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Review

Evolutionary View on Lactate-Dependent Mechanisms of Maintaining Cancer Cells Stemness and Reprimitivization

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Abstract: The role of lactic acid (lactate) in cell metabolism has been significantly revised in recent decades. Initially, lactic acid was attributed to the role of a toxic end product of metabolism, which accumulation in the cell and extracellular space leads to acidosis, muscle pain and other adverse effects. However, it has now become obvious that lactate is not only a universal fuel molecule and the main substrate for gluconeogenesis, but also one of the most ancient metabolites with signaling function, which has a wide range of regulatory activity. The Warburg effect described 100 years ago (that means intensification of glycolysis associated with high lactate production), which is characteristic of many malignant tumors, confirms the key role of lactate not only in physiological conditions, but also in pathologies. The study of lactate's role in the malignant transformation becomes more relevant in the light of the "atavistic theory of carcinogenesis," which suggests that tumor cells return to a more primitive hereditary phenotype during microevolution. In this review, we attempted to summarize the accumulated knowledges about the functions of lactate in cell metabolism and its role in the process of carcinogenesis, and to consider the possible evolutionary significance of the Warburg effect.

Keywords: lactate; lactic acid; glycolysis; carcinogenesis; malignant tumors; evolutionary oncology

1. Introduction

In recent years, interest in malignant neoplasms as a biological phenomenon found in almost all multicellular species has increased, indicating its deep evolutionary roots dating back to the dawn of multicellularity. In 1929, Theodore Boveri suggested that malignant neoplasms represented a type of atavism or reversion to a more primitive hereditary phenotype. In a series of research papers, Boveri's idea of malignant tumors as a type of atavism was developed into a detailed theory of the onset and progression of neoplasms, the provisions of which are confirmed by philostratigraphy, a statistical approach used to study the evolutionary age of genes involved in carcinogenesis [1]. Phylostratigraphic analysis originates from the pioneering work of Domazet-Loso [2]. The researchers determined the age of 'cancer-associated genes' using a series of compilations of such sequences and found two peaks in which the representation of the genes was the highest compared to the age distribution of all other human genes. The first peak was observed in the era of unicellularity before the emergence of eukaryotes. The second peak was correlated with the emergence of multicellular organisms. The most important observation was that the genes associated with carcinogenesis, and younger

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than ~400 million years, were presented to a much lower extent, which confirms the main thesis about the ancient evolutionary roots of malignantly transformed cells. Indeed, it is well known that genes responsible for cellular cooperation in multicellular organisms (e.g., signaling, adhesion, angiogenesis, and migration) are the first to be damaged during carcinogenesis and lead to loss of regulatory functions. Furthermore, the existence of a close relationship between carcinogenesis and the early stages of embryogenesis is generally recognized. Indeed, as the tumor progresses, its cells dedifferentiate in the direction of "stemness" and, in general, more resemble cells at an early stage of embryogenesis or unicellular forms [3].

A team of Australian researchers applied philostratigraphy method to RNA transcript sequencing data from The Cancer Genome Atlas for seven solid tumors divided into 16 age categories [4]. They found that genes that emerged during evolution in unicellular forms were overexpressed in human malignant tumors, while the expression of genes that appeared in the stage of the emergence of multicellularity, on the contrary, was suppressed. At the same time, overexpression of genes associated with unicellularity was caused by a serious dysregulation of control structures that occurred during the evolutionary transition to multicellularity. It should be noted that this "atavism" cannot be regarded simply as a reprimitivization to unicellularity. Tumor cells are most likely to follow the path of reprogramming the links between gene networks that control processes in unicellular organisms and those that control processes in multicellular organisms [4].

The anaerobic glycolysis arose in evolution even before the appearance of eukaryotes and thus is one of the most ancient methods of energy supply to the cell [5]. Tumor cells effectively use the glycolysis to obtain a large amount of lactate, a metabolite that serves not only as cells fuel, but also allows to reprogram the cells of the tumor microenvironment in such a way as to create the most favorable conditions for the growth and metastasis of malignant cells [6]. The study of the role of lactate in the malignant transformation becomes even more relevant in the light of the “atavistic theory of carcinogenesis”, which suggests that tumor cells return to a more primitive hereditary phenotype during microevolution. In this review, we have tried to summarize the knowledge about the functions of lactate in cell metabolism and its role in the process of carcinogenesis, and to consider the possible evolutionary significance of the Warburg effect.

2. Results

2.1. The evolutionary significance of the intensification of glycolysis.

Another argument in favor of the “atavistic theory of carcinogenesis”, which we talked about above, is the fact that tumor cells use glycolysis, but not oxidative phosphorylation, as the main metabolic pathway for glucose utilization: 100 years ago, in the 1920s, Otto H. Warburg, examining sections of tumor tissues, found that malignant cells predominantly convert glucose into lactate even under conditions of adequate cellular oxygenation (i.e., in the absence of hypoxia) [7]. Warburg associated such a metabolic feature of tumor cells, which he called "aerobic glycolysis", with damage to mitochondria and pointed out that such a change in metabolism is a key sign of malignant cell transformation [8]. However, the Warburg effect is currently associated with metabolic reprogramming that accompanies tumor transformation and is necessary for the rapid growth and proliferation of tumor cells, maintaining their stem properties, avoiding an immune system response, and survival [9]. Thanks to numerous studies in recent years, the signaling and regulatory properties of lactate have become known, which to some extent reveal the mechanisms of cell malignancy and allow us to look at their evolutionary significance. The view of the role of lactate in cell metabolism has undergone significant

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changes in recent decades: from the misconception of lactate as a "metabolic waste" and a toxic end product of glycolysis that must be neutralized, researchers have come to understanding the critical role of lactate in metabolism and its regulation. Moving between cells-producers (drivers) and cells-consumers (recipients), lactate performs at least three key functions: 1) the main source of energy for mitochondrial respiration; 2) the main precursor for gluconeogenesis; and 3) a signaling molecule involved, among other things, in the metabolic reprogramming of cells [10]. At the same time, the signaling function of lactate is of the greatest interest in terms of studying the mechanisms of malignant transformation: on the one hand, lactate, a metabolite of glycolysis, is one of the most ancient signaling molecules that performs regulatory functions in both eu- and prokaryotes [11]; on the other hand, the action of lactate is amazingly versatile. As described below, lactate is a key oncometabolite that provides energy for tumor cells, transformation of the tumor microenvironment, and thus growth, invasion, and metastasis of transformed cells.

2.2. *Lactic acid causes acidification of the extracellular space of the tumor and suppresses antitumor immunity.*

A well-known hallmark of tumor cells is their ability to evade or block the immune response. In view of the relatively young evolutionary age of adaptive immunity (less than 500 million years), the theory of "carcinogenesis as a process of regression to ancestral forms" predicts that the adaptive immune response should be inhibited shortly after the initiation of carcinogenesis [12]. One of the mechanisms by which a tumor escapes an immune response is the creation of an acidic microenvironment, which contributes to the suppression of the inflammatory response and activation of immune cells [13]. Lactate formed in large amounts in tumor cells (Warburg effect) is co-transported with protons (H^+) from tumor cells through monocarboxylate transporters (MCT1 and MCT4), which, on the one hand, leads to lactate accumulation (up to 45 mM), and the other is to reduce the pH of the tumor microenvironment [7]. In turn, acidification leads to the restriction of the production of interferon gamma (IFN-γ) by T-cells infiltrating the tumor, and prevents the activation of NK cells, which ultimately contributes to the avoidance of the immune response by the tumor and inhibits its growth [14]. In addition, various studies have shown that lactic acid can enhance toll-like receptor 4 (TLR 4) signaling, nuclear factor (NF)-κB-dependent gene regulation, and the proinflammatory function of macrophages, although other studies have shown opposite effects [15]. It has been demonstrated that high concentrations of lactate can stimulate the polarization of anti-inflammatory M2 macrophages through HIF1α stabilization or through epigenetic regulation through histone lactylation [16]. Lactic acid may also play a critical role in the phenotype and functionality of dendritic cells (DCs) by: (1) reducing the basal expression of CD1 (a major histocompatibility complex class I (MHC I) molecule that triggers the immune response), (2) maintaining a tolerogenic phenotype, characterized by reduced secretion of IL-12 and increased secretion of IL-10 in response to TLR stimulation, and (3) impaired migratory response to chemokines [17]. In addition, low pH values of the tumor microenvironment lead to a decrease in the production of cytokines and to the loss of cytotoxic effector functions of T-cells without affecting their viability [18]. A high concentration of lactic acid in the tumor microenvironment disrupts the [H^+] gradient between T-cells and their environment, reducing monocarboxylate transporter 1 (MCT1) mediated export of lactic acid from T cells. This inhibits the proliferation of effector T-cells. At the same time, neutralization of the acidic environment, for example, using bicarbonate therapy, increases tumor infiltration by T-cells and slows down its growth, and also contributes to an increase in the effectiveness of therapy with immune checkpoint inhibitors (anti-PD1/PDL1 therapy) [19–21].

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On the other hand, high concentrations of lactate in cells stimulate adenosine transporters and adenosine is secreted into the extracellular space. In turn, the lactate anion activates the key enzyme of adenosine catabolism and thus prevents adenosinergic immunosuppression in the tumor microenvironment [21]. Therefore, the question remains whether lactate performs a protective function or is a factor of aggression in tumor growth.

2.3. Lactate serves as a fuel molecule for proliferating cells.

For many physiological and pathological processes, the functioning of the lactate shuttle mechanism is well known, where lactate-anions are exported from one cell and imported into other cell for serving as a substrate for gluconeogenesis or ATP synthesis. The significance of this mechanism is especially important for tumor cells that coexist under conditions of both normoxia and hypoxia. Thus, glycolysis-dependent tumor cells under hypoxic conditions export lactic acid to the extracellular space, from where it is captured by well-oxygenated tumor cells and used to produce ATP through cellular respiration. Anaerobic glucose oxidation allows cells to adapt well to the hypoxic environment found in tumors but is also characteristic of ancient living organisms that lived in anoxic environments prior to the environmental oxygenation event occurred about 800 million years ago. The Warburg effect observed in tumors can be seen as a return to the ancient hypoxic roots of early multicellular life [22], and maybe to the metabolic profile FUCA (The First Universal Common Ancestor). It can be said that malignantly transformed cells form their tissue microenvironment in such a way as to recreate “atavistic” niches favorable for tumor growth, in which they can win competition with healthy cells.

2.4. Lactate promotes angiogenesis during tumor growth.

Lactate produced by tumor cells promotes endothelial cell activation and angiogenesis through HIF (hypoxia inducible factor)-dependent and HIF-independent mechanisms. Both mechanisms involve the import of lactate by tumor microenvironment cells through the MCT1 transporter and the subsequent inhibition of prolyl hydroxylase, which protects HIF from proteasomal degradation and promotes activation of proangiogenic IL-8 or induces VEGF expression to promote vascular growth. Thus, HIF-1α can be stabilized not only under hypoxic conditions, but also under normoxic conditions with the help of lactate. The accumulation of lactate allows tumor cells to activate the expression of pro-carcinogenic genes due to the transcriptional activity of HIF-1α, regardless of oxygen supply [23].

2.5. Lactate promotes cell migration, metastasis, and secretion of tumor exosomes.

Cell migration is a mandatory step in the processes of carcinogenesis and metastasis. Lactate is one of the factors required for endothelial cell migration [24], glioma cells [25] by inducing transcription of transforming growth factor b2 (TGF-b2). In addition, the release of lactate and protons from tumor cells into the extracellular environment (microenvironment) and, as a result, acidification of the microenvironment promotes the secretion of exosomes by tumor cells [26]. Exosomes are microvesicles containing microRNAs, enzymes, structural proteins, and other molecules required for metabolic reprogramming of the cells in microenvironment. Captured exosomes can induce epigenetic changes or carry oncogenes and onco-microRNAs, which contribute to the rearrangement of the metabolism of the cells surrounding the tumor and further tumor growth.

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Mechanisms of lactate influence on carcinogenesis induction and tumors progression suggest activation of the "hyaluronic system" in tumor-associated fibroblasts, stimulation of VEGF and HIF-1α [27]. It has been shown that not only HIF-1α induces the expression of glycolytic enzymes and therefore regulates glycolysis, but also metabolites of glycolysis (e.g., pyruvate) and TCA (e.g., oxaloacetate, succinate, and fumarate) can activate HIF-1α when they accumulate in the cell (because of violation of the ratios between glycolysis and oxidative phosphorylation) [28]. Among other things, pyruvate prevents aerobic degradation of HIF-1α and enhances the expression of HIF-1α-activated genes, including erythropoietin, VEGF, glucose transporter-3 (GLUT-3), and aldolase A [29]. In turn, VEGF expression is one of the key events in angiogenesis and subsequent hematogenous migration of cancer cells [30].

2.6. Lactate is a universal signaling molecule.

The ability of lactate to act as a signaling molecule has been known since the discovery of the lactate receptor GPR81, which is expressed in adipocytes, muscle, immune, nerve and cancer cells. The GPR81 receptor belongs to a type of G protein-associated receptor, the subfamily of hydroxycarboxylic receptors (HCARs), while the HCAR1 subtype to which GPR81 belongs is considered to be the most evolutionarily conserved of all other HCAR receptor subtypes [31]. Activation of GPR81 receptors in adipose tissue leads to inhibition of lipolysis in adipocytes, indicating a synergistic effect of lactate with insulin and its potential association with the development of obesity [32]. Thus, in experiments in mice, it was shown that mice knocked out for the gene encoding GPR81 gain weight to a much lower extent when kept on a high-lipid diet compared to wild-type mice [33].

Lactate has important functions in the nervous system. The recent discovery of extensive expression of GPR81 in the brain and retina has revealed many new mechanisms of action of lactate and its role as a signaling molecule in the processes of angiogenesis in the nervous system, regulation of neuronal excitation, and neuroprotection [34,35].

Several studies are devoted to the investigation of the role of lactate in inflammation. GPR81 expression has been shown to be reduced in adipocytes and endothelial cells under conditions of inflammation. At the same time, the expression of GPR81 in immune cells is associated with protection against inflammation and suppression of innate immune responses [36]. Interestingly, activation of GPR81 in the myometrium during pregnancy reduces its LPS-induced inflammation and thus the risk of preterm birth and infant mortality [37]. The role of GPR81 in the suppression of innate immunity has been confirmed by several studies. For example, in the work of Ranganathan et al. [36] it was found that the signaling pathway through GPR81 activation in intestinal dendritic cells and macrophages leads to suppression of inflammation in the intestine due to the induction of T-regulatory cells secreting IL-10 and blocking of pro-inflammatory Th1/Th17-cells.

Based on the many functions of lactate as a signaling molecule, one should expect its important participation in the process of carcinogenesis. Indeed, in recent years there has been more and more evidence of the critical role of lactate-activated signaling pathways through binding to GPR81 in the regulation of tumor growth (fig. 1).

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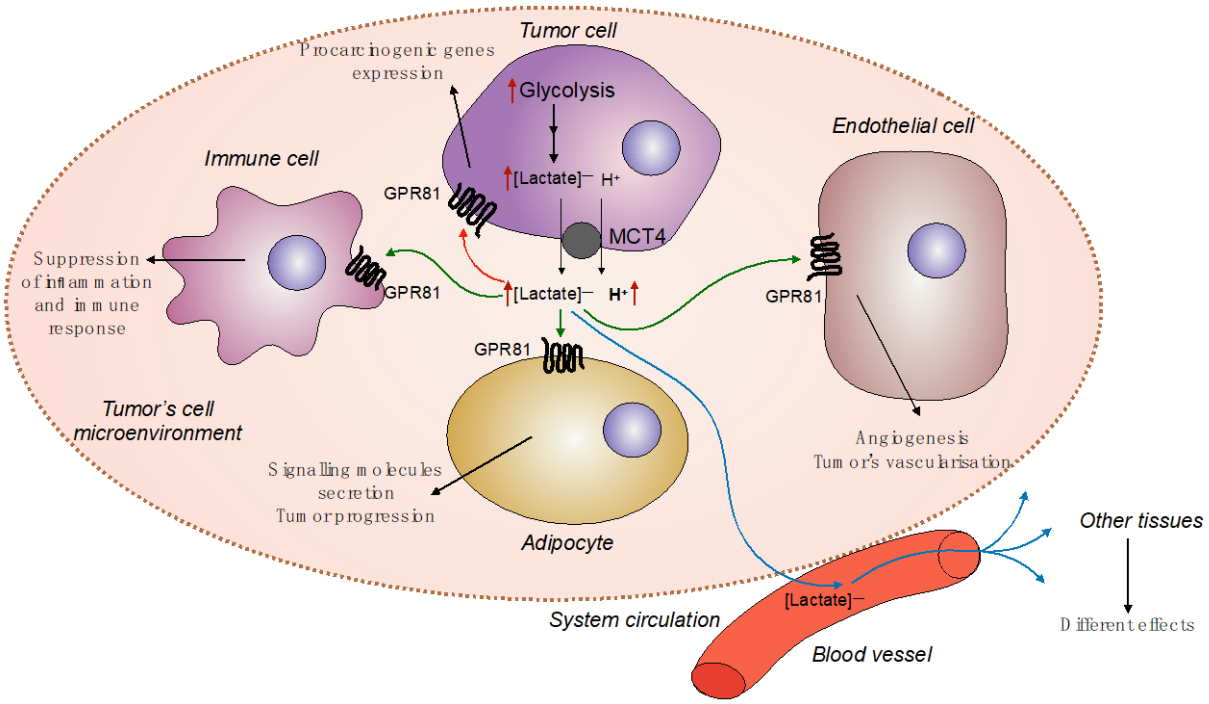

Figure 1. Lactate signaling function in malignant transformation process. Intensification of glycolysis in tumor cells lead to high lactate production; lactate-anions along with protons co-transported through MCT4-transpoters in extracellular medium providing acidification of tumor's microenvironment and high extracellular lactate concentration. Lactate-anions act through autocrine (red arrow), paracrine (green arrows) and endocrine (blue arrows) mechanisms. By binding with GPR81 receptors on different types of cells lactate promotes expression of prooncogenic genes, immune suppression, angiogenesis, tumor cells proliferation and some other effects.

The expression of GPR81 expression in malignant tumors was first demonstrated in 2014 [38]. The receptor has been shown to be activated in many types of neoplasms, despite low expression in benign cells from the same tissues. In this work, the investigators found that downregulation of GPR81 in pancreatic cancer cell lines significantly reduced the expression of the lactate transporters MCT1 and MCT4 and their chaperone protein CD147. Surprisingly, GPR81 knockdown resulted in a significant decrease in mitochondrial activity and a marked increase in cell death. The importance of GPR81 for tumor cell survival has also been demonstrated in breast cancer. Among all types of tumors, GPR81 is mostly expressed in breast cancer, especially positive for estrogen receptor (ER) [39]. In the study [39] it has been shown that GPR81 expression in MCF-7 cells increases the production of pro-angiogenic amphiregulin through a PI3K/Akt/cAMP-dependent pathway. In addition to breast and pancreatic cancer, the pro-oncogenic function of GPR81 is also associated with the development of hepatocellular carcinoma, cervical squamous cell carcinoma, and lung cancer [40]. Thus, GPR81 activation is associated with enhanced DNA repair (due to increased expression of DNA repair proteins - BRCA1, nibrin and DNA-dependent protein kinases) and resistance to chemotherapy [41]. In addition, GPR81 enhances doxorubicin chemoresistance in HeLa cells by increasing the expression and activity of the drug transporter ABCB1 [41]. At the same time, an increase in DNA repair and drug resistance is associated with the activation of PKC-ERK signaling pathways that obey GPR81. In addition to regulating angiogenesis, DNA repair, and chemoresistance, GPR81 activation is also required for tumor cells to evade an immune response, since activation of the receptor increases the expression of membrane-bound PD-L1 on the surface of lung cancer cells [40]. In particular, downstream signal-

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ing through the Gi/o protein results in the translocation of TAZ/TEAD to the PD-L1 promoter and subsequent induction of PD-L1 expression in vitro. Thus, lactate generated by tumor cells becomes a universal promoter of tumor growth due to its ability to induce autocrine effects, including activation of GPR81 expressed on tumor cells themselves. Interestingly, lactate itself induces the expression of GPR81 in cancer cells by transcriptional activation involving the Snail/EZH2/STAT3 transcription complex [42].

On the other hand, lactate is a paracrine regulator that binds to receptors on cells of the tumor microenvironment. It was found that GPR81 is expressed in tumor-infiltrating immune cells or adipocytes, which make up a significant mass of the mammary gland, and therefore are involved in the creation of a tumor microenvironment in breast cancer [43]. Since adipose tissue performs an important endocrine function, activation of GPR81, including through the tumor-derived lactate, can lead to the release of many cytokines and other regulatory factors that affect angiogenesis, vascularization, and tumor growth. However, the exact mechanisms and effects of lactate binding to its receptors remain to be elucidated.

Malignant cells are usually characterized by the presence of a set of distinctive features that arise both as a result of loss and gain of certain functions in cells. It is noteworthy that these signs do not occur *ab initio*; new functions that tumor cells acquire have always been encoded in the genome, since they play key roles in processes such as ensuring genetic diversity, embryogenesis, wound healing, etc., however, before tumor transformation, they are in a latent state [44].

Looking on how tumor cells use lactate, an evolutionarily ancient metabolite, and the process of glycolysis for their proliferation, growth, and metastasis, we can observe an action of ancient metabolic mechanisms for adaptation and further evolution cancer cells.

3. Discussion

Despite the numerous evidence of the procarcinogenic effect of lactate and lactatemia as a marker of an unfavorable prognosis for the course of oncological disease, in our opinion, not everything is so unambiguous in assessing the role of lactate in tumor growth. Currently, researchers are coming to understand the protective function of lactate and the possible compensatory mechanism of lactatemia to overcome stressful conditions in the body. For example, sodium lactate infusions have been found to improve recovery after prolonged exercise in athletes [48]. Intravenous administration of lactate (in the form of sodium lactate) provides an energy substrate, spares glucose, and has a slightly alkalizing effect on blood pH. Lactate is suggested to be used as an anti-inflammatory agent for infections, injuries, burns, hepatitis, pancreatitis, sepsis; wound healing agent; glycemic regulator; source of energy in case of myocardial injury; neuroprotective agent in cerebral ischemia [49]. Thus, a high level of lactate can compensate for the effects of acute or chronic hypoxia, inflammation, or injury. Lactatemia, observed in many tumor diseases, is possibly associated with the activation of ancient evolutionary defense mechanisms aimed at combating metabolic disorders. However, tumors began to use this mechanism for their own purposes - the acquisition of stem properties, rapid proliferation and metastasing. It remains to be seen whether this phenomenon can be called a case of antagonistic pleiotropy.

4. Conclusions

Summarizing current knowledge about the functions of lactate, the following key positions can be distinguished:

1. The processes of lactate oxidation, together with glycolysis, maintain the redox potential in the cytosol and mitochondria [45], which in turn is a very important evolutionarily conservative homeostatic constant.

2. Lactate is a universal and ancient signaling molecule. Lactate regulates the level of protein expression, the secretion of signaling molecules, cell proliferation and differentiation, immune surveillance, inflammatory response, the functioning of transporters and receptors, lipolysis, and gluconeogenesis, the content of polyADP-ribose, and the regulation of prolyl hydroxylases, and consequently, it is involved in the remodeling of the ECM.

3. Lactate is a universal fuel molecule for rapidly growing tissues and activated cells [46]. Stemness and hypermetabolism are always provided by high lactate production.

4. Lactate has a neuroprotective function during hypoxia [47], which is unconditional is the most important evolutionary mechanism.

5. Lactate provides stemness and unlimited cell growth, which is used by malignant tumors for their initiation and progression.

6. High lactate production may have protective effect for compensating pathological conditions such as hypoxia, inflammation, injury, tissues destruction.

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