

The Warburg effect and its cancer therapeutic implications

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Abstract Increased aerobic glycolysis in cancer, a phenomenon known as the Warburg effect, has been observed in various tumor cells and represents a major biochemical alteration associated with malignant transformation. Although the exact molecular mechanisms underlying this metabolic change remain to be elucidated, the profound biochemical alteration in cancer cell energy metabolism provides exciting opportunities for the development of therapeutic strategies to preferentially kill cancer cells by targeting the glycolytic pathway. Several small molecules capable of inhibiting glycolysis in experimental systems have been shown to have promising anticancer activity *in vitro* and *in vivo*. This review article provides a brief summary of our current understanding of the Warburg effect, the underlying mechanisms, and its influence on the development of therapeutic strategies for cancer treatment.

Keywords Warburg effect · Glycolysis · Inhibitor · Mitochondria · Cancer therapeutics · Drug resistance · Reactive oxygen species (ROS) · Oncogene · Hypoxia

Introduction

Otto H. Warburg, a Nobel Prize winner for the discovery of cytochrome *c* oxidase and his important work on mitochondrial respiration and cellular metabolism, was the first to report that liver cancer cells, compared to normal liver

tissue, exhibited an increase in glycolytic activity in the presence of oxygen (Warburg and Negelein 1924). His subsequent work showed that this increase in glycolysis not only exists in solid tumor cells but also in leukemia cells cultured in the presence of plenty oxygen. These consistent observations led Warburg to suggest that such metabolic alteration in cancer cells was due to respiratory injury leading to aerobic fermentation, a critical event regarded by him as “the origin of cancer cells” (Warburg 1956). Interestingly, Warburg showed that this increased glycolytic activity was similar to that observed in early embryonic cells, suggesting that cancer cells may somehow resume a primitive metabolic pattern (Warburg 1956). It is now known that cancer cells show various degrees of increase in glycolysis, depending on the cell types and cell growing conditions. Under aerobic conditions, some tumor cells produce as much as 60% of their ATP through glycolysis (Nakashima et al. 1984), whereas normal cells seem to generate most of their ATP through the mitochondrial oxidative phosphorylation, using glucose, fatty acids, and other metabolic intermediates as the energy sources. The positron emission tomography (PET), widely used in clinical diagnosis of cancer, is based on the fact that cancer cells have elevated glucose uptake. Although increased glycolysis has been consistently observed in cancer cells, whether such metabolic shift is a symptom or cause of cancer still remains controversial. It is clear, however, that the increased dependency of cancer cells on glycolytic pathway for ATP generation represents an important metabolic difference between the normal and malignant cells, and may serve as a biochemical basis for developing therapeutic strategies to preferentially kill cancer cells. The original observations by Warburg have had a profound influence on our understanding of cancer biology, clinical diagnosis, and cancer treatment.

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The Warburg effect: mechanistic perspectives

In recent years, extensive studies have been conducted to investigate the molecular mechanisms underlying the Warburg effect and its potential clinical applications. Although the exact reasons why tumor cells exhibit elevated glycolysis and use this primitive and less energy-efficient pathway to generate ATP are still unclear, accumulating lines of evidence suggest that multiple mechanisms likely contribute to the overall increase in glycolysis in cancer cells. As illustrated in Fig. 1, these mechanisms include (1) mitochondrial DNA (mtDNA) mutations and deletions, (2) nuclear DNA (nDNA) mutations or abnormal gene expression, (3) oncogenic transformation, and (4) influence of the tumor microenvironment.

Role of mtDNA

Mitochondria are semi-autonomous intracellular organelles, which play essential roles in production of ATP, generation of reactive oxygen species (ROS), regulation of apoptosis, and conversion of various metabolic intermediates. The human mitochondrial DNA (mtDNA) is a double-stranded circular helix of 16,539 base pairs, which encodes 13 protein components of the mitochondrial respiratory chain complexes. It is believed that the high rates of mtDNA mutations observed in cancer cells may lead to mitochondrial malfunction and decrease the cellular ability to generate ATP through oxidative phosphorylation (Carew and Huang 2002; Singh 2004; Brandon et al. 2006). Furthermore, dysfunction of mitochondrial respiratory chain may also increase electron leakage, leading to increased generation of ROS. This speculation led Carew et al. 2003 to use primary human leukemia cells isolated from patients to examine mtDNA mutations and their correlation with alteration in cellular ROS and mitochondrial mass. It was found that mtDNA mutations in leukemia cells were closely associated with increased ROS generation and altered sensitivity to drug treatment (Carew et al. 2003), and such functional abnormalities seem to be associated with an increase of mitochondria mass (Carew et al. 2004).

It should be pointed out, however, that mtDNA mutations are unlikely to cause a complete defect in mitochondrial oxidative phosphorylation. In fact, most cancer cells, upon exposure to oxygen, exhibit various degrees of oxygen consumption, suggesting that their mitochondrial oxidative phosphorylation is retained to some extent. In certain cancer cells, especially the fast-growing tumors, ATP generation through oxidative phosphorylation is active and supplies a large portion of the total cellular energy (Rodriguez-Enriquez et al. 2000; Marin-Hernandez et al. 2006). Thus it is important to recognize

that elevated glycolysis in cancer cells does not necessarily suggest that cancer cells only use glycolytic pathway for ATP generation. It is likely that the Warburg effect reflects a metabolic state in cancer cells where ATP generation through mitochondrial oxidative phosphorylation is compromised and insufficient to support the active cellular activity, and thus the cells become more dependent on increased glycolysis for ATP supply. This does not suggest that cancer cells solely rely on glycolysis as the only pathway to generate ATP. The possible reasons for compromised mitochondrial function in cancer cells are shown in Fig. 1. Because oxidation of a glucose molecule through mitochondrial respiratory generates 36 ATP, which is 18 times of the ATP amount produced by glycolysis (2 ATP/glucose), a small decrease in mitochondrial respiration would require a substantial increase in glycolysis to maintain a constant ATP supply for the active cancer cell

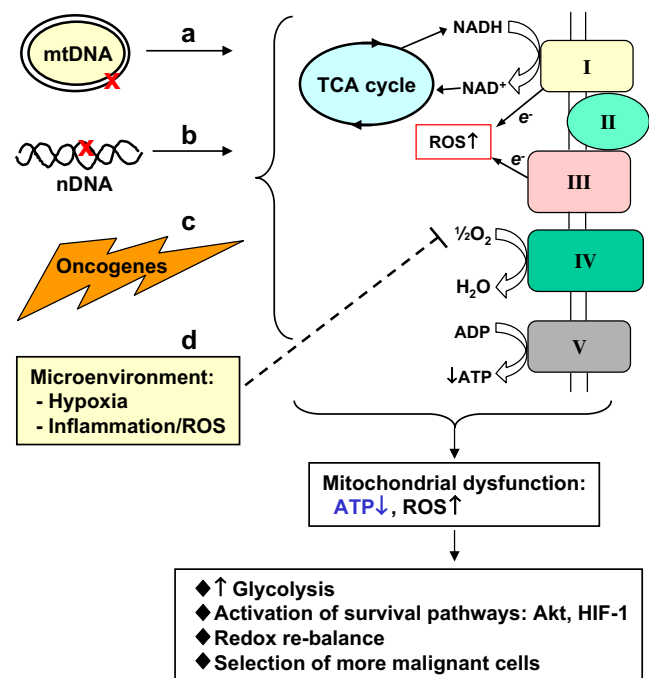


Fig. 1 Possible mechanisms contributing to decreased mitochondrial respiration and increased glycolysis in cancer cells. **a** Mutations and deletions of mitochondrial DNA (mtDNA) affect the mtDNA-encoded respiratory chain components, leading to mitochondrial dysfunction, decreased ATP generation, and increased ROS generation due to electron leakage from the respiratory chain. **b** Mutations in nuclear DNA (nDNA) or aberrant expression of certain nuclear genes may suppress mitochondrial respiratory function and/or the tricarboxylic acid (TCA) cycle, and promote glycolysis. **c** Expression of certain oncogenic molecules such as *Ras*, *Bcr-Abl*, and *Akt* can attenuate respiration and enhance glycolysis. **d** Hypoxia in tumor tissue microenvironment decreases the availability of oxygen for oxidative phosphorylation, whereas ROS generated in inflammatory tissue environment may inhibit the redox-sensitive mitochondrial respiratory chain components. The letters I–V indicate respiratory chain complexes I–V, respectively

metabolism. Interestingly, mitochondrial respiratory malfunction results in a change in cellular NADH/NADPH ratio, leading to a redox-mediated activation of the Akt survival pathway (Pelicano et al. 2006a).

Role of nDNA abnormalities

Mutation or abnormal expression of certain nuclear genes is another mechanism contributing to mitochondrial malfunction in cancer cells. Nuclear DNA encodes for the majority of the mitochondrial protein components. For instance, succinate dehydrogenase (SDH) and fumarate hydratase (FH) are encoded by nuclear genes and play important roles in TCA cycle and mitochondrial complex II function. Germline mutations in SDH and FH are associated with certain tumors (Gottlieb and Tomlinson 2005). SDH regulates apoptosis, and a reduction in SDH activity may lead to increased ROS generation (Albayrak et al. 2003; Ishii et al. 2005). Accumulation of succinate due to SDH deficiency causes an inhibition of HIF-1 α prolyl hydroxylase leading to the stabilization and nuclear translocation of HIF-1 α in normoxic condition (Selak et al. 2005). HIF-1 α is an important transcriptional factor that promotes the expression of glycolytic enzymes.

Over-expression of glycolytic enzymes including hexokinase II, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), enolase 1, pyruvate kinase, and lactate dehydrogenase has been observed in a variety of cancer cells (Altenberg and Greulich 2004; Mathupala et al. 2006). TKTL1, a transketolase-like enzyme that regulates the glucose metabolic flow into the pentose phosphate pathway, is highly expressed in various human cancer tissues (Coy et al. 2005; Langbein et al. 2006). The upregulation of pentose phosphate pathway will not only generate more glyceraldehydes-3-phosphate for the energy-yielding phase of the glycolytic pathway, but also produce pentose-5-phosphate and NADPH to support tumor cell growth.

A recent study suggests that p53 can directly transactivate the expression of SCO2 (synthesis of cytochrome c oxidase 2), which is essential for the assembling of mitochondrial cytochrome c oxidase complex for normal respiratory function, and a loss of p53 results in a decrease of mitochondrial respiration (Matoba et al. 2006). Furthermore, p53 induces the expression of TIGAR (TP53-induced glycolysis and apoptosis regulator), leading to a decrease of fructose-2,6-bisphosphate and suppression of glycolysis (Bensaad et al. 2006). These observations suggest that the tumor suppressor p53 can regulate the balance between oxidative phosphorylation and glycolysis by multiple mechanisms, and that cancer cells lacking functional p53 may have reduced respiratory activity and thus become dependent on glycolysis to generate ATP, even in the presence of oxygen.

Role of oncogenic signals

It has been known for some time that oncogenes including Ras, Src, PI3K/Akt, and Bcr-Abl may promote glycolysis and attenuate mitochondrial respiration. For instance, Ras or src transfection stimulates glucose uptake, increases glycolysis, and inhibits oxygen consumption (Flier et al. 1987; Biaglow et al. 1997). Cells expressing H-ras exhibit increased glycolysis and become more sensitive to the glycolytic inhibitor 2-deoxyglucose (Ramanathan et al. 2005). Activation of the PI3K/Akt/mTOR pathway promotes glucose uptake, enhances hexokinase activity, and increases glycolysis (Burgering and Coffey 1995; Gottlob et al. 2001; Rathmell et al. 2003). A recent study showed that combination of glycolysis inhibitor and mTOR inhibitor synergistically suppressed glucose uptake and severely depleted cellular ATP, leading to significant enhancement of cell killing in cancer cells (Xu et al. 2005a). The oncogene Bcr-Abl seems to promote the Warburg effect, which can apparently be reversed by the Bcr-Abl inhibitor Gleevec (Gottschalk et al. 2004; Serkova and Boros 2005).

Role of tumor microenvironment

Without structure abnormalities of the mitochondria, respiration can still be compromised if the availability of oxygen becomes limited. In human cancer, especially in solid tumors, hypoxia is frequently observed when the tumor mass reaches a certain size that exceeds the capacity of blood supply. Adaptation to the respiratory suppression owing to oxygen depletion causes tumor cells to switch to glycolysis for ATP production, accompanied by increased generation of lactate and acidification of tumor microenvironment, which leads to a further selection of malignant cells (Gatenby and Gillies 2004). HIF-1 α is a key molecule that mediates cellular response to hypoxia and can activate a set of genes involved in angiogenesis, glucose uptake, and glycolysis (Harris 2001). A recent study suggests that HIF-1 α may mediate a metabolic switch from mitochondria respiration to glycolysis by trans-activating pyruvate dehydrogenase kinase 1, which in turn inactivates pyruvate dehydrogenase leading to suppression of the TCA cycle (Kim et al. 2006). Although increased glycolysis in response to hypoxia does not exactly represent the classical Warburg effect (increased aerobic glycolysis), pharmacological agents that inhibit glycolysis will impact not only cancer cells exhibiting the Warburg effect, but also the cancer cells in hypoxic environment.

ROS generated in tumor tissue microenvironment due to chronic inflammation may also have negative effects on mitochondrial respiration and force the cancer cells to adapt

active glycolysis for ATP generation. Although it has been known for some time that many respiratory chain components and enzymes of the TCA cycle are sensitive to inhibition by ROS, the exact role of ROS in the tumor tissue environment in causing mitochondrial malfunction and its contribution to the Warburg effect still remain to be explored. The increased ROS generation within the cancer cells may also inhibit the respiratory chain and suppress oxidative phosphorylation. Interestingly, it has been suggested that aerobic glycolysis by proliferating cells may be a protective mechanism against reactive oxygen (Brand and Hermfisse 1997).

The Warburg effect: therapeutic implications

The original observation by Warburg that cancer cells exhibit increased glycolysis provides an important biochemical basis for the design of anticancer therapeutic strategies and new anticancer agents. As discussed above, the increase in glycolytic activity in cancer cells reflects a significant mitochondrial dysfunction due to various factors, and suggests that the malignant cells may depend more on glycolysis for ATP generation. In contrast, normal cells with competent mitochondrial respiration may be able to use alternative energy sources such as fatty acids and amino acids to generate ATP in the mitochondria and thus better tolerate glycolytic inhibition. This has led to the development of a therapeutic concept to inhibit glycolysis as a strategy to preferentially kill cancer cells (for review, see Pelicano et al. 2006b). As illustrated in Fig. 2, the glycolytic pathway is a series of metabolic reactions catalyzed by multiple enzymes or enzyme complexes. Some of these enzyme components represent possible targets for the development of glycolytic inhibitors as potential anticancer agents.

Potential therapeutic targets

Among the glycolytic enzymes, hexokinase (HXK), phosphofructokinase (PFK), GAPDH, and lactate dehydrogenase (LDH) represent potential therapeutic targets with known chemical inhibitors, although many of the small-molecule inhibitors may have only limited target specificity (Fig. 2).

HXK Hexokinase converts glucose to glucose-6-phosphate, the first rate-limiting step in the glycolysis pathway. In human cells, there are four isoforms of hexokinase (I–IV) with different patterns of tissue expression, subcellular localization, and catalytic/regulatory properties (Sebastian et al. 1999; Wilson 2003). HXK II is mainly found in normal skeletal muscle and adipose tissues, and is overexpressed in various cancer cells (Shinohara et al. 1991; Mathupala et al. 1995; Tian et al. 2005). Interestingly, the

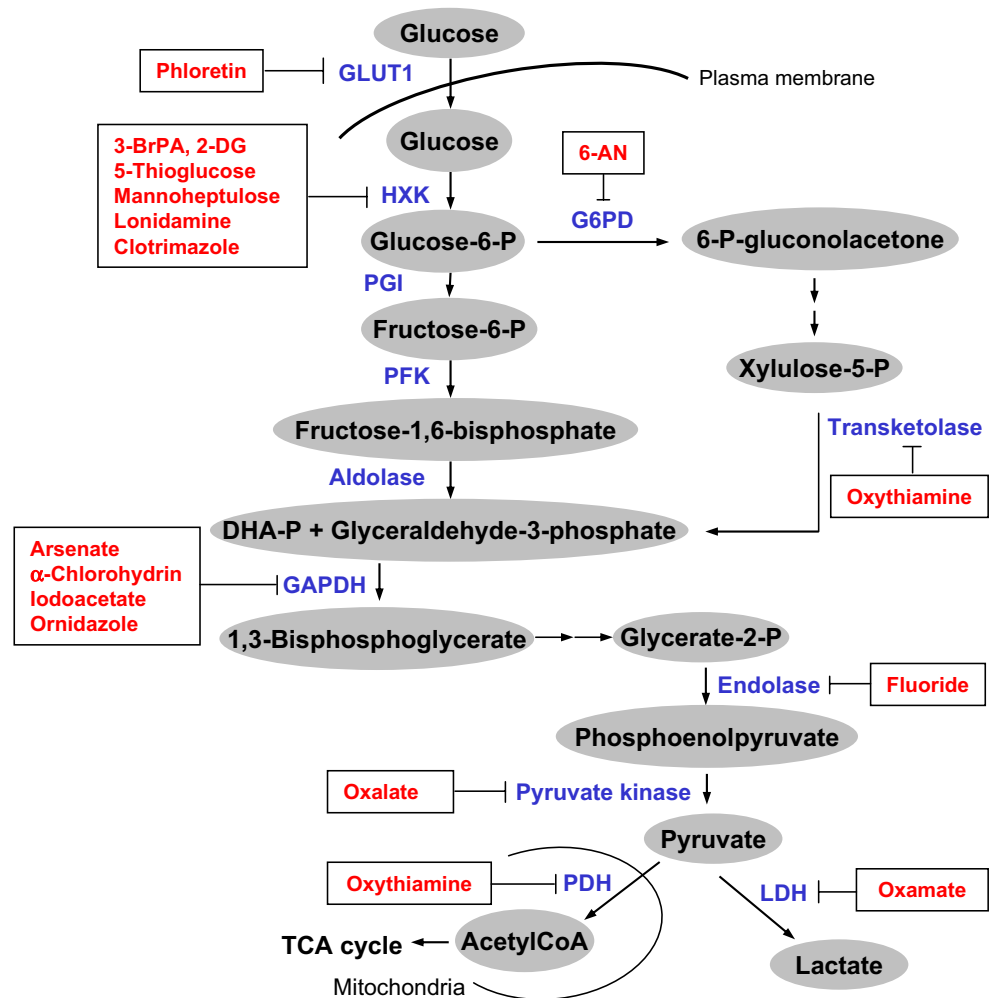
percentage of hexokinase binding to the mitochondria is also significantly increased in certain cancer cells (Arora and Pedersen 1988). HIF-1 and mutant p53 may promote HXK II expression (Pedersen et al. 2002). Because glucose-6-phosphate is a common metabolic intermediate for glycolysis and the pentose phosphate pathway, inhibition of HXK will have profound effects on both pathways and the down-stream mitochondrial metabolism. In addition to the enzymatic activity, the mitochondria-associated HXK II seems to play an important role in regulating apoptosis (Robey and Hay 2006). Thus, it is possible that agents targeting HXK II may also abolish the anti-apoptotic property of this molecule. Due to the important roles of HXK II in both cancer energy metabolism and apoptosis, this molecule is considered an attractive target. Known inhibitors of hexokinase include 2-deoxyglucose, 3-bromopyruvate, 5-thiogluconate and mannoheptulose.

PFK Phosphofructokinase catalyses the rate-limiting phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate, which is an energy-consuming step in glycolysis. This metabolic step is subjected to a complex allosteric regulation involving ATP as a negative regulator and fructose-2,6-bisphosphate as a positive regulator. Targeting this regulatory mechanism by suppression of PFKFB3 isozyme, which controls the cellular level of fructose-2,6-bisphosphate and thus affects the glycolytic flow, has been proposed as a strategy to preferentially kill Ras-transformed cells (Chesney 2006).

GAPDH This enzyme is encoded by a housekeeping gene and catalyses the key redox reaction in the glycolytic pathway by converting glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate, coupled with a reduction of NAD^+ to NADH. In addition to this enzyme activity, GAPDH also affects multiple cellular functions including membrane vesicular formation, nuclear tRNA transport, and DNA replication/repair (Sirover et al. 2005). Known inhibitors of GAPDH include α -chlorohydrin, Ornidazole, and iodoacetate. In addition, the pentavalent arsenate can induce arsenolysis during the GAPDH-catalyzing reaction and abolish ATP generation.

LDH LDH catalyzes the conversion of pyruvate to lactate, coupled with the oxidation of NADH to NAD^+ . Since NAD^+ is required for the glycolytic step catalyzed by GAPDH, regeneration of NAD^+ by the LDH-catalyzed reaction is important to maintain glycolysis, especially when mitochondrial respiration is compromised. Oxamate is a metabolic inhibitor of LDH. Interestingly, a recent study showed that a knockdown of LDH-A in tumor cells by shRNAs led to a stimulation of mitochondrial respiration, a decrease of cell proliferation under hypoxia, and a suppression of tumorigenicity (Fantin et al. 2006).

Fig. 2 Relationship between glycolytic pathway, the pentose phosphate pathway, and mitochondrial respiration. The enzymes that may be considered as potential targets for anticancer agents are indicated, and the relevant chemical inhibitors are also indicated. *GLUT1*, glucose transporter 1; *HXK*, hexokinase; *PGI*, phosphoglucose isomerase; *PFK*, phosphofructokinase; *DHA-P*, dihydroxyacetone phosphate; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *LDH*, lactate dehydrogenase; *PDH*, pyruvate dehydrogenase; *G6PD*, glucose6-phosphate dehydrogenase; *3-BrPA*, 3-bromopyruvate; *2-DG*, 2-deoxyglucose; *6-NA*, 6-aminonicotinamide



As shown in Fig. 2, inhibition of glycolysis by blocking *HXK* and *PFK* would also abolish pyruvate generation, and thus significantly decrease the supply of pyruvate to mitochondria for further metabolism through the TCA cycle and oxidative phosphorylation. In contrast, inhibition of *LDH* would mainly block the production of lactate from pyruvate, which can then be transported into mitochondria for ATP generation if the enzymes and protein machinery for the TCA cycle and respiratory chain are functional. As such, inhibition of *LDH* would selectively affect cancer cells with severe mitochondrial defects, and would be less toxic to normal cells. However, as discussed above, many cancer cells still maintain various levels of mitochondrial oxidative phosphorylation activity, and it is possible that inhibition of *LDH* alone would be insufficient to cause lethal depletion of ATP in these cancer cells. On the other hand, inhibiting the upstream enzymes (*HXK* or *PFK*) can block glycolysis and also decrease pyruvate supply for mitochondrial ATP generation, and thus has a more severe impact on energy production. In this case, toxicity to normal cells is a potential concern, although cells with

normal mitochondria in the presence of oxygen may still use other energy sources such as fatty acids and amino acids to generate ATP.

Transketolase Since certain metabolic intermediates of the pentose phosphate pathway can merge into the energy-yielding phase of the glycolytic pathway through the reaction catalyzed by transketolase (Fig. 2), an isoenzyme of transketolase, *TKTL1*, has been shown to overexpress in cancer cells (Coy et al. 2005; Langbein et al. 2006), and may serve as a potential anticancer target. Oxythiamine is a thiamine antagonist capable of inhibiting transketolase as well as pyruvate dehydrogenase. This compound has been shown to have significant anticancer activity (Rais et al. 1999).

Glycolytic inhibitors with anticancer activity

Based on the Warburg hypothesis, multiple groups have investigated the possibility of developing compounds

capable of inhibiting glycolysis or using the high glycolytic activity in cancer cells as a drug delivery mechanism to preferentially kill the malignant cells. Several compounds have been extensively evaluated *in vitro* and *in vivo*. The following compounds exhibit promising activity and are currently in different phases of drug development.

2-Deoxyglucose 2-Deoxyglucose is known to inhibit glucose metabolism. It is phosphorylated intracellularly by HXK to generate 2-deoxyglucose-phosphate, which accumulates inside the cells and inhibits hexokinase, presumably by a product-mediated inhibition. At the millimolar concentration range, 2-Deoxyglucose causes a depletion of ATP and cell death, especially in cancer cells with mitochondrial respiratory defects or cells in hypoxic environment (Liu et al. 2001, 2002; Maher et al. 2004). Several 2-halogenated glucose analogs seem to exhibit similar effect (Lampidis et al. 2006). Animal studies showed that 2-deoxyglucose, as a single agent, did not have significant therapeutic activity at the dose range of 500–2,000 mg/kg in mice, whereas its combination with doxorubicin or taxol enhanced therapeutic activity (Maschek et al. 2004). 2-Deoxyglucose has entered now clinical trials for cancer treatment in combination with other agents. A clinical evaluation suggests that this compound at the doses up to 250 mg/kg seems safe for use in combination with radiation therapy in patients with brain tumor (Singh et al. 2005). The high concentrations of 2-deoxyglucose required to produce cytotoxicity and the presence of high levels of competing glucose *in vivo* might affect the therapeutic activity of this compound.

3-Bromopyruvate (3-BrPA) 3-Bromopyruvate (3-BrPA) is a halogenated pyruvate ($\text{Br-CH}_2\text{-CO-COO}^-$) and a strong alkylating agent toward the free SH groups of cysteine residues in proteins. It has been used as a glycolytic inhibitor to kill protozoan parasite *Trypanosoma brucei*, which generates ATP mainly through glycolysis (Barnard et al. 1993). This early study showed that 3-BrPA is an inhibitor of the trypanosomal glyceraldehyde-3-phosphate dehydrogenase. More recent studies demonstrated that this compound is a potent inhibitor of HXK and also affects mitochondrial respiration, leading to ATP depletion and the death of cancer cells (Ko et al. 2001; Geschwind et al. 2004; Xu et al. 2005b). It is not surprising that 3-BrPA may affect multiple enzymes owing to its strong alkylating property. *In vivo*, 3-BrPA showed promising anticancer activity in rabbit hepatocellular carcinoma model without significant toxicity to normal liver cells (Geschwind et al. 2002; Ko et al. 2004). Importantly, 3-BrPA induced severe depletion of cellular ATP and massive cell death in cancer cells with mitochondrial defects or under hypoxia, which usually causes a decrease in cellular sensitivity to many

other anticancer agents (Xu et al. 2005b). The same study also showed that ATP depletion induced by 3-BrPA may disable the ATP-dependent multi drug-exporting pumps, and effectively kill cells with MDR phenotype. Thus, 3-BrPA possesses promising anticancer property in that it is effective against hypoxic cancer and may overcome multi-drug resistance. It should be noted, however, that although 3-BrPA is more potent than 2-deoxyglucose, this compound is unstable in solution and still requires 100 μM to be effective *in vitro*. Development of new analogs and drug combination are plausible means to improve anticancer activity. In fact, combination of glycolytic inhibitor with rapamycin, an inhibitor of mTOR has been shown to exhibit synergistic activity (Xu et al. 2005a). 3-BrPA and its analogs merit further evaluation as new anticancer agents.

Lonidamine Lonidamine is known for its ability to inhibit aerobic glycolysis in cancer cells, presumably by inhibiting the mitochondria-bound hexokinase (Floridi et al. 1981). *In vitro* studies showed that this compound caused depletion of cellular ATP in a dose-dependent manner (Floridi et al. 1998). Similar to 3-BrPA, lonidamine is effective in both doxorubicin-resistant and sensitive cells, and is able to enhance the activity of several alkylating agents (Rosbe et al. 1989). This compound has been evaluated in clinical trials for treatment of solid tumors including advanced breast, ovarian, lung cancer and malignant brain tumors, and exhibits some encouraging results (for review, see Di Cosimo et al. 2003). However, in a phase III trial, time to progression in metastatic breast cancer patients treated with epirubicin is not improved by adding lonidamine (Berruti et al. 2002).

Glufosfamide The development of glufosfamide represents a unique therapeutic strategy that takes advantage of elevated glucose uptake in cancer cells. This compound is a conjugate of glucose and ifosfamide, an alkylating agent currently used in clinical treatment of cancer. Glufosfamide is preferentially uptaken by cancer cells through SAAT1 glucose transporter, which is overexpressed in cancer cells. Once inside the cells, glufosfamide releases the toxic ifosfamide to kill the cells. Glufosfamide exhibits anticancer activity in experimental models, and has entered clinical trials for treatment of human cancers including lung and pancreatic cancer (Briasoulis et al. 2003; Giaccone et al. 2004).

Summary remarks

The Warburg effect (increased aerobic glycolysis) has been observed in a variety of cancer cells, and represents a fundamental alteration in energy metabolism associated with malignant transformation. This likely reflects a unique bio-energetic state of cancer cells where ATP requirements

increase due to active cellular function and the mitochondrial ATP generation is insufficient due to intrinsic and/or extrinsic alterations, leading to an increase in glycolysis. Whether this metabolic alteration is a symptom or a cause of cancer still remains as a matter of debate, the important observations made by Warburg have had a profound influence on our understanding of cancer biology and the development of cancer therapeutic approaches. Inhibition of glycolysis as a strategy to preferentially kill cancer cells and the development of glycolytic inhibitors as anticancer agents are important areas of research based on Warburg's hypothesis. Several glycolytic inhibitors with promising anticancer activity are currently at various stages of pre-clinical and clinical development. These inhibitors may be particularly useful for the treatment of hypoxic cancer. It should be pointed out, however, that many cancer cells still possess various degrees of mitochondrial respiration activity, and do not exclusively rely on glycolysis as the sole pathway to generate ATP. A clear understanding of the complex metabolic alterations and regulatory mechanisms in cancer cells is essential for the successful development of new therapeutic agents and effective regimens for cancer treatment.

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