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# The Warburg Effect: Evolving Interpretations Of An Established Concept

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## Abstract

Metabolic reprogramming and altered bioenergetics have become emerged as a hallmark of cancer and an area of active basic and translational cancer research. Drastically upregulated glucose transport and metabolism in most cancers regardless the oxygen supply, a phenomenon called the Warburg effect, is one of major focuses of the research. Warburg speculated that cancer cells, due to defective mitochondrial oxidative phosphorylation (OXPHOS), switch to glycolysis for ATP synthesis, even in the presence of oxygen. Studies in the recent decade indicated that while glycolysis is indeed drastically upregulated in almost all cancer cells, mitochondrial respiration continues to operate normally at rates proportional to oxygen supply. There is no OXPHOS-to-glycolysis switch but rather upregulation of glycolysis. Furthermore, upregulated glycolysis appears to be for synthesis of biomass and reducing equivalents in addition to ATP production. The new finding that a significant amount of glycolytic intermediates are diverted to the pentose phosphate pathway (PPP) for production of NADPH has profound implications in how cancer cells use the Warburg effect to cope with reactive oxygen species (ROS) generation and oxidative stress, opening the door for anti-cancer interventions taking advantage of this. Recent findings in the Warburg effect and its relationship with ROS and oxidative stress controls will be reviewed. Cancer treatment strategies based on these new findings will be presented and discussed.

### Keywords

The Warburg effect; metabolism reprogram; glucose transport; ROS; HIF; MYC

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## Introduction

A wave of new evidence indicates that not only gene mutations but also metabolic reprogramming play important roles in cancer [1–6]. In certain cases, the reprogramming of cell metabolism may even participate in the initiation of tumorigenesis [7–9]. The alterations of metabolism and energetics, within which glucose and adenosine triphosphate (ATP) are prominent players, have been recognized in recent years as an emerging hallmark of cancer [10]. Actually, the importance of metabolic alteration in cancer cells was recognized long ago. In the 1920s, Otto Warburg, a German biochemist, demonstrated that unlike normal tissues, cancer cells always upregulated glycolysis even when oxygen was abundant [11–13]. This phenomenon of so-called aerobic glycolysis became known as the Warburg effect [14–20].

Warburg hypothesized that existing mitochondrial dysfunction disrupts oxidative phosphorylation (OXPHOS) pathway therefore, cancer cells have to switch from OXPHOS to glycolysis for ATP generation [14, 18]. As glycolysis is much less efficient than OXPHOS for producing ATP, it has to be greatly upregulated so that sufficient ATP will be synthesized. However, this hypothesis has been challenged in recent years due to findings that upregulated glycolysis in many cancers is not accompanied by detectable mitochondrial defects or OXPHOS disruptions [21, 22]. In addition, new evidence revealed that the upregulation of glycolysis is not just for ATP synthesis, but also for synthesis of biomass such as ribonucleotides [23] and amino acids [24] as well as reduced nicotinamide adenine dinucleotide phosphate (NADPH) production [25], which can remove reactive oxygen species (ROS) generated by cancer cells' accelerated metabolism under hypoxic conditions [25, 26]. Thus, the Warburg effect appears to be a strategic move made by cancer cells not only to cope with multiple urgent requirements simultaneously for growth, and proliferation in an ever-changing microenvironment under numerous material limitations, such as shortages of oxygen and nutrients; but also to reduce ROS and therefore oxidative stress in cancer cells.

Although the Warburg effect was specifically described for metabolic changes in cancer cells, the phenomenon (aerobic glycolysis) was also observed in rapidly proliferating normal cells such as stimulated lymphocytes and mitotic and proliferating fibroblasts [27–32]. This dramatic physiological change in normal cells is due to the temporary higher demands in metabolic material and energy for completing the cell proliferation process. The fact that aerobic glycolysis is present in E coli, yeasts, normal proliferating cells as well as almost all cancer cells [27–32], suggests that this is an evolution-selected metabolic strategy conserved among cells to meet special needs during cell proliferation and most cancer cells exploit this strategy because of their constant needs for rapid growth and proliferation.

## Brief History And Current Interpretations Of The Warburg Effect

In the early 1920s, after partially elucidating the metabolic pathways of glycolysis and OXPHOS for ATP synthesis, Otto Warburg and his co-workers developed an *ex vivo* system to measure energy metabolism of cancer tissue slices with a thickness of approximate 200–300 μm isolated from Flexner-Jobling rat liver carcinoma using then newly developed

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quantitative measurement techniques. He and his coworkers meticulously measured $O_2$ uptake and lactic acid production by the tumor slices and calculated the amount of glucose consumed by cancer slices. He observed that, compared to normal tissues, cancer slices used approximately 10 times more glucose in their energy metabolism and produced a large amount lactate from upregulated glycolysis. Interestingly and surprisingly, the approximately ten-fold upregulation of glycolysis persisted even when the cancer slices were assayed in the presence of normal $O_2$ pressure [11–13, 18]. From this observation, Warburg concluded that the upregulated and persistent glycolysis was likely to be a forced action taken by cancer cells to switch to glycolysis for producing sufficient ATP to compensate for ATP loss due to dysfunctional OXPHOS resulting from mitochondrial defects [14, 18].

In recent decades, it has been recognized that cancer metabolism is an integral part of cancer biology. Research done in the last 10–15 years has confirmed the near-universal prevalence of the Warburg effect in cancers. What was observed and measured by Warburg more than 90 years ago was mostly and quantitatively correct. However, his theory regarding the reason for cancer cells to upregulate glycolysis has been challenged, because in many cancers, aerobic glycolysis is upregulated without mitochondrial dysfunction (no identifiable mitochondrial gene mutations) or OXPHOS disruption [18, 21, 22]. In these cancers, OXPHOS continues as normal and produces as much ATP as OXPHOS in normal tissue under the same oxygen pressures [18, 21, 22]. Therefore, the upregulation of glycolysis may be a strategic metabolic action made by cancer cells for the purpose of balancing the functional needs of cancer cells, primarily in synthesis of biomass: ribose for RNA and DNA [23], amino acids for proteins [24], fatty acids as precursors for components of plasma and intracellular membranes [27, 33] as well as reducing equivalents (NADPH) for reducing ROS and oxidative stress [25]. Glycolytic ATP synthesis seems to be of no higher priority because cancer cells, regardless of their oxygen supply, do not suffer an ATP shortage. The debate on the functional roles of upregulated glycolysis in the Warburg effect is ongoing, and interpretation is evolving alongside exciting new findings. Of note, the metabolic reprogramming observed in cancer cells is also found in normal proliferating cells for the same requirements in increased biosynthesis of nucleic acids, amino acids, and fatty acids [27–32].

Because of drastically upregulated glycolysis, more glucose is transported into cells and thus more pyruvate is produced in an average cancer cell than in normal cells. Limited by the capacity of OXPHOS and regulated by lactate dehydrogenase (LDH), pyruvate comparable to the amount in normal cells enters the mitochondrial TCA cycle. A lingering misconception about the Warburg effect is that OXPHOS in cancer cells is greatly reduced compared to normal cells. In fact, OXPHOS in most cancers is normal and a similar amount of ATP is produced by OXPHOS. Different from what Warburg theorized, there is no switch from OXPHOS to glycolysis in cancer cells, rather, glycolysis is upregulated even in the presence of normal oxygen pressure (normoxia) because of higher demands for biosynthesis. Under hypoxia, due to the limited oxygen availability, less pyruvate enters the TCA cycle and thus more pyruvate is converted into lactate. The excess lactate is secreted into the intratumoral space by hypoxic cancer cells [18, 20].

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## ROS Balancing And The Warburg Effect

ROS act as a double-edged sword for cancer cells. An elevated but controlled ROS level is required for cancer growth and proliferation [34]. ROS are involved in tumor angiogenesis [35, 36], in ligand-independent transactivation of receptor tyrosine kinase [37, 38], as well as in promoting invasion and metastasis of cancer cells [39, 40].

However, ROS are also a major contributor to oxidative damage [41]. Thus the cellular level of ROS must be vigorously maintained within certain ranges so that they will only promote cancer cell growth and proliferation without causing severe oxidative damage and even cell death. ROS production in cancer cells is elevated due to oncogenic stimulation and increased metabolic activities [34]. The Warburg effect, which leads to the production of NADPH and thus a proper redox status, becomes an important survival mechanism for cancer cells.

One important step of glycolysis reprogramming that leads to The Warburg effect is the switch in isoform of pyruvate kinase (PK), which catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate as the last step of glycolysis. Many types of cancer cells use the M2 isoform of pyruvate kinase (PKM2) instead of the M1 isoform of the enzyme (PKM1) as normal tissues do [42–44]. This is surprising because cancer cells drastically upregulate glycolysis; yet PKM2 is less active than PKM1. This paradoxical phenomenon was explained in recent years. The major reasons for upregulating glycolysis in cancers are not only for synthesis of ATP but also for synthesis of biomass (nucleic acids and proteins) and NADPH [25, 26]. By using PKM2, cancer cells upregulate glucose transport and the early steps of glycolysis without overproducing pyruvate. Instead, using the slower PKM2 leads to accumulation of earlier glycolytic intermediates, diverting them to glycolysis-connected subsidiary biosynthesis pathways such as hexosamine, PPP, and amino acids. One major purpose for upregulating glucose transport and early steps of glycolysis appears to be for cell biomass production and achieving metabolic balance among ATP production, biomass synthesis, as well as the control of oxidative stress resulted from ROS generation [25, 26]. The switch to PKM2 results in accumulation of PEP, which functions as a feedback inhibitor of the glycolytic enzyme triosephosphate isomerase (TPI). This in turn activates PPP, increasing antioxidative metabolism by producing more NADPH, reducing ROS, and amplifying the inhibitory effect of PKM2 [25, 26]. In addition, ROS and PKM2 form a negative feedback loop to maintain ROS in a tolerable and functional range (Fig. 1). PKM2 can be specifically oxidized on cysteine 358 by hydrogen peroxide ($H_2O_2$), an ROS, which leads to reduction in its activity and pyruvate production as well as augmentation of flux of glycolytic intermediates into PPP [25, 26].

Although the role of the Warburg effect in generating additional NADPH via the pentose phosphate pathway (PPP) for coping with higher ROS levels in cancer cells was elucidated in the past ten years [45], the higher steady-state levels of superoxide and $H_2O_2$ in cancer cells relative to normal cells were reported as early as 35 years ago [46–51]. Based on these earlier experimental results, it was hypothesized and has been validated that the increased glucose metabolism in cancer cells is for compensating increased fluxes of $H_2O_2$ produced in mitochondria by producing higher amounts of both NADPH as a co-factor for $H_2O_2$

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metabolism and pyruvate for directly scavenging $H_2O_2$ in a deacetylation reaction to form acetic acid and $H_2O$ [46, 52–56].

Superoxide and $H_2O_2$, two common ROS, are known to be increasingly produced primarily by mitochondria in cancer cells. One possible mechanism for the increased ROS production in cancer cells is that some alterations in the assembly of electron transport chain complexes lead to stoichiometric mismatches. Such mismatches result in increased residence time of electrons on sites of the complexes that mediate electron reductions of $O_2$ and production of increased amount of superoxide and $H_2O_2$ [57]. The increased fluxes and steady-state levels of ROS in cancer cells versus normal cells have significant impacts on tumorigenesis and metabolic reprogramming. It can not only lead to uncontrolled growth and the inability of cancer cells to differentiate but also to increased genomic instability. The resulting additional genetic mutations caused by ROS and other factors may significantly influence the progression of tumors and the evolution of metabolic reprogramming of cancer cells.

## ROS-Involved Gene Regulation And The Warburg Effect

Besides direct impact, ROS can also indirectly contribute to the Warburg effect via its involvement in regulation of gene expression. One well-studied ROS-regulated gene is hypoxia-inducible factors (HIF) [58–60]. The uncontrollable growth and proliferation of cancer cells as well as abnormal vasculogenesis lead to deficiency of oxygen supply and local hypoxia in tumors [61]. The resulting condition triggers the increased expression of HIF [61, 62]. There are three members (isoforms) in the HIF family: HIF1, HIF2, and HIF3 with HIF1 and HIF2 better studied and HIF3's functions poorly understood. Among the three, HIF1 is the only one that is ubiquitously expressed and the most relevant to cancer [61, 62]. HIF1, like all HIFs, consists of an oxygen-dependent α-subunit and a constitutively expressed β-subunit. Under normoxia, HIF1α is constitutively synthesized and hydroxylated by prolyl hydroxylases (PHDs). Hydroxylated HIF1α is recognized by the von Hippel-Lindau protein (VHL) and its associated ubiquitinase, resulting in proteolytic degradation in proteasomes [62, 63]. Under hypoxia, reduced oxygen supply diminishes the activity of PHDs, which are further inhibited by ROS released from stressed mitochondria that operate under reduced OXPHOS. ROS oxidize and inactivate the ferrous ion located in the active site of PHDs such that they become unable to modify and thus stabilize HIF1α, which binds to HIF1β to form a stabilized HIF1. HIF1 complex then binds and transactivates genes involved in glucose transport, glycolysis, pH regulation, and vasculogenesis, allowing cancer cells to rapidly adapt to hypoxia [61, 63, 64]. HIF functions as a master regulator for the initiation and maintenance of the Warburg effect at the level of gene expression. Recently, PKM2 is found to be a PHD-induced coactivator for HIF [65], adding another link between the Warburg effect and HIF.

Another well-documented gene that is regulated by HIF and contributes to the Warburg effect is the *MYC* proto-oncogene [66]. While it is not directly regulated by ROS, activated MYC, the protein product of the *MYC* gene, can either work together with HIF or independently in regulating glycolysis and OXPHOS. The *MYC* gene is a classical immediate early serum response proto-oncogene under vigorous transcriptional control [66, 67]. Approximately 30% of all human cancers show deregulated *MYC* gene expression [68].

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MYC is a transcription factor that regulates genes involved in glucose metabolism by binding to and regulating virtually all glycolytic enzyme genes as well as numerous genes involved in mitochondrial biogenesis [61, 69]. One of the key roles of MYC in normal cells is to stimulate glycolytic flux for OXPHOS [66, 67]. In cancer cells, working together with HIF and PKM2, constitutively active MYC upregulates glycolysis to ensure sufficient metabolic intermediates for synthesis of biomass and reducing equivalents needed by cancer cells [25, 26, 70]. Compared to HIF, MYC appears to be more involved in the transcriptional regulation of genes participating in energy generation and cell growth and proliferation. Both HIF and MYC activate hexokinase 2 (HK2) and pyruvate dehydrogenase kinase 1 (PDK1), leading to augmented glycolytic rates and conversion of glucose to lactate [61]. Furthermore, HIF1 and MYC independently activate glucose transporter 1 (GLUT1) and lactate dehydrogenase A (LDHA), resulting in increased glucose influx and higher glycolytic rates [61]. The roles of HIF1 and MYC in the regulation of the Warburg effect are schematically shown in Figure 1.

## Oxygen Supply, ATP Synthesis And The Warburg Effect

The Warburg effect is a dynamic process, in which the weight of OXPHOS relative to glycolysis in total ATP synthesis is constantly adjusted in response to cancer cells' microenvironments, particularly oxygen supply rate. Oxygen pressures ($pO_2$) in cancer cells are lower than those in normal cells of the same tissue origin and are different in different tumor types, ranging from very low mmHg to slightly above 10 mmHg as compared to 160 mmHg in the air and approximately 40 mmHg in the vein [71–73]. Normoxia and hypoxia are relative concepts without absolute standards because hypoxic $pO_2$ in one cancer type may be normoxic in another type. Inside of a single tumor, $pO_2$ is also different from one location to another depending upon the abundance of blood vessels to that location. Even more complicated, $pO_2$ of one location of a tumor can also be changing during tumorigenesis. This is because the vascular structure inside of a tumor is constantly forming and dying, leading to reported phenomena of intermittent and cyclic hypoxia inside tumors [74–77]. Cancer cells in tumors are heterogeneous with respect to their oxygen supply and ATP synthesis rate. Normoxic cancer cells produce as much as 50% more ATP than normal cells by fully oxidizing a molecule of glucose through OXPHOS (with 36 ATP molecules produced), while simultaneously metabolizing approximately another 10 glucose molecules to lactate through upregulated glycolysis and producing an additional ~20 ATP molecules during the process [18]. By comparison, anoxic cancer cells metabolize about 13 glucose molecules to produce only 26 ATP molecules exclusively through glycolysis. However, a large proportion of cancer cells in tumors lives and grows in varied hypoxic conditions between these two extremes. These heterogeneous cells produce more ATP than anoxic ones but significantly less ATP than normoxic cancer even normal cells [18]. Exactly how much ATP they can produce depends on their oxygen and glucose availabilities. On average, cancer cells in a tumor produce about 10% more ATP than normoxic normal cells [18], a value indicative of the heterogeneity of cancer cells within a tumor. While a sub-population of cancer cells has reduced ATP production due to a lack of oxygen and severely depressed OXPHOS rates, these cells do not appear to suffer from a lack of ATP. It is presently unclear as to how these hypoxic cancer cells are capable of securing sufficient levels of ATP

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to maintain growth and proliferation. One possible explanation is that high ATP-producing normoxic cancer cells and possibly even stromal cells release ATP while low-ATP producing hypoxic cancer cells take up the released ATP from the intratumoral space to supplement their intracellular ATP pool (Fig. 2). As a result, the intracellular ATP concentration of hypoxic cancer cells are elevated to such levels that they are capable of performing all the biological functions required for survival, growth, proliferation and even cell movement required for invasion and metastasis [78, 79].

Mammalian cells, including cancer cells, are known to release ATP under certain conditions [80–84]. Uptake of ATP by animal cells has been speculated [85–88] but has not been experimentally demonstrated. While interstitial ATP concentrations in normal tissues are maintained between 1–1000 nM depending upon tissue type [89–94], intratumoral ATP levels are in the range of several hundred M [95–98], which suggests ATP is readily available for use by hypoxic cancer cells. However, ATP is a charged and thus hydrophilic molecule, unable to cross the cell membrane without the help of a transporter. Since no plasma-membrane-associated ATP transporter has been found, it has long been presumed that extracellular ATP does not enter cells. There has been no direct evidence or identified mechanism for ATP uptake by cancer cells until a recent study demonstrated that normal pancreatic cells transformed with an oncogenic form of Kras gene ($Kras^{onc}$) drastically upregulated macropinocytosis [99], a type of endocytosis that engulfs extracellular fluid and nonspecifically takes up extracellular molecules in the fluid. When the macropinocytosis inhibitor EIPA was used to treat nude mice with xenografted tumors of the transformed cells, it substantially reduced tumor growth [99] indicating that the transformed cells use macropinocytosis to take up extracellular nutrients to support their growth *in vivo*. Because macropinocytosis, commonly named “large-scale fluid drinking”, is nonspecific for the molecules it internalizes [100, 101], many extracellular molecules including the highly concentrated ATP should be taken up by this process. These reports [80–88, 95–99] and our prevous finding of that extracellular ATP increased intracellular ATP levels and increased cancer cell growth and survival [102] led to the hypothesis that low ATP-producing hypoxic cancer cells likely solve the problem of ATP deficiency by upregulating macropinocytosis to bypass the lack of an ATP transporter for acquiring extracellular ATP. They internalize intratumoral ATP, hypothetically released from stromal cells and/or normoxic cancer cells (Fig. 2). The uptake of extracellular ATP also theoretically reduces intracellular ATP synthesis, ROS production and oxidative stress as well as increases survival of cancer cells under conditions of hypoxia. The hypothesis is supported by our recent finding that treating cells with ATP, in the range of the reported extracellular ATP concentrations in several cancer types [60–65], induced intracellular ATP concentration by 50% to 100% via macropinocytosis and other endocytic processes [103]. The increase of ATP was not observed in their non-cancerous cell counterparts, suggesting the extracellular ATP-caused intracellular ATP elevation was a capability associated with these cancer cell lines. In addition, the extracellular ATP substantially contributes the cancer cells’ resistance to tyrosine kinase inhibitor (TKI) anticancer drugs such as sunitinib [103]. The mechanism of the resistance might be the direct competition between intracellular ATP, which was in higher concentration in the presence of extracellular ATP, and TKIs. All these indicate not

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only extracellular ATP can enter cancer cells but also significantly contribute to cancer cell survival, growth and drug resistance to TKIs.

## Warburg Effect At The Tumor Microenvironment Scale

Cancer cells in a tumor nodule are far from a homogeneous population and several levels of heterogeneity exist among cancer cells in tumors. First, cancer cells in tumors are often genetically heterogeneous among tumors in the same individual or even within a single tumor. As tumors grow, cancer cells within a tumor can accumulate additional genetic mutations and create further genetic diversity [104, 105]. Second, cancer cells in a tumor are also metabolically heterogeneous primarily due to their distances to intratumoral blood vessels, which determines the relative levels of oxygen and nutrient supplies to the cells. Because of the differences in oxygen and nutrient supply, these cancer cells exhibit different rates of mitochondrial respiration and different degrees of reliance on aerobic glycolysis [18]. Third, a subpopulation of cancer stem cells (CSCs) have been identified and isolated from different cancers. Similar to normal stem cells (SCs), CSCs show full self-renewal capability and the Warburg effect (upregulated aerobic glycolysis) [106–109]. Different from SCs, CSCs can differentiate into only non-CSC cancer cells. Forth, the tumor heterogeneity is not only spatial but also temporal. Tumorigenesis is an ever-changing dynamic process. Oxygen and nutrient status of a region in a tumor can change during tumor development and normoxic cancer cells in a tumor at a given time can become hypoxic at another time and vice versa [74–77]. Genetic and metabolic heterogeneities can further communicate with each other, partially determining the fate of tumor development. These diversities exert differential metabolic pressures on heterogeneous cancer cells in a tumor. As a result, different cancer cells exhibit different levels of the Warburg effect depending upon their oxygen and nutrient status as well as their communications with other cancer cells and stromal cells.

Cancer is a mixed population of cancer cells and stromal cells, which include cells of hematopoietic and mesenchymal origins. All these cancer and non-cancer cells plus the extracellular matrix (ECM) form the tumor microenvironment [110–112]. Stromal cells of hematopoietic origin include T and B lymphocytes, natural killer (NK) cells as well as macrophages, neutrophils, and myeloid-derived suppressor cells (MDSCs) [111]. The role of T cells is either tumor-promotion [113] or tumor-elimination [114] depending upon the exact tumor microenvironmental context. For example, stimulated T lymphocytes are able to release large amount ATP into extracellular space, potentially modulating cancer metabolism through purinergic receptor-mediated signaling [115]. T cells and macrophages, interacting with cancer cells through cytokines, can launch tumor-protective and tumor growth-promoting inflammatory responses [116]. Similarly, each of the other constituent cell types of hematopoietic origin may have either a positive or negative effect on tumor development. Stromal cells of mesenchymal origin include fibroblasts, myofibroblasts, mesenchymal stem cells (MSCs), adipocytes and endothelial cells. Among these cells, adipocytes are found to secret hepatocyte growth factor (HGF) to promote tumor growth [117] and endothelial cells play roles in angiogenesis and cancer cell dissemination [118, 119]. Thus, all of these stromal cells directly or indirectly affect cancer metabolism and

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participate in metabolic reprogramming of cancer cells, including regulating the Warburg effect.

Although cancer cells are competing with stromal cells for limited resources such as oxygen and nutrients and energy in the form of ATP, they also form symbiotic and cooperative relationships with one another. The outcome of such overall competition and cooperation results in the formation of a commonly acceptable microenvironment for the best survival of the tumor by transforming normal stromal cells into tumor-friendly stromal cells to serve the needs of cancer cells and tumors. One such example is stimulated lymphocytes release large amounts of ATP into extracellular space [120], potentially creating an ATP-rich environment for both direct energy intake and for purinergic receptor-mediated signaling to regulated metabolisms of cancer cells. This theory is supported, at least in part, by the observation that intratumoral ATP concentrations are $10^3$ to $10^4$ times higher than those of normal tissues [95–98]. Also, immune stromal cells also use cell surface enzymes CD39 and CD73 to dephosphorylate ATP into AMP and adenosine, respectively [115, 121], creating an immunosuppressed environment within tumors. Working in coordination, CD39 and CD73 create and adjust a "purinergic halo" surrounding immune stromal cells to modulate signaling events mediated by purinergic receptors by regulating the duration, magnitude and composition of the halo [115, 121]. Through purinergic signaling, immune stromal cells mediate immunological and inflammatory responses such as immunoescape and cancer cell killing impairment in tumors. In addition, CD39/CD73 system is also found overexpressed on the surface of cancer cells [115], suggesting that this system also utilize high intratumoral ATP concentration to regulate cancer cell metabolism including the Warburg effect.

## The Warburg Effect As A Potential Target For Cancer Treatment

As presented in the Figure 1, some major characteristics of the Warburg effect are: (i) increased expression of glucose transporters and thus an increased uptake of glucose. (ii) Increased PPP-catalyzed NADPH production. (iii) Altered activities of glycolytic or glycolysis-related enzymes (such as HIF/MYC induced activation of HK2, LDHA and PDK1; and the switch from PKM1 to a less active PKM2). (iv) Increased lactate production. Some of these characteristics have been or could potentially be targeted for developing therapeutics for cancer treatments (Fig. 2) (Table 1). For example, inhibiting glucose transport should lead to shortage of glucose supply to cancer cells, thus slowing down cancer metabolism and biomass synthesis and forcing cancer cells to stop growing and undergo apoptosis. Up to 90% of all cancers substantially upregulate GLUTs and glucose metabolism as demonstrated by PET-scans of cancer patients [122–126]. Cancer cells are “addicted” to glucose and are more sensitive to changes in glucose transport and glucose supply than normal cells [102, 127]. At least twelve glucose transporters (GLUTs) have been identified [128–133]. Among them, GLUT1[134–139], GLUT2 [140, 141] and GLUT3 [142] are the most relevant to cancer. In fact, many GLUT inhibitors have been in studies. More inhibitors for GLUT1 have been developed than for other GLUTs due to its near-universal upregulation in cancer and better knowledge of its protein structure (Fig. 2). Reported GLUT1 inhibitors include GLUT1 antibody [143], fasentin [144, 145], apigenin [146–148], genestein [149–152], oxime-based inhibitors [153], STF-31 [154], WZB117 [67] and shRNA interfering expression of GLUT1 [155]. While all these GLUT1 inhibitors

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showed some inhibitory effect in different cultured cancer cell lines, STF-31 [154], WZB117 [102], and GLUT1 RNAi [155] also inhibited cancer growth *in vivo*. STF-31 inhibited tumor growth in a renal cell carcinoma animal model [154]; WZB117 reduced cancer growth rate by more than 60% in nude mice bearing human lung cancer (A549 cells) [102], and RNAi inhibition of GLUT1 prevented myeloproliferation [155]. These three *in vivo* studies clearly demonstrate anticancer efficacy of GLUT1 inhibitors in animal models.

In addition to GLUT inhibitors, drugs targeting enzymes involved in regulation of glycolysis are also in development. 2-deoxy-glucose (2DG) is a hexokinase inhibitor competing with glucose and 2DG has been widely studied as a potential anti-cancer drug [156]. As detailed previously, PKM2 used in cancer cells is less active than PKM1 used in normal tissues [42–44]. Thus, multiple activators of PKM2 have been studied for their ability in reducing upstream PPP-mediated anabolism and suppressing tumorigenesis [157, 158]. The activity of LDHA is closely related with NADH consumption. FX-11, as a selective inhibitor of LDHA, induced oxidative stress and inhibited tumor progression [159]. PPP not only produces metabolic intermediates for biomass synthesis, but also generates NADPH as reducing agents. Glucose-6-phosphate dehydrogenase (G6PD) catalyzes the critical step of generating NADPH in PPP. 6-aminocicotinamide (6-AN), as an inhibitor of G6PD, induced oxidative stress and inhibited the growth of cancer cells [160]. AZD3965, as an inhibitor of MCT1, blocked the transport of lactate into cancer cells and therefore inhibited the lactate-fueled respiration in cancer cells [161]. Dichloroacetate (DCA), an inhibitor of PDK1, indirectly activated mitochondrial respiration and inhibited cancer growth [162]. A recent clinical trial of DCA showed some durable anticancer efficacy in the treatment of non-Hodgkin's lymphoma [163]. Finally a group of inhibitors suppress tumor growth by targeting PI3K/AKT signaling pathway [164–167], which can regulate glycolysis and the Warburg effect by altering levels and activities of key glycolytic proteins and enzymes such as GLUTs, HK, and PFK [2, 16, 87].

Many of above-described agents could inhibit cancer cell growth by decreasing metabolic precursors for glycolysis and synthesis of biomass, reducing equivalents, and generating "absolute nutritional hunger"; their "side effect" on ROS production should not be ignored. Overproduction of ROS leads to excessive oxidative stress and cancer cell death. Inhibiting glucose transport reduces glucose supply, forcing cancer cells to reduce OXPHOS and resulting in increased ROS generation. Starvation and oxidative stress induced by glucose transport inhibition produce severe one-two punches to cancer. The key for the success of this approach is to produce more harm to cancer cells than benefits. An initial assault must be severe such that cancer cells suffer irreversible damage and cell death before they can shift to other energy and/or carbon sources such as glutamine. Tolerable ROS and oxidative stress ranges are also relative and can be adjusted by cancer cells. The details of the regulation of these two processes must be learned before we can identify and formulate a more effective anticancer strategy by combining inhibition of glucose transport and glucose metabolism as well as ROS dysregulation. Besides potential side effect from ROS, targeting the Warburg effect may encounter some other challenges. First, CSCs have at least three known features that may interfere cancer therapy targeting glucose metabolism or the Warburg effect. (a) CSCs show upregulated aerobic glycolysis as non-CSC cancer cells [51–

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54]. (b) CSCs are slower cycling compared to the non-CSC cancer cells in the same tumors [33]. Thus, it is likely that CSCs have lower glucose metabolic rate or reliance on glucose metabolism relative to non-CSC cancer cells. (c) CSCs overexpress the ATP-binding cassette transporters (ABCs), which play crucial roles in multi-drug resistance (MDR) [33]. As ABCs are ATP-binding and ATP-consuming, reducing ATP production or ATP internalization processes such as macropinocytosis may contribute to fighting drug resistance and the overall strategy of targeting CSCs. Simultaneous reduction of intratumoral (extracellular) ATP concentration and intracellular ATP synthesis and creation of an ATP-poor microenvironment should be a novel anticancer and anti-CSC strategy. Second, both normal proliferating cells and cancer cells upregulate aerobic glycolysis [27–29]. Targeting the Warburg effect (glycolytic proteins and/or enzymes) will not only inhibit cancer cells, but also normal proliferating cells. The latter (lymphocytes under clonal expansion for example) perform some important normal and urgently needed physiological functions. For this reason, infant and child cancer patients should not be treated with the Warburg effect-targeting therapeutics, as they are growing with large quantities of proliferating cells in their body. For similar reasons, cancer patients undergoing immunotherapy (with potential immune cell clonal expansion going on) should be excluded from the simultaneous anti-Warburg effect therapy. Last, the strategy may not be very effective for those cancers that rely more on glutamine rather than glucose for their metabolic needs. Profiles of glucose metabolism of tumors from cancer patients should be determined before the anti-Warburg effect therapy strategy is adopted.

## Conclusion

Cancer metabolism research in the past decade has substantially enhanced our understanding and changed the interpretations of the Warburg effect. Much more than Warburg initially speculated and in addition to glycolytic ATP synthesis, aerobic glycolysis also contributes to synthesis of biomass and reducing equivalents and plays a significant and varied role in cancer biology. The connection between the Warburg effect and cancer cell redox homeostasis has been established. The regulation of glycolysis and other glycolysis-connected metabolic pathways are well understood. However, many differences among various cancer types are recognized, with respect to their different genetic mutations; the heterogeneity among cancer cells within a single tumor; and their oxygen and nutritional supplies. Although still in an exploratory stage, targeting the Warburg effect nevertheless already has yielded promising results. With improved knowledge and better understanding of the Warburg effect, a therapy in combination with oncogenic and metabolic targets with redox manipulation is likely to generate synergistic anticancer effects and become one future anti-cancer strategy.

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## Highlights

ROS Balancing And The Warburg Effect

ROS-Involved Gene Regulation And The Warburg Effect

Oxygen Supply, ATP Synthesis And The Warburg Effect

Warburg Effect At The Tumor Microenvironment Scale

The Warburg Effect As A Potential Target For Cancer Treatment

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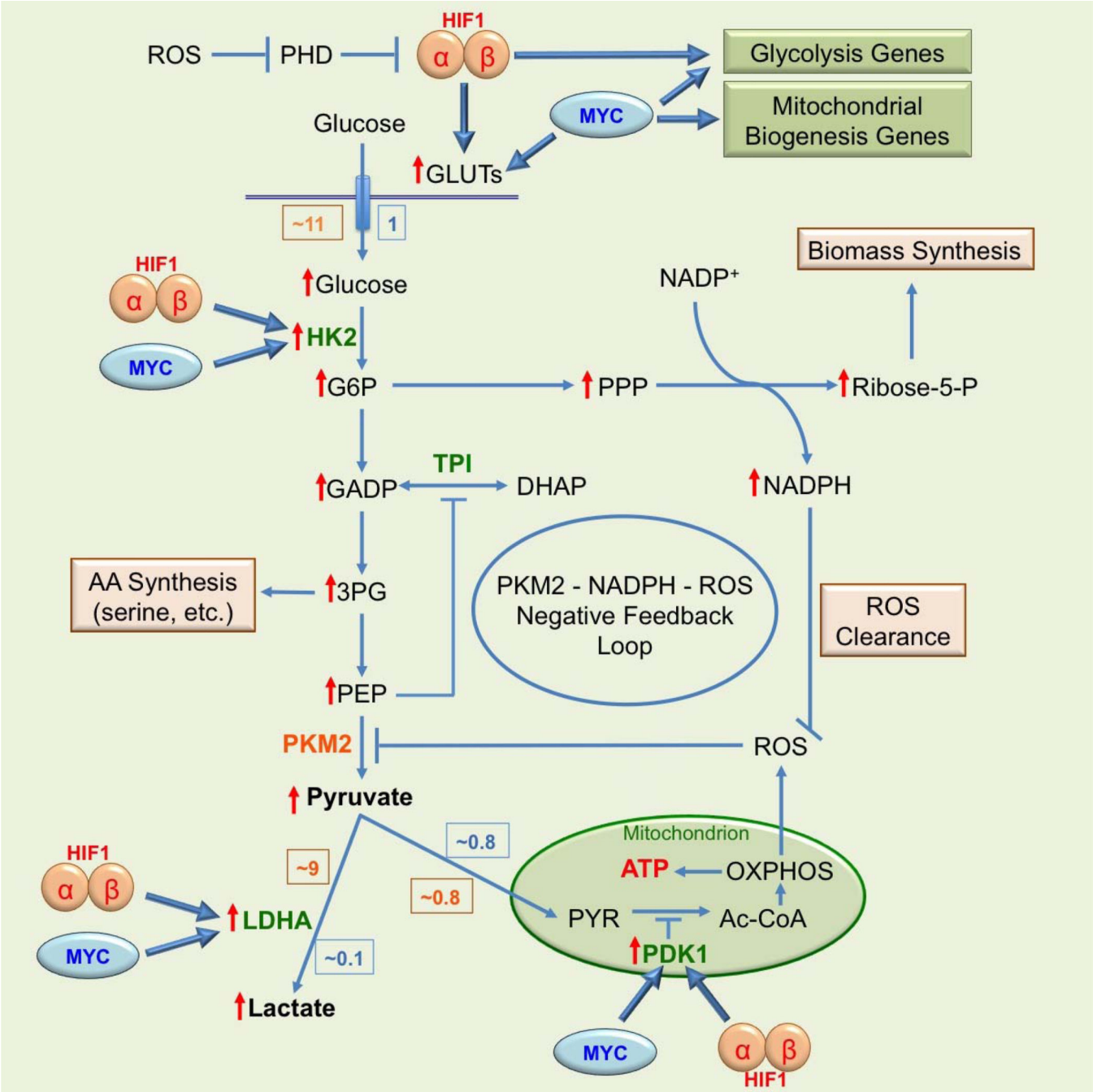


**Figure 1. The Warburg effect with its extended functions and regulations**

Relative amount of glucose consumption and its metabolic products in normal (blue box) and cancer (orange box) under normoxic condition are shown and compared. Red ↑ indicates an elevated level in cancer cells. The enzymes in green function in both normal and cancer cells; and the enzyme in orange functions mainly in cancer cells.

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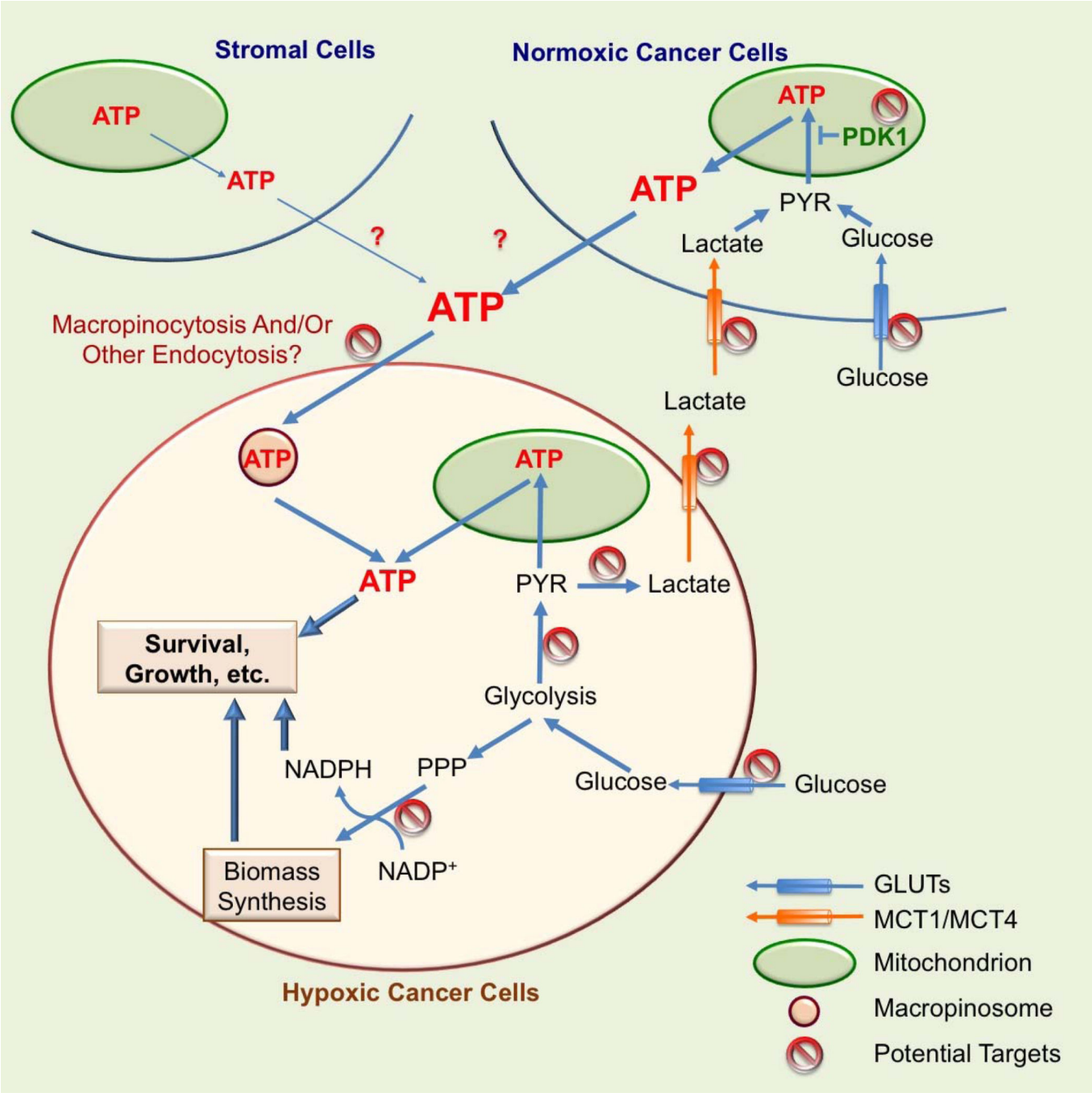


**Figure 2. Potential drug targets in ATP-sharing model**

According to this model, a symbiotic relationship exists among cancer and stromal cells in a tumor. Normoxic cancer cells and stromal cells recruited by hypoxic cancer cells release ATP into intratumoral space, leading to a large intratumoral ATP concentration increase. Highly concentrated intraturmoral ATP is then internalized by hypoxic cancer cells through macropinocytosis and/or other endocytic processes, supplementing the intracellular ATP pool in hypoxic cancer cells. Meanwhile, cancer cells uptake and release of lactate through

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transporters MCT1 and MCT4. Potential targets for anticancer therapeutic intervention in this model are shown by symbol .

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**Table 1**

Therapeutics targeting the Warburg effect in cancers

| Process | Target | Compound | Effect | Status | References |
|---|---|---|---|---|---|
| Glucose transport | GLUT1 | WZB117, STF-31 | Inhibits GLUT1 | Preclinical | [102, 154] |
| Glycolysis | HK | 2DG | Inhibits HK | Clinical trials discontinued | [156]<br>NCT00633087<br>NCT00096707<br>NCT00247403 |
| | PKM2 | TEPP-46 | Activates PKM2 and inhibits PPP | Preclinical | [157, 158] |
| | LDHA | FX11 | Inhibits LDHA | Preclinical | [159] |
| PPP | G6PD | 6-AN | Induces oxidative stress | Preclinical | [160] |
| Lactate transport | MCT1 | AZD3965 | Inhibits uptake of extracellular lactate | Phase I | [161]<br>NCT01791595 |
| Mitochondrial function | PDK1 | DCA | Inhibits PDK1 | Phase I-II | [162, 163]<br>NCT00566410<br>NCT01111097<br>NCT00540176 |
| AKT signaling pathway | AKT | AZD5363 | Inhibits AKT activity | Phase I-II | [164]<br>NCT01895946<br>NCT01353781<br>NCT01692262<br>NCT02077569<br>NCT01226316 |
| | | GDC0068 | | Phase I | [165]<br>NCT01090960 |
| | | GSK2141795 | | Phase I completed | [166]<br>NCT00920257<br>NCT01266954 |
| | | GSK2110183 | | Phase I–II completed Phase II | [166]<br>NCT00881946<br>NCT01531894 |

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| Process | Target | Compound | Effect | Status | References |
|---|---|---|---|---|---|
| | | MK-2206 | | Phase I-II | [167]<br>NCT01283035<br>NCT01277757<br>NCT01253447<br>NCT01231919<br>NCT01604772<br>NCT01258998<br>NCT01307631<br>NCT01349933<br>NCT01481129<br>NCT01802320<br>NCT01260701<br>NCT01425879<br>NCT01319539<br>NCT01226316 |