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Targeting Cancer Metabolism - Revisiting the Warburg Effects

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After more than half of century since the Warburg effect was described, this atypical metabolism has been standing true for almost every type of cancer, exhibiting higher glycolysis and lactate metabolism and defective mitochondrial ATP production. This phenomenon had attracted many scientists to the problem of elucidating the mechanism of, and reason for, this effect. Several models based on oncogenic studies have been proposed, such as the accumulation of mitochondrial gene mutations, the switch from oxidative phosphorylation respiration to glycolysis, the enhancement of lactate metabolism, and the alteration of glycolytic genes. Whether the Warburg phenomenon is the consequence of genetic dysregulation in cancer or the cause of cancer remains unknown. Moreover, the exact reasons and physiological values of this peculiar metabolism in cancer remain unclear. Although there are some pharmacological compounds, such as 2-deoxy-D-glucose, dichloroacetic acid, and 3-bromopyruvate, therapeutic strategies, including diet, have been developed based on targeting the Warburg effect. In this review, we will revisit the Warburg effect to determine how much scientists currently understand about this phenomenon and how we can treat the cancer based on targeting metabolism.

Key words: Energy metabolism, Warburg effects, Cancer metabolism, Mitochondria

INTRODUCTION

During the early 19th century, Dr. Otto Warburg demonstrated the enhancement of O_2 uptake and subsequent rapid cell division upon fertilization. He hypothesized that cancer cells might also take up more O_2 than normal cells (1). However, subsequently, Warburg and his co-workers noticed that rat liver carcinoma did not take up more O_2 than normal liver tissue, and that, even in the presence of O_2, such tissue produced lactic acid (2,3). In 1956, Warburg first reported that cancer cells exhibit high rates of glucose uptake and lactic acid production even in the presence of oxygen (4). It seemed that cancer cells preferred aerobic glycolysis to oxidative phosphorylation (OxPhos). Warburg also initially suspected that cancer cells displayed impaired respiration due to the functional defects in mitochondria (5), whereas the findings from his own laboratory (3) and those of others (6,7) indicated otherwise. Although the observations of Chance and Weinhouse (6,7) contradicted Warburg's argument of mitochondrial defects in cancers, many studies over the past several decades have documented oncogenic nuclear and mitochondrial DNA mutations in proteins involved in respiration. Moreover, remarkably, after more than half a century's research, the Warburg effect stands true for most types of cancer cells and has become the seventh hallmark of cancer cells besides the following: 1) persistent growth signals, 2) evasion of apoptosis, 3) insensitivity to antigrowth signals, 4) unlimited replicative potential, 5) angiogenesis, and 6) invasion and metastasis (8,9). The exact reasons for, and physiological value of, atypical metabolism in cancer remain to be elucidated. People generally believe that the Warburg effect will confer growth advantages on tumor cells, including the provision of faster production of ATP, amino acids for protein synthesis, nucleic acids for DNA duplication, and lipids for cell bio-membrane synthesis that might be needed in cell proliferation, as well as generate an acidic environment, which is harmful to normal cells but has no effect on tumor cells (10), and produce less

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reactive oxygen species (ROS) so that the genome of cancer cells may elude damage by a high concentration of ROS, resulting in apoptosis resistance in tumor subjects (11,12). Currently, the Warburg effect and its causes have caught the attention of scientists because people believe that a better understanding of the mechanisms of the Warburg effect might ultimately lead to more effective treatments for cancer. Indeed, numerous publications have proposed different models; thus, a comprehensive and clear cause of the Warburg effect might be on the horizon. Moreover, some anti-cancer drugs have also been developed as applications of the switch from oxidative phosphorylation to glycolytic metabolism in cancer (13), besides the diagnosis and detection of metastasis using F-18 fluorodeoxyglucose (FDG) positron emission tomography (PET).

1. Relationship between tumor-specific glucose metabolism and genetic changes. When normal differentiated tissues are in the presence of oxygen, one glucose molecule can generate up to 36 ATP molecules. Glycolysis is the primary metabolic pathway in the cytoplasm that converts glucose to two pyruvate molecules. This process releases two ATP and two reduced nicotinamide adenine dinucleotide (NADH) molecules. Pyruvate is then transported to the mitochondria and is converted to an acetyl group that comes along with coenzyme A to form the acetyl-CoA complex. Acetyl-CoA then joins the Krebs cycle in the mitochondrial matrix. The net result is one ATP, three NADH and one reduced flavin adenine dinucleotide ($FADH_2$). The electron transport chains (I, II, III, IV) are electron transporters inserted into the inner mitochondrial membrane that transport electrons from NADH and $FADH_2$ to oxygen. In the pathway, protons are impelled from the mitochondrial matrix to the intermembrane space, and oxygen is the final acceptor for conversion to water molecules. Regarding oxygen reduction, ROS are produced in complex I, II, and III (14). The energy is deposited in the form of a proton gradient between the intermembrane space and matrix, and the energy will finally be converted to ATP (15). The completed process results in the yield of 36 ATP molecules. However, under conditions where oxygen is limited, glucose undergoes anaerobic metabolism, which is the partial oxidation of glucose to pyruvate, and is reduced to lactate (in human) or alcohol (in bacteria). NADH becomes oxidized, and 1-NAD^+ is regenerated for glycolysis. Only two ATP molecules are yielded in anaerobic metabolism. Interestingly, the Warburg effect results in atypical metabolism, indicating that cancer cells mostly convert glucose into lactate even in the presence of oxygen. This characteristic is shared with normal proliferating cells. The net energy yield in anaerobic glycolysis is two ATP molecules, whereas the yield in oxidative phosphorylation is thirty-six. This observation leads to the paradox: why is the pathway that produces less ATP selected in such high-demand cells and is the Warburg effect the consequence of genetic changes in cancer or the cause of cancer? As mentioned above, a very common characteristic of cancer cells described by Otto Warburg is that the cancer cell exhibits increased glycolytic metabolism compared with normal cells. Using the dbEST database for the expression of genes and expressed sequence tags [U.S. NCBI, National Institutes of Health (16)], it was found that genes involved in glycolysis are overexpressed in 24 different types of cancers, representing more than 70% of all human cancer cases (17). Although the key factors/pathways underlying the cancer metabolic phenotype remain to be elucidated, several mechanisms have been proposed base on the epigenetic changes in proto-oncogenes and tumor suppressor genes in the multistep process of carcinogenesis. In this session, we classified those mechanisms into 4 types: 1) mitochondrial changes—defect in oxidative phosphorylation, 2) changes in the metabolome or metabolite pools that facilitate glycolytic flux, 3) hypoxia-induced switch from oxidative mitochondrial respiration to glycolysis and 4) coordinated regulation of proteins that control glycolytic flux.

1-1. Mitochondrial changes—defect in oxidative phosphorylation: Otto Warburg had mentioned that respiration must be defective in cancers because high levels of O_2 cannot suppress the production of lactic acid by cancer cells (18,19). Thus, are mitochondrial defects sufficient and necessary for tumorigenesis? With the discoveries of oncogenic mutations in mitochondrial metabolic enzymes, it is now untenable to deny the role of mitochondria in tumorigenesis (20,21). Indeed, cancer cell mitochondria were reported to show a reduction in DNA, a lower transcription rate and an accumulation of genomic mutations and deletions (Table 1). Mutation in certain mitochondrial genes may disrupt the electron transport chains, thereby decreasing oxidative phosphorylation. The mitochondrial genome is particularly susceptible to mutation for several reasons. Electrons may escape or leak from electron transport complexes, mainly at complexes I and III, to react with molecular oxygen, forming superoxide radicals (O_2^-) and other reactive oxygen species that damage mitochondrial DNA. Mitochondrial DNA is supercoiled, circular and prone to breakage. Moreover, there are few repair mechanisms for mitochondrial DNA.

1-1-1. Succinate dehydrogenase (SDH) and fumarate hydratase (FH); SDH is involved in the TCA cycle, catalyzes the conversion from succinate to fumarate, and releases one molecular-reduced flavin adenine dinucleotide. SDH mutations are commonly found in paraganglioma, gastric stromal tumors, and childhood T-cell acute leukemia (22,23). Germline mutations in SDH seem to be closely associated with human head and neck paragangliomas (23). These facts suggest that SDH mutations may provide a growth advantage in the initial stages of tumorigenesis. FH, an enzyme similar to SDH, also catalyzes the reaction from

Table 1. Mitochondrial genetic changes in cancers

Cancer Type	Mitochondrial Changes	Percentage of Cases
Colorectal cancer	12S rRNA, 16S rRNA, ND1, ND4L, ND5, Cytochrome b, COX I, COX II and COX III genes. Most are T → C or G → A transitions	70%
Ovarian cancer	D-loop, 12S rRNA, 16S rRNA and cytochrome b mutation. Most are T → C or G → A transitions	60%
Breast cancer	Mutations in the D-loop region of mitochondrial genome	60%
	16S rRNA, ND1, ND2, ND4, ND5, Cytochrome b and ATPase 6	< 15%
Hepatocarcinoma	Mutations in the D-loop	Frequent
Gastric cancer	Deletion of mtDNA	54%
	Insertions/deletions in the D-loop region or transitions in ND1, ND5 and COX I	44%
Esophageal adenocarcinomas or Barrett's esophagus	D-loop alterations	40%
Esophageal carcinoma	D-loop mutation	5%
Renal cell carcinoma	A 264-bp deletion of the ND1	100%
	Loss of mtDNA and mRNA coding for subunit the ND3 gene	-
	Loss of ATP synthase activity in Complex V	100%
Pancreatic cancer cell lines	12S rRNA, 16S rRNA, ND1, ND2, COX I, COX II, ATPase 6, COX III, ND4, ND4L, ND5, ND6, Cytochrome b as well as the non-coding D-loop region. Also 6-fold to 8-fold increase in the mtDNA mass.	100%
Prostate cancer	D-loop region, 16S rRNA and NADH subunit	18.75%
Brain tumors	mtDNA highly amplified	87%
Thyroid cancer	mtDNA alterations in the genes coding for Complex I and comlex IV of the respiratory chain	the mtDNA common deletion was identified in 100% of Hurthle cell tumors, 33.3% of adenomas, and in 18.8% of non-Hurthle cell papillary carcinomas.
Hematologic malignancies	Described mutations in cytochrome b, cytochrome c oxidases I and II and ATPase 8; increased mutations in the mitochondrially-encoded COX I and COX II genes	-

ND - mitochondrially encoded NADH dehydrogenase; COX I-III - cytochrome oxidase subunit I-III; mtDNA - mitochondrial DNA; rRNA - ribosomal RNA; NADH - reduced form of nicotinamide adenine dinucleotide (Adapted from (9)).

fumarate to malate. FH mutations have been observed in several types of malignant tumors in different tissues and organs, such as uterine leiomyomatosis, cerebral cavernomas, and breast cancer (24). Mutations in SDH and FH promote increased levels of succinate and fumarate, which inhibit prolyl hydroxylases that are responsible for the O_2-dependent modification of hypoxia inducible factor-1α (HIF-1α) and its degradation. In this regard, HIF-1α needs to be hydroxylated by proline hydroxylases (PHDs), a family of α-ketoglutarate (α-KG)-dependent enzymes before undergoing degradation under normal oxygen conditions (25,26). During the process of HIF-1α hydroxylation, the substrate of α-KG is oxidized, with the generation of succinate as a product (27). Moreover, subsequent tests verified that fumarate could inhibit PHD2 (28), while succinate could reduce the enzymatic activity of PHD3 (29). Therefore, even in the presence of normal levels of O_2, these mutations are thought to constitutively increase the production of HIF-1α to levels that trigger tumorigenesis (29). HIF activation under non-hypoxic conditions would thus inhibit the Pasteur effect and induce the Warburg effect in cancer cells. HIF consists of two subunits: an α subunit usually located in the cytoplasm (HIF-1α) and a β subunit located in the nucleus (HIF-1β). The inhibition of PHD promotes HIF-1α to enter into the nucleus and integrate with HIF-1β to form heterodimers, thus promoting the expression of a

series of HIF target genes, including genes encoding glucose transporters (GLUTs) (30), glycolysis enzymes such as pyruvate dehydrogenase kinase (PDK) (31,32), lactate dehydrogenase A (LDH-A), and myc (33). The enhancement of these HIF-target genes synergistically promotes the Warburg effect, allowing cancer cells to gain growth advantages (29,34,35).

1-1-2. P53, ATPase inhibitor protein (IF1) and isocitrate dehydrogenase (IDH); A nuclear gene that regulates mitochondrial respiration is the tumor suppressor p53 (TP53, also known as p53)-inducible gene synthesis of cytochrome c oxidase-2 (SCO2) (36). SCO2 is critical for regulating the cytochrome c oxidase (COX) complex, the major site of oxygen utilization in oxidative phosphorylation. SCO2 connects p53 to mitochondrial respiration and is responsible for the Warburg effect (36). Another possible mechanism for decreased oxidative phosphorylation is the overexpression of the ATPase inhibitor protein (IF1) (37). A decrease in H^+-ATP synthase (β-F1-ATPase) is a proteomic signature of decreased oxidative phosphorylation and characteristic of cancer cell bioenergetics, which can predict the prognosis of colon, lung, and breast cancer. The level of this protein is inversely correlated with the glycolytic rate in cancer cells (38). When oxidative phosphorylation becomes inefficient or defective, the loss of mitochondrial ATP removes the inhibition on glycolysis, providing a compensatory mechanism to generate ATP. In addition to the familial cancer syndromes associated with OxPhos mutations, somatic mutations of IDH1 (cytosolic form) and IDH2 (mitochondrial form) have been shown in low-grade gliomas and normal acute myelogenous leukemia (20,39,40). The catalytic inactive form of IDH1 and IDH2 was initially believed to cause the loss of function that led to diminished conversion of isocitrate to α-ketoglutarate, a metabolic intermediate that is required for the degradation of HIF-1α or HIF2α (also known as EPAS1) (41). However, the stabilization of HIF-1α by mutant IDH1 or IDH2 has not been independently confirmed. Despite the loss of oxidative phosphorylation, the mitochondria remain essential in the processing of intermediate metabolites for various pathways involving carbohydrates, amino acids and fatty acids. Cancer cells depleted of mitochondrial DNA by treatment with ethidium bromide (42) continue to maintain mitochondrial mass. Moreover, a recent study demonstrated that cancer development and metastasis require mitochondria DNA (43).

1-2. Changes in the metabolome or metabolite pools that facilitate glycolytic flux: Fructose 2,6-bisphosphate is an important metabolite that stimulates glycolysis by a potent positive allosteric effect on PFK-1 and inhibits gluconeogenesis by blocking fructose 1,6-bisphosphatase (FBPase-1) (44). The p53-inducible enzyme TP53-induced glycolysis and apoptosis regulator (TIGAR) is a FBPase-2 that functions to lower fructose 2,6-bisphosphate levels, thereby inhibiting glycolysis by decreasing the activity of PFK-1 and enhancing the activity of FBPase-1 (45). Because FBPase-1 activity is reduced in many tumor cells (often due to the loss of p53 function and the resultant downregulation of TIGAR expression (45,46)), the fructose 1,6-bisphosphate level remains persistently elevated, and the "brake" on glycolysis is removed. However, the levels of TIGAR expression in various cancer types have not been examined. Nevertheless, fructose 1, 6-bisphosphate may be the key metabolite that increases the activity of PKM2, leading to a drastic increase in forward flux through glycolysis. Three other characteristics of the metabolome restricting glycolysis are the accumulation of pyruvate, accumulation of reduced hydrogen, and depletion of NAD^+ as glycolysis proceeds. Increased glycolysis alters the glycerol-3-phosphate and malate-aspartate shuttles, reducing the transport of H^+ into the mitochondrial intermembrane space and requiring the cancer cells to oxidize NADH to regenerate NAD^+ in the cytosol by LDH. c-MYC induces the increase in LDH type A (LDH-A) expression (47), and LDH-A, which converts pyruvate to lactate, plays a key role in carcinogenesis (48). The reduction in LDH-A using short hairpin RNAs stimulates oxidative phosphorylation and decreases the mitochondrial membrane potential. The tumorigenicity and ability to proliferate under hypoxia are decreased in LDH-A-deficient cells (48). The reduction in the transport of H^+ into the mitochondrial intermembrane space in cancer cells exhibiting the Warburg phenomenon also presents a challenge in intracellular pH (pHi) regulation. In normal cells, the Na^+-driven Cl^-/HCO^{3-} exchanger (NHE1) and Na^+-independent Cl^-/HCO^{3-} exchanger are primarily responsible for the maintenance of pHi. In cancer cells, high NHE1 activity increases pHi and acidifies the extracellular space. The increased pHi facilitates glycolysis, and the resulting lactate is transported out of the cancer cells via the H^+/lactate cotransporter (49). Other proteins involved in pHi regulation include the monocarboxylate carriers that transport bicarbonate anions and carbonic anhydrase. Carbonic anhydrase IX is a hypoxia-inducible transmembrane protein and the only tumor-associated carbonic anhydrase isoenzyme known (50,51); it is needed for the growth and survival of cancer cells under both normoxia and hypoxia (52).

1-3. Hypoxia-induced switch from oxidative mitochondrial respiration to glycolysis: The solid tumor microenvironment is characterized by a disorganized microvasculature (53), increased interstitial pressure (54) and the presence of hypoxic, a lack of oxygen zones (55). The absence of oxygen shuts down oxidative mitochondrial respiration, forcing the cancer cells to switch on glycolytic metabolism for bioenergy. The lack of mitochondrial ATP will remove the inhibition of PFK-1 and PKM2, leading to increased forward flux through glycolysis. PFKFB1-4 genes are responsive to hypoxia in vivo, indicating a physiological role for glycolysis in the adaptation to hypoxia (56). There is also evidence for the overexpression of a specific spliced iso-

form of PFKFB-4 mRNA in cancer cells under hypoxic conditions (57). Overall, hypoxia activates HIF to induce adaptive responses, including angiogenesis, glycolysis, and pH regulation (58). The hypoxic microenvironment in which the cancer cells thrive may constitute a selection pressure to select for tumor cell clones with high glycolytic metabolism, as the cells evolve through the carcinogenic process (59).

1-4. Coordinated regulation of proteins that control glycolytic flux:

1-4-1. Regulation of cancer cell metabolism by the PKB; The PKB (Protein kinase B/Akt) signaling pathway has been shown to promote continued cell growth and coordinates the necessary metabolic changes to support cell growth by increasing glucose uptake, glycolysis and ATP production. Activation of PKB signaling may be a factor leading to the switch to glycolytic metabolism in cancer (52) (Fig. 1). PKB directly and/or indirectly regulates the transcription (60) and translation (61) of GLUT1, which functions in glucose uptake. PKB also activates hexokinase 2 (HK2) association with the mitochondria, promoting the phosphorylation of glucose to glucose 6-phosphate, to be metabolized via glycolysis or the pentose phosphate pathway, and the mitochondria-associated HK2 is involved in the inhibition of apoptosis (62,63). Moreover, PKB regulates de novo fatty acid synthesis and the usage of fatty acid for β-oxidation. It phosphorylates ATP citratelyase (ACL), stimulating the cleavage of citrate to oxaloacetate and acetyl-coenzyme A (Ac-CoA) to supply downstream de novo fatty acid synthesis (64). Phosphoinositide 3-kinase (PI3K) and PKB suppress the expression of the β-oxidation enzyme carnitine palmitoyltransferase 1A (CPT1A), and the modulation of CPT1A expression by PI3K/PKB signaling is the mechanism to suppress β-oxidation during cell growth (65). The mammalian target of rapamycin (mTOR), a downstream effector of the PI3K/PKB pathway, is situated at the crossroads of signaling pathways and is an integration center for signals bringing the coordinated regulation of nutrient uptake, energy metabolism, cell growth, proliferation, and cell survival (66,67). mTOR is regulated by AMP-activated protein kinase (AMPK) (the cellular energy sensor), tuberous sclerosis 1 & 2 (TSC1-TSC2) complex, and Ras homolog enriched in brain (RHEB) (68). Most importantly, mTOR is an upstream activator of HIF-1α in cancer cells (69), which is a subunit of a transcription factor that upregulates the expression of nearly all of the genes involved in

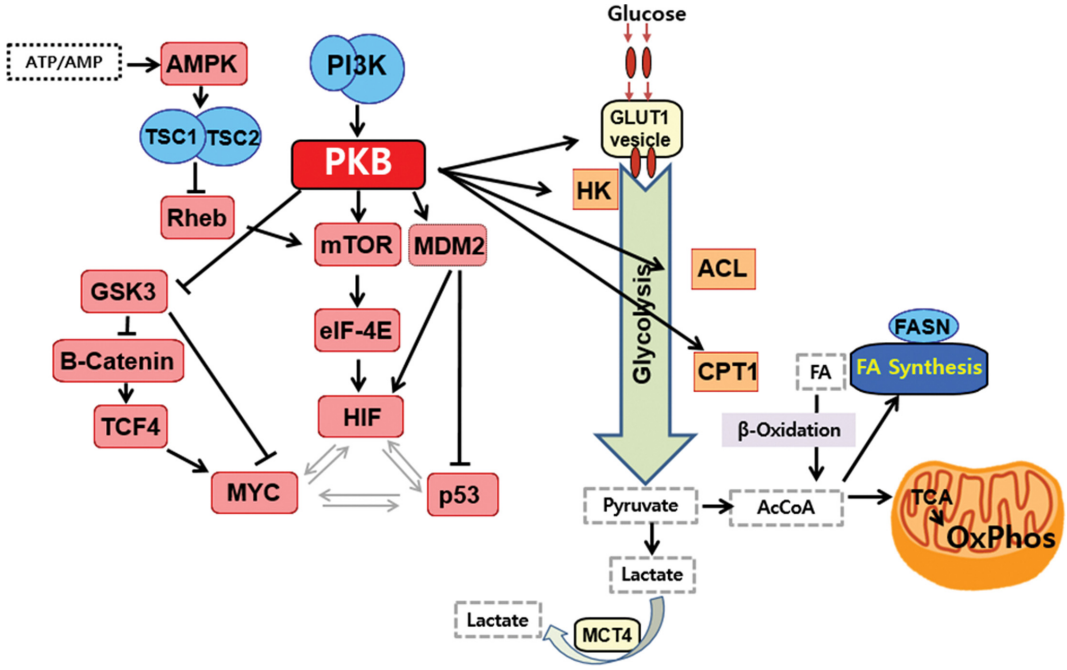

Fig. 1. The PI3K/PKB/mTOR signaling pathway regulates cancer cell metabolism. PKB upregulates glycolysis by affecting glucose transporter 1 (GLUT1) and activating hexokinase 2 (HK2) association with the mitochondria. Moreover, PKB regulates de novo fatty acid synthesis and the usage of fatty acid for β-oxidation. It phosphorylates ATP citratelyase (ACL) to supply downstream de novo fatty acid synthesis (64). Phosphoinositide 3-kinase (PI3K) and PKB suppress the expression of the β-oxidation enzyme carnitine palmitoyltransferase 1A (CPT1A), suppressing β-oxidation and impairing mitochondria. mTOR, a downstream effector of the PI3K/PKB pathway, is regulated by AMP-activated protein kinase (AMPK; the cellular energy sensor), the tuberous sclerosis 1 & 2 (TSC1/TSC2) complex, and Ras homolog enriched in brain (RHEB). Most importantly, mTOR is an upstream activator of HIF-1α in cancer cells (69), which is a subunit of a transcription factor that upregulates the expression of nearly all of the genes involved in the glycolytic pathway (See details in the text (Section 1-4-1)). Arrows represent stimulation/activation, and ends represent inhibition.

the glycolytic pathway (32).

1-4-2. Regulation of glycolysis by a triad of transcription factors; Three transcription factors, c-MYC, HIF-1 and p53, regulate the flux of glucose through the glycolytic pathway (Fig. 2). The transport of glucose into cancer cells is controlled by glucose transporters, including GLUT-1, which are regulated by HIF-1. Hexokinases are important enzymes that regulate glycolysis, and HK2 is the isoform expressed specifically in skeletal muscle, adipocytes and cancer cells. HK2 regulates the first step in glycolysis (70). HK2 is regulated by p53 as well as HIF-1. The upstream regulatory element of the HK2 gene contains a carbohydrate response element (ChoRE) and response elements for protein kinase A, protein kinase C, HIF-1 and p53 (71,72). In cancer cells, the HK2 gene is amplified, activated, and induced by multiple signal transduction cascades, and the overexpressed HK2 binds to the outer membrane of mitochondria. HIF-1 is the major transcription factor regulating the transcription of most of the enzymes in the glycolytic pathway, from glucose to lactate (32) (Fig. 2). The promoter regions of the genes of these enzymes have been shown to have HIF-1 regulatory elements (71,72). Pyruvate may also regulate the levels of glycolytic enzymes by preventing the oxygen-induced degradation of HIF-1α protein, thus activating HIF-1 (73). The enzyme that is very important in regulating the pyruvate level is LDH-A, and the cis-acting elements of its gene promoter resemble the core of the ChoRE and E-box (5-CAGGTG-3), and they overlap with the consensus binding sites for both c-MYC and HIF-1. Increased activities of HIF-1 and/or c-MYC upregulate glycolytic enzyme genes, leading to an increased glycolytic capacity in cancer cells (71). Another enzyme regulated by HIF-1 and c-MYC is pyruvate dehydrogenase kinase-1 (PDK-1), which inhibits pyruvate dehydrogenase by phosphorylation, stopping the conversion of pyruvate to acetyl-CoA, thus depleting the fuel supply for oxidative phosphor-

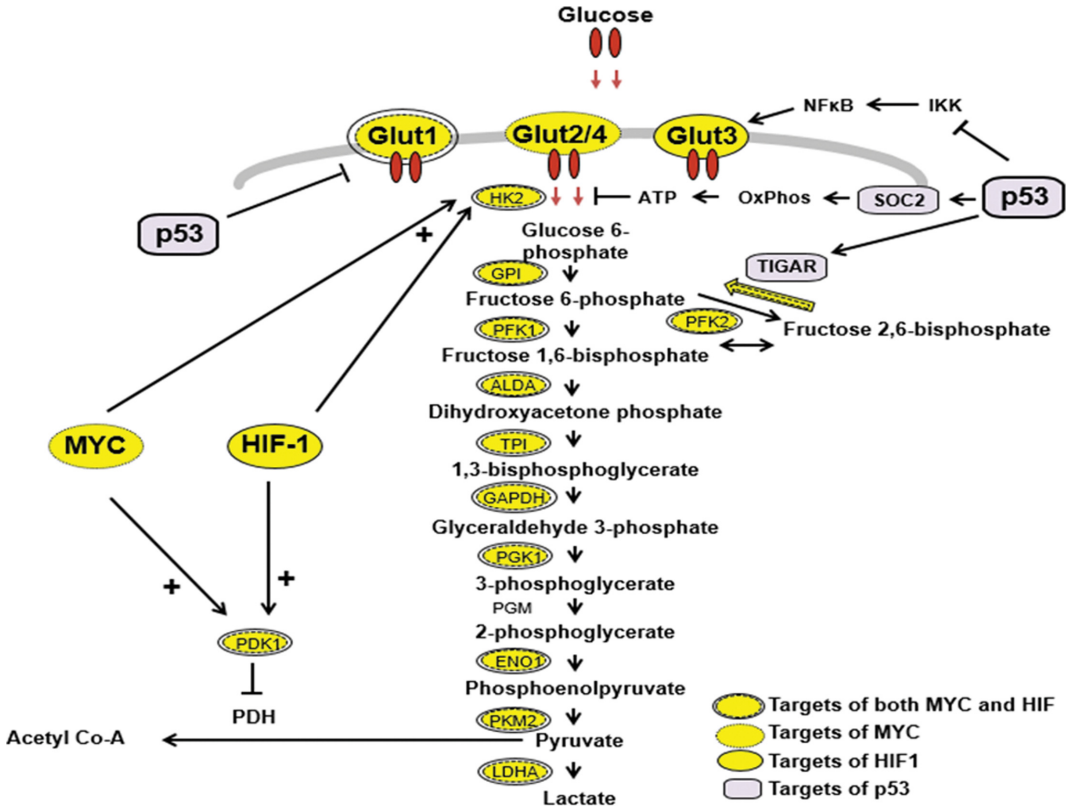

Fig. 2. c-MYC, HIF-1 and p53 regulate glycolytic metabolism. The Warburg phenomenon is due, at least in part, to the upregulation of genes coding for glucose transporters and glycolytic and regulatory enzymes mediated by the increased activity of the transcription factors c-MYC and HIF-1 in cancer cells, and the coordinated loss of regulatory proteins due to the loss of p53 function. Loss of p53 function also leads to the activation of GLUT-3 transcription via NFκB. Arrows represent stimulation/activation, and ends represent inhibition. + indicates synergism. HK2, hexokinase type 2; GPI, glucose phosphate isomerase; PFK1, phosphofructokinase 1; PFK2, phosphofructokinase 2; ALDA, aldolase A; TPI, triose phosphate isomerase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PGK1, phosphoglycerate kinase 1; PGM, phosphoglycerate mutase; ENO1, enolase 1; PKM2, pyruvate kinase type M2; LDH-A, lactate dehydrogenase type A: PDK1, pyruvate dehydrogenase kinase-1; TIGAR, TP53-induced glycolysis and apoptosis regulator; SCO2, synthesis of cytochrome c oxidase-2; IKK, I-kappa-B kinase; NF-κB, nuclear factor-kappa-B; GLUT, glucose transporter (154-158).

ylation (74). p53 controls the balance between oxidative respiration and glycolysis through two important p53-inducible genes (TIGAR (46) and SCO2 (36)) as discussed above. p53 represses the transcriptional activity of GLUT1 and GLUT4 gene promoters by direct DNA binding, leading to a decrease in glucose uptake (75). Another recent report has demonstrated that the inhibitory effect of p53 on I-kappa-B kinase (IKK) dampens the positive feedback loop between glycolysis and IKK-nuclear factor-kappa-B (NF-κB) signaling (76), and the loss of p53 will activate NF-κB to transcriptionally activate the expression of GLUT3 and increase the rate of aerobic glycolysis (76). p53 also induces ubiquitination and the degradation of phosphoglycerate mutase (PGM), and the loss of p53 results in an increase in the PGM protein level, resulting in enhanced glycolysis (77). Therefore, it has become clear that this triad of transcription factors, HIF-1, c-MYC and p53, are responsible for a coordinated shift in cancer cell metabolism from oxidative phosphorylation to glycolysis.

2. Lactic acid-mediated tumor progression. The accumulation of an enzyme facilitates the high conversion rate of pyruvate to lactate, and LDH-A has been observed to be critical in the development and progression of cancer. Following production, lactate is transported out of the cancer cell and across the plasma membrane by monocarboxylate transporter (MCT) family proteins. MCTs are proton-linked transporters and are involved primarily in the transport of monocarboxylic acids. MCT 1, an import transporter, is utilized in oxidative tumors to shuttle exogenous lactate as an energy source. The shuttling of lactate by MCT 1 into endothelial cells drives the CXCL8 pathway, enabling endothelial cell migration to stimulate angiogenesis. Lactate also drives the process of angiogenesis by promoting the production of VEGF (78). Furthermore, MCT1 shuttling of lactate stimulates IL-8 mRNA expression in endothelial cells,

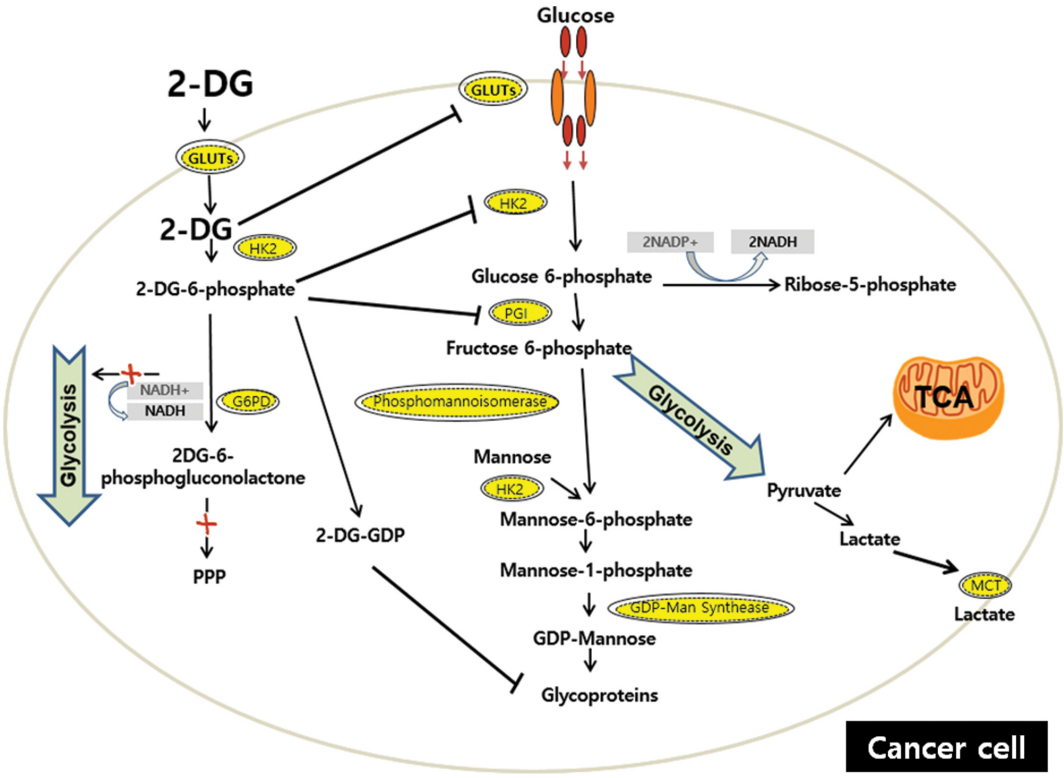

Fig. 3. The targets of 2-DG. Similar to glucose, 2-DG is taken up through GLUTs and then is phosphorylated by HK to form 2-DG-6-P, and 2-DG competitively inhibits GLUTs. 2-DG-6-P cannot be further metabolized via glycolysis but accumulates in the cell and noncompetitively inhibits HK and competitively inhibits PGI. NADPH generation is inhibited. Only one molecule of NADPH can be generated from the conversion of 2-DG-6-P to 2-DG-6-phosphogluconolactone. 2-DG structurally resembles mannose and undergoes conversion into 2-DG-GDP, which interferes with the N-linked glycosylation of proteins. The inhibition of N-linked glycosylation induces the accumulation of unfold/misfolded proteins in the ER, resulting in ER stress and constant cell apoptosis. Intracellular glucose can promote glycosylation because its metabolic product F-6-P can be used in the mannose glycosylation pathway. However, upstream glucose metabolism is inhibited by 2-DG, which may not allow exogenous glucose to restore the interrupted N-linked glycosylation. GLUTs, glucose transporters; HK, hexokinase; PGI, phosphoglucose isomerase; G-6-PD, glucose-6-phosphate dehydrogenase; GDP, guanosine diphosphate; MCT, monocarboxylate transporter.

a finding that is also important for angiogenesis (79). In addition, MCT4 releases lactate from glycolytic tumors (80). Overall, as a common feature of malignant cells, increased glucose uptake and lactate production, even under normoxic conditions, is involved in cancer survival and proliferation via several mechanisms, including evading the immune system and modulating cell motility. Furthermore, the accumulation of lactate in cancer has been demonstrated to possess clinical relevance as a prognostic marker (81).

3. Potential cancer therapy to target the Warburg effects.

3-1. 2-Deoxy-D-glucose (2-DG): 2-DG, a synthetic glucose analog in which the C-2-hydroxyl group is replaced by hydrogen, has been extensively and thoroughly investigated in both scientific and clinical studies since the early 1950s (82). Although 2-DG has been identified as a potential anti-cancer agent by interfering with various biological process, including the depletion of cellular energy, intensification of oxidative stress, interference with N-linked glycosylation, and induction of autophagy (83,84), the mechanism by which 2-DG prevents cancer is commonly thought to be via the inhibition of glycolysis. 2-DG competitively inhibits glucose uptake because it is transferred by GLUTs. After entering the cell, 2-DG is phosphorylated by HK to form 2-deoxy-D-glucose-6-phosphate (2-DG-6-P), which cannot be further metabolized via glycolysis but accumulates and noncompetitively inhibits HK and competitively inhibits PGI (82,85,86). Because 2-DG inhibits the first critical steps at the beginning of glucose metabolism, both glycolysis and OxPhos may be partially disrupted (87). These events lead to decreased ATP production, a blocked cell cycle, decreased and inhibited cell growth and even cell death (84,88,89). Decreased intracellular ATP production leads to an increase in the AMP/ATP ratio, which activates and increases AMPK, resulting in elevated catabolic metabolism via the phosphorylation of downstream targets, such as mTOR (90) (see 1-1-4). In addition, decreased ATP sensitizes cells to extrinsic (death receptor-mediated) apoptosis (87,91) via binding of tumor necrosis factor (TNF) ligand family members, including TNF-related apoptosis-inducing ligand (TRAIL), to their cognate transmembrane death receptors (92). Defects in p53

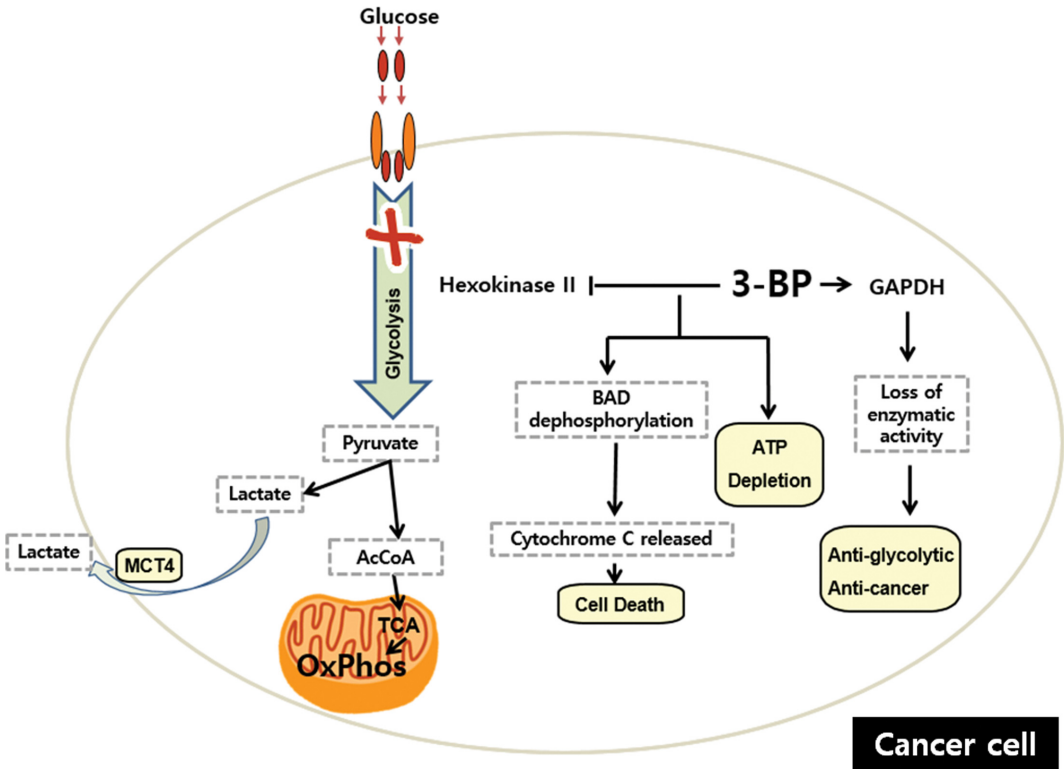

Fig. 4. DCA "switch on" mitochondria in cancer. The multi enzyme pyruvate dehydrogenase complex (PDC) is located in the mitochondrial matrix and catalyzes the rate-limiting step in the aerobic oxidation of glucose, pyruvate, alanine and lactate to acetyl CoA, a substrate of the TCA cycle. Thus, PDC is the key mediator of OxPhos. The upstream effectors of the PDC include the following: the family of pyruvate dehydrogenase kinase (PDK) isoforms that phosphorylate and inactivate PDC; the pyruvate dehydrogenase phosphatase (PDP) isoforms that dephosphorylate the PDC and restore catalytic activity. Dichloroacetic acid (DCA), a structural analog of pyruvate, stimulates PDC activation by inhibiting PDKs, thereby maintaining the PDC in its unphosphorylated form. Moreover, DCA increases PDC activity by inhibiting the turnover of the complex, although the mechanism remains unclear.

are closely related to reduced ATP induced by 2-DG because p53 can act as an effective energy sensor of decreased ATP and restore the ATP level by promoting oxidative phosphorylation (82). HIF also reduces the efficiency of 2-DG and induces resistance by increasing glucose transporters and several glycolytic enzymes; therefore, the down-regulation of HIF sensitizes cells to the detrimental effect of 2-DG (82,93). However, energy deprivation itself is not sufficient for the anti-cancer effect of 2-DG. First, ATP is necessary for both intrinsic and extrinsic-mediated apoptosis, and apoptotic cells generally have elevated levels of ATP (82). This suggests that 2-DG may paradoxically exert a cytoprotective effect. Second, 2-DG treatment, mimicking glucose deprivation, does not predispose some cells to death because they maintain OxPhos function and utilize alternative carbon sources, such as fatty acids and amino acids, to synthesize ATP under normoxic conditions (94). In addition, treatments that rescue cells from 2-DG-induced cell death, including mannose and Bcl-2 overexpression, do not reverse the depletion of ATP (88).

3-2. Dichloroacetic acid (DCA): DCA's therapeutic potential was foreshadowed by research in the 1950s and 1960s on the pharmacology of various ionic complexes of dichloroacetate (95). These studies led to the discovery of the DCA moiety as a molecule capable of eliciting significant effects on carbohydrate and lipid metabolism in experimental diabetes (96) and, soon thereafter, to the landmark report of its stimulatory effect on the pyruvate dehydrogenase complex (PDC) (97). These early findings led to an interest in DCA as an anti-diabetic and lipid-lowering drug (98), with potential for treating these conditions as well as myocardial and cerebrovascular ischemia (99,100) and acquired (101,102) and congenital (103-105) forms of lactic acidosis, the latter, in particular, due to loss-of-function mutations in the PDC (106). The multi enzyme PDC is located in the mitochondrial matrix and catalyzes the rate-limiting step in the aerobic oxidation of glucose, pyruvate, alanine and lactate to acetyl CoA, and is thus integral to cellular energetics (107-110). The PDC is the key mediator of OxPhos. Respiration would be severely impaired if the activities of the two initiating enzymes of mitochondrial glucose and fatty acid metabolism were inhibited or if further reactions in the β-oxidation pathway were blocked. In humans, a family of PDK isoforms reversibly phosphory-

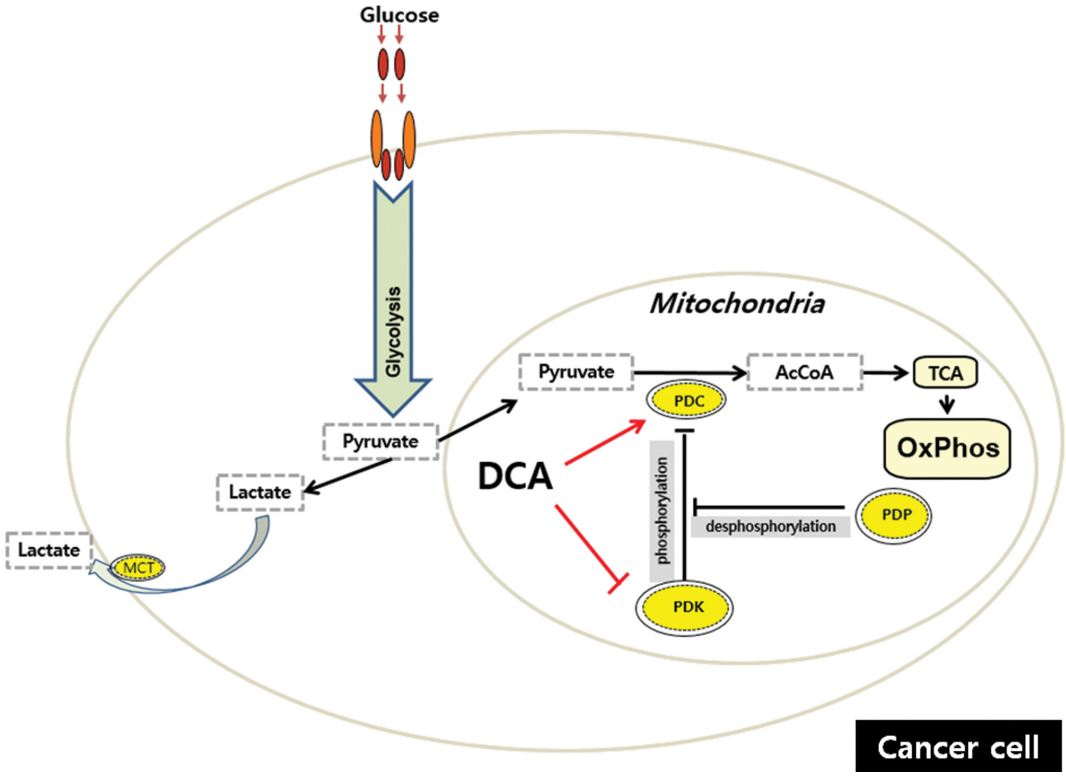

Fig. 5. Effect of 3-bromopyruvate (3-BP) on cell metabolism and survival. 3-BP dampens glycolysis by inhibiting hexokinase II and impeding the production of ATP. Furthermore, glycolysis inhibition caused by 3-BP leads to the dephosphorylation of Bcl-2-associated death promoter protein (BAD) at Ser112. Consequently, BAX, a protein required by BAD, is displaced and localized to the mitochondria, altering the mitochondrial membrane permeability and resulting in the release of cytochrome c and subsequent cell death. In addition, a main target of 3-BP is the pyruvylation of glyceraldehydes-3-phosphate dehydrogenase (GAPDH), which is associated with the loss of GAPDH enzymatic activity, resulting in the anti-glycolytic and anti-cancer effects.

lates, and inactivates, the PDC, while two pyruvate dehydrogenase phosphatase (PDP) isoforms dephosphorylate the complex and restore catalytic activity (111-113). The important thing is that the stimulatory effect of DCA on tumor cell metabolism is mediated by decreasing the expression of one or more PDK isoforms (114,115). DCA, a structural analog of pyruvate, stimulates PDC activation by inhibiting PDKs, thereby maintaining the PDC in its unphosphorylated form. PDK2 is the most ubiquitously expressed kinase and is most susceptible to inhibition by DCA (110). The molecular interactions between DCA and PDKs have been studied extensively (116-119). In general, DCA appears to bind to a hydrophobic pocket in the N-terminal domain of PDK and, in the presence of ADP, disrupts the binding of the kinase to the lipoyl (E2) domain of the PDC (120). At least for the PDK1 isoform, DCA induces a conformational change in the protein that alters both nucleotide-binding and lipoyl-binding pockets, inhibiting the catalytic activity of the kinase (116). PDK inhibition by DCA occurs rapidly in vivo because the stimulatory effect of the drug on PDC activity is measurable within 15~30 min of a single oral or parenteral dose and wanes within 12~24 hr after administration (121,122). The increase in enzyme activity is associated temporally with significant decrements in circulating pyruvate and lactate concentrations (121). The fall in the blood lactate level is a useful surrogate marker for DCA's effect on the PDC and is dose-dependent up to at least a 50-mg/kg dose in humans (123). The other mechanism by which DCA increases PDC activity is by inhibiting the turnover of the complex. This was first demonstrated in rat liver following repeated in vivo dosing, whereby total and unphosphorylated PDC increased as a function of dose and was not prevented by pharmacological inhibitors of transcription or translation (121). Such an apparent increase in enzyme stability was also observed in the primary cultures of fibroblasts from PDC-deficient patients (124,125). The molecular mechanism accounting for the stabilization of the PDC by DCA is unknown. However, it is reasonable to postulate that it may be linked to the drug-induced change in the phosphorylation state of the complex because there is considerable precedent for decreased turnover in response to changes in a protein's phosphorylation state (126-131). Regardless of the precise mechanism, stabilization of the PDC by DCA is consistent with the protracted lactate-lowering and other dynamic effects of the drug, observed with its repeated administration in humans that persist long after DCA is cleared from plasma (98).

3-3. 3-Bromopyruvate (3-BP): 3-BP has been shown to have the ability to dampen glycolysis by inhibiting hexokinase II (132). An experiment using a rat model with breast cancer highlighted the significant decrease of tumor FDG uptake (77%) while that of normal cells was negligible, indicating the strong anti-glycolytic effect of 3-BP (133). In another experiment using a hepatocellular carcinoma rat model, it was hypothesized that, due to the increased level of lactate in many cancer cells, lactate transporters are elevated and cannot discriminate the structurally similar 3-BP from lactate, permitting the entry of 3-BP into the cells and impeding the production of ATP (134). The Bcl-2-associated death promoter protein (BAD) is a pro-apoptotic protein that regulates glycolysis and apoptosis (135). Experiments have shown glycolysis inhibition caused by 3-BP leads to concentration- and time-dependent BAD protein at Ser112 dephosphorylation. After 8 hr of incubation, complete dephosphorylation was observed. Consequently, BAX, a protein required by BAD in the formation of apoptosis-preventing complexes, is displaced and localized to mitochondria, changing mitochondrial membrane permeability. Cytochrome c is then released and causes cell death (135). Further investigation shows that cancer cell death mediated by 3-BP also relies on the pyruvylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which was identified as the main target of 3-BP. This pyruvylation is associated with the loss of GAPDH enzymatic activity, leading to the anti-glycolytic and anti-tumoral effects (136). In addition, the cytotoxicity caused by 3-BP is not dependent on p53 and proved to result in massive cell death when incorporated with cisplatin and oxaliplatin (137). More importantly, a report has demonstrated that 3-BP did not cross the blood-brain barrier when the [^{14}C]3-BP uptake was measured in the brain tissue, a finding that is crucial because the brain normally has a high glucose consumption to maintain its normal functions (138). However, 3-BP is unstable and only exhibits the inhibition of glycolysis at a high concentration (139).

3-4. Diet and cancer: Diet has frequently been connected to long life. Reducing the caloric intake can influence aging, and its mechanism is being actively researched. A possible mechanism for this event appears to involve mTOR inhibition and sirtuin activation (140,141). In addition, diet is thought to be a major contributory factor in the development and progression of cancer. Epidemiological reports have emphasized that a population with low-sugar and low-fat consumption exhibits a lower incidence of cancer (142). Likewise, mice fed a diet containing low carbohydrate and high protein showed the reduction of cancer development as well as the inhibition of tumor growth (143). Links between diabetes and cancer have also been established. For example, diabetic patients treated with metformin, a drug that improves insulin sensitivity, have a reduced cancer incidence (144,145). In addition, metformin and other anti-diabetic drugs inhibit transformation (146). However, confirmation is needed that these drugs indirectly regulate hormones or directly promote cell death. High-fat diets have been linked to cancer development (147). The expression of monoacylglycerol lipase (MAGL) is also correlated with tumor aggressiveness and an invasive phenotype. MAGL acted on the release of free fatty acids. siRNA-mediated reduction of MAGL led to the inhi-

Table 2. Therapeutics targeting the Warburg effect in cancers

Process	Target	Compound	Effect	Status	References
Glucose transport	GLUT1	WZB117, STF-31	Inhibits CLUT1	Preclinical	(159,160)
Glycolysis	HK	2-DG	Inhibits HK	Clinical trials discontinued	(94)
	PKM2	TEPP-46	Activates PKM2 and inhibits PPP	Preclinical	(161,162)
	LDHA	FX11	Inhibits LDHA	Preclinical	(163)
PPP	G6PD	6-AN	Induces oxidative stress	Preclinical	(164)
Lactate Transport	MCT1	AZD3965	Inhibits uptake of extracellular lactate	Phase I	(165)
Mitochondrial function	PDK1	DCA	Inhibits PDK1	Phase I-II	(166,167)
PKB signaling pathway	PKB	AZD5363	Inhibits PKB activity	Phase I-II	(168)
		GDC0068		Phase I	(169)
		GSK2141795		Phase I completed	(170)
		GSK2110183		Phase I-II completed Phase II	(170)
		MK-2206		Phase I-II	(171)

(Adapted from (172)).

bition of invasiveness and tumor growth (148). Caloric restriction has also been known to reduce cancer incidence (149). A potential explanation is that altered levels of insulin and IGF-1 by caloric restriction function as growth factors for tumors. However, most of the studies have been performed on laboratory rodents and need to be verified in humans. Therefore, an adapted diet together with chemotherapy might be a novel way to treat cancer in the future (150,151).

CONCLUSION AND PERSPECTIVES

It has been about nine decades since the proposal of Otto Warburg concerning the metabolism of cancer cells. Unlike normal cells that undergo glycolysis and oxidative phosphorylation in the presence of oxygen, proliferating and cancer cells exhibit an increased uptake of glucose and increased rate of glycolysis and, predominantly, undergo lactic acid fermentation. This metabolism finding had raised controversy over the role of mitochondria in cancer initiation, development and metastasis. While Warburg's effect demonstrated that mitochondria seem to be damaged because of nonessential OxPhos in cancer, recent studies have indicated that mitochondria are somehow required, at least in certain steps of cancer development (43). Moreover, mitochondria function as an intracellular factory or storage of danger for cytochromes and ROS. In other words, mitochondria are the master regulators of cell fate: healthy, cancer or death! (152,153). Whether the Warburg phenomenon is the consequence of genetic dysregulation in cancer or the cause of cancer remains unknown. However, there are certainly strong links between the genetic factors and Warburg effects, suggesting several mechanical signaling pathways and the break down of some potential pharmacy compounds that target metabolic factors. Therapies targeting cancer cell metabolism are emerging and promising; however, they are currently limited by their broad effects on different tissues. It is anticipated that further research will be aimed at developing therapeutics that may provide improved targeting for aberrant components of cancer cell metabolism.

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