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Emerging Metabolic Targets in Cancer Therapy

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1. ABSTRACT

Cancer cells are different from normal cells in their metabolic properties. Normal cells mostly rely on mitochondrial oxidative phosphorylation to produce energy. In contrast, cancer cells depend mostly on glycolysis, the aerobic breakdown of glucose into ATP. This altered energy dependency is known as the "Warburg effect" and is a hallmark of cancer cells. In recent years, investigating the metabolic changes within cancer cells has been a rapidly growing area. Emerging evidence shows that oncogenes that drive the cancer-promoting signals also drive the altered metabolism. Although the exact mechanisms underlying the Warburg effect are unclear, the existing evidence suggests that increased glycolysis plays an important role in support malignant behavior of cancer cells. A thorough understanding of the unique metabolism of cancer cells will help to design of more effective drugs targeting metabolic pathways, which will greatly impact the capacity to effectively treat cancer patients. Here we provide an overview of the current understanding of the Warburg effect upon tumor cell growth and survival, and discussion on the potential metabolic targets for cancer therapy.

2. INTRODUCTION

Cancer cells have a fundamentally different bioenergetic metabolism from that of non-neoplastic cells, with the latter relying mostly upon the process of mitochondrial oxidative phosphorylation, consuming glucose and oxygen in order to produce energy. In contrast, cancer cells depend primarily upon aerobic glycolysis, defined as the aerobic breakdown of glucose into the energy-storing molecules of adenosine triphosphate (ATP), even in the presence of oxygen. The increased dependency upon the glycolytic pathway is known as the Warburg effect, which is a hallmark of cancer cell metabolism. This phenomenon was first described and reported by Warburg over 80 years ago; leading him to hypothesize that cancer develops as a direct result of defects in oxidative phosphorylation (1-8). However, an accumulating body of evidence has shown that mitochondrial function is not impaired in most cancer cells. Thus, today's description of the Warburg effect is defined as an increase in aerobic glycolysis that is not necessarily correlated with permanent mitochondrial dysfunction (9).

Glycolysis generates only 2 ATPs per molecule of glucose, whereas oxidative phosphorylation produces up to 36 ATPs upon complete oxidation of one molecule of glucose. The question immediately arises as to why cancer cells would switch to a less efficient form of energy production. One proposed hypothesis describes an increase in aerobic glycolysis as an adaptation to the intermittent hypoxia found in pre-malignant lesions. This "glycolytic phenotype" has been suggested to confer a selective growth advantage, thereby promoting unconstrained proliferation and invasion (10). However, Vander Heiden *et al.* proposed a different explanation, with hypoxia as a late-occurring event that may not be a major contributor to the glycolytic switch. They further explain that the inefficient production of ATP is a problem only when resources are scarce, usually not the case for proliferating tumor cells. Secondly, increased glycolysis meets the building block (e.g., lipids, amino acids and nucleotides) requirements that are necessary for cell survival above and beyond the production of ATP by cancer cells (11). Further evidence comes from ^{18}F-deoxyglucose positron emission tomography (FDG-PET), which is a widely used cancer diagnostic imaging technique in the clinic. It reveals that both primary and metastatic human cancers show increased FDG uptake (10, 11), supporting that altered glucose metabolism is an early-occurring event in cancer development.

Investigating the bioenergetic metabolic changes within cancer cells has been a rapidly growing area of research. Emerging evidence shows that oncogenes that drive the cancer-promoting signal transduction pathways also are capable of further driving altered cellular metabolism. Although the exact molecular mechanisms underlying the Warburg effect are not completely clear, the existing evidence suggests that increased glycolysis plays an important role in the metabolic mechanisms utilized by the malignant phenotype. A more thorough understanding of the unique metabolic demands of cancer cells will help with the design of more effective and specific drugs which are capable of selectively targeting the pathways that are involved in cancer cell metabolism. Thus, it may open a new therapeutic avenue which will greatly impact the capacity to effectively treat cancer patients. In this review, we provide an overview of the current understanding of the Warburg effect upon tumor cell growth and survival; further discuss potential emerging metabolic targets of glucose metabolism for cancer therapy.

3. THE ENERGY REQUIRMENTS FOR TUMOR CELL SURVIVAL

Normal cellular physiology is capable of utilizing mitochondrial oxidative phosphorylation to maximal efficiency, producing ATP which in turn is responsible for 70-90% of total cellular oxygen consumption (8). However, tumor cell neovasculature has been shown to be intrinsically inefficient in carrying oxygen and other nutrients to viable tumor cells resulting in cancer cell hypoxia and low nutrient levels, especially in solid tumors (12, 13). Under hypoxic conditions, a reduction in mitochondrial oxygen consumption by tumor cells causes a compensatory increase in glycolysis for production of ATP (14), which allows for continued survival and even further cellular proliferation (15).

In the cellular metabolic shift from mitochondrial oxidation to glycolysis, hypoxia-inducible factor -1 (HIF-1) has been shown to play a crucial role by activating a set of genes involved in angiogenesis, glucose uptake, and glycolysis (15). HIF-1 is a heterodimeric transcription factor composed of a constitutively expressed HIF-1 beta subunit and an O_2-regulated HIF-1 alpha or HIF-2 alpha subunit (16). In the presence of oxygen, the tumor suppressor, Von Hippel-Lindau (VHL), binds to HIF-1 alpha or HIF-2 alpha, which is subsequently targeted for ubiquitination and proteasomal degradation (17). However, in the absence of oxygen, VHL-induced ubiquitination and degradation dramatically declines, with HIF-1 stabilized and further activated. Hypoxia activated HIF-1 also has been shown to induce the expression of nearly all the enzymes in the glycolytic pathway, as well as gene expression of the glucose transporters GLUT1 and GLUT3 (18), which results in increased anaerobic glycolysis and ATP production.

Recently, several groups (19-21) have reported that HIF-1 not only stimulates glycolysis, but also actively downregulates mitochondrial oxygen consumption by inducing pyruvate dehydrogenase kinase (PDK) 1 and 3, both of which inactivate pyruvate dehydrogenase (PDH), an enzyme involved in the citric acid cycle, also known as the tricarboxylic acid cycle (TCA cycle). Although PDK1 and PDK3 have similar biological functions, PDK3 harbors the highest enzyme activity among all PDKs and is the only enzyme that is not inhibited by high concentrations of pyruvate. Overexpression of HIF-1alpha in cancer cells is occurs with high concentrations of pyruvate, with the unique feature that PDK3 continuously inhibits of PDH activity and the subsequent shutdown of mitochondrial respiration. Considering the potential importance of PDK3

in the metabolic switch of cancer cells, the PDK3 gene has been proposed as a new target of inhibition for potential therapy in cancer patients (20). In addition, under hypoxic conditions, perturbation in electron transport results in increased production of reactive oxygen species (ROS), which may pose a barrier for cell survival. An active suppression of mitochondrial respiration avoids this excess production of ROS (19). These findings may provide a more rational explanation for how cancer cells adapt to hypoxia through an active metabolic shift from mitochondrial respiration to glycolysis.

4. THE METABOLIC REQUIREMENTS OF TUMOR CELL PROLIFERATION

Warburg originally hypothesized that cancer cells were forced to rely on glycolysis due to an unidentified mitochondrial defect, with subsequent studies revealing that most cancer cells actually possess normal mitochondrial function (11). It is easier to understand why tumor cells switch to glycolysis within a hypoxic microenvironment. However, high rates of aerobic glycolysis (the classical Warburg effect) also were commonly observed in a wide variety of tumor histologies. Vander Heiden proposed that the switch was probably due to the large metabolic demands of tumor cells in addition to ATP production (11, 22). Such large metabolic requirements for cell proliferation, rather than efficient ATP production, result in a logical switching to glycolysis, which provides a host of intermediary compounds, such as lipids, amino acids, and nucleotides, for the synthesis of the required components for cell proliferation.

5. METABOLISM-REGULATING SIGNALING MOLECULES, ENZYMES AND TRANSCRIPTION FACTORS

5.1. PI3K-Akt-mTOR pathway

The PI3K-Akt-mTOR pathway is an important signal transduction pathway which is overactivated in many types of human malignancies, both sporadic and hereditary (9). PI3K is divided into 3 classes, class I, class II and class III, with class I further sub-divided into class Ia and class Ib. Class Ia is composed of a p110 catalytic subunit and a p85 regulatory subunit. There are three variants of the p110 catalytic subunit, described as p110 alpha, p110 beta and p110 delta. The gene encoding the p110 alpha subunit, PIK3CA, is amplified and overexpressed in several ovarian cancer cell lines and mutated in a variety of other cancers including colorectal, glioblastoma and gastric. PI3K is directly activated by both Ras and growth-factor receptor tyrosine kinases, which are mutationally activated in a majority of human cancers. PTEN is a negative regulator of PI3K, with the activation of PI3K leading to the phosphorylation of phosphatidylinositol-4, 5-bisphosphate (PIP2) to generate phosphatidylinositol-3, 4, 5-bisphosphate (PIP3). PIP3 is a second messenger that activates the downstream kinase AKT. Through TSC and RHEB, AKT activates the mammalian target of rapamycin (mTOR). Activation of mTOR leads to protein translation, including HIF1alpha protein synthesis (23-25).

AKT is a major regulator of cellular energy metabolism, stimulating aerobic glycolysis in cancer cells and rendering them dependent upon aerobic glycolysis for both growth and survival (26). AKT also enhances glycolysis via multiple other cellular mechanisms. First, AKT increases glucose uptake by increasing the expression of glucose transporters and by promoting membrane translocation of the glucose transporters. Second, AKT may indirectly activate the glycolysis rate-limiting enzyme phosphofructokinase-1 (PFK1) by directly phosphorylating and activating phosphofructokinase-2 (PFK2). Third, AKT also activates mTOR, another master regulator gene of cellular metabolism. The mTOR gene promotes HIF-1alpha abundance, resulting in HIF-1alpha-associated glycolytic gene expression, such as glucose transporter, HKII and LDH (27).

AKT also increases oxidative phosphorylation by enhancing the coupling efficiency between glycolysis and oxidative phosphorylation through the stimulation of mitochondrial hexokinase (HK) which is associated with the voltage-dependent anion channel (VDAC) and mitochondria. Rapidly proliferating tumor cells have both anabolic and catabolic demands, with AKT-induced glycolysis providing intermediates for building blocks. AKT-increased oxidative phosphorylation may also provide large amounts of ATP for protein and lipid synthesis. Therefore, AKT activation provides selective advantages to tumor cells by increasing both glycolysis and oxidative phosphorylation. In this sense, AKT may serve as a "Warburg kinase" that can be specifically targeted to change cancer cell energy metabolic requirements for therapeutic benefit.

5.2. Hypoxia-inducible factor 1 (HIF1)

As mentioned above, HIF1 has been sown to regulate many enzymes in the glycolytic pathways, which results in increased anaerobic and aerobic glycolysis and ATP production. In addition, HIF-1alpha is over-expressed in many human cancers, such as breast, ovarian, cervical and lung (28, 29). Hypoxia and genetic alterations can lead to HIF-1alpha over-expression, which is further associated with increased mortality in patients with brain, breast, cervical, oropharynx, ovarian and uterine cancer. By contrast, HIF-1alpha over-expression has been associated with decreased mortality for patients with head and neck and non-small-cell lung cancer (30). Furthermore, *in vivo* studies show that increased levels of HIF-1alpha are associated with increased xenograft tumor growth, whereas inhibition of HIF-1 activity markedly impaired tumor growth (30). Therefore, HIF-1 is an excellent metabolic target for cancer therapy. Currently, major efforts have been made to identify specific inhibitors for HIF-1.

5.3. c-Myc

The transcription factor c-Myc is encoded by the proto-oncogene c-*myc*, which belongs to the *myc* family of genes (c-*myc*, L-*myc,* s-*myc*, and N-*myc*). The *myc* genes are commonly translocated and amplified, which contribute to the genesis of many human cancers, and deregulated expression of the c-Myc protein has been found in about 70% of all human tumors (18). As a DNA-binding protein,

c-Myc contains a helix-loop-helix leucine zipper motif and acts as either a transcriptional activator or repressor. After forming a heterodimer with Max, c-Myc is then capable of binding to E boxes (CACGTG) to transactivate specific genes. The Myc-Max hetreodimer also can inhibit the expression of other genes by further forming a trimer with the transcription factor Miz 1 or Sp1 (31).

It's surprising that about 30% of all known genes in humans can be directly bound by c-Myc. However, only a fraction of these genes are actually up or down-regulated by it, because c-Myc must cooperate with other transcription factors, such as E2F1 and HIF-1, to regulate genes involved in nucleotide and glucose metabolism. The conserved core set of c-Myc target genes seem to be involved in ribosomal and mitochondria biogenesis, regulation of cell cycle, glucose metabolism and glutamine metabolism. Moreover, more studies indicate that the c-Myc gene is definitively linked to altered cellular metabolism and tumorigenesis (32).

Shim *et al* first reported on the link between c-Myc gene expression and the regulation of glucose metabolism in 1997 (33). In this report, *LDH-A*, which encodes Lactate dehydrogenase-A (LDH-A), the enzyme that catalyzes the conversion of pyruvate to lactate, was identified as a target gene of c-Myc. It was found that c-Myc induced LDH-A gene expression, which resulted in increased lactate production. Moreover, induction of LDH-A was found to be necessary for c-Myc-mediated transformation, implicating a potential role of c-Myc and LDH-A in tumor growth. Subsequent studies revealed that many other glycolysis-associated genes are directly regulated by c-Myc, including the glucose transporter, *GluT1*, hexokinase 2 (*HK2*), phosphofructokinase (*PFK*) and enolase 1 (*ENO 1*) (34, 35). Hence, c-Myc is able to stimulate a variety of genes with different functions that ultimately result in increased transport of glucose and activation of glycolysis (32).

Several other recent studies have shown that c-Myc not only induces genes involved in glycolysis, but also induces many other genes involved in glutamine metabolism. The glutamine transporters ASCT2 and SN2 have been indentified as direct c-Myc target genes (36). A proteomic analysis of human B-lymphocytes shows high levels of mitochondrial glutaminase (GLS) protein compared to control lymphocytes. GLS is the first enzyme that catalyzes the conversion of glutamine to glutamate and its activity correlates with the growth rate of tumors. However, unlike ASCT2 and SN2, the GLS gene is under indirect transcriptional control by c-Myc. It has been found that c-Myc induces the expression of GLS through suppression of miR-23a and miR-23b, which directly target the GLS mRNA 3'- UTR (37).

Glutamine is another major nutrient consumed by cancer cells besides glucose, and some tumor cells have been reported to exhibit a high rate of glutamine metabolism, although this was not seen universally in cancer cell lines. Yuneva *et al* (38) have shown that glutamine deprivation, but not glucose deprivation, selectively induces apoptosis in some, but not all, human cells with high levels of Myc gene expression. Wise *et al* (36) have shown that a human glioma cell line with an amplification of the Myc gene was unable to survive in glutamine-deficient medium even in the presence of sufficient glucose. Further studies revealed that Myc-transformed glioma cells use glutamine, not glucose, as the oxidizable substrate to maintain the TCA cycle and overall cell viability. These results are in concordance with others, showing that (39) Myc-transformed cells exhibit increased mitochondrial mass and rate of O_2 consumption. Overall, the Myc gene not only promotes glycolysis, but also stimulates mitochondrial glutaminolysis to maintain mitochondrial function.

5.4. Metabolic enzymes

5.4.1. Hexokinase (HK)

HK catalyzes the conversion of glucose to glucose-6-phosphate, the first and rate-limiting step in the glycolytic pathway. There are four isoforms of HK (I-IV) in mammals, with different subcellular localization, catalytic and regulatory properties (40). In cancer cells, HK (primarily HKII) is over-expressed and bound to the outer mitochondrial membrane via the porin-like protein VDAC (41-43). Mitochondrial-bound HKIIplays an important role in preventing tumor apoptosis (44).

5.4.2. Pyruvate kinase (PK)

PK catalyzes the irreversible transfer of a phosphate group from phosphoenolpyruvate (PEP) to ADP, producing pyruvate and ATP. This step in glycolysis is essentially irreversible under normal physiologic conditions. Therefore, PK is also considered a rate-limiting enzyme in the glycolytic pathway. Growth factor signaling pathways, which play a key role in anabolic metabolism and cell proliferation, are known to be initiated by tyrosine phosphorylation of signaling proteins, which are commonly increased in many tumors. Mammalian PK has four isoenzymes (M1, M2, L and R) which are present in different cell types. The L and R isoforms exist in the liver and red blood cells, while the M1 isoform exists in most normal adult tissues with constitutively high activity and the M2 isoform is only expressed during embryonic development. Recently, PKM2 has been indentified as a phosphotyrosine-binding protein (45).

It has been shown that the embryonic isoform PKM2 is expressed predominantly in tumor cells with low activity (46), and exclusive PKM2 expression in tumor tissues indicates its potential critical role in cancer cell metabolism. Recently, Christofk *et al* revealed an important role for PKM2 expression in aerobic glycolysis and tumor growth (47). Their data showed that stable "knockdown" of the PKM2 gene in the human cancer cell lines resulted in decreased rates of glucose metabolism and reduced cell proliferation. Furthermore, the endogenous replacement of the PKM2 gene with mouse PKM1 or mouse PKM2 genes revealed that the M1 rescue cells showed more oxygen consumption and less lactate production than the M2 rescue cells.

These findings indicated that the M1 rescue cells prefer to metabolize glucose by oxidative phosphorylation

rather than rely on aerobic glycolysis. Additionally, nude mice injected with M1 rescue cells were shown to have a reduced ability to form tumors when compared with mice injected with M2 rescue cells. Moreover, the tumors that arose from a 50/50 mixture of M1 and M2 rescue cells only expressed the mouse PKM2 rescue protein, indicating that most of the tumor was derived from the M2-expressing cells. Taken together, the data from this report reveal that the switch of pyruvate kinase expression from an adult M1 isoform to the embryonic M2 isoform in tumor cells is required in order to cause the metabolic switch to aerobic glycolysis. Lastly, PKM2 expression appears to provide a selective growth advantage to tumor cells (47).

It should be noted though that not all tumor types are dependent on PKM2-mediated aerobic glycolysis for growth, this is primarily due to the existence of other energy sources such as fatty acids and glutamine. In support of this concept, the authors also tested a non-invasive breast cell line, MCF-7, which is known to have a low aerobic glucose consumption rate. The results showed the switch of PKM2 to PKM1 in MCF-7 cells has no significant effect upon oxygen consumption and lactate production. However, the mechanism by which more pyruvate was converted to lactate, rather than metabolized in the mitochondria, is still unknown.

5.4.3. Lactate dehydrogenase (LDH)

LDH catalyzes the conversion of pyruvate to lactate, coupled with the oxidation of NADH to NAD^+, which is important in order to maintain cellular glycolysis. Each enzymatically functional LDH molecule consists of four subunits, with two types of subunits designated as M (LDH-A gene product) and H (LDH-B gene product). Human cells contain five different LDH isozymes as a result of the different combinations of H and/or M subunits: LDH1 (H4); LDH2 (MH3); LDH3 (M2H2); LDH4 (M3H); LDH5 (M4) (33), with LDH-A identified as a target of both c-Myc and HIF-1 (18). Targeting LDH-A re-sensitizes Taxol-resistant cancer cells to Taxol, indicating that LDH-A plays an important role in the Taxol resistance of human breast cancer cells (48). Experimental knockdown of LDHA-A in tumor cells by shRNAs resulted in the stimulation of mitochondrial respiration, a decrease of cell proliferation under hypoxic conditions and suppression of tumorigenicity. These findings demonstrated that LDH-A plays an important role in tumor maintenance (22).

It has been reported that LDH-A knockdown by shRNA in the fumarate hydratase (FH) knockdown background results in increased apoptosis via ROS production. This also was shown to result in a significant reduction in tumor growth in a xenograft mouse model, indicating that LDH-A inhibition might serve as a therapeutic strategy for treating patients with hereditary leiomyomatosis and renal cell cancer (HLRCC) (49). LDH-A inhibition by siRNA or by a small molecule inhibitor, FX11 [3-dihydroxy-6-methyl-7-(phenylmethyl)-4-propylnaphthalene-1-carboxylic acid] has also been investigated. FX11 can bind and inhibit human LDH-A enzyme activity. siRNA against LDH-A or FX11 reduced ATP levels and induced a significant oxidative stress and cell death. Importantly, FX11 inhibits tumorigenesis in human lymphoma and pancreatic cancer xenografts, with LDH-A identified as a significant candidate target gene for further therapeutic development (50).

5.4.4. Pyruvate dehydrogenase kinase (PDK)

Pyruvate dehydrogenase complex (PDC) catalyzes the oxidative decarboxylation of pyruvate to produce acetyl-CoA, which enters the TCA cycle to generate ATP. PDC consists of three enzymes, E1, E2 and E3, with the E1 enzyme named pyruvate dehydrogenase (PDH), which is responsible for the rate-limiting reaction of catalyzing pyruvate to acetyl-CoA. PDH activity is regulated by pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase (PDP), with PDK phosphorylating the α-subunit of PDH, which in turn leads to the inhibition of its own activity. There are four isozymes of PDK in mammals, PDK1-4, (20, 51).

5.4.5. Succinate dehydrogenase (SDH)

SDH is a mitochondrial TCA cycle enzyme complex consisting of four subunits encoded by four nuclear genes (*SDHA*, *SDHB*, *SDHC* and *SDHD*), which form the complex II of the electron transport chain (ETC) (52). SDH couples the oxidation of succinate to fumarate in the TCA cycle with electron transfer to the terminal acceptor ubiquinone which prevents the excessive formation of reactive oxygen species (53). There appears to be growing evidence indicating the importance of an SDH deficiency in tumorigenesis. Indeed, several germline mutations in subunits *B, C*, or *D* of the *SDH* genes have been indentified in renal cell carcinoma, papillary thyroid cancer, paraganglioma and pheochromocytoma (54). A down-regulation of *SDHD* also has been reported in gastric and colon carcinoma (55).

Numerous other reports reveal that *SDH* germline mutations result in the loss of SDH enzymatic activity and the induction of HIF-1alpha or HIF-2alpha.Selak *et al* show that the inhibition of SDH leads to an accumulation of succinate in cells, linking SDH inhibition to HIF-1alpha (54). Additionally, HIF-alpha prolyl hydroxylase (PHD), an enzyme which catalyzes the conversion of alpha-ketoglutarate and O_2 to succinate and CO_2 , has been shown to be inhibited by increased succinate levels in SDH-inhibited cells, eventually leading to HIF-1alpha stabilization. In other words, SDH inhibition may mimic cellular hypoxic conditions through stabilization and activation of HIF-1alpha, leading to elevated glycolysis and other changes in angiogenesis, metastasis and metabolism.

5.4.6. Fumarate hydratase (FH)

FH is present in both the cytosol and mitochondria. However, the exact role and function of the cytosolic isoform still unknown. The mitochondrial isoform of FH acts within the TCA cycle and hydrates fumarate to form malate. It does not appear to have any known role in the ETC. Germline mutations of the FH gene have been identified in hereditary leiomyomatosis and renal cell cancer (HLRCC) families, and similar to the SDH gene mutations, the FH gene mutations are loss-of-function mutations that cause an absence of or truncated FH protein.

There also may be substitutions or deletions of highly conserved amino acids, which eventually result in the absence or inhibition of FH enzymatic activity (56).

FH deficiency has been shown to be associated with elevated HIF-1alpha or HIF-2 alpha protein levels in HLRCC tumor samples or FH siRNA-treated cells (57). Fumarate has been found to be an inhibitor of PHD, resulting in the induction of HIF stabilization through the prevention of HIF hydroxylation and VHL recognition (58). Thus, these data provide a reasonable explanation for the correlation between FH deficiency and up-regulated gene expression of HIF-1alpha or -2 alpha in HLRCC tumors. Lastly, it has been observed that FH deficiency in HLRCC induces a metabolic switch to glycolysis through the stabilization of HIF-1, which is likely due to the known regulatory role of HIF on the genes involved in the glycolytic pathway.

5.4.7. Isocitrate dehydrogenase 1 (IDH1)

The IDH1 gene produces an enzyme that is localized within the cytoplasm and peroxisomes. This cytosolic enzyme catalyzes the oxidative carboxylation of isocitrate to alpha-ketoglutarate, accompanied by the generation of nicotinamide adenine dinucleotide phosphate (NADPH). A recent integrated genomic analysis of a low-grade human glioblastoma multiforme sample (59) identified a point mutation at codon 132 of the *IDH1* gene at a high frequency. The affected amino acid, R132, is known to be an evolutionarily conserved residue located within the isocitrate binding site. Although the exact effect of this alteration is unknown, some enzymatic studies have shown that the *in vitro* replacement of R132 (arginine) with glutamate, an alteration not observed in patients, caused a catalytically inactive enzyme (60).

Also Parsons correlated patients with *IDH1* mutations with prognosis, showing that patients with IDH1 mutations had a significantly improved prognosis (59). A second study by Yan *et al* (61) revealed several *IDH2* gene mutations in human glioma samples. Of note, it is interesting that all the identified *IDH2* gene mutations are localized at residue R172, which is the exact analogue of the R132 residue in IDH1, both within the isocitrate binding site. However, this data is not sufficient to conclude that the mutations are inactivating, furthermore, the effects of these mutations upon cellular metabolism are also unclear.

Given the role of IDH1 and IDH2 in producing alpha-ketoglutarate, which is also the substrate of PHD, Thompson *et al.* (62) have proposed that inactivation of IDH1, IDH2, or both might induce HIF-1 stabilization due to diminished production of cytosolic alpha-ketoglutarate. Moreover, both IDH1 and to a lesser extent, IDH2, contribute to cytosolic NADPH production in cells. NADPH is required for macromolecular synthesis and redox control, which eventually promote cell survival and growth (63). So there is the possibility that alterations in NADPH production caused by IDH1 or IDH2 mutation may affect cell proliferation via this metabolic aberration in NADPH production.

6. p53

p53, the most extensively studied tumor suppressor gene, is centrally involved in metabolic re-programming during malignant transformation. The metabolic regulation of cells by p53 has proven to be complex, with still many unanswered questions. Thus, we will focus on a single aspect of p53 function, that of modulation of the glycolytic pathway. As reported by Schwartzenberg *et al*. (64), wild-type p53 represses *GLUT1* and *GLUT4* glucose transporter gene transcription in a tissue-specific manner, with the inhibitory effects abolished by point mutations within the p53 gene.

Another report by Kawauchi *et al* (65) shows a loss of p53 which in turn causes an increase in the rate of aerobic glycolysis. Additionally, they show an up-regulation of GLUT3 through the activation of NF-kappaB.

Phosphoglycerate mutase (PGM) is an enzyme involved in glycolysis and the activity of PGM is known to be markedly up-regulated in many cancers, enhancing the glycolytic flux (66, 67). Although the regulation of PGM is poorly understood, the potential link between PGM and p53 function has been observed in MEF cells. Since wild-type p53 has the ability to down-regulate PGM, mutation of p53 in tumors could promote glycolysis via its effects upon PGM levels (68). Recently, Bensaad and his colleagues (69) reported on a direct role for p53 involvement in cellular metabolism. They identified *TIGAR* (*T*P53-*i*nduced *g*lycolysis and *a*poptosis *r*egulator) as a novel p53-inducible gene, with the functional protein sharing functional sequence similarities with the enzyme 6-phosphofructo-2-kinase / frucotse-2, 6-biophosphatase (PFK-2/FBPase-2), a member of the PGM family. PFK-2/FBPase-2 is a bi-functional enzyme with both kinase and bisphosphatase activities, and the similarity between TIGAR and PFK-2/FBPase-2 is limited to the bisphosphatase domain of FBPase-2. TIGAR can function in a manner similar to FBPase-2 to lower fructose 2, 6-bisphosphate (Fru-2, 6-P_2) levels in cells, resulting in the inhibition of glycolysis and redirection of glucose toward the pentose phosphate pathway to produce NADPH.

7. HSF1

Heat shock factor (HSF1) is a transcriptional factor that has a critical role in the regulation of the heat shock response in eukaryotes (70). Although not well understood, the functions of HSF1 are beyond the classical induction of heat shock responses. Recent reports have shown that HSF1 may play an important role in cancer development, with Dai *et al.* showing that downregulation of HSF1 led to decreased glycolysis in mouse embryonic fibroblasts (71-74).

However, our group has found that overexpression of the oncogene ErbB2 leads to increased glucose uptake, lactate production and decreased oxygen consumption in multiple human breast cancer cell lines. ErbB2 overexpression increases LDH-A expression and its activity through the upregulation of HSF1, resulting in

Table 1 Selective targets of the Warburg effect in current clinical trials

Target	Inhibitor/Activator	Metabolic effect	Targeted tumor types	Stage of therapy development
Hexokinase	2-DG	Inhibits glycolysis	Advanced solid tumor(e.g. breast, lung, gastric and prostate)	Phase I/II
Hexokinase	Lonidamine	Inhibits glycolysis	Benign prostatic hyperplasia	Phase III
Hexokinase	3-BrPA	Inhibits glycolysis	N/A	Preclinical
Pyruvate kinase	TLN-232	Inhibits glycolysis	Refractory metastatic renal cell carcinoma	Phase II
PDK1	Dicholoroacetate	Reactivates PDH	Recurrent and /or metastatic solid tumors	Phase I/II
HIF-1alpha	PX-478	Inhibits HIF signaling	Advanced solid tumors and lymphoma	Phase I
PI3K	GDC-0941	Inhibits PI3K	Advanced solid tumors	Phase I
AKT	Perifosine	Inhibits AKT	Breast, head and neck, lung, kidney, prostate, glioma, leukemia, sarcoma, myeloma	Phase II
mTORC1	Temsirolimus	Inhibits mTORC1	Renal cell carcinoma	FDA approved
PI3K/mTOR	BEZ235	Inhibits PI3K/mTORC1 and PI3K/mTORC2 signaling	Advanced solid tumors	Phase I
AMPK	Metformin	Activates AMPK	Solid tumors and lymphoma	Phase I/II

increased glycolysis. This pathway (ErbB2/HSF1/LDH-A) may play a major role in regulating glucose metabolism in cancer cells. In addition, compared to their counterparts, ErbB2-over-expressing cancer cells are more sensitive to the glycolysis inhibitors, 2-DG and oxamate. This is important for the future design of ErbB2 targeted therapeutics for breast cancer and other ErbB2-over-expressing cancers (75).

8. THERAPEUTIC IMPLICATIONS IN CANCER THERAPY

There are two strategies to target the Warburg effect in cancer cells. One is direct inhibition of glycolysis by targeting glycolytic enzymes and the second is via the indirect inhibition of the signaling pathways which regulate cellular metabolism.

8.1. Inhibition of metabolic enzymes

The glycolytic pathway is a series of metabolic reactions catalyzed by multiple enzymes or enzyme complexes. Some of these enzymes represent possible targets for the development of glycolytic inhibitors as anti-cancer agents, such as HK, phosphofructokinase, PK and LDH.

8.1.1. HK inhibitors

Due to the important roles of hexokinase in both glycolysis and apoptosis, this enzyme is one of the main targets for glycolytic inhibition in cancer treatment. Representative inhibitors of HK, such as 2-deoxyglucose (2-DG), lonidamine and 3-bromopyruvate (3-BrPA), are in pre-clinical and early phase clinical trials and are discussed below.

2-DG is a glucose analog that acts as a competitive inhibitor of glucose metabolism. It is phosphorylated by HK to 2-deoxyglucose-phosphate. This product, unlike glucose-6-phosphate, cannot be further catalyzed by phosphohexokine isomerase. Therefore, it is accumulated inside the cells and inhibits HK, with 2-DG causing a depletion of ATP concomitant with cell cycle inhibition and cell death (76). Interestingly, incubation of rat brain astrocytes with 2-DG leads to an approximately 35% decrease in the amount of HK associated with mitochondria (77). *In vitro* studies have clearly shown that cancer cells in a hypoxic microenvironment are very sensitive to 2-DG (76, 78, 79), further showing that 2-DG significantly increases the anti-cancer efficacy of adriamycin and paclitaxel in mice-bearing human osteosarcoma and non-small cell lung cancers xenograft models. However, 2-DG alone did not have a significant effect upon tumor growth *in vivo* (80). Thus, combination therapy was examined with either chemotherapy or radiotherapy as the next approach, with several early phase trials examining various combinations with 2-DG (1, 9, 81) (Table 1). Singh *et al* examined the oral administration of 2-DG at doses of up to 250mg/kg combined with large fractions of radiation (5 Gy /fraction/week), finding that it was considered safe and well-tolerated in patients with glioblastoma multiforme. (81).

2-DG induces Akt phosphorylation through PI3K, with this event being independent of LKB1/AMPK signaling activation, glycolysis inhibition or epidermal growth factor receptor signaling. This indicates that the combination of PI3K/Akt inhibition in 2-DG-based chemotherapy/radiotherapy may result in enhanced therapeutic efficacy (82). Recently, it has been reported that 2-DG activates not only the PI3K/Akt pathway, but also other prosurvival pathways such as the MEK-ERK and the insulin-like growth factor1 receptor (IGF1R) pathways. Combination therapy with 2-DG and an IGF1R inhibitor was found to induce synergistic colon cancer cell killing (83). The action of 2-DG can be rapidly reversed by high levels of glucose *in vivo* and therefore, glycolysis inhibition by 2-DG cannot be considered total (84).

Lonidamine is a derivative of indazole-3-carboxylic acid and is an orally administrated anti-cancer agent. This agent is capable of inhibiting glycolysis in cancer cells, likely through the inhibition of mitochondria-bound HK (85). This compound also lowers cellular ATP levels (86) and enhances the cytotoxicity of several alkylating agents in human breast cancer MCF-7 cells. Lonidamine is currently being examined in both phase I and II clinical trials in combination with other anti-cancer agents against breast, ovarian and lung cancer, as well as recurrent glioblastoma multiforme (87-90) (Table 1).

3-BrPA is a bromo-halogenated derivate of pyruvate and is considered a classic HKII inhibitor, causing

ATP depletion and massive cell death in cancer cells (91-93). 3-BrPA effectively kills colon cancer and lymphoma cells with mitochondrial respiration defects or under hypoxic conditions. In addition to depleting ATP stores, 3-BrPA causes a rapid dephosphorylation of BAD at Ser^{112}, re-localization of BAX to the mitochondria and the release of cytochrome c to the cytosol which leads to cell death by apoptosis. Importantly, multidrug-resistant cells also are sensitive to 3-BrPA, indicating that energy deprivation may be an effective mechanism to overcome such resistance (93). *In vivo* studies show that 3-BrPA has promising therapeutic activity in a rabbit liver cancer model when the compound was given by direct intra-arterial injection (94, 95). Combinations of 3-BrPA with other inhibitors are currently under active investigation in a number of tumor types. Xu *et al* have examined a combination of 3-BrPA with rapamycin, an mTOR inhibitor, showing a synergistic cytotoxic effect in human lymphoma and leukemia cells (96). Cao *et al* examined a combination of 3-BrPA with geldanamycin, a HSP90 inhibitor, again showing a synergistic anti-cancer effect in chemotherapy-resistant pancreatic cancer (97). Although 3-BrPA has shown some promising results *in vivo,* it is currently not being examined in human experimental clinical trials (Table 1).

8.1.2. PKM2 inhibition

It has been reported that knockdown of PKM2 gene expression by shRNA and replacement with PKM1 reduced the tumorigenicity of human lung cancer cell lines in a nude mouse xenograft model (47). TLN-232 is a synthetic cyclic heptapeptide and targets PKM2 (Thallion Pharmaceuticals, Montreal, Quebec, Canada). A Phase II clinical trial has been successfully completed in refractory metastatic renal cell carcinoma patients. (51, 84) (Table 1).

There are several approaches to develop PKM2 inhibitors. One approach is to develop synthetic peptide aptamers. The peptide aptamers specifically bind to PKM2, but not to PKM1, which fix PKM2 in the dimer form, resulting in its inactivation. The PKM2-binding aptamers have been shown to moderately reduce the proliferation rate of the human U-2 OS osteosarcoma cell line (98, 99). Another approach is to fix PKM2 in the active tetrameric form. It has been reported that the expression of a PKM2 Y105F mutant in H1299 cells leads to reduced tumor cell growth. PKM2 is a phosphotyrosine binding protein and tyrosine kinases may phosphorylate PKM2, such as FGFR1, BCR-ABL, JAK2 and FLT3. Phosphorylation of PKM2 at Y105 disrupts formation of the active tetrameric form by releasing the cofactor, FBP. The Y105F mutant cannot be phosphorylated and fix PKM2 in the tetrameric form, leading to the inhibition of tumor growth due to a lack of precursors for the synthesis of cellular building blocks (45, 46, 100).

Unlike PKM1, PKM2 contains a unique region for allosteric regulation. Selective targeting of this allosteric site of PKM2 may allow for isoform selective inhibition of PKM2 over PKM1. Recently, Vander Heiden *et al.* developed a systematic method of screening a library of more than 100,000 small molecules, identifying several potential target molecules capable of selective PKM2 inhibition. These molecules induce decreased glycolysis and increased cell death (25). A third approach is to develop antibody-like agents, like TEM8-Fc, which is comprised of 200 amino acid residues (N-terminus) of the human tumor endothelial marker 8 (TEM8) and 232 amino acid residues from the Fc portion of human IgG1. This antibody-like agent was shown to suppress the growth and metastasis of a xenograft model of human tumors in nude mice (LS-180, MCF-7 and HepG2). Importantly, TEM8 was found to bind to PKM2, thus, representing a novel antibody-like molecule in the management of solid tumors that may act by trapping PKM2. However, extensive pre-clinical studies will be necessary before TEM8-Fc can enter clinical trials (101).

Lastly, a combination of PKM2 shRNA and cisplatin was recently examined in a human xenograft lung cancer model. Guo *et al* found that this combination significantly induced apoptosis and inhibited cell proliferation, leading to inhibited tumor growth. This finding suggests that this combination may have some clinical efficacy when translated into early phase clinical trials (102).

8.1.3. PDK inhibitors

It has been reported that the knockdown of PDK1 by shRNA lowers PDH phosphorylation, reactivates PDC activity and decreases invasiveness in human head and neck squamous cell carcinoma. Importantly, siPDK1 led to dramatic reduction of tumor growth in a xenograft nude mouse model (103). Induction of PDK3 by HIF-1 promotes the metabolic switch and resistance to cisplatin and paclitaxel (20)(104, 105). There are a few PDK inhibitors that have been examined, such as dicholoroacetate (DCA), AZD7545 and Radicicol. DCA is a pyruvate analog that is orally available and binds to the N-terminal region of PDK. This binding results in the inhibition of PDK, re-activation of PDH, a metabolic shift from glycolysis to glucose oxidation, induction of apoptosis and inhibition of tumor growth (106-109). DCA is already in clinical trials for the treatment of patients with recurrent and /or metastatic solid tumors (9, 51) (Table 1).

8.2. Inhibition of metabolism-regulating signaling pathways

8.2.1. Inhibition of the HIF-1alpha signaling pathway

HIF-1alpha is not only associated with the up-regulation of glycolytic enzymes (110), but also directly transactivates the gene encoding PDK1 (19). These specific properties make HIF-1alpha an important target for cancer therapy. Currently, investigators are evaluating several agents which selectively target HIF-1alpha, with some showing very good anti-tumor effects (9). PX-478 is an HIF-1alpha inhibitor, which reduces HIF-1alpha protein levels under both normoxic and hypoxic conditions. It inhibits HIF-1alpha translation, decrease HIF-1alpha mRNA and increases HIF-1alpha polyubiquitination, thus inhibiting HIF-1 activity. It also inhibits the expression of HIF-1 target genes including glucose transporter 1 (GLUT1) and vascular endothelial growth factor (VEGF). Most importantly, PX-478 shows potent anti-tumor activity against several human tumor xenografts in mice, such as

colon, small cell lung, prostate, breast and pancreatic cancer. The anti-tumor activity of PX-478 correlates with the *in vitro* findings of a xenograft model of HIF-1alpha.(111, 112). Currently, PX-478 is in Phase I clinical trials for advanced solid tumors and lymphoma (9)b (Table 1).

Acriflavine is a small molecule inhibitor of HIF-1 activity identified by a cell-based screening assay. It binds directly to HIF-1alpha and HIF-2alpha and inhibits HIF-1 dimerization and transcriptional activity. Acriflavine has been shown to reduce tumor growth and vascularization in a prostate cancer xenograft mice model (113). Other inhibitors, such as HSP90 inhibitor (17-AAG), topoisomerase inhibitors, YC-1 and 2ME2, have the ability to decrease HIF-1alpha levels, inhibit expression of VEGF and other HIF-1 target genes, and reduce tumor growth and vascularization *in vivo*. However, it seems that none of these drugs are able to specifically inhibit HIF-1. Ongoing screens should lead to the identification of more selective HIF-1 inhibitors.

8.2.2. Inhibition of the PI3K-Akt-mTOR pathway

PI3K/Akt/mTOR pathway plays an important role in tumor cell metabolism. Hence, the inhibition of PI3K, Akt and/or mTOR is a promising target for cancer therapy.

8.2.2.1. PI3K inhibitors

Wortmannin and LY294002 were the first drugs to target PI3K, however, due to their high level of toxicity in animal studies, are unable to be expanded into human clinical trials. There are several other newer agents being developed with less toxicity, such as the PI3K inhibitor, GDC-0941. This agent is a potent, selective, orally bioavailable inhibitor of PI3K and is currently being evaluated in Phase I clinical trials (23) (Table 1). It has been reported that GDC-0941 inhibits the proliferation of both trastuzumab-sensitive and -insensitive cells. PTEN loss occurs commonly in breast tumors and has been suggested to cause trastuzumab resistance. GDC-0941 effectively inhibits proliferation of Her2-amplified breast cancer cells that are resistant to trastuzumab due to PTEN loss. GDC-0941 is also effective in treating Her2-amplified, trastuzumab-resistant tumors with PIK3CA mutation *in vivo*. Combinations of GDC-0941 and trastuzumab synergistically inhibit proliferation and induce apoptosis of breast cancer cells and trastuzumab-sensitive tumors *in vivo* (114). The combination therapy of GDC-0941, trastuzumab and pertuzumab appears to have a synergistic effect upon growth inhibition and suppression of AKT-MAPK, ERK and MEK effector signaling pathways. GDC-0941 also renders Her2-amplified BT474M1 cells and tumor xenografts more sensitive to docetaxel. These results indicate that GDC-0941 is efficacious in pre-clinical models of breast cancer. Combinations of GDC-0941 and Her2-directed treatment regimens may further augment the efficacy of these agents in a synergistic fashion in breast cancer patients (115).

8.2.2.2. Akt inhibitors

Akt is the major downstream target of PI3K, with several Akt inhibitors in current development. To date, the best developed and characterized Akt inhibitor is perifosine. Perifosine is an alkylphospholipid which blocks the translocation of Akt to the cell membrane thereby inhibiting the growth of a variety of human tumor cell lines (lung, breast, prostate, colon cancer and melanoma). Perifosine also has been reported to sensitize tumor cells to cell cycle arrest and apoptosis induced by radiation *in vitro* and *in vivo*. The synergistic anti-proliferative effect has been shown in tumor cells when perifosine was used in combination with traditional chemotherapeutic agents, such as doxorubicin in MM cells, etoposide in leukemia cells and temozolomide in glioma cells. Perifosine is currently being evaluated in Phase II clinical trials in breast, head and neck, lung, kidney and prostate cancer and glioma, leukemia, sarcoma and multiple myeloma (116) (Table 1).

8.2.2.3. mTOR inhibitors

mTOR inhibitors can be divided into two groups: the rapalogues and the small molecule mTOR inhibitors. Rapamycin is the prototype of the rapalogues and three others are under investigation in clinical trials: temsirolimus, everolimus and deforolimus. Rapalogues bind to FK506-binding protein 12 (FKBP12) and form a complex, which then binds to and inhibits mTORC1. Rapalogues have been shown to have modest anti-tumor activity against a wide range of malignancies, including renal cell carcinoma, mantle cell lymphoma, hepatocellular cancer, glioblastoma multiforme and breast cancer. In a Phase II clinical trial utilizing the single-agent temsirolimus, substantial anti-tumor activity was exhibited in patients with relapsed mantle cell lymphoma (117). A phase II study of temsirolimus in heavily pre-treated patients with locally advanced or metastatic breast cancer also found modest anti-tumor activity (118).

In a recently completed phase III randomized trial comparing interferon alpha with or without temsirolimus in patients with metastatic renal cell carcinoma, an overall improvement in survival was noted in the group receiving the combination. (119). Based on this and other studies, temsirolimus has been approved by the FDA for the treatment of patients with renal cell carcinoma (Table 1). However, even in this tumor type, mTOR inhibitors as monotherapy have shown only modest effects. One possible explanation for this may be due to a negative feedback loop from mTORC1, through S6K1, to upstream PI3K. Drugs targeting both mTORC1 and mTORC2 also have been developed, such as Torin1 and PP242, however, these are only under investigation in pre-clinical trials at this time.

8.2.2.4. Dual PI3K/mTOR inhibitors

Recently, a new generation of drugs has been developed which can target both the mTOR complexes and PI3K pathway, examples being BEZ235 and XL765. These compounds are in phase I clinical trials (Table 1). BEZ235 is an imidazo [4, 5-c] quinoline derivative, which binds to the ATP-binding cleft of PI3K and mTOR, resulting in inhibition of the kinase activity of these enzymes. It has been reported that BEZ235 is able to effectively and specifically block the dysfunctional activity of the PI3K pathway, inducing G1 arrest, both *in vitro* and *in vivo*. This compound appears to be well-tolerated, orally administered

and seems to enhance the efficacy of other anti-cancer agents when used in combination (120). Higher doses of BEZ-235 are able to prevent Akt activation due to mTOR inhibition. It also inhibits the PI3K/mTOR axis and results in anti-proliferative and anti-tumor activity in cancer cells with activating PI3K mutations (121). BEZ235 also was found to potentially inhibit VEGF-induced angiogenesis and microvessel permeability both in non-neoplastic and in BN472 mammary tumors (122). Interestingly, monotherapy with single-agent BZ235 has little effect upon Kras-mutated lung cancers, but when it is combined with a MEK inhibitor, there was marked synergy in shrinking these same Kras-mutant tumors (123).

8.2.2.5. AMPK activator

Metformin (N', N'-dimethylbiguanide) is a drug that has been used for decades to treat type II diabetes. Epidemiological studies of diabetic patients showed that metformin treatment is associated with a reduced risk of cancer. Metformin activates the AMPK/mTOR pathway in tumor cells and leads to inhibition of cell growth. Both *in vitro* and *in vivo* studies have shown that metformin exhibits anti-tumor activity in several cancer cell lines including breast, ovary, pancreas, lung and prostate cancer cells. Interestingly, it has been reported that metformin-mediated AMPK activation results in a reduction of translation initiation in breast cancer cells. Another interesting study showed that low doses of metformin (0.1-0.3 mM) inhibited cellular transformation and selectively killed cancer stem cells in four breast cancer cells. In addition to its effect upon AMPK/mTOR, metformin also has direct effects on cancer cell metabolism. In addition to metformin increasing glucose uptake and glycolysis, it also inhibits mitochondrial complex I, resulting in ATP reduction. Metformin is currently being tested in Phase I and II clinical trials (9, 124) (Table 1). Combinations of metformin and doxorubicin, a chemotherapeutic agent, kill both cancer stem cells and non-stem cancer cells in culture. Importantly, this combination treatment reduces tumor mass and prevents relapse much more effectively than either drug alone in a xenograft mouse model. These results provide the basis for using the combination of metformin with other chemotherapeutic agents (125).

9. TARGETING THE WARBURG EFFECT MAY OVERCOME TUMOR DRUG RESISTANCE

As one of the most fundamental metabolic alterations during malignant transformation, the Warburg effect undoubtedly has important therapeutic implications. Moreover, cancer cells with mitochondrial defects or under hypoxic conditions, which always exhibit extremely high glycolytic rates, are frequently associated with cellular resistance to conventional anti-cancer drugs and radiation therapy (126). There are an increasing number of reports showing that targeting the Warburg effect may overcome tumor resistance to commonly used chemotherapeutic agents.

3-bromopyruvate (3-BrPA) is a potent inhibitor of hexokinase II and effectively inhibits glycolysis (91). Xu *et al* (93) reported that cancer cells with respiratory defects, which usually were less sensitive to common anti-cancer agents, could be effectively killed by inhibition of glycolysis using 3-BrPA. Further studies show that treatment with 3-BrPA results in severely depleted ATP levels in cancer cells, especially in cells with respiratory defects, leading to rapid dephosphorylation of BAD at Ser112, a glycolysis-apoptosis integrating molecule, and re-localization of BAX to the mitochondria, with associated massive tumor cell death. More importantly, they found that cancer cells, including colon cancer and lymphoma cells, were more sensitive to 3-BrPA under hypoxic conditions than under normoxic conditions, with multidrug-resistant (MDR) cells still remaining sensitive to 3-BrPA. Overall, this study indicated that the inhibition of glycolysis in cancer cells is an effective strategy to overcome the drug resistance associated with mitochondrial defects and hypoxic conditions.

As mentioned above, LDH-A plays a key role in glycolysis by catalyzing the conversion of pyruvate to lactate in cytosol. A recent study (48) from our laboratory investigated the role of LDH-A in acquired Taxol resistance in multiple human breast cancer cells lines. Compared with Taxol-sensitive cells, taxol-resistant cancer cells showed increased LDH-A expression in both protein and mRNA levels and higher LDH-A activity, which led to increased lactate production and higher glycolytic rates. Furthermore, the knockdown of LDH-A by specific siRNA resulted in the re-sensitization of Taxol-resistant cells to Taxol. The Taxol-resistant cells also showed a higher sensitivity to Taxol with the addition of the LDH inhibitor, oxamate. These results further confirm that inhibition of glycolysis may indeed overcome drug resistance in cancer cells.

Lastly, HIF-1-mediated up-regulation of PDK1 and PDK3 in cancer cells induces a metabolic switch from mitochondrial respiration to glycolysis by inactivation of the PDH enzyme complex (19-21). Lu *et al (20)* also reported hypoxia-induced upregulation of PDK3 caused increased resistance to anti-cancer drugs, which was abolished in PDK3 knockdown cells. Further studies have shown that double knockdown of PDK1 and PDK3 led to more cell death under cellular hypoxic conditions, demonstrating PDK1 and PDK3 played an additive effect in HIF-induced drug resistance. These data strongly indicate that inhibition of PDK1, PDK3 (or both) may sensitize cancer cells to anti-cancer drugs.

10. FUTURE DIRECTIONS

Accumulating evidence supports that cancer is not only a disease of genetics, but also a disease of dysregulated bioenergetic metabolism. Advances in our understanding of the complex cellular and molecular mechanisms involved with the Warburg effect have provided new insight into the development of selective and specific agents that are presently being evaluated in several clinical trials. In this review, we provide a broad overview of the Warburg effect and its interactions with tumor cell growth, along with therapeutic implications of the Warburg effect in cancer treatment. Although extensive research

continues, our current knowledge on tumor specific bioenergetic metabolism provides only a small glimpse into this complex area of cancer research. In order to develop more effective treatment for patients with altered metabolism, scientists and physicians need to combine efforts in order to pursue the remaining challenges that have hindered the development of metabolism-targeting therapies for decades. Importantly, more resources and effort should be invested in further study of the mechanisms which underlie the altered bioenergetic metabolism in cancer cells. A deeper understanding of the mechanisms of the Warburg effect will facilitate identifying key signaling nodes to be targeted for cancer therapy. Current available glycolytic inhibitors are generally not very potent, and high doses are required, which may cause high levels of systemic toxicity which may inhibit its use in humans. Therefore, searching for more potent and specific glycolytic inhibitors becomes an urgent task. There is accumulating evidence for cross-talk between metabolic regulation and signaling pathways. This suggests that the combination of direct inhibitors of glycolysis and inhibitors of signaling pathways is a rational approach to treat cancer. With today's powerful genomic, proteomic, metabolomic, and imaging technologies, it has become feasible to identify factors that contribute to the dysregulated metabolic profiles of individual patient. This information then can be used as biomarkers to monitor the therapeutic effectiveness of metabolism-targeting drugs and to potentially develop strategies that are tailored for the individual patient and are based on the unique metabolic profile of each patient. In addition, altered bioenergetic metabolism starts at an early stage of cancer development and it has been well accepted that the dysregulated metabolism may give cancer cells a selective advantage for growth and survival. Thus, it is predictable that targeting metabolism may prevent or hinder the cancer development of this process.

Although increased aerobic glycolysis has been generally accepted as a metabolic marker of cancer cells, most cancer cells still possess mitochondrial function and do not rely on glycolysis as the sole pathway to produce energy. Thus, glycolytic pathway inhibition is not the only therapeutic approach, and it will be important to further understand alternative anti-metabolic approaches, such as targeting mitochondrial metabolism, inhibition of the pentose phosphate pathway, inhibition of fatty acid synthesis, targeting amino acid metabolism and targeting tumor acidification. In conclusion, a better and deeper understanding of the metabolic alterations and regulatory mechanisms in cancer cells will help us successfully develop selective and effective therapeutic agents for patients who suffer from cancer.

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