Review

Cancer Research

The Tumor Metabolic Microenvironment: Lessons from Lactate

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Abstract

The extracellular milieu of tumors is generally assumed to be immunosuppressive due in part to metabolic factors. Here, we review methods for probing the tumor metabolic microenvironment. In parallel, we consider the resulting available evidence, with a focus on lactate, which is the most strongly increased metabolite in bulk tumors. Limited microenvironment concentration measurements suggest depletion of glucose and modest accumulation of lactate (less than 2-fold). Isotope tracer measurements show rapid lactate exchange between the tumor and circulation. Such exchange is catalyzed by MCT transporters, which cotransport lactate and protons (H^+). Rapid lactate exchange seems at odds with tumor lactate accumulation. We propose a potential resolution to this paradox. Because of the high pH of tumor cells relative to the microenvironment, H^+-coupled transport by MCTs tends to drive lactate from the interstitium into tumor cells. Accordingly, lactate may accumulate preferentially in tumor cells, not the microenvironment. Thus, although they are likely subject to other immunosuppressive metabolic factors, tumor immune cells may not experience a high lactate environment. The lack of clarity regarding microenvironmental lactate highlights the general need for careful metabolite measurements in the tumor extracellular milieu.

Introduction

Cancerous tumors are heterogeneous mixtures of cells and extracellular matrix (1–3). In addition to the driver cancer cells, tumors frequently contain fibroblasts, immune cells, adipocytes, and endothelial cells (1–5). The diverse cell types within the tumor may support or antagonize one another through direct cell–cell interactions or paracrine signaling. In addition, the cell types may cooperate or compete metabolically. For example, one cell type may release a metabolite that feeds another cell type (6–8). Alternatively, several cell types may fight for access to a single limiting nutrient (9–12). Such nutrient competition could potentially dictate the effectiveness of antitumor immune response.

Although it is clear that tumors are nutrient avid, the extent to which they have a distinct metabolic milieu remains less well proven. The interstitial space, or interstitium, refers to extracellular tissue space (excluding the lumen of blood and lymphatic vessels), including both the extracellular matrix and the interstitial fluid (13, 14). The metabolic composition of the tumor interstitium is determined by: (i) nutrient uptake from the blood supply into the tumor, (ii) nutrient uptake and waste excretion by tumor cells, and (iii) drainage by veins and lymphatics. In normal tissues, rapid exchange of nutrients between the interstitium and blood stream provides cells with reliable nutrient access and waste removal. Establishment of a distinct tumor metabolic microenvironment depends on nutrient consumption and waste production within the tumor overwhelming exchange with the circulation. This could in principle occur due either to the tumor's heightened metabolic activity or to poor perfusion. Here, we review methods for probing the tumor metabolic microenvironment and the somewhat contradictory evidence arising from them. We then propose a potential mechanism for tumor lactate accumulation, which suggests that lactate may localize within tumor cells and therefore not rise to immunosuppressive levels in the extracellular milieu.

Nutrient utilization

What metabolic activity within tumors might yield a distinct microenvironment? The answer goes back to the work of Otto Warburg, who demonstrated avid glucose fermentation into lactate by cancer cells. This raises the potential for the tumor microenvironment to be glucose poor and lactate rich. More recently, glutamine has been recognized as a key substrate for cancer cells, which often in turn secrete glutamate. This phenomenon is in part an artifact of cell culture media containing high cystine, which drives glutamate export via the xCT transporter (15); nevertheless, glutamine depletion is another potential outcome of heightened cancer cell metabolism (16). Other important cancer cell substrates to support biomass and energy production include acetate (17, 18), branched chain amino acids (19), non-essential amino acids (7), and even the canonical tumor waste product lactate (20, 21). In addition, tumor cells can release immunosuppressive metabolites, such as adenosine (22) or the tryptophan catabolite kynurenine (23).

Changes to the extracellular metabolic environment can impact cancer by altering the growth, survival, or phenotype of both cancer cells and other tumor cells, including immune cells. Cancer cells themselves are typically dependent on glucose, and also often glutamine, to grow. Hence, a nutrient-poor microenvironment might slow cancer growth. Unfortunately, the same

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nutrients play a critical role in immune response (24). For example, glucose is critical to T-cell proliferation and effector function, with genetic experiments showing the essentiality of T-cell glucose uptake via glucose transporter-1 (GLUT1) to mounting of an effective immune response (25). Conversely, lactate can drive T cells toward an immunosuppressive Treg phenotype (26). Similar phenomena occur in macrophages, where the pro-inflammatory M1 macrophages have high glucose consumption, whereas immune suppressive M2 macrophages do not, thriving on either lactate or fatty acids (27). Moreover, amino acids, including glutamine, arginine, and serine, are important nutrients both for cancer cells and for mounting an effective immune response (28–30). Thus, metabolism by both cancer and immune cells within the tumor microenvironment holds the potential for local nutrient depletion and waste buildup. These metabolic changes could advantageously limit tumor growth, while disadvantageously impairing tumor immune response. But does this really happen to a meaningful extent?

pH and pO_2, key regulators of metabolism within the tumor microenvironment

H^+ and O_2 are not canonical metabolites, but pH and pO_2 are key players in the tumor metabolic microenvironment (31–33). Both H^+ and O_2 play a dual role in tumor metabolism. On one hand, they are metabolic products/reactants, whose levels may directly impact reaction flux and thermodynamics. On the other hand, they regulate the expression and activity of important metabolic enzymes (32, 33).

Extracellular acidification has been shown in tumors through several measurement methods, initially by insertion of electrodes (34, 35). Although a direct measurement approach, probe insertion might induce tissue damage (34). Accordingly, noninvasive techniques such as magnetic resonance spectroscopy (MRS), MRI, and positron emission tomography (PET), using both endogenous and exogenous compounds as pH reporters, were developed (36–38). Compared with normal tissue, where $pH_{intracellular} \approx 7.2$ and $pH_{extracellular} \approx 7.4$, these measurement approaches consistently showed an inverted pH gradient in tumors. The intracellular tumor pH is slightly more basic than in normal tissues, although extracellular pH is more acidic ($pH_{intracellular} > 7.4$, $pH_{extracellular} \approx 6.5$-7). Biochemically, this inverted gradient is maintained by the action of various transporters, for example, Na^+/H^+ exchangers, H^+/K^+-ATPases, and Na^+/HCO_3^-cotransporters, in combination with carbonic anhydrases (e.g., CAIX and CAXII), and monocarboxylic transporters (MCT), which cotransport H^+ and monocarboxylic acid compounds such as lactate (31, 32). The maintenance of this inverted gradient has been suggested to provide a dual benefit for cancer cells: (i) intracellular alkalization facilitates increased glycolysis, thereby promoting cancer cell proliferation, especially in hypoxia (39) and (ii) extracellular acidification hampers the development of a proper immune response (40).

Tumor hypoxia involves oxygen partial pressure (pO_2) below 15 mm Hg (41). Hypoxic and/or anoxic areas are estimated to occur in 50% to 60% of locally advanced solid tumors and are heterogeneously distributed within the tumor mass (42). As in the case of pH measurements, initial tumor pO_2 measurements were made using electrodes (43, 44). A variety of approaches are, however, now available. These include measurements of endogenous proteins, such as HIF1α–target proteins CAIX or GLUT1, whose levels rise in hypoxia. In addition, compounds that selectively stain hypoxic cells (e.g., pimonidazole) can be injected. More direct measurements can be made by implanting paramagnetic crystals (whose interaction with oxygen is measured by electron paramagnetic resonance) or oxygen-sensitive fluorescent probes attached to a fiber-optic cable (OxyLite). Moreover, MRI and PET methods have been developed for noninvasive measurement of tumor oxygenation. PET methods usually rely on 2-nitroimidazole compounds (e.g., ^{18}F-fluoromisonidazole). They can enter the cells by passive diffusion where they can be retained due to the formation of reactive species under reducing conditions in a pO_2-dependent manner (42, 45, 46). Primary tumors in the cervix and breast have been shown to have significantly decreased median pO_2 values (8 and 10 mmHg, respectively) compared with normal tissue in the same organ ($pO_2 = 42$ and 65 mmHg, respectively). Tumor hypoxia ($pO_2 < 10$ mmHg) has been identified as an adverse factor in several tumor types with increased risk of metastasis and decreased overall survival (42).

In summary, both hypoxia and the maintenance of an inverted pH gradient ($pH_{intracellular} > pH_{extracellular}$), are well-established features of the tumor microenvironment that may favor tumorigenesis by rewiring tumor metabolism and by suppressing antitumor immunity.

Metabolite concentration changes in tumors

To understand the tumor metabolic milieu, it is of course necessary to look more broadly than O_2 and H^+. Fortunately, advances in mass spectrometry (MS) and NMR now enable routine measurement of several hundreds of metabolites (as well as yet larger numbers of lipids) from resected tumor specimens. Tissue and tumor samples are usually flash-frozen, ground into powder, and extracted, and the extract analyzed (47, 48). Although providing valuable information about the overall metabolite levels within the tumor, these methods do not measure the microenvironment per se.

Moreover, even measurement of overall tumor metabolite levels can be challenging, because metabolite concentrations may rapidly change upon cutting off the tumor blood supply, making fast resection and freezing of metabolism critical. In addition, MS is subject to substantial day-to-day variation in absolute signal intensity. This can be ideally accounted for using isotopic internal standards, but due to the limited availability and stability of such standards, it is often more practical to normalize to the median metabolite signal intensity in a given sample run. In addition, to interpret the data, choice for proper comparator specimen is critical. The most intuitive comparison is between tumor and benign adjacent tissue, and this works well in cases where the tumor and tissue of origin have equivalent cellular density (e.g., liver, kidney, pancreas), but less so when they do not (e.g., lung, breast; refs. 47, 48).

Perhaps due to these technical challenges, or perhaps due to intrinsic biological variability (e.g., across tissues of origin, oncogenic driver mutations, and better and worse-perfused tumor regions; refs. 19, 49–51), tumor metabolomic analyses have given diverse results. Meta-analyses have identified a general trend for most proteogenic amino acids to be increased in tumors (52). In our own studies of pancreas cancer, however, we found a more interesting pattern, with increased concentrations of amino acids used purely for protein synthesis, but lower concentrations of those used also as nucleotide precursors, with glutamine being the most depleted metabolite (53). This pattern of metabolite

levels is consistent with pancreas cancer acquiring amino acid substantially through protein catabolism, highlighting the potential to link metabolite levels to the biology of specific tumor types (53, 54).

In contrast with the inconsistent results for most metabolites, the most consistently upregulated metabolite across diverse tumors is lactate. Moreover, glucose is one of the most consistently downregulated metabolites. Thus, modern technology has confirmed the centrality of the Warburg effect in tumor metabolism (Fig. 1A; refs. 52, 55). A simple interpretation of these bulk tumor measurements is that tumors avidly convert glucose into lactate, which accumulates in the tumor. But do available data support lactate accumulation in the tumor interstitial fluid rather than inside tumor cells? Is the lactate that accumulates into the tumor locally produced?

Direct sampling of tumor interstitial fluid

Tumor interstitial fluid can in principle be sampled by inserting a probe into the tumor *in vivo* or by isolation of interstitial fluid from a piece of resected tumor. Direct interstitial fluid sampling requires implanting a capillary probe with a semipermeable membrane into the tumor. Such methods have been employed more extensively for extracellular neurotransmitter measurements and have had a substantial impact in neuroscience. In one approach, known as ultrafiltration, negative pressure in the probe is used to collect a filtrate of interstitial fluid. Alternatively, in microdialysis, the capillary probe is filled with carrier fluid, into which contents of the interstitial fluid diffuse (56, 57). In ultrafiltration, the nature of the sampled metabolites depends only on the molecular weight cutoff of the semipermeable membrane, but application of negative pressure might mechanically disrupt surrounding cells. In microdialysis, negative pressure is avoided, but carrier fluid could potentially alter ion-coupled metabolite exchange processes and thus the metabolic milieu (57). In both methods, probe implantation requires a surgical intervention, limiting clinical use. Despite their potential imperfections, these methods offer an unparalleled opportunity to obtain extracellular fluid directly from tumor. Key observations can be validated with complementary methods, such as electrochemical probes used in neuroscience for the *in vivo* analysis of brain interstitial fluid (58). The biggest current limitation is the paucity of available data. To our knowledge, only two studies have applied the microdialysis technique to sample tumor interstitial fluid in human patients with cancer. In head and neck cancer, tumors showed about a 2-fold increase in lactate levels compared with mucosa, whereas adequate data were not available to assess glucose in the tumor versus normal mucosal microenvironment (59). In astrocytoma, compared with adjacent brain, interstitial lactate was modestly increased (1.6-fold) and glucose trended lower, but was not significantly depleted (60). No significant changes were found for other measured metabolites (glutamate, glycerol, and pyruvate). Importantly, the measured microenvironmental lactate was lower than those in tumors overall and lower than the levels applied to modulate immune cell function in cell culture experiments (Fig. 1B).

Ex vivo purification of tumor interstitial fluid

Given the technical challenges of probe-based sampling, there is interest in measuring interstitial fluid in resected tumor specimens. In one approach, known as tissue elution, the tissue is cut into small pieces (1–3 mm^3), washed, and incubated in PBS at 37°C in a humidified CO_2 incubator for 1 to 24 hours (61). Although controls have shown that tissue elution does not result in substantial protein leakage, it seems almost inevitable that cells will continue to excrete metabolites. Thus, although the measurements may reveal interesting aspects of tumor metabolism, they seem ineffective for measuring the tumor microenvironment.

More promising, but still problematic, is tissue centrifugation, in which fresh tissue is washed to remove any residual blood, blotted dry, and transferred to a microcentrifuge tube over a nylon mesh. By applying a controlled centrifugation force (e.g., <424 g), the interstitial fluid is collected at the bottom of the tube, whereas the rest of tissue components, including intact cells, are retained (62, 63). Key concerns in this approach are alteration of the microenvironment during sample collection and handling, and leakage of metabolites due to the centrifugal force, a problem that we have observed in preliminary efforts to apply the method. Using this method, which may be skewed by glucose consumption during the tissue collection or handling, glucose concentration in tumor interstitial fluid has mainly been found to be lower than blood or normal tissue (Fig. 1C). The magnitude of the change is highly variable, ranging from insignificant (64) to over 10-fold decreased (24, 65–67). Corresponding lactate data are in most cases not available.

Thus, it appears that some (and perhaps most) tumors consume glucose sufficiently rapidly to outpace perfusion and create a local, glucose-poor microenvironment. Whether extracellular lactate levels increase appreciably is less clear, with reported levels not reaching those known to inhibit immune response, although this may vary with tumor type and region.

Flux measurements and the tumor metabolic microenvironment

Fluxes provide an orthogonal viewpoint on the tumor metabolic environment: What is being consumed? What is being made? And how fast is exchange between the blood stream and the tumor? For glucose, such analyses have confirmed rapid consumption. But for lactate, they have raised new questions.

Measurement of glucose uptake flux using ^{18}F-fluorodeoxyglucose (^{18}F-FDG)-PET is an important clinical tumor imaging modality. ^{18}F-FDG is an analog of glucose that can be taken into cells and retained by phosphorylation, but not further metabolized. Accumulation of ^{18}F-FDG is a hallmark of glycolytic cells and the avid uptake of glucose by the tumor aligns well with the observation that tumors (both in bulk and in interstitial fluid specifically) are glucose poor (Fig. 1D; ref. 68).

Beyond the PET, other techniques can be employed to probe *in vivo* tumor metabolic fluxes. These include (i) arteriovenous blood composition differences, (ii) stable isotope tracers coupled to either MS or NMR detection, and (iii) hyperpolarized MRI. Nutrients are delivered to tissues (and tumors) by arterial blood and waste is removed by venous blood. The difference in metabolite levels between the incoming and outgoing blood (A/V difference) provides a quantitative measure of net metabolite production and consumption by the tumor. Although conceptually simple, this approach requires obtaining mixed venous blood draining the tumor, which can be difficult given the disorganized nature of tumor vasculature (69, 70). Early tumor A/V difference studies by Otto Warburg showed that Jensen's sarcoma transplanted into rats consumed glucose and produced lactate (71). Net glucose consumption by tumors has been confirmed in a wide

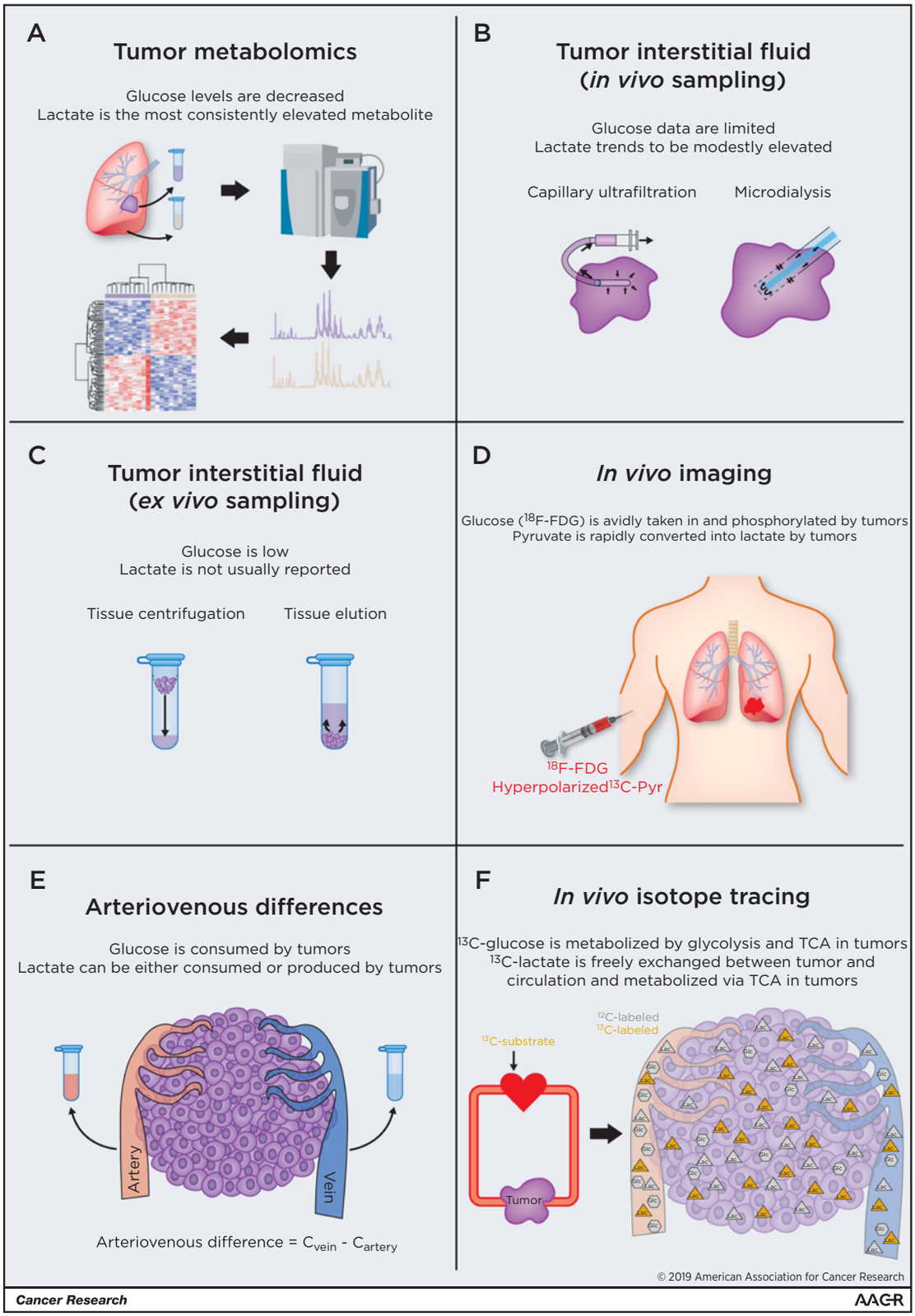

Figure 1.
Experimental approaches for understanding the tumor metabolic microenvironment and summary of associated literature data for glucose and lactate.

range of animal models (72–74). In contrast, these studies suggest that tumors may either produce or consume lactate (Fig. 1E). One apparent determinant is the systemic lactate concentration, with a trend for tumors to consume lactate when the incoming lactate concentration is high, and excrete it when the incoming arterial concentration is lower (72, 73).

In addition to these animal experiments, we are aware of two human studies that measured A/V differences across the tumor. In colon cancer, tumors consumed glucose and essential amino acids; showed no consistent uptake or excretion of ketone bodies or free fatty acids, and consistently secreted ammonia, alanine, and lactate (75). In head and neck cancer, all tumors took up glucose, pyruvate, and ketone bodies while lactate was excreted by some and consumed by other tumors (76). Thus, at least some tumors seem to be net lactate consumers.

Infusions of isotope-labeled nutrients provide a means to probe their metabolic fates within the tumor, and to determine the sources of key metabolic products (21, 48, 77, 78). Recent experiments using isotope tracers *in vivo* are substantially revamping our understanding of tumor metabolism. For example, in contrast with the Warburg effect, which focuses exclusively on glucose's catabolism into lactate, tracing experiments using ^{13}C-labeled glucose have shown that tumors actively metabolize carbon from infused glucose into the TCA cycle via both pyruvate carboxylase and pyruvate dehydrogenase, with rates in lung cancer exceeding those in benign adjacent pulmonary tissue (51, 79–82). Even more surprisingly, tumors not only produce lactate, but they also uptake circulating lactate to be used as TCA substrate (Fig. 1F; refs. 20, 21, 51). Indeed, quantitative analysis suggests that, in several murine tumor models and about half of human non–small cell lung tumors, the main way infused glucose enters the tumor TCA cycle is via circulating lactate: The exchange of lactate between the tumor and circulation is so rapid that little of the pyruvate made from glucose via the tumor's upregulated glycolytic pathway actually makes it directly into the TCA cycle. Instead, in these cases, most tumor pyruvate is converted into lactate and excreted, with most of the pyruvate in the tumor coming from circulating lactate made elsewhere in the body (21). Analogous studies of additional human tumor types are needed. Thus, although many tumor cells produce lactate (32, 83), such lactate may be largely released into the circulation, rather than locally feeding other tumor cells.

Consistent with the rapid transport of pyruvate and lactate into and out of the tumor, and their rapid interconversion within tumors, hyperpolarized [1-^{13}C]pyruvate is converted more rapidly to lactate in tumors compared with benign adjacent tissue: Upon infusion of hyperpolarized pyruvate into patients with prostate cancer, biopsy-proven cancerous regions show increased levels of hyperpolarized [1-^{13}C]lactate and an elevated ratio of [1-^{13}C]lactate/[1-^{13}C]pyruvate (84). Collectively, these isotope tracer studies are difficult to align with the finding that lactate is the most elevated metabolite in human tumors (and the common assumption that lactate is elevated in the microenvironment): How can a uniquely high concentration of lactate be maintained within the tumor, if lactate is mixing rapidly between the tumor and circulation?

A potential resolution to the "lactate paradox": the role of pH in lactate transport

We believe that the biochemistry of MCT transporters can explain the coexistence of (i) rapid exchange between tumor and circulating lactate and (ii) elevated lactate within the tumor. Much as phosphorylation is used by the cells to favor glucose uptake and intracellular retention, we propose that pH is similarly used by cancer cells to maintain their lactate pools.

Lactic acid is transported across membrane through a family of MCTs (85). MCTs cotransport a lactate anion (Lac^-) and H^+. Accordingly, at equilibrium, the product of Lac^- and H^+ concentrations (i.e., $[Lac^-][H^+]$) has to be equal among different compartments linked by MCT transporters. Thus, by maintaining low H^+ concentration (high pH), tumor cells accumulate lactate. Specifically, although normal cells typically have an intracellular pH about 0.2 U lower than the extracellular pH, tumor cells may have an intracellular pH about 0.8 U higher than the microenvironment pH. If the lactate concentration in the microenvironment is equivalent to that in the circulation (e.g., due to leaky capillaries), this could lead to a lactate concentration in tumor cells nearly 10-fold higher than in normal cells, based solely on the thermodynamic consequences of the H^+-coupling of MCT transporters (Fig. 2A). The potential for pH gradients to drive lactate into cells has been previously described in the brain (86). An open question remains the mechanism by which high H^+ concentration is maintained in the tumor microenvironment. Nevertheless, pH gradients, through mechanisms unrelated to the Warburg effect, may drive lactate accumulation in tumors.

Speed of lactate exchange versus net transport by MCTs

Another aspect of the biochemistry of MCT transporters may also help to maintain lactate and pH concentration gradients within the tumor while allowing for a fast isotope exchange between tumor and circulating lactate pools: MCT transporters can exchange lactate across the membrane (i.e., importing one molecule of lactate, and exporting a different one) or trade lactate for another MCT substrate like pyruvate, faster than they can net transport lactate in either direction. The underlying biophysics involves the MCT catalytic transport cycle (Fig. 2B). Transporters bound to both H^+ and Lac^- (or another MCT carbon substrate) rapidly undergo a conformational change that exposes the ligands to the opposite site of the membrane. But transporter lacking substrate undergoes the conformational change slowly. Thus, MCT's are more efficient at transporting Lac^- back and forth across the cell membrane than at dissipating lactate concentration gradients (85). Therefore, MCTs are expected to equilibrate labeling patterns (i.e., mixing of lactate molecules of different origin) and relative monocarboxylate levels (e.g., lactate:pyruvate ratio) faster than they dissipate lactate concentration gradients.

Conclusions

Accumulation of immunosuppressive metabolites in the tumor milieu, in particular lactate, is commonly assumed but poorly proven. *In vitro* experiments have shown that lactic acid is a potent inhibitor of antitumor T cells and favors the development of tumor permissive (i.e., immunosuppressive) T_{reg} cells (26, 40, 87). But, limited direct measurements of interstitial fluid lactate suggest relatively modest accumulation, substantially less than levels used in cell culture studies. A/V

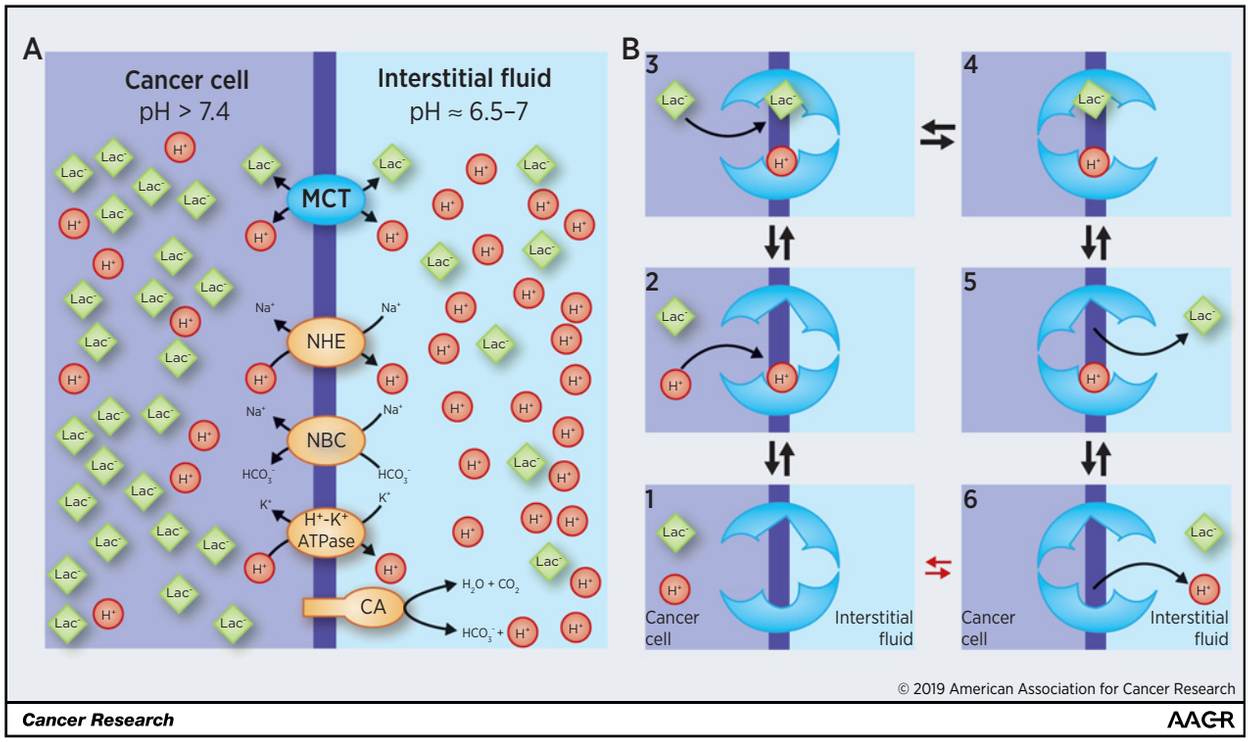

Figure 2.

The role of MCT and pH in lactate transport. **A,** Cancer cells maintain a high intracellular pH, relative to the microenvironment through several mechanisms, including the increased expression and activity of acid extruding plasma membrane transporters, for example, Na^+/H^+ exchangers (NHE), H^+/K^+-ATPases, and Na^+/HCO_3^- cotransporters (NBC), which work in concert with carbonic anhydrases (CA). The generated pH gradient favors the accumulation of lactate inside the cell, as monocarboxylate transporters (MCT) cotransport lactate and H^+, so low intracellular H^+ concentration favors lactate flux into the cell (to equilibrate the product of $[H^+] \times [Lac^-]$ across the membrane). **B,** To mediate lactic acid transport, MCTs bind and release the ligands in an ordered way. The transporters bind sequentially H^+ and Lac^-. Then a conformational change exposes the ligands to the opposite site of the membrane, Lac^- and H^+ are sequentially released, and finally the empty transporter returns to the initial position. The return of the empty transporter to the initial position is the rate-limiting step in the transport process. Therefore, MCTs transport Lac^- back and forth across the membrane more rapidly than they net transport lactate.

gradient measurements suggest that some tumors actually net consume lactate. Isotope tracer studies argue for equilibration of lactate between the circulation and tumor microenvironment (i.e., that the tumor microenvironment is well-perfused from the perspective of lactate, if not from the perspective of oxygen). Even though lactate accumulates within tumor cells, such lactate is "hidden" from immune cells and unlikely to impact immune response. This does not discount the importance of lactate dehydrogenase A expression in cancer cells, which has been shown experimentally to favor tumor immune escape (87), perhaps by enabling rapid cancer glucose consumption. Indeed, even if lactate is mainly in tumor cells, not the environment, low glucose, oxygen, and/or pH in the microenvironment may nevertheless be immunosuppressive (24, 40, 65, 66, 88).

Can we extract any general conclusion about the metabolic composition of tumor microenvironment and its significance for tumor growth and progression? The strongest conclusion is that we need to more carefully characterize the tumor metabolic milieu. This is particularly critical because, due to active transport processes, the metabolic composition of the microenvironment may differ markedly from that of the bulk tumor. Direct interstitial fluid sampling followed by metabolomic analysis seems a valuable step in this direction.

Note Added in Proof

Metabolomics analysis of murine tumor interstitial fluid (isolated via centrifugation) was recently reported, with lactate concentration indistinguishable from plasma (89).

Disclosure of Potential Conflicts of Interest

J.D. Rabinowitz has ownership interest (including stock, patents, etc.) in Agios, Raze Therapeutics, L.E.A.F, Kadmon Pharmaceuticals, Bantam Pharmaceuticals and is a consultant/advisory board member for Kadmon Pharmaceuticals, Agios, L.E.A.F., Bantam Pharmaceuticals, Raze Therapeutics. No potential conflicts of interest were disclosed by the other authors.

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