

# Mitochondrial Metabolism and Cancer

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Historically, it has been assumed that glycolytic metabolism, not mitochondrial metabolism, is essential for tumor cell proliferation. However, most tumor cells have functional mitochondria, and recent studies suggest that the citric acid cycle (TCA) cycle intermediates are precursors for synthesis of nucleotides, lipids, and amino acids. Here we review the accumulating evidence that mitochondrial metabolism plays an essential role in tumor cell proliferation.

**Key words:** mitochondria; ROS; glycolysis; Warburg effect; ATP; NADPH; TCA cycle; glutamine

## Introduction

Cancer arises when cells undergo uncontrolled proliferation due to gain of function of oncogenes and loss of function of tumor suppressors. There are six major alterations that normal cells undergo to become tumor cells.<sup>1</sup> These alterations consist of “self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis”.<sup>1</sup> Many researchers now propose a seventh alteration, a change in cellular metabolism, is required for tumorigenesis.<sup>2</sup> Tumor cells need to generate an ample amount of ATP for energy and *de novo* synthesis of nucleotides, lipids, and proteins for rapid proliferation. Historically, glycolysis has been thought to be the central metabolic pathway regulating tumor cell metabolism.

Over 80 years ago, Otto Warburg first observed that tumor slices consume glucose at a higher rate than normal tissue.<sup>3</sup> This high rate of aerobic glycolysis is now known as the

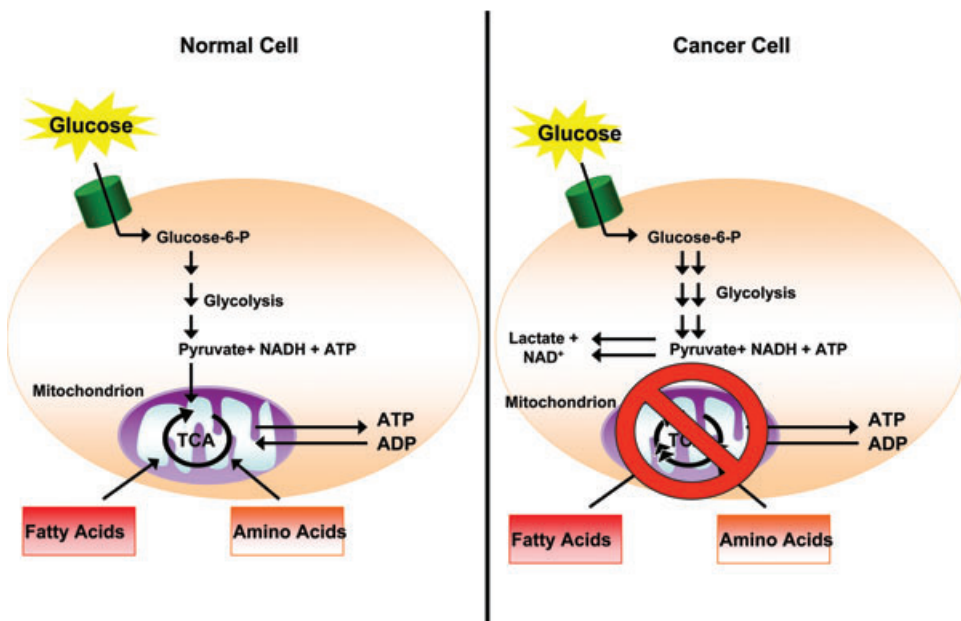
“Warburg effect.” Warburg hypothesized that the increase in glycolysis under normal oxygen conditions arose from a deficiency in respiration.<sup>4</sup> Since this seminal finding, many reports have documented the Warburg effect in a variety of different tumors.<sup>5</sup> In fact, the clinical application of positron emission tomography (PET), which uses a glucose analogue to detect a significant increase in glucose uptake in tumors as compared to other tissue,<sup>6–8</sup> is based upon Warburg’s initial observation. While the increase in glycolysis is a hallmark of tumorigenicity, the function of mitochondrial metabolism in cancer remains contentious in cancer research. In this review we will discuss the role of the mitochondrial metabolism in cancer.

## Role of Glycolysis in Anabolism

The high rate of glycolysis observed in cancer cells can provide these cells with several advantages. Glycolysis generates ATP with lower efficiency, but at a faster rate, than oxidative phosphorylation. This enhanced speed of ATP generation may be beneficial for rapidly proliferating cells.<sup>9</sup> Additionally, the high rate of glycolysis in cancer cells can also provide cells with glycolytic intermediates necessary for the

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## Warburg's Hypothesis for Origins of Cancer



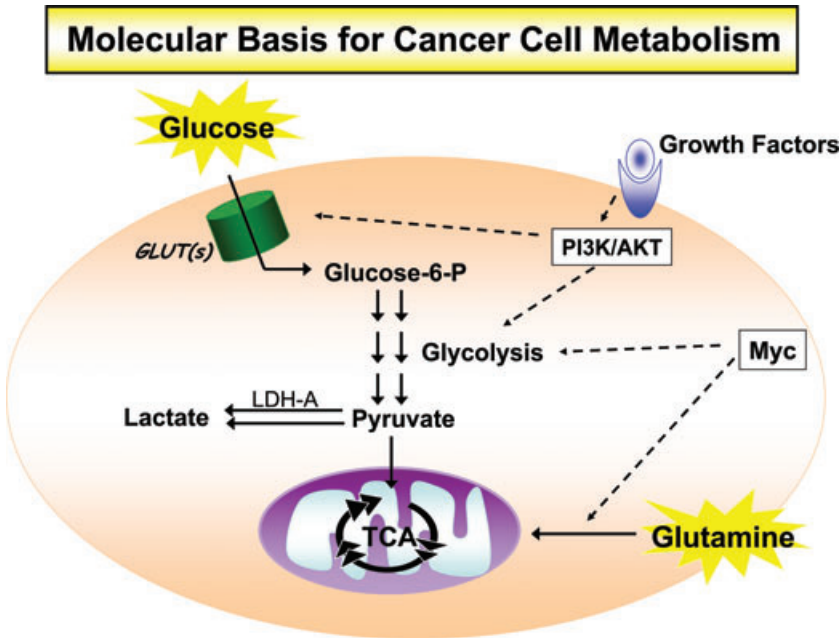
**Figure 1.** Otto Warburg's theory on the origins of cancer postulates that tumor cells have defects in mitochondrial oxidative phosphorylation and therefore rely on high levels of aerobic glycolysis (the Warburg effect) as the major source for ATP to fuel cellular proliferation.<sup>3,4</sup> This was in contrast to normal cells which primarily utilize oxidative phosphorylation for growth and survival.

pentose phosphate pathway to generate NADPH and ribose-5-phosphate, used for the regeneration of reduced glutathione and in biosynthesis of nucleic acids.<sup>2</sup> NADPH is also used for anabolic pathways such as lipid and cholesterol synthesis. Since Warburg's initial discovery, an abundance of research has focused on discerning the mechanism by which cancer cells increase glycolysis.

### Molecular Basis of the Warburg Effect

Warburg's hypothesis was that mitochondria are injured in tumor cells, resulting in an increase in glycolysis under aerobic conditions (Fig. 1). However, multiple studies have demonstrated that mitochondria are not dysfunctional in tumor cells.<sup>10</sup> Over the past decade, studies identified a gain of function in oncogenes, loss

or mutation of tumor suppressors, and the activation of the phosphoinositide 3-kinase (PI3K) to be major regulators of the high levels of aerobic glycolysis observed in tumor cells. The PI3K pathway is frequently activated in tumor cells through loss of its negative regulator PTEN (Fig. 2). PI3K activation through AKT can increase glucose uptake and utilization.<sup>11</sup> Oncogenes, such as Ras or Myc, stimulate glycolysis through induction of glycolytic enzymes and glucose transporters.<sup>12</sup> The glycolytic enzyme lactate dehydrogenase (LDH-A) is an important target of oncogenes in increasing glycolysis. LDH-A converts pyruvate to lactate with concomitant oxidization of NADH to NAD<sup>+</sup> (Fig. 2). In normal cells, under aerobic conditions where LDH expression is low, the glycolytic-produced NADH is shuttled to the mitochondria, where it is oxidized to NAD<sup>+</sup> by the mitochondrial electron transport chain and, subsequently, shuttled back to the cytosol.



**Figure 2.** In the past decade there have been multiple mechanisms to account for the increase in aerobic glycolysis observed in tumor cells. These include the activation of PI3K-AKT signaling pathway and the induction of glycolytic enzymes by oncogenes such as Myc. Interestingly, Myc also upregulates enzymes responsible for glutamine catabolism by mitochondria.

As  $\text{NAD}^+$  is essential in driving glycolysis, the overexpression of LDH-A in tumor cells allows for NADH to be quickly oxidized to  $\text{NAD}^+$  in the cytosol, thereby enhancing glycolytic flux under aerobic conditions. Indeed, the ectopic expression of LDH-A alone in noncancerous cells is sufficient to increase glycolytic flux.<sup>13</sup> In contrast to oncogenes, the activation of the tumor suppressor p53 leads to the downregulation of glycolysis.<sup>14–16</sup> p53 activation also leads to an increased rate of mitochondrial respiration by inducing expression of SCO2 (synthesis of cytochrome oxidase 2).<sup>17</sup> Thus, the gain of oncogenes, loss of the tumor suppressor p53, and activation of AKT cooperate to enhance glycolytic flux in cancer cells.

### Confusing Role of HIF-1 and the Warburg Effect

The transcription factor HIF-1 enhances glycolysis by increasing the expression of genes that encode glycolytic enzymes and glu-

cose transporters. HIF-1 also regulates mitochondrial respiration by increasing the expression of pyruvate dehydrogenase kinase (PDK1).<sup>18,19</sup> PDK1 inactivates pyruvate dehydrogenase (PDH), the enzyme responsible for converting pyruvate into acetyl-CoA, thus reducing the delivery of acetyl-CoA to the tricarboxylic acid cycle and the levels of NADH and FADH<sub>2</sub> delivered to the electron transport chain. HIF-1 is composed of two subunits: an oxygen-sensitive HIF-1 $\alpha$  subunit and a constitutively expressed HIF-1 $\beta$  subunit. Under normal oxygen conditions HIF-1 $\alpha$  is polyubiquitinated and targeted for degradation by an E3 ubiquitin ligase complex that contains the von Hippel–Lindau tumor suppressor protein (pVHL), elongin B, elongin C, Cul2, and Rbx.<sup>20</sup> Many investigators assume that HIF-1 is responsible for the increased glycolytic phenotype observed in tumor cells. However, HIF is only stabilized and functional at low oxygen levels, and the definition of the Warburg effect is the presence of high levels of glycolysis under normal oxygen conditions. Thus,

HIF-1 likely contributes to the Warburg effect in cancer cells only when it is aberrantly active under normoxic conditions (such as renal clear cell carcinoma) that exhibit a loss of VHL protein<sup>21</sup> or prostate cancer cells where the PI3K/AKT pathway activates HIF-1 under normoxic conditions through repression of FOXO3a and stimulation of mTOR.<sup>22–25</sup>

### **Role of Glycolysis in Tumor Cell Proliferation and Growth**

In a seminal study, Dang and colleagues demonstrated that lactate dehydrogenase A (LDH-A), which converts pyruvate to lactate, is a c-Myc target gene.<sup>13</sup> Decreasing LDH-A levels through antisense LDH-A expression reduces soft agar clonogenicity of c-Myc-transformed cells, and knockdown of LDH-A by short hairpin RNA (shRNA) in mouse mammary tumor cells led to a significant reduction in xenograft tumor formation.<sup>26</sup> Furthermore, genetic or pharmacologic strategies to inhibit PDK1 prevents pyruvate conversion into acetyl-CoA to fuel mitochondrial metabolism, decreasing tumor growth.<sup>27,28</sup> At first glance, these studies seem to indicate that tumor cell proliferation is dependent solely on glycolysis. However, certain tumor cells can grow in culture in the absence of glucose as long as there are sufficient substrates to feed the pentose phosphate pathway.<sup>29</sup> Thus, the high levels of aerobic glycolysis might be required to fuel the pentose phosphate pathway for biosynthetic pathways. In support of this hypothesis, transformed human mesenchymal stem cells display an upregulation of ribose 5-phosphate isomerase A (RPIA), which produces ribose 5-phosphate and leads to an increase in enzymes involved in DNA synthesis, suggesting that the pentose phosphate pathway is upregulated in transformed cells.<sup>30</sup> Furthermore, Cantley and colleagues found pyruvate kinase M2 (PKM2), an isoform of pyruvate kinase that regulates the final step of glycolysis, is overexpressed in tumors, and that

inhibition of PKM2 reduces xenograft tumor growth of human lung cancer cells.<sup>31</sup> PKM2, unlike PKM1, is induced into a low activity state by phosphotyrosine—protein-binding allowing for glycolytic intermediates to be diverted to anabolic processes as opposed to ATP generation.<sup>32</sup> These studies suggest that an alternate explanation for the Warburg effect is that high glycolytic flux is required to provide continuous glycolytic intermediates for the pentose phosphate pathway to generate nucleotides for rapidly proliferating tumor cells.

### **Role of Mitochondrial Metabolism in Tumor Cell Proliferation**

Warburg's initial hypothesis was that cancer originates from irreversible injury to respiration followed by an increase in glycolysis to replace the ATP lost from defective oxidative phosphorylation.<sup>4</sup> According to Warburg, this metabolic shift from oxidative phosphorylation to glycolysis converts highly differentiated cells into undifferentiated cells that proliferate as cancer cells. Although the observation that tumor cells exhibit high levels of aerobic glycolysis has been corroborated, the role of mitochondria in tumor cells has been contentious. As Sidney Weinhouse noted in his critique of Warburg's 1956 paper "On the Origin of Cancer Cells": "It is recognized by all, including Warburg, that despite their high glycolysis, oxygen consumption is not quantitatively diminished; by and large, a representative group of tumors absorb oxygen about as rapidly as a comparable group of non-neoplastic tissues" (pp. 267).<sup>33</sup> Thus, the phenomenon of increased glycolysis could not be explained by a respiratory defect alone.

Although some have argued that a decrease in mitochondrial metabolism and respiratory rate is essential for tumor cell proliferation, multiple investigators have demonstrated that mitochondria are indeed functional in most tumor cells. Oncogenes, such as the Myc, can upregulate genes important in mitochondrial

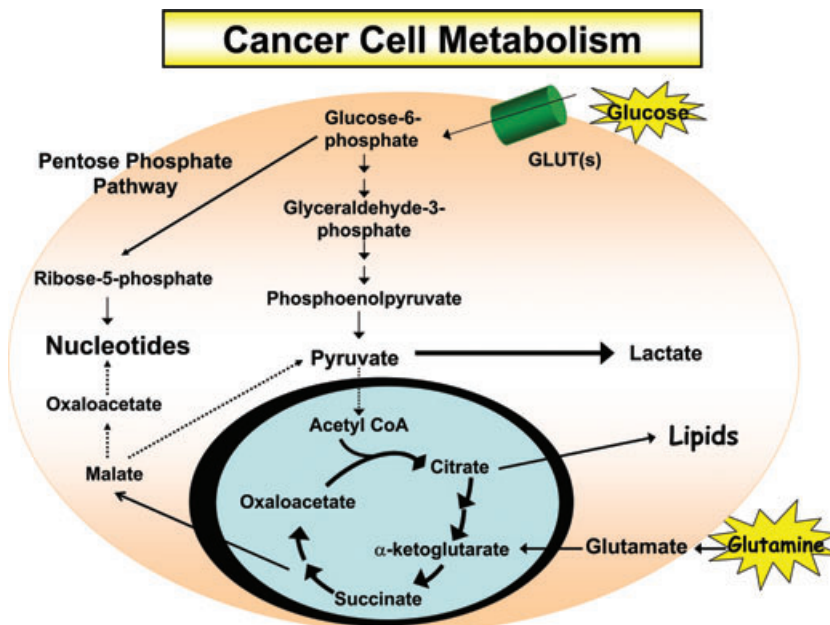
biogenesis, mtDNA transcription, and OXPHOS function. The transformation of human mesenchymal stem cells with retroviruses encoding for hTERT, HPV-16 E6 and E7, SV40 small T antigen, and an oncogenic allele of H-ras increases their dependency on OXPHOS for energy production.<sup>30</sup> Mouse embryonic stem cells with high mitochondrial membrane potential are able to form teratomas, while those with low membrane potential undergo differentiation.<sup>34</sup> Interestingly, the excess lactate produced by hypoxic cells can be transported to neighboring normoxic cells, where it can be taken up by the monocarboxylate transporter 1 (MCT1) and converted to pyruvate to fuel mitochondrial metabolism.<sup>35</sup> Inhibition of MCT1 results in the consumption of large amounts of glucose rather than lactate in normoxic tumor cells resulting in limited availability of glucose to the hypoxic tumor cells. This causes a combined glucose and oxygen deprivation-induced cell death of hypoxic tumor cells.

Studies performed over 25 years ago show that glutamine, not glucose, is the important substrate for growth and proliferation of HeLa cells in culture.<sup>36</sup> Glutamine is converted to glutamate by glutaminase and is subsequently converted to  $\alpha$ -ketoglutarate by glutamate dehydrogenase or the aminotransferases to fuel the TCA cycle (glutaminolysis). Indeed, early reports indicated that the higher the level of aminotransferase activity in a cell correlated with higher rates of cell proliferation.<sup>37</sup> Ehrlich rat hepatoma cells oxidize glutamine at higher rates than any other amino acid.<sup>38,39</sup> Aminooxyacetic acid, an inhibitor of aminotransferases, prevents glutamine stimulation of mitochondrial respiration in tumor cells.<sup>40</sup> HL-60 leukemic cancer cells require glutamine and not glucose for anchorage-independent growth in soft agar.<sup>41</sup> Furthermore, when rat hepatoma cells were grown in culture in the presence of galactose they grew equally as well as when grown in glucose.<sup>42</sup> Galactose entry into glycolysis proceeds through the Leloir pathway and occurs at a significantly lower rate than glucose

entry into glycolysis. Cells incubated in galactose media rely exclusively on glutamine-driven mitochondrial metabolism for growth and survival.<sup>36,42</sup> The residual levels of glycolytic intermediates produced by galactose metabolism are funneled into the pentose phosphate pathway. Collectively, these classical biochemical studies reveal glutamine to be an important metabolic substrate for tumor cells.

Recent reports from Thompson and colleagues have demonstrated that Myc induces a transcriptional program that promotes glutaminolysis and causes cells to utilize glutamine as a bioenergetic substrate. The demand for glutamine far exceeds the requirement for nucleotide synthesis or nonessential amino acid pool maintenance.<sup>43</sup> Myc transformed cells exhibited a high rate of glutamine consumption that was required to replenish TCA cycle intermediates through generation of  $\alpha$ -ketoglutarate.<sup>44</sup> Not surprisingly, the Myc transformed cells undergo cell death upon glutamine deprivation.<sup>44,45</sup> The molecular basis for Myc-dependent glutaminolysis can be explained by the observation that Myc enhances glutaminase expression by suppressing the microRNA 23a/b (miR-23a/b).<sup>46</sup> Silencing glutaminase in Myc-expressing cells leads to reduced cell proliferation; this defect can be partially rescued with oxaloacetate, a TCA cycle intermediate, suggesting that glutamine is required in these cells for mitochondrial metabolism and cell growth.<sup>46</sup> It is unknown whether other oncogenes also stimulate glutaminolysis similar to Myc.

What are the benefits of glutaminolysis? The high proliferating rate of cancer cells creates not only a demand for ATP but also a demand for lipid, nucleotide, and protein synthesis. Thus, glutamine can serve as a substrate for the TCA cycle to fulfill the requirements for the building blocks for cancer cell proliferation. The TCA cycle intermediate citrate is exported to the cytosol where ATP citrate lyase (ACL) converts citrate into acetyl-CoA, which serves as a precursor for lipid biosynthesis. Inhibition of ACL prevents xenograft



**Figure 3.** We propose that feeding substrates to the TCA cycle through amino acids such as glutamine are critical for the generation of ATP, lipids, and ROS. The major role of glycolysis in cancer cells is to provide substrates to the pentose phosphate pathway for nucleotide synthesis.

tumor growth of human lung cancer cells. Another TCA cycle intermediate malate can be exported into the cytosol, where it can be decarboxylated to produce pyruvate and NADPH by malic enzyme (ME). NADPH is then used for the regeneration of reduced glutathione and for lipid synthesis. In the cytosol, malate can be converted to oxaloacetate, which subsequently becomes aspartate through aminotransferases. Pyrimidines are synthesized from aspartate and carbamoyl-phosphate in the cytoplasm. This raises the possibility that glutaminase or aminotransferases could serve as therapeutic targets to prevent tumor growth. Indeed, blocking aminotransferases by aminooxyacetic acid prevents xenograft tumor growth of MDA-MB-231 breast cancer cells.<sup>47</sup>

### Conclusion: Integrated View of Cancer Cell Metabolism

Cancer cells have evolved to “get the best of both worlds” with respect to energy

metabolism. Tumors cells concomitantly increase glucose metabolism, leading to generation of ATP, NADPH, lactate, and nucleic acids, and also utilize glutamine-fueled OXPHOS, leading to generation of ATP, NADPH, amino acids, nucleic acids, and lipids. Experimental evidence from carbon labeling metabolic studies demonstrates that glycolysis, the Krebs cycle, the pentose phosphate pathway, and nucleotide biosynthesis are all coordinately enhanced in tumor cells.<sup>48</sup> The gain of function of oncogenes, loss of tumor suppressors, and the activation of PI3K pathway coordinate the increase in glycolytic metabolism and mitochondrial metabolism in cancer cells. We hypothesize that the major role of *aerobic* glycolysis in cancer cells is to provide glycolytic intermediates to the pentose phosphate pathway for nucleotide synthesis, while mitochondrial metabolism provides not only ATP, but also TCA cycle intermediates that serve as building blocks for lipids, amino acids, and nucleotide synthesis (Fig. 3). The best evidence to date for this hypothesis comes from studies done

almost 30 years ago demonstrating that HeLa tumor cells can grow in the absence of glucose with ample glutamine if they are provided a substrate such as uridine to generate ribose-5-phosphate.<sup>29</sup> It remains to be determined whether most tumor cells are similar to HeLa cells in that they rely on glucose metabolism only for the generation of ribose-5-phosphate and utilize mitochondrial metabolism to fulfill the other major requirements for proliferation.

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### Conflicts of Interest

The authors declare no conflicts of interest.

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