Fundamentals of the Warburg Effect in Cancer

Pi Xian Zhang¹, Ya Qi Xing², Yong Dong Niu²

¹Department of Biochemistry and Molecular Biology, Shantou University Medical College, Shantou, Guangdong 515041, China;

²Department of Pharmacology, Shantou University Medical College, Shantou, Guangdong 515041, China

Abstract: As the fundamental energy unit of most cells, the ATP generated from glucose is vital to maintain biological processes such as the synthesis of proteins and nucleic acids. However, a common feature of cancer cells is an altered metabolism with increased glucose uptake and the fermentation of glucose to lactate even in the presence of normal mitochondria. This phenomenon is known as the ‘Warburg effect’ (now termed aerobic glycolysis). One simple explanation for this effect is that it permits cells to more easily produce energy to support rapid growth. It can also help to meet the biosynthetic requirements for pyruvate as a major building block. As a well-known metabolic hallmark of cancer cells, the role of the Warburg effect in oncology is a newly-emerging area of growing interest. In this perspective, we provide some new understanding of these mechanisms based on recent progress in research on cancer metabolism. The better understanding of these mechanisms will support the development of new therapeutic strategies that take into account tumor nutrition and energy metabolism.

Key words: Warburg effect; Cancer; Glycolysis; ATP; Lactate

Introduction

Limitless replicative potential and deregulated metabolism are universally accepted as hallmarks of carcinogenesis [1]. The rapid proliferation of cancer cells requires high amounts of nutrients and energy, and elevated biosynthetic activity to generate bio-macromolecules during each stage of the cell cycle. It is well known that cancer cells have a greater need for energy and building block molecules, which are necessary for the synthesis of macromolecular components during cell proliferation. It has long been considered that the altered metabolism, especial energy metabolism, in tumor cells is required to facilitate their rapid growth and duplication.

In 1923, Otto Warburg observed that cancer cells proliferate with accelerated glycolysis and excessive lactate formation, even when supplied by enough oxygen, although these processes only occurred in normal cells when there was insufficient oxygen [2-4]. According to Warburg’s estimation, the arterial glucose uptake in tumor cells is about 47%~70% compared to 2%~18% in normal tissues, with tumor cells converting 66% of glucose to lactate [5]. Cancer cells prefer aerobic glycolysis to oxidative phosphorylation during ATP production [6,7]. This phenomenon was subsequently called the “Warburg effect” based on his initial observations [4,8]. In Warburg’s opinion, this change might result in the impaired respiration and functional defects of mitochondria in cancer cells [7,9]. The Warburg effect has subsequently been observed in more than 70% of human cancers, including colorectal cancer [10], breast

cancer [11], lung cancer [12], and glioblastoma [13,14]. Additionally, ubiquitous overexpression of glycolysis-related genes has been noted in various human cancers [15-17]. The lactate released from cancer cells was deemed to be a byproduct of cancer metabolism, and might be the basis of cell migration and metastasis [18-20], immune escape [21-24], poor survival [19], angiogenesis [20,25-27] and even overall cancer development [4,28]. The origins of lactate formation are still incompletely understood and are probably influenced by multiple factors [29-32], while the exact roles and the mechanisms underlying the Warburg effect in carcinogenesis are still being explored. Here, we focus on the mechanism of lactate production and discuss the possible regulation of the Warburg effect in cancer.

Mitochondrial Dysfunction in Cancer Cells?

In Otto Warburg’s opinion, aerobic glycolysis, which releases lactate under normal oxygen conditions, was a preferential manner to meet the energy requirements for cancer cells’ rapid growth and due to the mitochondrial dysfunction common in cancer cells [7,9]. For each molecule of glucose consumed, 2 ATP are produced by glycolysis, while 32 ATP molecules are produced by the oxidative phosphorylation pathway. Therefore, although the ATP generation via aerobic glycolysis is less efficient than oxidative phosphorylation, cancer cells are able to quickly produce sufficient energy by consuming huge quantities of glucose. Although inefficient, this allows cancer cells to maintain their abnormal biological functions, including rapid proliferation.

The factors that drive cancer cells to choose such an inefficient pathway, and the biological significance of the Warburg effect, have remained elusive. It was theorized that the increased rate of glycolysis could compensate for the energy requirements for cancer cells’ rapid growth.

Correspondence author: Yong Dong Niu, Department of Pharmacology, Shantou University Medical College, No.22 Xinling Road, Shantou, Guangdong 515041, China; Tel: +86 754 8890 0432; Email: ydniu@126.com

This speculation was supported by later evidence [33-37], and was considered to be a mechanism responsible for the fast energy production and biosynthesis. Nevertheless, this theory has been challenged by many investigators. Early in 1967, Weinhouse S et al. observed that the glycolmetabolism of slow-growing rat hepatocytes was typically supported by oxidation [29]. Not only in cells with normal mitochondrial function [30-32], but also some tumor cells, have been reported to use oxidative phosphorylation as the major ATP supplier, regardless of the metabolic rate of glycolysis [38,39]. The mitochondrial membrane potential ($\Delta\Psi_m$) is mainly generated by the end-step of ATP production. NADH or FADH₂ (Dihydroflavine-adenine dinucleotide) transfers electrons to oxygen molecules via the electron transport chain to form water, NAD⁺ or FAD²⁺, thus releasing protons from the mitochondrial matrix outside of the mitochondrial inner membrane. As a result, the $\Delta\Psi_m$ can function as a powerful index for assessing mitochondrial activity [40,41]. When compared with the adjacent normal tissues, tumors always exhibit elevations in the mitochondrial membrane potential [42-47]. This suggests that oxidative phosphorylation may be preferred over glycolysis for efficient ATP production. Therefore, Warburg's theory about mitochondrial malfunction is not a reasonable explanation for the dominance of aerobic glycolysis in cancer cells. Thus, additional research has been performed to try to elucidate the main driving factor(s) underlying the high lactate release in tumor cells.

Pyruvate Dehydrogenase Complex (PDC)-the Major Controller of Lactate Production in Tumor Cells

It is well known that the canonical fate of pyruvate is to be converted into lactate through glycolysis (Figure 1) or completely oxidized to carbon dioxide, water and 32 ATPs by oxidative phosphorylation. Researchers have tried to explore the primary pathway by which cancer cells undergo pyruvate metabolism and the major factors controlling this process. Recent evidence has indicated that the pyruvate dehydrogenase complex (PDC) is crucial. The aerobic oxidation of glucose doesn't produce lactate, while glucose glycolysis does. The fundamental switch between these processes depends on the activity of the PDC, which catalyzes the rate-limiting oxidative decarboxylation of pyruvate into acetyl-CoA. PDC is only active in aerobic oxidation, not in glycolysis [48-50]. The major effect of PDC activity is controlling the flux of acetyl CoA converted from glucose. When the pathway is blocked, excessive pyruvate will return to the cytoplasm and form lactate and NADH.

Some recent studies demonstrated that there was inhibition of PDC activity in most tumors [51-55]. Inhibition of PDKs could induce PDC to reverse the Warburg effect [56]. On the other hand, enhancing the expression of pyruvate dehydrogenase kinase, PDK, could inactivate PDC, facilitating lactate generation from pyruvate [57-59]. Additionally, Mi-

chelle Potter reported that when cancer cells were cultured in high (25 mM) and low (1 mM) glucose conditions, different extracellular acidification rates were observed. Cancer cells cultivated in the high (25 mM) glucose condition had a high and dispersive extracellular acidification rate, while those cultured with 1 mM glucose showed a very low and similar extracellular acidification rate [60]. This result suggested that treatment with a high concentration of glucose will lead to high levels of lactate production in cancer cells, which means that most pyruvate converted from glucose does not enter the mitochondrion to produce ATP, but is instead located in the cytoplasm, where it forms lactate. These findings also indicated that the activity of PDC is aberrant in cancer cells.

Figure 1 Glycolysis and the oxidative phosphorylation pathway. When ingested glucose is converted into pyruvate, two pathways exist to utilize it: glycolysis, as shown by the red arrows, and oxidative phosphorylation, as shown by the green arrows. The most important controller is pyruvate dehydrogenase complex, PDC. GLUT: glucose transporter; LDHA: lactate dehydrogenase A; MCT: monocarboxylate transporter; PDC: pyruvate dehydrogenase complex; TCA: tri-carboxylic acid cycle; NADH: nicotinamide adenine dinucleotide hydride; FADH₂: flavin adenine dinucleotide hydride; ETC: electron transport chain; ATP: adenosine triphosphate

However, the major molecules controlling the PDC were unclear. The acetylation and succinylation of the PDC had recently been reported [61,62]. However, the phosphorylation of the PDC seems to be more important for its activity. PDKs are over-expressed in almost all kinds of cancers [63-73], and several studies have shown that PDKs play a major role in the metabolic adaptations that occur during the acquisition of the tumor metabolic phenotype [74-77].

The Effects of NADH, and Their Importance in Controlling Lactate Production

NADH is an allosteric inhibitor of PDC that can directly or indirectly restrain PDC activity. Additionally, it is an activator of PDKs. More importantly, NADH is required to produce lactate (Figure 2), as follows:

Catalyzing enzymes are crucial for the fate of NADH. However, the end controller is the molecular balance, not the enzyme activity. For example, the reaction to produce lactate does not depend on LDHA activity, but rather the concentrations of reactants and products. Increased pyruvate, NADH and decreased lactate will promote the reaction to produce lactate. Therefore, it has been speculated that a high level of NADH in the cytoplasm could drive increased lactate production. In support of this, the production of active NADH in the cytoplasm and its

importance for ATP production have been reported [47,78-80].

Kim SY et al. found that cytosolic 10-formyltetrahydrofolate dehydrogenase (ALDH1L1) produces abundant cytosolic NADH, which could be transported to the mitochondria via the malate-aspartate shuttle (MAS) in non-small-cell lung cancer cells (NSCLC). ALDH1L1 knockdown was found to reduce ATP production by 60%. And an ALDH inhibitor, gossypol, combined with phenformin, a mitochondrial complex I inhibitor, significantly suppressed tumor growth

Figure 2 The influence of NADH on the production of lactate. ① NADH, as a reactant, reacts with pyruvate to produce lactate and NAD⁺. ② NADH, as a direct allosteric inhibitor of PDC, increases lactate production. ③ NADH, as an inhibitor of PDC, activates PDK, which inhibits PDC by phosphorylating it.

Figure 3 A new proposed model of cancer energy metabolism. The major source of electron may be cytosolic NADH produced by metabolic enzymes such as ALDH. ETC: electron transport chain; FAO: fatty acid oxidation; MAS: malate-aspartate shuttle; TCA: tri-carboxylic acid cycle (This model originated from work by Kim SY et al. [79]).

[47,78,79]. Kim SY et al. named this change in metabolism the “Cytosolic NADH theory” (Figure 3) [79]. In this model, lactate is not an essential factor for cell proliferation.

Conclusion and Perspectives

The “Cytosolic NADH theory” suggests that the increased ATP production is from cytosolic NADH. This NADH originates from the conversion of glutamine and FAO during cancer cell proliferation. This indicates that the first choice of material for energy utilization in cancer cells is amino acids and fats, not glucose. Based on this viewpoint, some researchers speculated that the energy supply for the cellular proliferation of tumors is not derived from glucose in non-glycotropic cancers. This association between cytosolic NADH and energy metabolism has been reported in NSCLC [78,81]. Although the change in the lactate level was not described in NSCLC, these factors would all be expected to influence the release of lactate. Although these discoveries are fascinating, there is still a long way to go to determine whether this is a common phenomenon.

Increasing research about the Warburg effect has given insight into the interpretation of its functions in the energy metabolism of cancer cells. However, there is much work still needed to elucidate how rapid proliferation is directly or indirectly regulated by cell metabolism. These uncertainties also highlighted the need to enhance our understanding of the biology and functions of the Warburg effect and its possible therapeutic potential in carcinogenesis. Recently, regulating the nutrient intake of cancer cells has been considered a possible therapeutic strategy, which may involve the exploitation of glycolytic inhibitors or PKM-2 [82], PKM-1 [83], or metformin [84]. Obtaining a better understanding of the key principles and mechanisms underlying the Warburg effect in cancer cells will aid in the development of preventive and therapeutic approaches using dietary and pharmacological interventions.

Conflict of interests

The authors declare no conflict of interest.

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