

## Acidity of Microenvironment as a Further Driver of Tumor Metabolic Reprogramming

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### Abstract

In the last decade, experimental research has intensely focused on metabolic reprogramming of tumor cells, which contributes to cancer cell adaptation and survival in different and hostile microenvironments. Metabolic reprogramming consists of the switch of tumor cells from aerobic or anaerobic glycolysis to oxidative phosphorylation. A comprehensive vision of the metabolic scenario involving functionally different tumor cell subpopulations was proposed as a necessary premise to the design of new strategies of diagnosis and therapy. Special focus has been put on the role of acidosis of certain tumor regions, a very important although frequently neglected aspect.

Despite the progresses in cancer therapy, the escaping of tumor cancer cells from host defense and relapse of disease still represent main issues in tumor-bearing patients. Indeed, malignant cells are provided with a tremendous plasticity that they exploit to survive, replicate and invade in stressed microenvironments. Such plasticity allows cancer cells to easily modify their properties, including metabolism, switching back and forth from aerobic or anaerobic glycolysis to oxidative phosphorylation (OxPhos). It is well ascertained that a suitable metabolic profile of cancer cells is necessary to sustain tumor growth, local invasion and distant colonization. Thus, cancer metabolism needs to be considered in view of the design of new strategies to control tumor progression.

**Keywords:** "Warburg effect"; Glucose level; Hypoxia; Hypoxia-inducible factor-1 $\alpha$ ; Acidosis; Oxidative phosphorylation

### Tumor Microenvironment and Metabolic Reprogramming of Tumor Cells

#### Warburg effect

Proliferating tumor cells have been largely shown to adopt "aerobic glycolysis" as the main metabolic profile, the so-called "Warburg effect" [1]. Indeed, most cancer cells use huge amounts of glucose even when oxygen tension is high enough to sustain mitochondrial respiration in normal cells. A link between oncogenesis and glucose metabolism is the activating mutations in phosphoinositide 3-kinase (PI3K) or overexpression of the AKT oncogenes, promoting expression and localization of the high affinity glucose transporters on the plasma membrane [2]. This is followed by lactic acid fermentation in the cytosol and lactate export from the cell [3]. This alteration of glucose metabolism acquired practical importance in clinical settings following the development of 18-fluorodeoxyglucose-positron emission tomography imaging [4,5]. Furthermore, it is well recognized that a high serum level of lactate dehydrogenase (LDH) represents a biomarker of poor prognosis in different cancers [6].

When Warburg metabolism is favored, a relatively low level of pyruvate is metabolized in the mitochondria and the energy gain is only 2 ATP per molecule of glucose. Thus, fermentation of glucose to lactic acid is an inefficient pathway, but, very important, it is a fast energy supplier (about 100 folds faster than OxPhos). Moreover, the

aerobic glycolytic phenotype confers a significant proliferative advantage as it ensures biomass formation and DNA duplication, crucial aspects of proliferation [7]. Indeed, the glycolytic breakdown of glucose generates a number of substrates which turn into "anabolic" precursors for the synthesis of different compounds, such as glucose-6-phosphate for glycogen and ribose 5-phosphate, dihydroxyacetone phosphate for triacylglyceride and phospholipids, and pyruvate for alanine and malate [8]. Nucleotides, amino acids and lipids are indeed synthesized during anabolic reactions [9]. Cancer cells try to reduce the last step of glycolysis that is catalyzed by pyruvate kinase (PK) by up regulating the low-activity dimeric isoform M2 of PK, and thereby facilitating the accumulation of metabolites upstream of pyruvate [10]. In this respect, intermediate components of the glycolytic pathway appear to be more significant than its final product, e.g. pyruvate. To replenish the TCA cycle, due to the limited supply of pyruvate, cancer cells increase the consumption of glutamine [11]. The particular attitude of proliferating cancer cells to use aerobic glycolysis favors the development of a microenvironment in which lactate produced by tumor cells can be taken up by normal stroma cells to regenerate pyruvate, which can be extruded to refuel cancer cells [12].

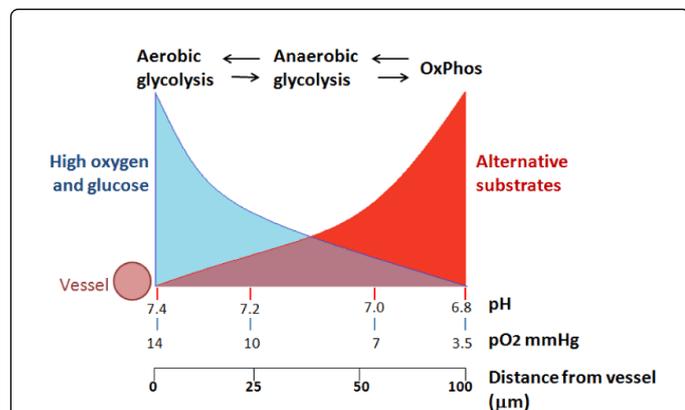
Therefore, dividing cancer cells, usually exposed to a relatively high oxygen tension, adopt a glycolytic metabolism, followed by increased glucose uptake and lactic acid production.

#### Hypoxia

Although the use of aerobic glycolysis leads to a lower oxygen usage, promoting oxygen availability for tumor cells located more distant from blood vessels [13], tumor growth within a disorganized

vasculature generates an hypoxic microenvironment. Experimental studies have demonstrated that partial pressure of oxygen is near zero between 100 to 200  $\mu\text{m}$  from a vessel (Figure 1), at the variance of glucose exhaustion which is longer, due to the higher diffusion rate of glucose [14]. Tumor cells need to adapt to the above change and this adaptation partly relies on the induction of hypoxia-inducible factor (HIF)-1 $\alpha$ , a transcription factor finely regulated at a protein level in an oxygen-sensitive manner [15]. HIF-1 $\alpha$ -related genes are critical in inducing reduction of cell proliferation combined with the development of a more aggressive and resistant phenotype, particularly potentiated by an elicited epithelial-to-mesenchymal transition (EMT) program [16]. Indeed, HIF-1 $\alpha$  promotion causes loss of E-cadherin [16] and the expression of the *met* proto-oncogene and TWIST transcription factor [17]. HIF-1 $\alpha$  also acts as a central player in tumor metabolism to support hypoxic tumor cell energy supply, driving an anaerobic glycolysis by upregulating glucose transporter isoform 1 (GLUT1), hexokinase (HK1 and HK2, which catalyze the initial and fundamental step of glycolysis), lactate dehydrogenase A (LDHA), and the lactate-extruding monocarboxylate transporter 4 (MCT4) [16,18]. Besides, carbonic anhydrase IX (CAIX) under the influence of HIF-1 $\alpha$  prevents the acidification of intracellular pH of cells under hypoxia [19]. HIF-1 $\alpha$  blocks pyruvate conversion into acetyl-CoA triggered by pyruvate dehydrogenase (PDH), by upregulating the PDH inhibitor PDH kinase 1 (PDK1) [20,21]. Thus, inhibiting PDH, HIF-1 $\alpha$  compromises the entrance into the oxidative metabolism. HIF-1 $\alpha$  can also be activated under normoxic conditions by oncogenic signaling pathways, including PI3K, and by mutations in tumor suppressor proteins, such as VHL (von Hippel Lindau), succinate dehydrogenase, fumarate hydratase [22]. c-Myc also cooperates with HIF-1 $\alpha$  in the promotion of glycolysis inducing HK2 and PDK1 [23].

The result of this metabolic change is that tumor microenvironment is enriched with H<sup>+</sup> and lactate generated by hypoxic cancer cells [24].



**Figure 1:** Metabolic reprogramming of tumor cells prompted by variability of tumor microenvironment. Data in part from Helmlinger et al. [72].

### Acidosis of tumor microenvironment

Both aerobic and anaerobic glycolysis used by proliferating and hypoxic tumor cells cooperate in the generation of an acidic extracellular pH [25], now considered an important hallmark of cancer to be investigated. The use of a new pH-sensitive positron emission tomography (PET) radiotracer allows *in vivo* determination of tumor

pH, which has been showed to be in the range of 6.4-7.0 for the most solid tumors [26]. We cannot exclude that a chaotic vasculature, low lymphatic drainage and high interstitial pressure contribute to the lowering of extracellular pH (pHe). It has been demonstrated that tumor cells are tolerant to a broad range of acidic pHe, while acidification of the microenvironment is toxic for adjacent normal cells [25].

Acidosis and lactic acidosis, generated by protons and lactate extrusion in the extracellular milieu, correlates with a poorer clinical prognosis [27,28]. We have demonstrated that acidity reduces proliferation together with stimulation of apoptosis resistance and an EMT profile in melanoma cells [29]. The EMT phenotype acquired by acidic melanoma cells was characterized by an increased invasiveness and the ability to promote lung colonization of non-acidic counterpart of cells, disclosing a new cooperation among different tumor cell subpopulations [30]. Robey et al. reported that orally-administered bicarbonate to tumor-bearing mice reduces cell dissemination, providing the evidence that increasing tumor pHe to a physiological level is crucial to abrogate progression to metastasis [30]. The acidic tumor microenvironment also represents a major driving force of resistant phenotype to chemo- and radio-therapy [31,32]. Indeed, both the uptake and efficacy of weak base drugs are reduced in low pH environment [33]. Moreover, lactate by itself has been found to induce motility, angiogenesis, radio-resistance and immune escaping, all features contributing to disease progression [34]. Beyond the aggressive features that acidosis confers to cancer cells, recent discoveries show that local acidity, either generated by high proton level or lactic acid, plays a role in tumor metabolic reprogramming, driving cancer cells from glycolysis to OxPhos [35]. Thanks to aerobic and anaerobic glycolytic pathways adopted by proliferating and hypoxic tumor cells, a residual oxygen tension in tissues may sustain an OxPhos metabolism, possibly at a “reduced rate”. It is known that a residual 3.5 mmHg tension of O<sub>2</sub> may permit mitochondrial activity (Figure 1) [36]. OxPhos is considered a major sink for protons able to control internal pH, quite different from protons generated during ATP hydrolysis produced by glycolysis, which are not consumed and generate acidosis. A low pHe generates a low pH of intracellular milieu able to contribute to the metabolic switch to OxPhos, inhibiting the HIF-1 $\alpha$ -dependent program through a PI3K/Akt/mTOR signal transduction pathway [37]. In accordance with this finding, it has been recently found that pHe influences hypoxia-related gene expression to a large extent [38,39]. Low pHe suppresses the hypoxia induced up-regulation of PDK1, the effector of PDH inhibition and entry into mitochondrial respiration [40,41]. Breast cancer cells, grown in an acidic medium, also express a reduced glycolysis and lactate production and an increased respiratory metabolism [42]. More recently, we proved that both acidosis and lactic acidosis induce OxPhos metabolism in melanoma cells and, importantly, metformin, a mitochondrial complex I inhibitor, selectively targets and kills acidic cancer cells in a dose-dependent manner [35]. Further, glucose exhaustion caused by glycolytic cells and accumulation of AMP-activated protein-kinase favor the metabolic shift of acidic cells toward OxPhos. When low-pH-adapted cells were grown in a high glucose medium, they maintain a higher pyruvate oxidation, indicating that Crabtree effect is not applicable to acidic cells [43]. To sustain resistance to apoptosis, migration and distant dissemination, all energy-demanding processes, and in the absence of a sufficient glucose supply (already used by proliferating and hypoxic cells), acidic cells use alternative substrates such as lactate, free fatty acids and amino acids. Accordingly, acidosis increases both glutaminolysis and fatty acid  $\beta$ -

oxidation in oxidative tumor cells contributing to the entry of metabolic intermediates into the TCA cycle and ATP generation [42]. Lactate also promotes glutamine uptake and metabolism in oxidative cancer cells [44].

LeBleu et al. [45] demonstrated that the bioenergetic phenotype of circulating tumor cells, endowed with a high migratory ability and EMT profile, uses OxPhos. This is of the utmost importance in view of our finding that acidic melanoma cells using an OxPhos metabolism are able to promote lung colonization of non-acidic/glycolytic melanoma cells intravenously injected into immunodeficient mice [29]. We may speculate that, when tumor cells reach their target organ thanks to the ability of acidic subpopulations to lodge and resist to new stressors, non-acidic/glycolytic cell subpopulations start to proliferate leading to a new colony. It is also possible that acidic/OxPhos cells, influenced by an adequate blood supply, which may remove protons and lactate from the microenvironment, reprogram their metabolic profile back to aerobic glycolysis, a key to proliferation. EMT plays a fundamental role during development as well as tumor metastasis. Liu et al., studying head and neck squamous cell carcinoma (HNSCC), found that histone H3K9 methyltransferase G9a is essential for the EMT-mediated metastasis [46]. Actually, G9a forms a complex with Snail and mediates Snail-induced transcriptional repression of E-cadherin and EMT [47]. G9a is also essential for the induction of EMT and cancer stem cells (CSC)-like properties in HNSCC [48]. Since recent observation indicates that apart from establishment and maintenance of H3K9me2, G9a also plays a critical role in the maintenance of DNA methylation at certain loci [49], both the epigenetic mechanisms that associated with histone and DNA methylation might be involved in the maintenance of the EMT-mediated metastasis ability in tumor cells. Therefore, G9a might be a promising target for identifying a subset of cancer cells that display CSC-like profile and it might be of interest investigating the expression levels of G9a in cancer cells undergoing the metabolic reprogramming.

To add complexity to the metabolic reprogramming of tumor cells, a metabolic symbiosis among different tumor cell subpopulations, generating and using lactate, may also take place and contribute to tumor cell dissemination [50]. A metabolic cross-talk has been demonstrated, called “reverse Warburg effect”, among cancer-associated fibroblasts (CAF) and prostate or breast cancer cells, identifying a scenario in which lactate generated by glycolytic CAF is used by tumor cells using a respiratory metabolism [51,52]. In addition, we have identified a possible metabolic cross-talk between acidic mesenchymal stem cells (MSC) and melanoma cells. We demonstrated that acidic MSC undergo a metabolic reprogramming to OxPhos, which in turn may favor the removal of lactate produced by glycolytic melanoma cells [53]. Lactate taken up by acidic stromal cells may work reducing acidification of tumor microenvironment and supporting tumor growth.

Overall, whereas the Warburg effect confers growth advantage to cancer cells when oxygen and glucose supply is sufficient, the OxPhos phenotype promoted by lactic acidosis prompts the use of alternative substrates and renders cancer cells more resistant and competent to progress locally and at a distance.

### **Perspective in diagnosis and therapy of acidic tumor microenvironment**

Several findings indicate that measuring pHe in tumors might acquire a great importance for the characterization of tumor

aggressiveness. Indeed, the influence that acidic microenvironment exerts on tumor progression and relapse, due to the reduced sensitivity of acidic cancer cells to radio- and chemotherapy [13], led to test several non-invasive optical imaging methods to monitor *in vivo* tumor pHe. Positron emission tomography was used for the imaging of acidic prostate tumors [54]. Experience was also made using electron paramagnetic resonance spectroscopy [55] and magnetic resonance (MR) spectroscopy [56]. Quite recently, Chen described a method, termed acido-CEST (chemical exchange saturation transfer) MR imaging, to provide information of tumor pHe in preclinical settings of breast carcinoma [57].

On the meantime, many efforts were put in developing combined therapeutic strategies to target the acidic subpopulations of tumors. Among these, are the alkalization of tumor microenvironment with the use of systemic buffers, such as sodium bicarbonate (NaHCO<sub>3</sub>) [30,58], or use of proton pump inhibitors, such as esomeprazole, which leads to the normalization of pHe [59]. A comprehensive review of pH regulation in tumors is reported by Neri and Supuran [60]. An additional attractive target of acidic cancer cells is CAIX, which is overexpressed in acidic cancer cells in a HIF-1 $\alpha$ -independent manner [61]. Several inhibitors have been developed, such as sulfonamides, sulfamates, and sulfamides, that bind to Zn<sup>2+</sup> ion-containing catalytic site of the enzyme, thus blocking its function [62]. These compounds are currently under investigation to test *in vivo* efficacy. Treatment of mice transplanted with CAIX-positive mammary tumor cells with novel CAIX specific inhibitors resulted in a significant reduction of tumor growth and metastatic dissemination [63]. Further, weakly-acidic drugs are designed to reach solid tumors in order to release toxins within the acidic microenvironment [64]. Additional delivery systems to selectively reach and target the acidic core of cancers have been developed. Among them, pH-sensitive liposomes, that release chemotherapeutic substances at an acidic pH [65], and the pH low insertion peptides (pHLIP), that consist of soluble peptides able to bind cell membrane in a pH-dependent way [66]. Another interesting strategy is to target the plasticity of cancer metabolic reprogramming with anti-metabolic drugs. Recently, different therapeutic approaches have been developed based on the targeting of OxPhos metabolism of cancer cells by inhibiting tumour-specific alterations of mitochondrial metabolism or by stimulating mitochondrial membrane permeabilization [67,68]. Among the several drugs tested, metformin, one of the most widely used antidiabetic agents, was found to be efficient in various cancers, such as prostate, breast, lung and pancreas [69]. Its anticancer effect is mediated through the activation of LKB1-AMPK pathway, resulting in the inhibition of the mammalian target of rapamycin (mTOR), that is associated with resistance to anticancer drugs [70]. Also, acidic tumor cells, including melanoma cells, seem to be responsive to metformin treatment [35]. Using osteosarcoma as a cancer model, Quattrini et al. [71] found that metformin amplifies the effects of cisplatin, pointing to a novel approach to therapy of tumors that is the combination of conventional therapy with metabolic inhibitors.

### **Conclusions**

In order to block local and distant dissemination of tumor cells, it is of critical importance to target the acidic regions of tumors to revert the aggressive phenotype that acidity confers to cancer cells. In view of the growing knowledge on tumor metabolism and its reprogramming stimulated by an acidic microenvironment, metabolism of acidic

cancer cells may represent an interesting target to improve diagnosis and therapy of tumors.

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