

Cancer's Molecular Sweet Tooth and the Warburg Effect

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Abstract

More than 80 years ago, the renowned biochemist Otto Warburg described how cancer cells avidly consume glucose and produce lactic acid under aerobic conditions. Recent studies arguing that cancer cells benefit from this phenomenon, termed the Warburg effect, have renewed discussions about its exact role as cause, correlate, or facilitator of cancer. Molecular advances in this area may reveal tactics to exploit the cancer cell's "sweet tooth" for cancer therapy. (Cancer Res 2006; 66(18): 8927-30)

Introduction

ATP required for normal cell proliferation and survival comes primarily from two sources. The first is glycolysis, which comprises a series of reactions that metabolizes glucose to pyruvate in the cytoplasm to produce a net of 2 ATP from each glucose. The other is the tricarboxylic acid (TCA) or Krebs cycle, which uses pyruvate formed from glycolysis in a series of reactions that donate electrons via NADH and FADH₂ to the respiratory chain complexes in mitochondria. With oxygen serving as the final electron acceptor, electron transfer creates across the mitochondrial inner membrane, a proton gradient of which the dissipation through ATP synthase forms 36 ATP per glucose molecule. With limited oxygen, such as with muscles that have undergone prolonged exercise, pyruvate is not used in the TCA cycle and is converted into lactic acid by lactate dehydrogenase (LDH) in a process termed anaerobic glycolysis (Fig. 1).

Many cancer cells consume glucose avidly and produce lactic acid rather than catabolizing glucose via the TCA cycle, which is key for generating ATP in nonhypoxic normal cells. The avid uptake of glucose by tumors is the foundation for the detection and monitoring of human cancers by fluorodeoxyglucose positron emission tomography. More than 80 years ago, Otto Warburg observed that thin slices of human and animal tumors *ex vivo* displayed high levels of glucose uptake and lactate production. The shift toward lactate production in cancers, even in the presence of adequate oxygen, is termed the Warburg effect or aerobic glycolysis (1). These observations have since been confirmed, although the nuances of aerobic glycolysis and its molecular underpinnings are still emerging. Tumors display aerobic glycolysis partly through activation of oncogenes or loss of tumor suppressors, which are then further enhanced by stabilization of the hypoxia-inducible factor (HIF) via adaptive response to hypoxic microenvironment or through pathways that stabilize HIF under nonhypoxic conditions.

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Recent advances in genomics and proteomics have provided insights into molecular mechanisms that contribute to the Warburg effect and tumorigenesis. In this review, molecular mechanisms that provide significant molecular insights into many aspects of the Warburg effect such as oncogenes (*AKT*, *MYC*, and *RAS*), tumor suppressors [succinate dehydrogenase (SDH) and fumarate hydratase (FH)], and the novel HIF pathway [pyruvate dehydrogenase kinase (PDK1)] will be discussed.

Oncogenes, Tumor Suppressors, and the Warburg Effect

To account for the conversion of glucose to lactate by cancer cells in the presence of oxygen, Warburg (1) speculated that tumor mitochondria are decreased or functionally impaired. In fact, Warburg suggested that impaired mitochondrial function contributes to tumorigenesis. Recent studies suggest that mutations affecting mitochondrial DNA (mtDNA) or enzymes of the TCA cycle might contribute to the Warburg effect. Mutations of mtDNA, which are prevalent in human cancers, could render the mtDNA encoded components of the respiratory chain complexes defective. Intriguingly, the subunits of the TCA cycle enzyme SDH (SDHB, SDHC, and SDHD) behave as classic tumor suppressors in pheochromocytoma or paraganglioma (2). Furthermore, FH is a tumor suppressor in leiomyoma and renal cell carcinoma. Although these observations suggest that mitochondrial function may be compromised by mutations in mitochondrial enzymes, there is no direct evidence that these mutations are sufficient for tumorigenesis. In this regard, there is no evidence that respiration is, in fact, less active in cancer cells than in normal cells. Hence, further studies are required to address whether impaired mitochondrial function sufficiently contributes to tumorigenesis.

Several oncogenes have been implicated in the Warburg effect. The *AKT* oncogene, which encodes a protein serine-threonine kinase, is associated with enhanced glucose uptake and aerobic glycolysis seemingly independent of HIF-1 (3). *AKT* mobilizes glucose transporters to the cell surface to enhance glucose uptake and activates hexokinase 2 (HK2) to phosphorylate and trap intracellular glucose. Through these effects, *AKT* is able to enhance glycolytic flux without affecting mitochondrial oxidative phosphorylation, thereby presumably contributing to the Warburg effect. The *MYC* oncogene, which is widely activated in human cancers, has also been implicated in the direct activation of aerobic glycolysis. The *Myc* transcription factor activates virtually all glycolytic enzyme genes and directly binds numerous glycolytic genes, including those encoding HK2, enolase, and LDHA (4). In immortalized rat fibroblasts, *Myc* is able to enhance aerobic glycolysis; however, activation of *MYC* in a human B cell model resulted in increased respiration that was associated with increased mitochondrial biogenesis (5). Elevated and sustained activation of *MYC*, however, is tightly associated with increased mitochondrial reactive oxygen species, which may cause mtDNA mutations that in turn contribute to dysfunctional mitochondria (6). Intriguingly,

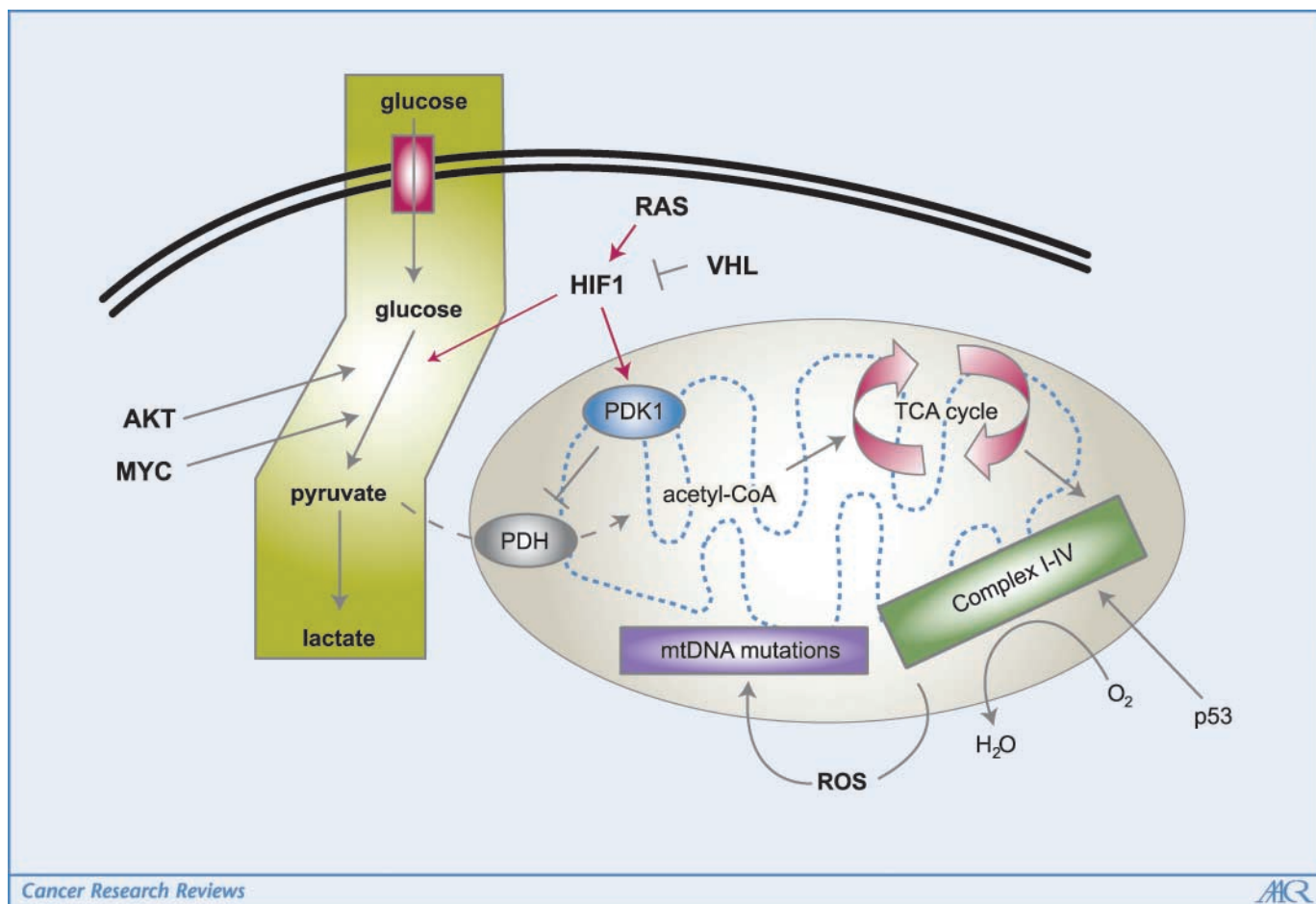


Figure 1. Molecular underpinnings of the Warburg effect. The Warburg effect (highlighted) describes the enhanced conversion of glucose to lactate by tumor cells, even in the presence of adequate oxygen that would ordinarily be used for oxidative phosphorylation. Activation of the *AKT* oncogene results in increased glucose transportation and stimulation of *HK2* activity, which enhances glycolytic rates. The *MYC* oncogene is depicted to activate glycolytic genes and mitochondrial biogenesis, which when sustained by high *MYC* levels can result in reactive oxygen species (ROS) production. ROS could, in turn, cause mtDNA mutations that render mitochondria dysfunctional. p53 is shown to stimulate respiration through activation of a component of the respiratory chain. In addition to hypoxic stabilization, HIF-1 is shown to be increased by RAS and loss of VHL, which mediates its degradation. HIF-1 transactivates glycolytic genes as well as directly activates the *PDK1* gene, which in turn inhibits PDH that catalyzes the conversion of pyruvate to acetyl-CoA. Acetyl-CoA is shown to enter the TCA cycle, which donates electrons to the mitochondrial respiratory chain complexes I to IV. Inhibition of PDH by PDK1 attenuates mitochondrial function, resulting in the shunting of pyruvate to lactate.

the tumor suppressor p53, which is frequently mutated in human cancers, also stimulates mitochondrial respiration by directly transactivating the *SCO2* gene for synthesis of cytochrome *c* oxidase 2 (7). *SCO2* is required for the assembly of the COXII (MTCO2) subunit into the cytochrome *c* oxidase complex, which is integral to the respiratory chain. Loss of either p53 or *SCO2* expression results in a switch from cellular respiration to aerobic glycolysis, suggesting that inactivation of p53 in human cancers may directly contribute to the Warburg effect. In aggregate, these observations suggest that oncogenes or tumor suppressors could independently or cooperatively contribute to the Warburg effect.

Tumor progression, as modeled by serial transduction of normal human cells with immortalizing and oncogenic events, is associated with the emergence of the Warburg effect (8). Through comprehensive multidimensional metabolic profiling of stepwise transformed primary human cells, highly tumorigenic cells transformed by hTERT, large T antigen, small T antigen, and oncogenic H-Ras were found to have high rates of lactate production with low mitochondrial mass. In contrast, cells lacking H-Ras but trans-

formed by the other three genes had high mitochondrial mass and a high oxygen consumption rate with lower lactate production. These results provide evidence that serial oncogenic activation transforms metabolism toward aerobic glycolysis.

Oncogenic Activation of HIF and Aerobic Glycolysis

In addition to oncogenic activation of aerobic glycolysis, the activation of HIF, a transcription factor that is stabilized in response to hypoxia, also significantly contributes to the conversion of glucose to lactate. HIF-1 consists of an oxygen sensitive HIF-1α subunit that heterodimerizes with HIF-1β to bind DNA. In high oxygen tension, HIF-1α is hydroxylated by prolyl hydroxylases (PHD) using α-ketoglutarate derived from the TCA cycle. The hydroxylated HIF-1α subunit is recognized by the von Hippel Lindau (VHL) protein and destined for degradation by proteasomes, such that HIF-1α is continuously synthesized and degraded under nonhypoxic conditions. Hypoxia is a pathophysiologic stimulus of anaerobic glycolysis through stabilization of HIF-1 and its direct transactivation of glycolytic enzyme genes. Hence,

adaptation to the hypoxic tumor microenvironment results in increased glucose uptake and lactate production.

In addition to hypoxia, oncogenic events have been linked to stabilization of HIF in the presence of adequate oxygen. Activation of the *Src* oncogene increased *in vivo* tumorigenicity as well as HIF-1 levels in nonhypoxic conditions (9). Oncogenic H-Ras has been reported to increase the level of HIF-1 (10), and phosphatidylinositol 3-kinase signaling may stabilize HIF-1 (9). In renal cell carcinoma, mutations in the VHL tumor suppressor disrupt its function, which is necessary for the oxygen-dependent prolyl hydroxylation and proteasomal degradation of HIF-1 (11). Moreover, mutations of the TCA cycle tumor suppressors, SDH and FH, have also been linked to the stabilization of HIF. In particular, prolyl hydroxylation of HIF-1 α requires α -ketoglutarate as a substrate, which is converted to succinate, such that deficiency of SDH or FH decreases α -ketoglutarate or increases succinate, thereby inhibiting the degradation of HIF-1 (12). Taken together, activation of certain oncogenic pathways stabilizes HIF-1 protein under nonhypoxic conditions, resulting in activation of glycolytic metabolism. However, activation of glycolytic flux alone would not account for the Warburg effect, which is also associated with diminished mitochondrial function that has been thought to decrease passively due to the lack of oxygen.

PDK1 and Aerobic Glycolysis

Two recent studies provide insight into the Warburg effect via a novel HIF-1-mediated mechanism that actively inhibits mitochondrial function (13, 14). PDK1, which is one of four family members, was identified as a direct HIF-1 target gene in hypoxic cells. PDK1 phosphorylates and inactivates the mitochondrial pyruvate dehydrogenase (PDH) complex. Suppression of PDH by PDK1 inhibits the conversion of pyruvate to acetyl-CoA, thereby attenuating mitochondrial function and respiration (Fig. 1). Because nonhypoxic stabilization of HIF through oncogenic events has been observed in many types of tumors, we hypothesize that PDK1 levels may be up-regulated in nonhypoxic tumor cells by HIF, which would divert pyruvate from PDH and result in the increased lactate production (Fig. 1). A recent immunohistochemical study further provided evidence that PDK1 expression is elevated in non-small-cell lung cancers (15). Intriguingly, PDH expression seems to be reduced in high PDK1-expressing cancer cells. These studies suggest that PDK1 activation may be a key regulatory switch contributing to the Warburg effect. In aggregate, the activation of oncogenes, such as *AKT*, *MYC*, and *RAS*, along with the stabilization of HIF can enhance aerobic glycolysis or the Warburg effect through increased glycolytic flux and attenuation of mitochondrial function.

Does Aerobic Glycolysis Participate in Tumorigenesis?

Aerobic glycolysis may provide cancer cells growth advantage in the tumor microenvironment (16). Although ATP production

through the glycolytic pathway is much less efficient stoichiometrically as compared with mitochondrial oxidative phosphorylation, activation of AKT could provide cells with sufficiently high glycolytic fluxes to maintain a higher than adequate level of ATP (17). In fact, excess pyruvate in cells with a high rate of glucose utilization is redirected toward lipid synthesis through ATP citrate lyase (18). As such, activation of aerobic glycolysis liberates cancer cells from oxygen dependency for ATP production, particularly in the hypoxic tumor microenvironment. This liberation, however, could be exploited for therapeutic purposes. For example, inhibition of ATP citrate lyase can suppress tumor cell growth (18). Inhibition of glycolysis can overcome tumor drug resistance (19). Furthermore, inhibition of HIF-1 or PDK1, which causes further mitochondrial oxygen depletion, renders cells more susceptible to anoxia-induced cell death or sensitivity to tirapazamine, a chemotherapeutic agent that is activated by the absence of oxygen (13).

Aerobic glycolysis may also participate in tumorigenesis, which is characterized by cellular immortalization and subsequent malignant transformation. Recent evidence suggests that increased aerobic glycolysis could contribute to immortalization of cells. A recent expression cloning study identified two glycolytic genes, encoding glucose phosphate isomerase and phosphoglycerate mutase, capable of immortalizing primary human cells, increasing glycolysis, and attenuating mitochondrial generation of reactive oxygen species that are known to cause cellular senescence (20). The loss of classic tumor suppressor genes encoding enzymes of the TCA cycle also suggests that aerobic glycolysis may significantly contribute to tumorigenesis; however, it should be noted that the stabilization of HIF-1 in these tumors may convey other advantages such as the induction of angiogenesis. In this regard, the Warburg effect could be considered as a positive modifier of cancer, such that it may not be causative but rather facilitates tumor progression.

In addition to metabolic advantages of increased aerobic glycolysis, the nonglycolytic functions of glycolytic enzymes may also contribute to tumorigenesis through the antiapoptotic effects of HK2, cell cycle-dependent transcriptional regulation by LDH and glyceraldehyde 3-phosphate dehydrogenase, and enhanced cell motility by glucose phosphate isomerase (autocrine motility factor; ref. 4). Taken together, the switch to glycolytic metabolism may contribute to tumor development through enhanced glycolytic flux and/or the multifaceted functions of glycolytic enzymes. It should be noted, however, that the role of normal mitochondrial function in tumorigenesis is not well defined, and hence the recent findings on cancer's molecular sweet tooth should not dissuade from the strife for a deeper understanding of mitochondrial function in cancer glucose metabolism.

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