nitrogen freezing of such biopsies are possible in the clinical setting, and that these specimens enable the analysis of a tissue’s momentary metabolic status (metabolic snapshot) (Walenta et al. 2000, 2016). We have demonstrated that in most cases, one tumor biopsy from the pathological routine can be representative of metabolic features of an entire tumor when compared with measurements from two or three biopsies from the same cancer (Walenta et al. 2016). Furthermore, we found that tissue concentrations of lactate do not change when biopsies were kept in liquid nitrogen for ten years (Walenta et al. 2016), which provides potential for long-term storage of metabolic tissue banks.

An increasing number of signaling pathways have been closely linked to cancer metabolism. As a result, the metabolic deregulation in tumors may be recognized as a complex network of interrelated pathways that is unpredictable in its functionality in individual tumors (Carroll et al. 2015). In contrast, there is a common readout of cancer cell metabolism integrating over its various signaling activities, i.e., the cellular efflux of lactate into the tumor microenvironment (Dhup et al. 2012, Hirschhaeuser et al. 2011, Luc et al. 2015). The clinical significance and implications of the extremely variable lactate concentrations in solid tumors were first identified by our group in 2000 (Walenta et al. 2000) using induced metabolic bioluminescence imaging (imBI). The imBI
technique allows for the quantification of various metabolites, such as glucose, lactate, pyruvate, ATP, glucose-6-phosphate, or D2-hydroxyglutarate, and for the assessment of the regional distribution of these metabolites within tissues of interest. The method has been developed in our laboratory on the basis of biochemical precursor studies, mainly on brain metabolism (Kim et al. 1993, Kricka 2000, Paschen 1985, Paschen et al. 1981). The current status of imBI and its advantages and limitations have been reviewed previously (see Walenta et al. 2014 for further methodological details).

The use of imBI has generated a huge amount of data in a wide range of experimental and clinical tumors. In all tumors studied, tissue concentrations of lactate showed the largest variability compared to all other metabolites investigated. Lactate concentrations ranged across tumors from 0 to 50 μmol/g of tissue, which corresponds to approximately 0–50 mM in a liquid phase. Considering that the physiological range of lactate in human blood is 0–2 mM, cancer cells survive in the face of exorbitant lactate concentrations combined with a severe metabolic acidosis (as discussed elsewhere). Even considering that blood lactate concentrations reach transient values of 10–15 mM during exhausting physical work, this metabolite can be cleared from blood within 30 minutes post-exercise. In contrast, cancer cells are chronically exposed to elevated lactate concentrations, acidic pH, and carbonic dioxide tensions up to 80 mmHg, which can be considered chronically pathophysiological conditions (summarized by Walenta & Mueller-Klieser 2004 and Walenta et al. 2000).

5.1. Clinical Relevance of Lactate Accumulation in the Tumor Microenvironment

During quantitative evaluation of tumor lactate concentrations and their clinical relevance, it appeared advantageous to classify tumors into high- and low-lactate cancers by separating the data values using the median lactate concentration as a limit between the two classes. Since the difference between the two lactate classes is most likely generated by different glycolytic activities of the tumor tissue, the terms “high-” and “low-glycolytic tumors” were eventually used in the literature as synonyms for “high-” and “low-lactate tumors,” respectively. Interestingly, the separating limit between high- and low-lactate tumors was invariably in a range of 10 ± 2 mM in all tumors investigated, i.e., in different independent studies, experimental and clinical settings, and tumor entities.

5.1.1. Patient survival. In most cancers investigated, high-lactate tumors were associated with reduced long-term survival or disease-free survival compared to their low-lactate counterparts (Walenta & Mueller-Klieser 2004; Walenta et al. 2000, 2004). In some tumor entities, such as head and neck cancer, the statistical probability of tumor recurrence was dramatically higher in high-versus low-lactate tumors (Brize et al. 2001). In line with high-lactate tumors, the expression of the hypoxia-inducible H+/lactate symporter MCT4 demonstrated the strongest deleterious impact on survival in two separate cohorts of 770 node-negative breast tumors (Doyen et al. 2014).

5.1.2. Incidence of metastasis. The emergence of metastasis is a primary clinical factor that limits patient survival. Incidence of early distant metastasis at first tumor diagnosis was significantly higher in high-lactate primary cancers compared to low-lactate primary cancers (summarized by Walenta & Mueller-Klieser 2004). It has been shown that lactate per se stimulates angiogenesis through activation of the VEGF/VEGFR2 pathway, which may support the metastatic process (Dhup et al. 2012, Porporato et al. 2012). Another pathophysiological mechanism
enhancing the formation of metastasis is the stimulation of tumor cell motility by lactate (Baumann et al. 2009, Goetze et al. 2011). At present, several G protein–coupled receptors (GPCRs), GPR4, GPR65, GPR68, GPR81, and GPR132, have been identified as putative lactate or proton sensors (Justus et al. 2013). While GPR81 was initially detected and classified as an orphan receptor in adipocytes (Cai et al. 2008), its function as not only a cell surface l-lactate receptor but also a hydroxyxycarboxylic receptor has been investigated in several cell types including malignant cells (see Romero-Garcia et al. 2016). However, in the context of the tumor acidic microenvironment, the proton-sensing GPR4, GPR65, GPR68, and GPR132 have received the greatest attention (Weiss et al. 2017). They are activated via the protonation of several histidine residues in response to an extracellular pH drop. Tumor acidity, generated from lactic and carbonic acids (Newell et al. 1993), transmits intracellular signals through G proteins coupled to either adenylate cyclase (GPR4, GPR65), phospholipase C (GPR68), or a presently unidentified effector (GPR132) (Justus et al. 2013, Seuwen et al. 2006).

Of great interest, two acidic-sensitive GPCRs, GPR132 and GPR65, both expressed in tumor-associated macrophages (TAMs), have now been recognized to exhibit a reciprocal interaction between cancer cells and macrophages for breast cancer (Chen et al. 2017) and melanoma (Bohn et al. 2018). Although the nature of acidic-activated GPR132 signaling is lacking, GPR132 activates the M2-like macrophage phenotype, which facilitates cancer cell migration, invasion, and metastasis (Chen et al. 2017). In contrast, acidic-activated GPR65 induces via cyclic AMP the transcriptional repressor ICER (inducible cyclic AMP early repressor) in tumor-associated macrophages, which leads to their functional polarization toward a noninflammatory phenotype and promotes tumor growth (Bohn et al. 2018).

5.1.3. Therapeutic resistance. In 2010, we published a collaborative study with Michael Baumann’s group on radioresistance in a large cohort of human head and neck cancer xenografts (Sattler et al. 2010), following standard clinical protocols for irradiation dose and fractionation scheme. Unlike many metabolic parameters investigated, lactate concentration was a dominant modulator of tumor response to irradiation, with the highest-lactate tumors being most resistant to treatment. Among other factors, this may be explained by the generation of a reductive milieu by high-glycolytic turnover rates; under these conditions, pyruvate can act as an antioxidant by nonenzymatic formation of acetate and concomitant scavenging of hydrogen peroxide (Salahudeen et al. 1991). Furthermore, it has been shown that the addition of exogenous lactate to endothelial cell cultures leads to an increase of the NAD(P)H:NAD(P) ratio and to the transcriptional control of several genes mediated by the redox-regulated transcription factor complex AP-1 (Hoffmann et al. 2001). In analogy to radioresistance caused by reductive milieu conditions, chemoresistance may occur with those drugs that are inactivated under these conditions, such as doxorubicin (Velaei et al. 2016).

Facing the significance of the tumor redox status for cancer therapy, we used imBI technology for structure-related quantitative redox imaging (Sattler et al. 2007). This is illustrated in Figure 4, which shows the histology of a human head and neck squamous cell carcinoma next to striated muscle (Figure 4a) and a color-coded map of lactate-to-pyruvate ratios (Figure 4b). The coded colors clearly mirror the intensively reduced redox state of the malignant versus normal tissue.

5.2. Noninvasive Detection of Lactate and Related Metabolites

Although invasive, the imBI technology has a unique combination of properties, including spatial resolution on a microscopic level, quantitative measurements of metabolites in absolute units (micromoles per gram of tissue), biochemical versatility with regard to a broad spectrum of possibly
detectable metabolites, the direct colocalization of metabolites and histological structure, and clinical applicability. Presently, these traits of imBI cannot be met by any of the up-to-date metabolic imaging techniques currently used experimentally or routinely in the clinic. Nevertheless, imaging techniques are urgently required and need to be advanced to improve our knowledge of human malignant disease, of comprehensive diagnosis, and of versatile, customized therapies. Numerous efforts and advances in noninvasive metabolic imaging have been reported in the recent literature, but only a few select examples of lactate imaging–related studies can be briefly mentioned here. Unlike glucose, tissue lactate fluxes have yet been detected by positron emission tomography (PET). However, a recent report showed that $^{18}$F-3-fluoro-2-hydroxypropionate can serve as an analog of lactate, which enables monitoring of cellular uptake of lactate by MCT1 in PET studies (Van Hee et al. 2017). Using a combination of modified PET and magnetic resonance spectroscopy (MRS) techniques, both glucose and lactate were identified as TCA cycle carbon sources in patient lung tumors (Faubert et al. 2017). Recently, in an H1-MRS study in patients with neuroepithelial tumors, Nakamura et al. (2018) were able to quantify tumor lactate content in relative terms and demonstrate that this quantification supported tumor grading. Lactate profiling of tumors in the clinic therefore appears to be an essential parameter in the progression toward improved anticancer therapies.

6. WARBURG EFFECT, CANCER, AND THERAPEUTIC APPROACHES

Although highly proliferative normal cells display an intense glycolytic fermentative phenotype similar to cancer cells, a key distinction in cancer is the loss of regulatory feedback loops. This unique glucose addiction phenotype (Kroemer & Pouysségur 2008), coupled with high-lactate tumors that serve as prominent biomarkers of low patient survival (Walenta & Mueller-Klieser 2004; Walenta et al. 2000, 2004), has prompted many investigators to abrogate glycolysis in tumors as a putative therapeutic approach.

6.1. The Warburg Effect is Dispensable for Cancer

Initial studies exploited the inhibition of glycolysis with 2-deoxy-glucose, a competitive inhibitor of glucose transport and an end product inhibitor of hexokinases and glucose-6-phosphate
isomerase (Hay 2016, Pouysségur et al. 1980, Pusapati et al. 2016), or with the alkylating metabolic inhibitor 3-bromopyruvic acid (Birsoy et al. 2013, Pedersen 2007). High toxicity of these and many other inhibitors targeting glycolysis has greatly prevented their use in the clinic (Augoff et al. 2015, Fortunato et al. 2018). Other investigators have instead explored inhibition, gene silencing, or disruption of specific downstream steps of glycolysis, namely lactate dehydrogenases A and B (LDHA/B) (Boudreau et al. 2016, Brand et al. 2016, Fantin et al. 2006, Le et al. 2010), or of the final step of glycolysis, lactic acid export via the H+/lactate symporters (MCT1 and MCT4) (Benjamin et al. 2018, Doherty et al. 2014, Granja et al. 2015, Le Floch et al. 2011, Marchiq et al. 2015, Renner et al. 2019). Surprisingly, we recently demonstrated that a dual genetic disruption of LDHA and LDHB was necessary to fully ablate the production of lactic acid in aggressive tumors (Zdralevic et al. 2018a). This could be attributed to compensatory adaptations that are induced in response to single-isoform LDHA/B knockouts, as observed in the prostate cancer cell line DU145 (Liu et al. 2018). The consequence of this genetic ablation of glycolysis via LDHA/B double knockout was only a twofold reduction of tumor growth rate in immune-incompetent mice and a complete dependence on OXPHOS (Zdralevic et al. 2018a). Remarkably, these findings obtained with human colon adenocarcinoma and mouse melanoma cells were similar to those obtained in vitro with pancreatic cancer cell lines exposed to GNE-140, a dual pharmacological inhibitor of LDHA/B (Boudreau et al. 2016, Zdralevic et al. 2018a). Therefore, in contrast to previous pharmacological studies, suffering from off-target effects, we concluded that the Warburg Effect is dispensable for tumor growth. This conclusion is in agreement with previous studies, which deleted the upstream glycolytic enzyme glucose-6P-isomerase (de Padua et al. 2017, Pouysségur et al. 1980, Zdralevic et al. 2018b).

6.2. Targeting Lactic Acid Export Offers High Therapeutic Promises: Why?

Although full disruption of fermentative glycolysis does not stop tumor development, in contrast, combined inhibition/disruption of MCT1/4, which severely reduces lactic acid export, imposes a marked reduction in tumor growth (Marchiq et al. 2015, Zdralevic et al. 2018a). The reason for this growth arrest is due to intracellular acidification that is known to block mTORC1 (Balgi et al. 2011, Chambard & Pouysségur 1986). Now, this growth arrest/cytostatic effect can be transformed into cell death (energy crisis) when MCT inhibition is combined with a short exposure to the mitochondrial complex I inhibitor phenformin (Benjamin et al. 2018, Marchiq et al. 2015, Parks et al. 2013a). Alternatively, glycolytic-null tumor cells, relying on OXPHOS and antioxidant response for survival, could be killed by ferropotosis through xCT (cystine-glutamate transporter) inhibition (Daher et al. 2019, Dixon & Stockwell 2019, Dixon et al. 2012).

The second reason for optimism with respect to therapeutic approaches controlling lactic acid production/export is that the recognized strategies evolved by way of glycolytic tumors to evade the immune system. Tumor acidity appears central to reducing T cell and natural killer cell activation, tumor infiltration, interferon gamma secretion, and reprogramming of TAMs into a non-inflammatory phenotype (Bohn et al. 2018, Brand et al. 2016, Colegio et al. 2014, Damgaci et al. 2018, Renner et al. 2019). Thus, a reduction in tumor acidity via inhibition of lactic acid export could prove valuable in future efforts to improve immune therapy strategies in the clinic (Pillai et al. 2019).

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LITERATURE CITED

Aasen T, Mesnil M, Naus CC, Lampe PD, Laird DW. 2016. Gap junctions and cancer: communicating for 50 years. *Nat. Rev. Cancer* 16:775–88

Adelroth P, Brzezinski P. 2004. Surface-mediated proton-transfer reactions in membrane-bound proteins. *Biochim. Biophys. Acta* 1655:102–15

Aly A, Mullins CD, Hussain A. 2015. Understanding heterogeneity of treatment effect in prostate cancer. *Curr. Opin. Oncol.* 27:209–16

Augoff K, Hryniwicz-Jankowska A, Tábola R. 2015. Lactate dehydrogenase 5: an old friend and a new hope in the war on cancer. *Cancer Lett.* 358:1–7

Balgi AD, Diering GH, Donohue E, Lam KK, Fonseca BD, et al. 2011. Regulation of mTORC1 signaling by pH. *PLOS ONE* 6:e21549

Baumann F, Leukel P, Doerfelt A, Beier CP, Dettmer K, et al. 2009. Lactate promotes glioma migration by TGF-β2-dependent regulation of matrix metalloproteinase-2. *Neuro-Oncology* 11:368–80

Becker HM, Deitmer JW. 2008. Nonenzymatic proton handling by carbonic anhydrase II during H⁺-lactate cotransport via monocarboxylate transporter 1. *J. Biol. Chem.* 283:21655–67

Becker HM, Hirnet D, Fecher-Trost C, Sultemeyer D, Deitmer JW. 2005. Transport activity of MCT1 expressed in *Xenopus* oocytes is increased by interaction with carbonic anhydrase. *J. Biol. Chem.* 280:39882–89

Becker HM, Klier M, Deitmer JW. 2010. Nonenzymatic augmentation of lactate transport via monocarboxylate transporter isoform 4 by carbonic anhydrase II. *J. Membr. Biol.* 234:125–35

Becker HM, Klier M, Schuler C, McKenna R, Deitmer JW. 2011. Intramolecular proton shuttle supports not only catalytic but also noncatalytic function of carbonic anhydrase II. *PNAS* 108:3071–76

Benjamin D, Robay D, Hindupur SK, Pohlmann J, Colembali M, et al. 2018. Dual inhibition of the lactate transporters MCT1 and MCT4 is synthetical lethal with metformin due to NAD⁺ depletion in cancer cells. *Cell Rep.* 25:3047–58.e4

Bissoy K, Possemato R, Lorbeer FK, Bayraktar EC, Thiru P, et al. 2014. Metabolic determinants of cancer cell sensitivity to glucose limitation and biguanides. *Nature* 508:108–12

Bissoy K, Wang T, Possemato R, Yilmaz OH, Koch CE, et al. 2013. MCT1-mediated transport of a toxic molecule is an effective strategy for targeting glycolytic tumors. *Nat. Genet.* 45:104–8

Bohn T, Rapp S, Luther N, Klein M, Bruehl TJ, et al. 2018. Tumor immunoevasion via acidosis-dependent induction of regulatory tumor-associated macrophages. *Nat. Immunol.* 19:1319–29

Boonekamp FJ, Dashko S, van den Broek M, Gehrmann T, Daran JM, Daran-Lapujade P. 2018. The genetic makeup and expression of the glycolytic and fermentative pathways are highly conserved within the *Saccharomyces* genus. *Front. Genet.* 9:504

Boudreau A, Purkey HE, Hitz A, Robarge K, Peterson D, et al. 2016. Metabolic plasticity underpins innate and acquired resistance to LDHA inhibition. *Nat. Chem. Biol.* 12:779–86

Bovenzi CD, Hamilton J, Tassone P, Johnson J, Cognetti DM, et al. 2015. Prognostic indications of elevated MCT4 and CD147 across cancer types: a meta-analysis. *Br. Med. Res. Int.* 2015:242437

Brand A, Singer K, Koebl GE, Kolituz M, Schoenhammer G, et al. 2016. LDHA-associated lactate acid production blunts tumor immunosurveillance by T and NK Cells. *Cell Metab.* 24:657–71

Branden M, Sanden T, Brzezinski P, Widengren J. 2006. Localized proton microcircuits at the biological membrane-water interface. *PNAS* 103:19766–70
Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, et al. 2001. Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. *Int. J. Radiat. Oncol. Biol. Phys.* 51:349–53

Brooks GA. 1985. Anaerobic threshold: review of the concept and directions for future research. *Med. Sci. Sports Exerc.* 17:22–34

Brooks GA. 2009. Cell-cell and intracellular lactate shuttles. *J. Physiol.* 587:5591–600

Cai TQ, Ren N, Jin L, Cheng K, Kash S, et al. 2008. Role of GPR81 in lactate-mediated reduction of adipose lipolysis. *Biochem. Biophys. Res. Commun.* 377:987–91

Cali C, Tauffenberger A, Magistretti P. 2019. The strategic location of glycogen and lactate: from body energy reserve to brain plasticity. *Front. Cell. Neurosci.* 13:82

Carroll PA, Diolaiti D, McFerrin L, Gu H, Dijkovic D, et al. 2015. Deregulated Myc requires MondoA/Mlx for metabolic reprogramming and tumorigenesis. *Cancer Cell* 27:271–85

Chambard JC, Pouysségur J. 1986. Intracellular pH controls growth factor-induced ribosomal protein S6 phosphorylation and protein synthesis in the G0→G1 transition of fibroblasts. *Exp. Cell Res.* 164:282–94

Chen P, Zuo H, Xiong H, Kolar MJ, Chu Q, et al. 2017. Gpr132 sensing of lactate mediates tumor-macrophage interplay to promote breast cancer metastasis. *Pnas* 114:580–85

Chiche J, Le Fur Y, Vilmen C, Frassineti F, Daniel L, et al. 2012. In vivo pH in metabolic-defective Ras-transformed fibroblast tumors: key role of the monocarboxylate transporter, MCT4, for inducing an alkaline intracellular pH. *Int. J. Cancer* 130:1511–20

Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, et al. 2014. Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature* 513:559–63

Cort CF, Cort GT. 1925. The carbohydrate metabolism of tumors. II. Changes in the sugar, lactic acid, and CO2-combining power of blood passing through a tumor. *J. Biol. Chem.* 65:397–405

Counillon L, Bouret Y, Marchiq I, Pouysségur J. 2016. Na+/H+ antiporter (NHE1) and lactate/H+ symporters (MCTs) in pH homeostasis and cancer metabolism. *Biochim. Biophys. Acta* 1863:2465–80

Daher B, Parks SK, Durivault J, Cormerais Y, Baidarjad H, et al. 2019. Genetic ablation of the cystine transporter xCT in PDAC cells inhibits mTORC1, growth, survival, and tumor formation via nutrient and oxidative stresses. *Cancer Res.* 79:3877–90

Damgaard S, Ibrahim-Hashim A, Enriquez-Navas PM, Pilon-Thomas S, Guvenis A, Gillies RJ. 2018. Hypoxia and acidosis: immune suppressors and therapeutic targets. *Immunology* 154:354–62

De Deken RH. 1966. The Crabtree effect: a regulatory system in yeast. *J. Gen. Microbiol.* 44:149–56

De Padua MC, Delodi G, Vučetić M, Durivault J, Vial V, et al. 2015. Disrupting glucose-6-phosphate isomerase fully suppresses the “Warburg effect” and activates OXPHOS with minimal impact on tumor growth except in hypoxia. *OncoTarget* 8:8763–37

DeNicola GM, Chen PH, Mullarky E, Sudderth JA, Hu Z, et al. 2015. NRF2 regulates serine biosynthesis in non-small cell lung cancer. *Nat. Genet.* 47:1475–81

Dhung S, Dadich RK, Porporato PE, Sonveaux P. 2012. Multiple biological activities of lactic acid in cancer: influences on tumor growth, angiogenesis and metastasis. *Curr. Pharm. Des.* 18:1319–30

Dixon SJ, Lemberg KM, Lamprecht MR, Skouta R, Zaitsev EM, et al. 2012. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 149:1060–72

Dixon SJ, Stockwell BR. 2019. The hallmarks of ferroptosis. *Annu. Rev. Cancer Biol.* 3:35–54

Doherty JR, Yang C, Scott KE, Cameron MD, Fallahi M, et al. 2014. Blocking lactate export by inhibiting the Myc target MCT1 disables glycolysis and glutamine synthesis. *Cancer Res.* 74:908–20

Dovmak TH, Hulikova A, Niederer SA, Vaughan-Jones RD, Swietach P. 2018. Normoxic cells remotely regulate the acid-base balance of cells at the hypoxic core of connexin-coupled tumor growths. *FASEB J.* 32:83–96

Dovmak TH, Saccomano M, Hulikova A, Alves F, Swietach P. 2017. Connexin-43 channels are a pathway for discharging lactate from glycolytic pancreatic ductal adenocarcinoma cells. *Oncogene* 36:4538–50

Doyen J, Trastour C, Ettore F, Peyrottes I, Toussant N, et al. 2014. Expression of the hypoxia-inducible monocarboxylate transporter MCT4 is increased in triple negative breast cancer and correlates independently with clinical outcome. *Biochem. Biophys. Res. Commun.* 451:54–61
Fantin VR, St-Pierre J, Leder P. 2006. Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance. *Cancer Cell* 9:425–34

Fauhert B, Li KY, Cai L, Hensley CT, Kim J, et al. 2017. Lactate metabolism in human lung tumors. *Cell* 171:358–71.e9

Flinck M, Kramer SH, Pedersen SF. 2018. Roles of pH in control of cell proliferation. *Acta Physiol.* 223:e13068

Fortunato S, Bononi G, Granchi C, Minutolo F. 2018. An update on patents covering agents that interfere with the cancer glycolytic cascade. *ChemMedChem* 13:2251–65

Fothing-Gilmore LA, Michels PA. 1993. Evolution of glycolysis. *Prog. Biophys. Mol. Biol.* 59:105–235

Frenkel DG. 2003. The top genes: on the distance from transcript to function in yeast glycolysis. *Curr. Opin. Microbiol.* 6:198–201

Gladden LB. 2004. Lactate metabolism: a new paradigm for the third millennium. *J. Physiol.* 558:5–30

Goetzte K, Walenta S, Ksiazkiewicz M, Kunz-Schughart LA, Mueller-Klieser W. 2011. Lactate enhances motility of tumor cells and inhibits monocyte migration and cytokine release. *Int. J. Oncol.* 39:453–63

Granja S, Marchiq I, Le Floch R, Moura CS, Baltazar F, Pouysségur J. 2015. Disruption of BASIGIN decreases lactic acid export and sensitizes non-small cell lung cancer to biguanides independently of the LKB1 status. *Oncotarget* 6:6708–21

Hagman A, Piskur J. 2015. A study on the fundamental mechanism and the evolutionary driving forces behind aerobic fermentation in yeast. *PLoS ONE* 10:e0116942

Halestrap AP. 2012. The monocarboxylate transporter family—structure and functional characterization. *IUBMB Life* 64:1–9

Halestrap AP. 2013. The SLC16 gene family—structure, role and regulation in health and disease. *Mol. Aspects Med.* 34:337–49

Hay N. 2016. Reprogramming glucose metabolism in cancer: Can it be exploited for cancer therapy? *Nat. Rev. Cancer* 16:635–49

Hirschhaeuser F, Sattler UG, Mueller-Klieser W. 2011. Lactate: a metabolic key player in cancer. *Cancer Res.* 71:6921–25

Hoffmann A, Gloe T, Pohl U. 2001. Hypoxia-induced upregulation of eNOS gene expression is redox-sensitive: a comparison between hypoxia and inhibitors of cell metabolism. *J. Cell Physiol.* 188:33–44

Hui S, Ghergurovich JM, Morsch RJ, Jang C, Teng X, et al. 2017. Glucose feeds the TCA cycle via circulating lactate. *Nature* 551:115–18

Hulikova A, Black N, Hsia LT, Wilding J, Bodmer WF, Swietach P. 2016. Stromal uptake and transmission of acid is a pathway for venting cancer cell-generated acid. *PNAS* 113:E5344–53

Jafri MS, Dudycha SJ, O’Rourke B. 2001. Cardiac energy metabolism: models of cellular respiration. *Annu. Rev. Biomed. Eng.* 3:57–81

Jamali S, Klier M, Ames S, Barros LF, McKenna R, et al. 2015. Hypoxia-induced carbonic anhydrase IX facilitates lactate flux in human breast cancer cells by non-catalytic function. *Sci. Rep.* 5:13605

Jeng KS, Chang CF, Jeng WJ, Sheen IS, Jeng CJ. 2015. Heterogeneity of hepatocellular carcinoma contributes to cancer progression. *Crit. Rev. Oncol. Hematol.* 94:337–47

Jeoung N, Wu P, Joshi MA, Jaskiewicz J, Bock CB, et al. 2006. Role of pyruvate dehydrogenase kinase isozyme 4 (PDK4) in glucose homeostasis during starvation. *Biochem. J.* 397:417–25

Justus CR, Dong L, Yang LV. 2013. Acidic tumor microenvironment and pH-sensing G protein-coupled receptors. *Front. Physiol.* 5:354

Kim JK, Haselgrove JC, Shapiro IM. 1993. Measurement of metabolic events in the avian epiphysseal growth cartilage using a bioluminescence technique. *J. Histochem. Cytochem.* 41:693–702

Kim JW, Tchernyshyov I, Senzena GL, Dang CV. 2006. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab.* 3:177–85

Kricka LJ. 2000. Application of bioluminescence and chemiluminescence in biomedical sciences. *Methods Enzymol.* 305:333–45

Kroemer G, Pouysségur J. 2008. Tumor cell metabolism: cancer’s Achilles’ heel. *Cancer Cell* 13:472–82

Le A, Cooper CR, Gouw AM, Dinavahi R, Maitra A, et al. 2010. Inhibition of lactate dehydrogenase A induces oxidative stress and inhibits tumor progression. *PNAS* 107:2037–42
Le Floch R, Chiche J, Marchiq I, Naiken T, Ile K, et al. 2011. CD147 subunit of lactate/H+ symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. *PNAS* 108:16663–68

Liu J, Chen G, Liu Z, Liu S, Cai Z, et al. 2018. Aberrant FGFR tyrosine kinase signaling enhances the Warburg effect by reprogramming LDH isoform expression and activity in prostate cancer. *Cancer Res.* 78:4459–70

Luc R, Tortorella SM, Ververis K, Karagiannis TC. 2015. Lactate as an insidous metabolite due to the Warburg effect. *Mol. Biol. Rep.* 42:835–40

Marchiq I, Le Floch R, Roux D, Simon MP, Pouységur J. 2015. Genetic disruption of lactate/H+ symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin. *Cancer Res.* 75:171–80

Martinez C, Kalise D, Barros LF. 2010. General requirement for harvesting antennae at CA and H channels and transporters. *Front. Neuroenerg.* 2:27

Mboge MY, Mahon BP, McKenna R, Frost SC. 2018. Carbonic anhydrases: role in pH control and cancer. *Metabolites* 8:19

McDonald PC, Swayampakula M, Dedhar S. 2018. Coordinated regulation of metabolic transporters and migration/invasion by carbonic anhydrase IX. *Metabolites* 8:20

Mitsushi Y, Taguchi K, Kawatani Y, Shihata T, Nukiwa T, et al. 2012. Nrf2 redirects glucose and glutamine into anabolic pathways in metabolic reprogramming. *Cancer Cell* 22:66–79

Nakamura H, Doi M, Suzuki T, Yoshida Y, Hoshikawa M, et al. 2018. The significance of lactate and lipid peaks for predicting primary neuroepithelial tumor grade with proton MR spectroscopy. *Magn. Reson. Med. Sci.* 17:238–43

Newell K, Franchi A, Pouységur J, Tannock I. 1993. Studies with glycolysis-deficient cells suggest that production of lactic acid is not the only cause of tumor acidity. *PNAS* 90(3):1127–31

Noor SI, Jamali S, Ames S, Langer S, Deitmer JW, Becker HM. 2018. A surface proton antenna in carbonic anhydrase II supports lactate transport in cancer cells. *eLife* 7:e35176

Palm W, Thompson CB. 2017. Nutrient acquisition strategies of mammalian cells. *Nature* 546:234–42

Papandreou I, Cairns RA, Fontana L, Lim AL, Denko NC. 2006. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab.* 3:187–97

Parks SK, Chiche J, Pouységur J. 2013a. Disrupting proton dynamics and energy metabolism for cancer therapy. *Nat. Rev. Cancer* 13:611–23

Parks SK, Cornerais Y, Pouységur J. 2017. Hypoxia and cellular metabolism in tumour pathophysiology. *J. Physiol.* 595:2439–50

Parks SK, Mazure NM, Counillon L, Pouységur J. 2013b. Hypoxia promotes tumor cell survival in acidic conditions by preserving ATP levels. *J. Cell Physiol.* 228:1854–62

Paschen W. 1985. Regional quantitative determination of lactate in brain sections. A bioluminescence method. *J. Cereb. Blood Flow Metab.* 5:609–12

Paschen W, Niebuhr I, Hossmann KA. 1981. A bioluminescence method for the demonstration of regional glucose distribution in brain slices. *J. Neurochem.* 36:513–17

Pedersen PL. 2007. Warburg, me and Hexokinase 2: multiple discoveries of key molecular events underlying one of cancers’ most common phenotypes, the “Warburg Effect”, i.e., elevated glycolysis in the presence of oxygen. *J. Bioenerg. Biomemb.* 39:211–22

Pedersen SF, Counillon L. 2019. The SLC9A-C mammalian Na+/H+ exchanger family: molecules, mechanisms, and physiology. *Physiol. Rev.* 99:2015–113

Pelletier J, Bellot G, Gounon P, Lacas-Gervais S, Pouységur J, Mazure NM. 2012. Glycogen synthesis is induced in hypoxia by the hypoxia-inducible factor and promotes cancer cell survival. *Front. Oncol.* 2:18

Pescador N, Villar D, Cifuentes D, Garcia-Rocha M, Ortiz-Barahona A, et al. 2010. Hypoxia promotes glycogen accumulation through hypoxia inducible factor (HIF)-mediated induction of glycogen synthase I. *PLOS ONE* 5:e9644

Pilai SR, Damaghi M, Marunaka Y, Spugnini EP, Fais S, Gillies RJ. 2019. Causes, consequences, and therapy of tumors acidosis. *Cancer Metastasis Rev.* 38:205–22

Porporato PE, Payen VL, De Saedeleer CJ, Preat V, Thissen JP, et al. 2012. Lactate stimulates angiogenesis and accelerates the healing of superficial and ischemic wounds in mice. *Angiogenesis* 15:581–92
Pouységur J, Dayan F, Mazure NM. 2006. Hypoxia signalling in cancer and approaches to enforce tumour regression. Nature 441:437–43

Pouységur J, Franchi A, Salomon JC, Silvestre P. 1980. Isolation of a Chinese hamster fibroblast mutant defective in hexose transport and aerobic glycolysis: its use to dissect the malignant phenotype. PNAS 77:2698–701

Pusapati RV, Daemen A, Wilson C, Sandoval W, Gao M, et al. 2016. mTORC1-dependent metabolic reprogramming underlies escape from glycolysis addiction in cancer cells. Cancer Cell 29:548–62

Renner K, Bruss C, Schnell A, Koehl G, Becker HM, et al. 2019. Restricting glycolysis preserves T cell effector functions and augments checkpoint therapy. Cell Rep. 29:135–50.e9

Romero-Garcia S, Moreno-Altimirano MM, Prado-Garcia H, Sanchez-Garcia FJ. 2016. Lactate contribution to the tumor microenvironment: mechanisms, effects on immune cells and therapeutic relevance. Front. Immunol. 7:52

Salahudeen AK, Clark EC, Nath KA. 1991. Hydrogen peroxide-induced renal injury. A protective role for pyruvate in vitro and in vivo. J. Clin. Investig. 88:1886–93

Sardet C, Franchi A, Pouységur J. 1989. Molecular cloning, primary structure, and expression of the human growth factor-activatable Na+/H+ antiporter. Cell 56:271–80

Sattler UG, Meyer SS, Quennet V, Hoerner C, Knoerzer H, et al. 2010. Glycolytic metabolism and tumour response to fractionated irradiation. Radiother. Oncol. 94:102–9

Sattler UG, Valenta S, Mueller-Klieser W. 2007. A bioluminescence technique for quantitative and structure-associated imaging of pyruvate. Lab Invest. 87:84–92

Semenza GL. 2017. Hypoxia-inducible factors: coupling glucose metabolism and redox regulation with induction of the breast cancer stem cell phenotype. EMBO J. 36:252–59

Seuwen K, Ludwig MG, Wolf RM. 2006. Receptors for protons or lipid messengers or both? J. Recept. Signal Transduct. Res. 26(5–6):599–610

Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, et al. 2008. Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J. Clin. Investig. 118:3930–42

Sukonina V, Ma H, Zhang W, Barisaghi S, Subhash S, et al. 2019. FOXK1 and FOXK2 regulate aerobic glycolysis. Nature 566:279–83

Swietach P. 2019. What is pH regulation, and why do cancer cells need it? Cancer Metastasis Rev. 38:5–15

Swietach P, Monterisi S. 2019. A barter economy in tumors: exchanging metabolites through gap junctions. Cancers 11:E117

Thacker SV, Nickel M, DiGirolamo M. 1987. Effects of food restriction on lactate production from glucose by rat adipocytes. Am. J. Physiol. 253:E336–42

Ullah MS, Davies AJ, Halestrap AP. 2006. The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1α-dependent mechanism. J. Biol. Chem. 281:9030–37

Van Hee VF, Labar D, Dehon G, Grasso D, Gregoire V, et al. 2017. Radiosynthesis and validation of (±)-[18F]-3-fluoro-2-hydroxypropionate ([18F]-FLac) as a PET tracer of lactate to monitor MCT1-dependent lactate uptake in tumors. Oncotarget 8:24415–28

Vander Heiden MG, Cantley LC, Thompson CB. 2009. Understanding the Warburg effect: the metabolic requirements of cell proliferation. Science 324:1029–33

Velaei K, Samadi N, Barazvan B, Soleimani Rad J. 2016. Tumor microenvironment-mediated chemoresistance in breast cancer. Breast 30:92–100

Walenta S, Mueller-Klieser WF. 2004. Lactate: mirror and motor of tumor malignancy. Semin. Radiat. Oncol. 14:267–74

Walenta S, Schroeder T, Mueller-Klieser W. 2004. Lactate in solid malignant tumors: potential basis of a metabolic classification in clinical oncology. Curr. Med. Chem. 11:2195–204

Walenta S, Voelken NF, Mueller-Klieser W. 2016. Lactate—an integrative mirror of cancer metabolism. In Metabolism in Cancer, ed. T Cramer, CA Schmitt, pp. 23–37. Cham, Switz.: Springer

Walenta S, Voelken NF, Sattler UGA, Mueller-Klieser W. 2014. Localizing and quantifying metabolites in situ with lumimometry: induced metabolic bioluminescence imaging (imBI). In Brain Energy Metabolism, ed. J Hirrlinger, HS Waagepetersen, pp. 195–216. New York: Humana
Walenta S, Wetterling M, Lehrke M, Schwickert G, Sundfor K, et al. 2000. High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. *Cancer Res.* 60:916–21

Walther V, Hiley CT, Shibata D, Swanton C, Turner PE, Maley CC. 2015. Can oncology recapitulate paleontology? Lessons from species extinctions. *Nat. Rev. Clin. Oncol.* 12:273–85

Warburg O. 1923. Experiments on surviving carcinoma tissue: methods. *Biochem. Z.* 142:317–30

Warburg O. 1956. On respiratory impairment in cancer cells. *Science* 124:269–70

Warburg O, Wind F, Negelein E. 1927. The metabolism of tumors in the body. *J. Gen. Physiol.* 8:519–30

Weiss KT, Fante M, Kohl G, Schrenl J, Haubner F, et al. 2017. Proton-sensing G protein-coupled receptors as regulators of cell proliferation and migration during tumor growth and wound healing. *Exp. Dermatol.* 26:127–32

Wykoff CC, Beasley NJ, Watson PH, Turner KJ, Pastorek J, et al. 2000. Hypoxia-inducible expression of tumor-associated carbonic anhydrases. *Cancer Res.* 60:7075–83

Zdralevic M, Brand A, Di Ianni L, Dettmer K, Reinders J, et al. 2018a. Double genetic disruption of lactate dehydrogenases A and B is required to ablate the “Warburg effect” restricting tumor growth to oxidative metabolism. *J. Biol. Chem.* 293:15947–61

Zdralevic M, Vucetic M, Daher B, Marchiaf F, Parks SK, Pouysségur J. 2018b. Disrupting the ‘Warburg effect’ re-routes cancer cells to OXPHOS offering a vulnerability point via ‘ferroptosis’-induced cell death. *Adv. Biol. Regul.* 68:55–63

Zhou R, Bansal N, Leeper DB, Pickup S, Glickson JD. 2001. Enhancement of hyperglycemia-induced acidification of human melanoma xenografts with inhibitors of respiration and ion transport. *Acad. Radiol.* 8:571–82
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Errata

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