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Lactate and Acidity in the Cancer Microenvironment

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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.

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, $CH_3CH(OH)COOH$, 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

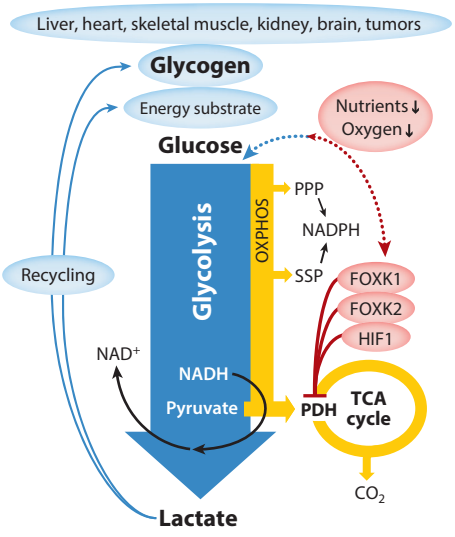

Figure 1

Fermentative glycolysis (*central blue arrow*) with massive recycling of lactate fueled into glycogen synthesis in several organs. The pentose phosphate pathway (PPP) and the serine/glycine synthetic pathway (SSP), which divert from the glycolysis pathway, are dependent on oxidative phosphorylation (OXPHOS, *yellow arrows*). Note that pyruvate oxidation by the pyruvate dehydrogenase complex (PDH) of the TCA (tricarboxylic acid) cycle is seriously compromised by the transcription factors HIF1 (hypoxia-inducible factor 1) and FOXK1/FOXK2, favoring lactate formation and regeneration of NAD^+. The double dotted arrow represents (*blue*) induction of glycolytic enzymes or (*red*) inhibition of pyruvate oxidation by the three transcription factors HIF1, FOXK1, and FOXK2.

Figure 2

Dr. Carl F. Cori (*standing*) and Dr. Gerty Theresa Cori (*seated*) in their lab in 1947 at the Washington University School of Medicine in St. Louis, Missouri. Photograph courtesy of the History of Medicine Collection (photographer unknown), US National Library of Medicine.

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

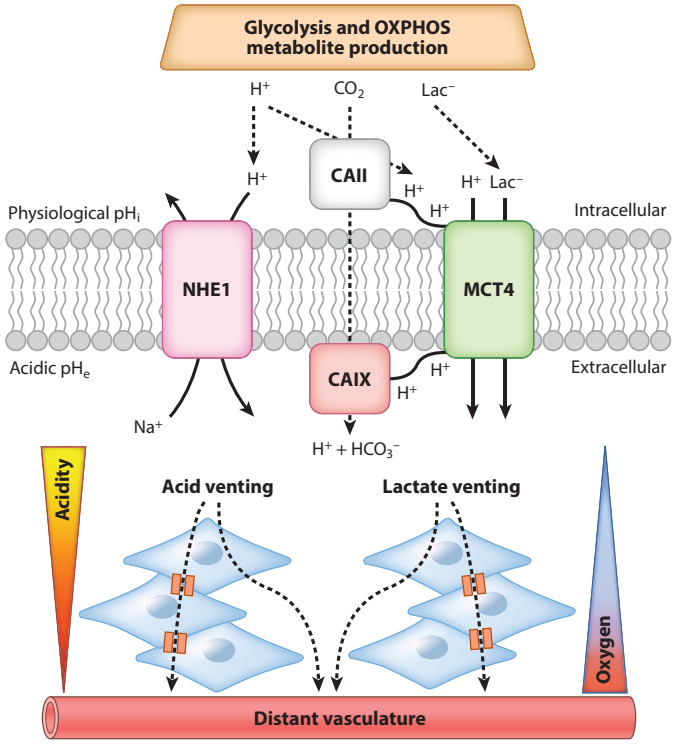

Figure 3

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_i 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 (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^+/lactate$^-$ 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_i is essential to retain virtually all cytoplasmic protein activity, with change in pH_i 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^+ in addition to membrane transporters that remove excessive H^+ to the extracellular space. The ubiquitously expressed NHE1 (Sardet et al. 1989) responds immediately to extrude cellular H^+ in exchange for Na^+ (reviewed in Counillon et al. 2016 and Pedersen & Counillon 2019). Other pH-regulating proteins including CAs and bicarbonate transporters are further implicated in pH_i regulation and buffering of metabolic acid production (Parks et al. 2013a). Thus, the efficient mechanisms of pH_i regulation in tumor cells could compete for free H^+ 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_i (Marchiq et al. 2015) and that forced expression of MCT4 in tumor xenografts increased both pH_i and glycolytic rates (Chiche et al. 2012), indicating that MCTs are required to complement other pH_i-regulating proteins during heightened metabolic activity. The importance of MCTs in maintaining a more alkaline pH_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_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_i indicates that MCTs contribute to the tumor cell pH_i value that is permissive for tumor cell survival and elevated proliferation.

Considering the potential for H^+ competition between transporters such as NHE1 and MCTs, it has been of great interest to understand how MCTs obtain the H^+ required for lactate transport. The fact that MCTs strip H^+ from the cytoplasm at a much greater rate (~15 times for MCT1) than would be predicted for H^+ 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_2 to HCO_3^- and H^+ and have thus received extensive attention in the context of tumor cell metabolism due to their ability to regulate both pH_e and pH_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^+ shuttle within the CA protein structure is utilized as an H^+-collecting antenna to provide a continuous stream of H^+ 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

(Parks et al. 2013b). 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_i 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-phophate, or D2-hydroxygluturate, 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_e, 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 invariantly 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 (Brizel 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 hydroxycarboxylic 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 4*a***) and a color-coded map of lactate-to-pyruvate ratios (**Figure 4*b***). 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

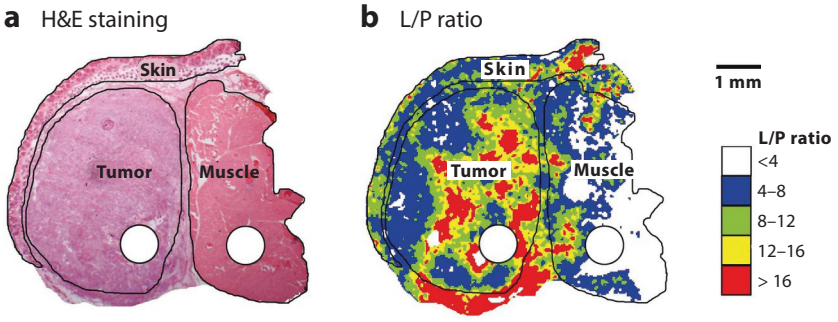

Figure 4

Representative quantitative redox imaging in a human head and neck squamous cell carcinoma. Adjacent serial tissue sections were used for (*a*) hematoxylin and eosin (H&E) staining, allowing for the identification of different tissue types, and (*b*) compiling a corresponding lactate-to-pyruvate (L/P) ratio image. For this purpose, separate lactate and pyruvate images (not shown) were obtained with the induced metabolic bioluminescence imaging (imBI) technique and were subsequently used to calculate a map of local L/P ratios. Figure adapted with permission from Sattler et al. (2007).

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 ferroptosis 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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