Review

Possible Therapeutic Targets Among the Molecules Involved in the Warburg Effect in Tumor Cells

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Abstract. *The majority of human tumors display a high rate of glycolysis under aerobic conditions. This phenomenon was recognized approximately seven decades ago and is known as the Warburg effect. Several key enzymes required to maintain this high level of glucose metabolism are found in tumor cells. The effects of the glycolytic enzymes are known to be directly or indirectly regulated by various signaling pathways, oncogenes, suppressor genes and transcription factors. Recent molecular biology studies have shown that multiple genetic alterations are related to tumor development. Therefore, these factors may be rational targets for cancer therapy. In this short review, we describe several important molecules that affect aerobic glycolysis and discuss their possible use as therapeutic targets for cancer.*

Various metabolic changes occur in tumor cells. Tumor cells have special metabolic requirements for their development into three-dimensional tumor masses. Among the metabolic changes exhibited by tumor cells, enhanced glucose metabolism and glucose dependence compared to normal tissue is particularly correlated with tumor aggressiveness and prognosis. Normal cells generate most of their ATP through glycolysis under anaerobic conditions and mitochondrial oxidative phosphorylation under aerobic conditions. Furthermore, normal tissues can use alternative energy sources, such as glucose, fatty acids, amino acids and other metabolic intermediates, to generate ATP in mitochondria. Tumor cells, however, generate as much as 60% of their ATP through glycolysis, regardless of the presence or absence of oxygen, and depend more on glycolysis for ATP generation. This phenomenon was originally observed by Otto Warburg and is now known as the Warburg effect (1) or "aerobic glycolysis". Aerobic glycolysis has been observed in a variety of tumor cells. It has been hypothesized that aerobic glycolysis has some advantages for tumor cells over oxidative phosphorylation. For instance, aerobic glycolysis has been shown to protect DNA from damage caused by oxygen radicals produced by oxidative phosphorylation and to generate ATP at a faster rate than oxidative phosphorylation (2).

The glycolytic pathway is a series of metabolic reactions catalyzed by multiple enzymes or enzyme complexes. The expression of many of these glycolytic enzymes is known to increase in tumor cells. Therefore, several glycolytic enzymes in this pathway have recently been considered as possible targets for the treatment of cancer and have gathered interest as possible targets for the development of glycolytic inhibitors as potential anticancer agents (3). Furthermore, recent molecular biology studies have identified signaling pathways, oncogene products, tumor suppressor proteins and transcriptional factors that directly or indirectly affect tumor cell energy metabolism. Therefore, these factors may also be rational targets for cancer therapy. In this short review, we focus on these factors as well as on glucose transporters and glycolytic enzymes (Figure 1), that have been shown to influence the Warburg effect.

Glucose Transporters and Glycolytic Enzymes Related to the Warburg Effect

Tumor cells exhibit a high rate of glycolysis, a phenomenon caused by the up-regulation of many glycolytic enzymes and the increased flux of glucose *via* glucose transporters (4). Each of these molecules is considered a possible therapeutic target. In this section, we address the function of glucose transporters and glycolytic enzymes and summarize the findings of recent studies of genetic and pharmacological interventions targeting these molecules (Figure 1).

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Glucose transporter (GLUT) and sodium-dependent glucose transporter (SGLT). Glucose is a hydrophilic compound and therefore cannot pass through the cell membrane lipid bilayer *via* simple diffusion. Instead, it requires a specific transporter to enter the cytosol. Two classes of hexose transporters are known to be glucose transporters: the GLUT and SGLT families. The GLUT family comprises of facilitative transporters that transport glucose along a concentration gradient. The expression of GLUT1 is increased in some tumor cells and is correlated with a poor prognosis (5). Recent studies have shown the possibility of inhibiting GLUT through the use of small interference RNA (siRNA), antisense nucleotides or antibodies to GLUT (6). The SGLT family comprises of Na^+/glucose transporters that transport glucose against a concentration gradient utilizing the sodium-electrochemical gradient and ATP. SGLT1 is expressed in some primary tumors and metastatic lesions (7). In a recent study, it was found that epidermal growth factor receptor (EGFR) associates with and stabilizes SGLT1 to promote glucose uptake into tumor cells (8). Therefore, EGFR, which participates in cell growth, migration and proliferation, seems to be not only an important mediator of intracellular signaling, but also an integral component of the active glucose transport system (9). This may explain the resistance of tumor cells to chemotherapeutic agents and tyrosine kinase inhibitors. Several novel strategies have recently been proposed to deliver chemotherapeutic agents into tumor cells *via* the actions of SGLT1 (10).

Hexokinase. Following cellular uptake *via* glucose transporters, glucose is converted to glucose 6-phosphate, the initial phosphorylated intermediate of the glycolytic pathway, by hexokinases. In human cells, there are four isoforms of hexokinase (I-IV) with different patterns of tissue expression (11). Among them, hexokinase II is overexpressed in various types of cancer cells. Mutant p53, v-akt murine thymoma viral oncogene homolog (AKT) and hypoxia-inducible factor-1 (HIF1) may promote the hexokinase II expression (12). Since glucose 6-phosphate is a common metabolic intermediate for glycolysis, hexokinase II plays a role in the initiation and maintenance of high rates of glucose catabolism in tumor cells. Therefore, inhibiting this molecule is considered an important target of therapy. Well-known hexokinase inhibitors include 2-deoxyglucose, 3-bromopyruvate, 5-thioglucose, mannoheptulose and lonidamine (13).

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH is encoded by a housekeeping gene and catalyzes the key redox reaction in the glycolytic pathway by converting glyceraldehyde 3-phosphate to 1,3-*bis*phosphoglycerate. In addition to its enzyme activity, GAPDH affects multiple cellular functions, including membrane vesicular formation, nuclear tRNA transport and DNA replication/repair (14). Known inhibitors of GAPDH include α-chlorohydrin, ornidazole and iodoacetate. In addition, pentovalent arsenate can induce arsenolysis during the GAPDH-catalyzing reaction and abolish ATP generation.

Pyruvate kinase (PK). PK plays an essential role in regulating the balance between glycolytic ATP generation and biosynthetic requirements in proliferating cells (15). PK isozyme type M2 (PK-M2), which converts phosphoenol-pyruvate into pyruvate, is highly active in its tetrameric form and less active in its dimeric form. The tetramer to dimer shift of PK-M2 controls the relative activity of glycolysis. Cancer cells express higher levels of PK-M2 over the more catalytically active PK-M1. The tetramer-dimer ratio of PK-M2 is regulated by ATP, fatty acids and fructose 1,6-*bis*phosphate (16). Fructose 1,6-*bis*phosphate is a key metabolite that increases the activity of PK-M2 in normal tissues, leading to a drastic increase in forward flux *via* glycolysis. The PK-M2 expression provides a proliferative advantage for cancer cells and enhances the transcriptional activity of HIF1 (17), raising the possibility that PK-M2 may be an attractive target for cancer therapy. Oxalate is a potent inhibitor of PK. A recent study showed that inhibiting PK-M2 activity using siRNA increases apoptosis in cell culture and can inhibit tumor cell growth (18).

Lactate dehydrogenase A (LDHA) and pyruvate dehydrogenase kinase (PDK). In tumor cells, the increased expression of LDHA and inactivation of pyruvate dehydrogenase (PDH) promote glycolysis *via* pyruvate to lactate conversion and suppression of the tricarboxylic acid (TCA) cycle. PDK inactivates PDH and prevents the movement of pyruvate into the mitochondrial matrix (19). The expressions of LDHA and PDK are increased in human cancer and their activity is directly regulated by a variety of oncogenes and transcriptional factors, including v-myc myelocytomatosis viral oncogene homolog (c-MYC) and HIF1α. LDHA has been used as a marker of neoplastic transformation. Inhibiting LDHA primarily blocks the production of lactate from pyruvate; thus, several glycolysis inhibitors, including the LDHA inhibitors FX11and oxamate, are in pre-clinical and clinical development (20).

Signaling Pathways, Oncogenes, Suppressor Genes and Transcriptional Factors Related to the Warburg Effect

In tumor cells, the activation of signaling pathways, proto-oncogenes and transcriptional factors and the inactivation of tumor suppressor genes are associated with glycolytic fueling, involving a metabolic switch from oxidative phosphorylation to glycolysis, thereby attenuating mitochondrial respiration (21). Recent molecular biological

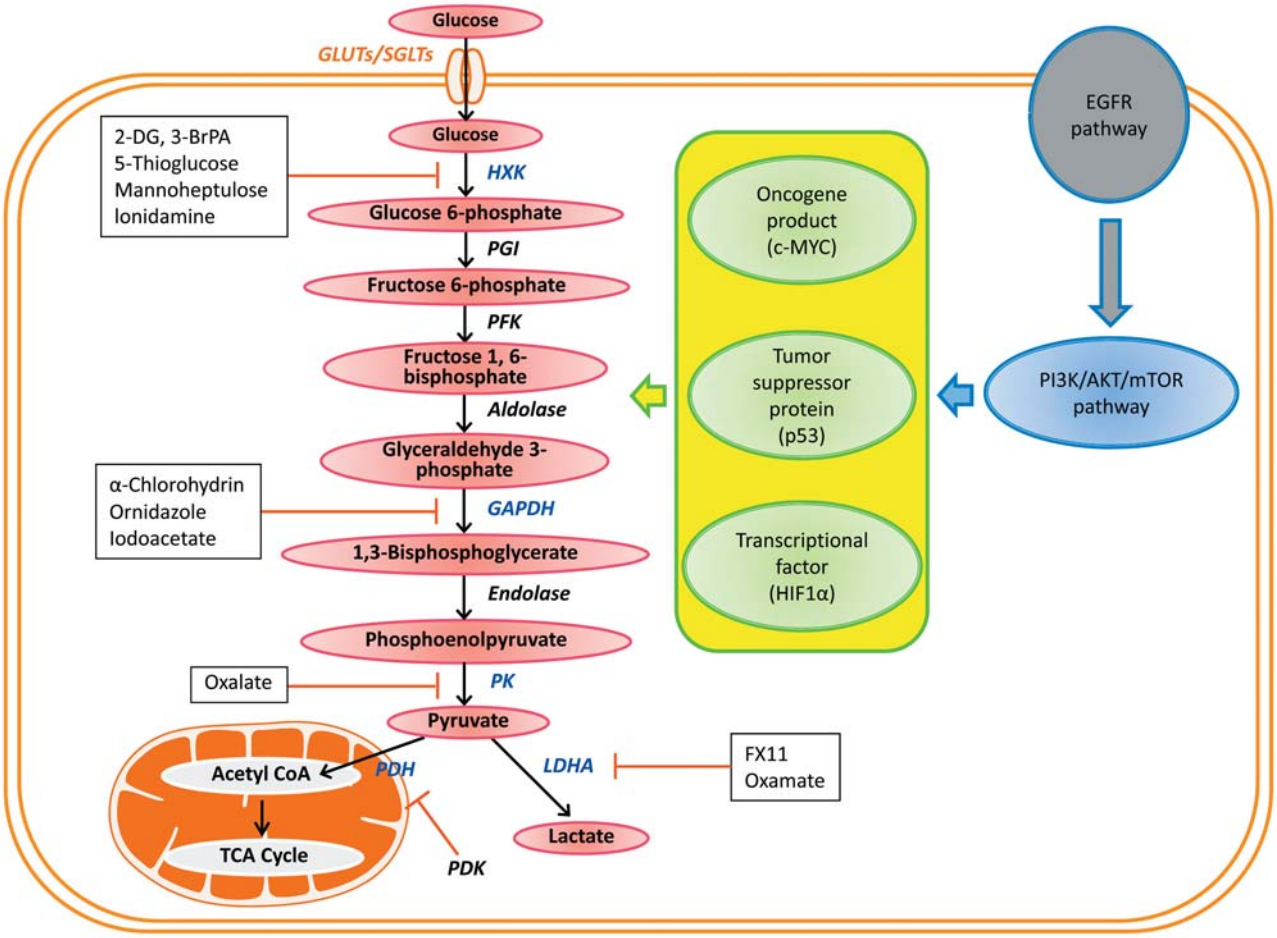

Figure 1. *Schematic representation of the relationships between the glycolysis pathway and signaling pathways, oncogenes, tumor suppressor genes and transcription factors. Glucose transporters (GLUT and SGLT) and the subsequent catabolism of glucose via the glycolytic pathway are shown. Aerobic glycolysis is in part due to up-regulation of genes coding glucose transporters and glycolytic enzymes. The glycolytic enzymes that are considered to be potential targets for anticancer agents are indicated in blue, and the relevant chemical inhibitors are noted. Activation of signaling pathways, transcriptional factors and oncogene products and inactivation of tumor suppressor proteins directly or indirectly regulate the glycolysis pathway and glycolytic enzymes. PGI: Phosphoglucose isomerase; PFK: 6-phosphofructokinase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PK: pyruvate kinase; PDH: pyruvate dehydrogenase; PDK: pyruvate dehydrogenase kinase; LDHA: lactate dehydrogenase A.*

studies have shown that these multiple genetic alterations are related to tumor development. Therefore, these factors may be rational targets for cancer therapy. In this section, we address the functions of signaling pathways, oncogenes, tumor suppressor genes and transcriptional factors affecting aerobic glycolysis and summarize the findings of recent studies of genetic and pharmacologic interventions targeting these factors (Figure 1).

Phosphatidylinositol 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) signaling pathway. Aberration and activation of the PI3K/AKT pathway is commonly observed in cancer cells. PI3K is activated *via* mutation or amplification in numerous types of cancers (22). Growth factor-dependent activation of PI3K leads to the activation of downstream effectors, including AKT and mTOR, that coordinate metabolic activities that support cellular biosynthesis. Enhanced PI3K/AKT signaling results in metabolic transformation *via* multiple pathways, including the increased expression of genes involved in glycolysis and stimulation of various glycolytic enzyme activities. AKT signaling activation regulates the transcription and translation of glucose transporters and glycolytic enzymes and increases their expressions. AKT has also been implicated in the regulation of glucose uptake. AKT-dependent stimulation of glycolytic enzymes, such as hexokinase and phosphofructokinase (PFK), drives glycolysis, thereby increasing the transcription of genes involved in glycolysis and lipogenesis, and enhances protein translation *via* an AKT-dependent mTOR activation (23). Therefore, activation

of AKT signaling may be sufficient to bring about the switch to glycolytic metabolism in tumor cells. PI3K and mTOR are located up- and downstream of AKT, respectively. mTOR is an upstream activator of HIF1α in tumor cells that functions as a transcription factor regulating the expressions of genes involved in the glycolytic pathway. Since the PI3K/AKT/mTOR pathway regulates the genes responsible for aerobic glycolysis, disruption of PI3K/AKT/mTOR signaling may switch the source of metabolic energy from glycolysis to oxidative phosphorylation and remove the survival advantage of cancer cells. In this context, it is very interesting that several mTOR inhibitors used in phase I/II trials, including RAD001, temsirolimus (CCI-779) and AP-23573, demonstrate antitumor activity and a generally tolerable safety profile (24-27).

Oncogene c-MYC. c-MYC is frequently found in various human tumors (28). The c-MYC protein participates in the regulation of cell proliferation, differentiation and apoptosis (29). c-MYC is an oncogenic transcription factor that stimulates the expression of glycolytic enzymes, such as LDHA, and reduces the amount of glucose carbon that enters the TCA cycle (30). Furthermore, c-MYC promotes glucose metabolism, and c-MYC-transformed cells have been demonstrated to be susceptible to glucose deprivation-induced apoptosis (31). c-MYC activation collaborates with HIF1 to confer metabolic advantages to tumor cells, allowing them to thrive in a hypoxic microenvironment (the Warburg effect) (32). Recently, c-MYC gene targets have emerged, although they have not been comprehensively characterized. Therefore, the development of therapeutics that inhibit the transcriptional properties of c-MYC has, thus far, eluded drug discovery efforts.

Tumor suppressor gene p53. P53 is a transcriptional factor that suppresses tumor development by regulating the expressions of genes inducing cell cycle arrest, apoptosis and senescence under stress conditions (33, 34). The activation of P53 indirectly decreases the activity of several glycolytic enzymes and increases the use of the TCA cycle and oxidative phosphorylation. Activated P53 protein inhibits the activity of the PI3K/AKT/mTOR pathway. In many types of tumor cells, P53 is mutated and inactivated, and the loss of the P53 functions leads to the Warburg effect. P53 induces a gene named TP53-induced glycolysis and apoptosis regulator (TIGAR). The expression of TIGAR leads to a decrease in the expression of fructose-2,6-*bis*phosphate and suppression of glycolysis (35). Therefore, the tumor suppressor P53 can regulate the balance between oxidative phosphorylation and glycolysis *via* multiple mechanisms, and tumor cells lacking functional P53 may exhibit reduced respiratory activity and thus become dependent on glycolysis to generate ATP, even in the presence of oxygen. P53 also represses the transcriptional activity of some *GLUT* gene promoters *via* direct DNA binding, leading to a decrease in glucose uptake (36). Mutated P53 stimulates the transcription of the hexokinase II promoter. A recent study demonstrated that loss of P53 activates nuclear factor-kappa B (NFκB) to increase the rate of aerobic glycolysis (37). In recent years, a number of strategies to reactivate mutant P53 with small molecules, such CP-31398 and PRIMA-1, have shown effective inhibition of aerobic glycolysis in tumor cells both *in vitro* and *in vivo* (38, 39).

Transcriptional factor HIF1α. Hypoxia induces the activation of HIF1α, an important transcription factor involved in glucose uptake and glycolysis that promotes the expression of glycolytic enzymes (40). HIF1α may mediate the metabolic switch from mitochondrial respiration to glycolysis by inducing and consecutively increasing the expressions of glucose transporters and glycolytic enzymes. The majority of glycolytic enzymes in the glycolytic pathway, from glucose to lactate, are regulated by HIF1α (41). HIF1α targets glucose enzymes, such as PDK1, hexokinase II, PFK1 and LDHA (42). HIF1α-dependent activation of PDK1 and LDHA and the resulting inactivation of the PDH complex contribute to a decreased flux in oxidative phosphorylation. In addition, HIF1α targets membrane transporters, such as GLUT1 and monocarboxylate transporter 4, that ensure both adequate glucose delivery into the cell and export of accumulated lactate out of the cell. Interestingly, HIF1α is stabilized by the activation of the PI3K/AKT/mTOR pathway (43, 44). Since HIF1 is such an important drug target, small-molecule inhibitors of HIF1, such as topotecan, NSC 644221, YC-1 and PX-478, have been identified using screening.

Conclusion

Recent studies have shown that several signaling pathways, oncogene products, tumor suppressor proteins and transcriptional factors are involved in the Warburg effect in tumor cells, indicating that these factors may be rational targets for tumor therapy. However, the drugs tested thus far have demonstrated only modest effects in tumor therapy. Why these drugs exhibit modest effects results from the complexity of metabolic pathways in cancer. Metabolic abnormalities involve several common pathways and transcriptional factors associated with cancer metabolism. Our recent studies demonstrated that the EGFR ligand and pathway in tumor cells are partially associated with metabolic abnormalities. Furthermore, some therapeutic drugs that attenuate the EGFR ligand and metabolic pathway exhibit synergistic inhibitory effects for tumor therapy. Since the EGFR pathway is upstream of the PI3K/AKT/mTOR pathway and transcription factors, such as HIF1α and P53,

which activate glycolytic enzymes, are associated with the expression of the EGFR ligand (45, 46), this pathway is considered to be an important therapeutic target in aerobic glycolysis. Several novel anticancer agents against heparin-binding EGF-like growth factor (HB-EGF), an EGFR ligand, have recently been developed (46). Therefore, it will be interesting to determine whether these agents also exert effects on metabolic abnormalities.

Conflicts of Interest

The Authors declare no conflict of interests.

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