

Review

How DNA methylation affects the Warburg effect

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Abstract

Significant enhancement of the glycolysis pathway is a major feature of tumor cells, even in the presence of abundant oxygen; this enhancement is known as the Warburg effect, and also called aerobic glycolysis. The Warburg effect was discovered nearly a hundred years ago, but its specific mechanism remains difficult to explain. DNA methylation is considered to be a potential trigger for the Warburg effect, as the two processes have many overlapping links during tumorigenesis. Based on a widely recognized potential mechanism of the Warburg effect, we here summarized the relationship between DNA methylation and the Warburg effect with regard to cellular energy metabolism factors, such as glycolysis related enzymes, mitochondrial function, glycolysis bypass pathways, the tumor oxygen sensing pathway and abnormal methylation conditions. We believe that clarifying the relationship between these different mechanisms may further help us understand how DNA methylation works on tumorigenesis and provide new opportunities for cancer therapy.

Key words: the Warburg effect; DNA methylation; aerobic glycolysis; mitochondria; reactive oxygen species

Introduction

The Warburg effect, also called aerobic glycolysis, was first discovered by Otto Warburg in the 1920s [1]. He observed that tumors required extremely high levels of glucose compared with surrounding normal tissues, and that glycolysis was significantly enhanced tumors, even in the presence of adequate oxygen [2].

It is apparent that the Warburg effect is beneficial to the proliferation and survival of tumor cells, but how it works remains unclear. Based on current opinions, we summarize five major factors that lead to the Warburg effect: (I) The need for rapid ATP synthesis. The energy demands of tumor cells increase rapidly, and the rates of glucose uptake and metabolism through aerobic glycolysis are much higher than those that can be achieved through oxidative phosphorylation (OXPHOS) alone [3]. Mitochondrial defects are thought to be another

important feature of the Warburg effect, although some researchers disagree. (II) The need for extensive biosynthesis [4-9]. Uncontrollably proliferating tumor cells require extensive biosynthesis, and glycolysis and its bypass route – the pentose phosphate pathway (PPP) produce large amounts of raw materials. (III) The need for redox balance in tumor cells. The OXPHOS pathway is one of the main sources of reactive oxygen species (ROS) production, which can cause devastating damage to tumor cells [10]. (IV) Stimulation by hypoxia. The accumulation of hypoxia-inducible factor (HIF) leads to deficiency in the aerobic respiratory response and to the activation of glycolysis in tumor cells. (V) The need for an acidic tumor microenvironment [11,12].

DNA methylation is a form of epigenetic modification of gene expression. In mammalian cells, DNA methylation commonly involves the addition of

a methyl group contributed by S-adenosyl-L-methionine (SAM) to CpG dinucleotides to create 5-methylcytosine (m⁵c), which is catalyzed by DNA methyltransferases (DNMTs)[13]. CpG islands (CGIs), which are characterized by a very high CpG densities and are often found in the promoter regions of genes, are typically hypomethylated. Methylation of CGIs results in transcriptional silencing. In normal cells, methylation ensures the proper gene expression regulation and stable gene silencing, but abnormal DNA methylation is a powerful cause of many tumors. Unlike that of DNA methylation, the mechanism of DNA demethylation has not been well elucidated. Studies have shown that ten-eleven translocation methylcytosine dioxygenases (TETs) can oxidize m⁵c to 5-hydroxymethylcytosine (hm⁵c) [14]. hm⁵c and its further oxidized derivatives are subsequently replaced with an unmodified cytosine by base-excision repair to achieve demethylation [15]. In myeloid leukemia and glioblastoma cells,

inhibition of TETs enzymes decreases the levels of hm⁵c increases DNA methylation [15-18].

It is frequently reported that changes in DNA methylation levels regulate the expression of key enzymes in glycolysis. DNA methylation is also reported to cause mitochondrial dysfunction in tumor cells. Tumor redox balance and the accumulation of HIF have also been widely reported to be associated with aerobic glycolysis, and DNA methylation has been found to play a regulatory role in these processes. The PPP and gluconeogenesis, as important bypass pathways of glycolysis, provide abundant raw materials for the rapid proliferation of tumor cells; DNA methylation can participate in the regulation of key enzymes and molecules in these processes, thus playing a potentially important role in aerobic glycolysis in tumor cells. In this review, we discuss how DNA methylation contributes to tumor aerobic glycolysis in different pathways (Figure 1) (Table 1).

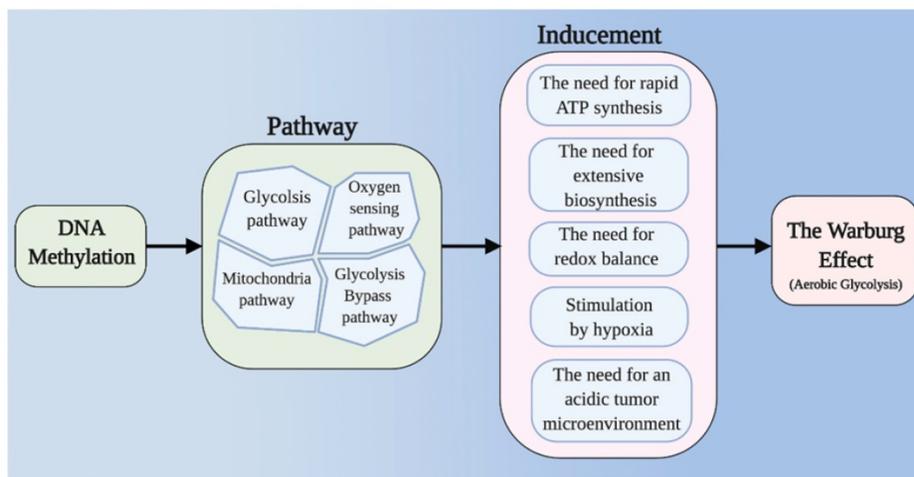


Figure 1. Main framework. The need for rapid ATP synthesis, biosynthesis, maintenance of redox balance and acidification of the tumor microenvironment are all internal causes of the Warburg effect, and HIF accumulation is an important extrinsic cause. Through different pathways (including the glycolysis pathway, the mitochondria pathway, the glycolysis bypass pathway, and the oxygen sensing pathway), DNA methylation can affect these causes of the Warburg effect.

Table 1. Targets of DNA methylation function in aerobic glycolysis.

Target	Pathway	Inducement	Cancer/cell type	Reference
GLUT-1	Glycolysis	Rapid ATP synthesis / Acidic microenvironment	Colorectal cancer	[26]
LDH	Glycolysis	Rapid ATP synthesis / Redox balance/ Acidic microenvironment	Breast cancer	[13]
PKM2	Glycolysis / PPP	Rapid ATP synthesis/ Redox balance/ Acidic microenvironment	Breast cancer / Pancreatic cancer	[37] [38]
HK	Glycolysis	Rapid ATP synthesis / Acidic microenvironment	HCC / Glioblastoma / Ovarian cancer	[39] [41] [42]
GAPDH	Glycolysis	Rapid ATP synthesis / Biosynthesis Requirement	HCC	[45]
mt-DNA	Mitochondria	Rapid ATP synthesis / Redox balance	Colorectal cancer	[63] [64] [65]
Mieap	Mitochondria	Rapid ATP synthesis / Redox balance	Colorectal cancer / Hepatoblastoma	[74]
TKTL1	Bypass pathway (PPP)	Biosynthesis Requirement / HIF accumulation	Head and neck squamous cell carcinoma	[82] [83]
NRF2	Bypass pathway (PPP)	Redox balance / Biosynthesis Requirement	Lung cancer cells / Glioma cells	[87] [88]
FBP1	Bypass pathway (Gluconeogenesis)	Glycolysis / Redox balance / Acidic microenvironment	NSCLC / HCC/ Breast cancer	[96] [97][98]
Wwox	Oxygen sensing pathway	HIF accumulation	Head and neck squamous cell carcinoma	[138]
PDK	Mitochondria	Rapid ATP synthesis / REDOX balance	Colorectal cancer	[77]
CITED4	Oxygen sensing pathway	HIF function	Breast cancer	[134]
LIMD1	Oxygen sensing pathway	HIF accumulation	Cervical carcinoma	[140]

Abbreviation: GLUT: glucose transport; LDH: lactate dehydrogenase; PK: pyruvate kinase; PK: pyruvate kinase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; Mieap: mitochondrial quality control protein; TKTL1: transketolase like-1; Nrf2: NF-E2-related factor 2; Nrf2: NF-E2-related factor 2; FBP: fructose-1,6-bisphosphatase; FBP: fructose-1,6-bisphosphatase; PDK: pyruvate dehydrogenase (PDH) kinase family; CITED4: Carboxy-terminal domain4; HNSCC: Head and neck squamous cell carcinoma; HCC: hepatocellular carcinoma; NSCLC: non-small-cell lung cancer.

DNA methylation regulates enzymes involved in glycolysis

The main feature of the Warburg effect is that cytoplasmic metabolism becomes the main source of energy instead of mitochondrial aerobic respiration. Although the amount of ATP synthesized by glycolysis per unit of glucose is small [19,20], the ATP synthesis rate is 10-100 times faster than that of complete oxidation in mitochondria; Thus, glycolysis can meet the rapid energy requirements of tumor cells [21]. DNA methylation can directly regulate glycolysis by affecting key enzymes in glycolysis.

Glucose transporter (GLUT)

Due to their rapid proliferation, malignant tumors require enormous amounts of glucose to meet their energy metabolism and anabolism needs. GLUT, a member of the glucose transporter family, is the first key molecule in tumor glucose metabolism. Upregulation of GLUT1 enhances glucose entry into tumor cells, which promotes aerobic glycolysis. Previous studies have shown the relationships between GLUT1 and poor prognosis and aggressiveness in many kinds of cancer, such as breast, kidney and stomach [22-25]. Lopez-Serra et al. demonstrated the occurrence of promoter CGI hypermethylation-linked inactivation of Derlin-3 (DERL3), a key gene in the endoplasmic reticulum-associated protein degradation pathway. The downstream targets of DERL3 include GLUT1, which means DERL3 is responsible for the degradation of GLUT1. Increases in the expression of GLUT significantly increase the uptake and transport of glucose and ultimately promote aerobic glycolysis. The final metabolites, lactate and pyruvate, acidify the tumor microenvironment and enhance tumor proliferation and invasion [26,27]. In addition to GLUT1, GLUT3 has also been reported to be related to DNA methylation. Sung et al. discovered a structural protein—caveolin-1(CAV-1), whose expression is reduced in the contexts of many human cancers [28]. With the promoter CpG site hypomethylation, the expression of CAV-1 is abnormally elevated, which stimulates GLUT3 transcription via the high-mobility group protein A (HMGA) binding site within the GLUT3 promoter, thus, upregulating glucose uptake and ultimately enhancing aerobic glycolysis [29].

Lactate dehydrogenase (LDH)

LDH, a crucial enzyme for aerobic glycolysis, converts pyruvate into lactate and oxidizes NADH to regenerate NAD⁺. LDH can guarantee the sustained utilization of NADH-generating glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and maintain

orderly aerobic glycolysis. LDH is a tetrameric enzyme consisting of 2 major subunits (A and B), that are encoded by 2 different genes, LDH-A and LDH-B [30]. Different numbers of LDH-A and LDH-B subunits can bind in tetramers to form 5 different isoenzymes (LDH-1 to LDH-5) [31]. As LDH-5 consists only of LDH-A and preferentially catalyzes the conversion of pyruvate into lactate, whereas LDH-1 consists only of LDH-B and catalyzes the conversion of lactate into pyruvate, the LDH-A/LDH-B ratio in tumor cells modulates lactate production [31]. Although HIF-1 α - and c-Myc-related pathways can promote LDH-A expression [30], many studies have demonstrated that DNA methylation regulates the LDH-A ratio by promoting LDH-B promoter region hypermethylation in breast and prostate cancer, while the demethylating agent 5-azacytidine can restore the mRNA levels [13]. Another study has reported that LDH-A is silenced in isocitrate dehydrogenase (IDH) mutant gliomas because of hypermethylation [32]. As the LDH-A/LDH-B ratio increases, it greatly promotes the conversion of lactate, which enhances aerobic glycolysis and tumorigenesis [33].

Pyruvate kinase (PK)

PK, a key rate-limiting enzyme in the glycolysis pathway [34], catalyzes the conversion of phosphoenolpyruvate and ADP to pyruvate and ATP. Because of mutually exclusive alternative splicing [35], PK is divided into two types [36]; the alternatively spliced isoform M2 (PKM2) contributes to the Warburg effect by promoting aerobic glycolysis, whereas the PKM1 isoform promotes OXPHOS. Some believe that PKM2 is often upregulated in tumors due to intron hypomethylation, and hence promotes the Warburg effect [13]. However, others have suggested that PKM2 stimulates the Warburg effect because of DNA methylation. Singh et al. reported that the intragenic DNA methylation-mediated binding of Brother of Regulator of Imprinted Sites (BORIS) at the alternative exon 10 of PK is associated with cancer-specific splicing that promotes the Warburg effect and breast cancer progression. Once DNA methylation is inhibited or the BORIS binding site is deleted, a splicing switch from the cancer-specific PKM2 to the normal PKM1 isoform occurs. In this case, glycolysis and the Warburg effect are inhibited, limiting rapid proliferation and growth of tumor cells [37]. In the same year, Xu et al. proposed another explanation based on mitochondria function related to the effects of coactivator-associated arginine methyltransferase 1 (CARM1), which interacts with and methylates PKM2 at three arginine residues

(R445, R447 and R455). Methylated PKM2 localizes to the mitochondria-associated endoplasmic reticulum membrane, through interaction with inositol 1,4,5-trisphosphate receptors (IP3Rs), decreasing mitochondrial membrane potential ($\Delta\Psi_m$) and Ca^{2+} uptake, which is essential for activating pyruvate dehydrogenase (PDH) to support OXPHOS and thus ultimately promoting aerobic glycolysis [38]. Hence, PKM2 methylation is an important regulator of the switch between OXPHOS and aerobic glycolysis in cancer cells. PKM2 can also further promote the aerobic glycolysis process in tumor cells by affecting the PPP, which will be described later. Therefore, the specific mechanism and status of PKM2 methylation in tumor cell metabolism are still worth studying.

Hexokinases (HKs)

HKs catalyze the first step in glycolysis, the ATP dependent phosphorylation of glucose to yield glucose-6-phosphate (G6P). Four major hexokinase isoforms, encoded by separate genes, are expressed in mammalian tissues: HK1, HK2, HK3, and HK4. HK2 is often expressed at high levels in tumor cells [39,40]. Hypomethylation of the promoter is responsible for the upregulation of HK2 in liver cancer and glioblastoma [41]; this upregulation then enhances aerobic glycolysis and tumor proliferation [42]. HK2 has also been found to be mediated by microRNAs in ovarian cancer, such as miR603 and miR145 [43,44], whose precursor genes are modulated by DNMT3a. Given these findings, microRNAs can be considered inhibitors of the Warburg effect. And the inhibitory effects of miR603 and miR145 can be enhanced by the use of 20(S)-Rg3, an inhibitor of DNMT3a.

GAPDH

GAPDH converts glyceraldehyde-3-phosphate (G3P) and NAD^+ into 1,3-bisphosphoglycerate (1,3-BPG) and NADH. It also plays an important role in aerobic glycolysis and has been found to be upregulated in the contexts of several kinds of cancer, but the exact cause of the upregulation remains unclear. Recently, Lei et al. proposed a new explanation for how GAPDH functions in the Warburg effect [45]. They focused on CARM1, which methylates GAPDH at R234 and inhibits its catalytic function in hepatocellular carcinoma (HCC). Therefore, aerobic glycolysis was repressed and the proliferation and growth of HCC cells are repressed.

Mitochondrial dysfunction caused by DNA methylation is a potential trigger for aerobic glycolysis

Mitochondria are the most important organelles for cell energy metabolism. As the end product of

glycolysis, pyruvate enters the mitochondria for further reactions to produce ATP and metabolites through the tricarboxylic acid (TCA) cycle and OXPHOS. Abnormalities in the structure or function of mitochondria are very common in tumors, such as mitochondrial DNA (mtDNA) damage in tumor cells, which affects mitochondrial respiration and energy synthesis [46,47]. There is abundant evidence suggesting mitochondrial dysfunction is a potential trigger for aerobic glycolysis [48-50] (Figure 2).

Abnormalities in mtDNA

MtDNA accounts for only a very small proportion of the total cellular DNA [51-53], but its gene expression products play crucial roles in mitochondrial and cellular functions. MtDNA encodes several important proteins related to the mitochondrial respiratory chain [54,55]. Therefore, changes in mtDNA status will directly affect mitochondrial function and ultimately glycolysis status. Whether methylation of mtDNA exists has long been debated in the growing field of tumor metabolism research [56-60]. Shock et al. first reported the presence of m5c and hm5c in mitochondria which might be derived from mtDNA cytosine methylation, and the existence of mitochondrial DNA methyltransferase 1 (mt-DNMT1) which bound to mtDNA and modifies the mitochondrial genome and function [61]. Sunil et al. further identified the DNMT1 in mitochondria as isoform 3 [62]. Marianne et al. first directly proved the potential functionality of this molecule in tumor cells [63] and some other studies have also suggested a direct relationship between mtDNA methylation and tumors [64,65]. Notably methylation of mtDNA generally leads to OXPHOS dysfunction [66]. The methyl donor SAM is also important to mtDNA methylation [67]. The SLC25A26 gene encodes the SAM mitochondrial carrier (SAMC) which catalyzes the import of SAM into mitochondria [68]. In cancer cells, upexpression of SAMC increases mitochondrial SAM levels, promotes mtDNA methylation, leads to decreased expression of key respiratory subunits, and decreases the release of cytochrome B, thus impairing mitochondrial OXPHOS and ROS balance [69]. The glycolysis function then is further strengthened for compensation in tumor cells, even in the presence of oxygen.

Abnormalities in mitochondrial health

Another cause of mitochondrial dysfunction is the accumulation of unhealthy mitochondria. The mitochondrial quality control protein—Mieap can induce intramitochondrial lysosome-like organelle that plays a critical role in eliminating oxidized

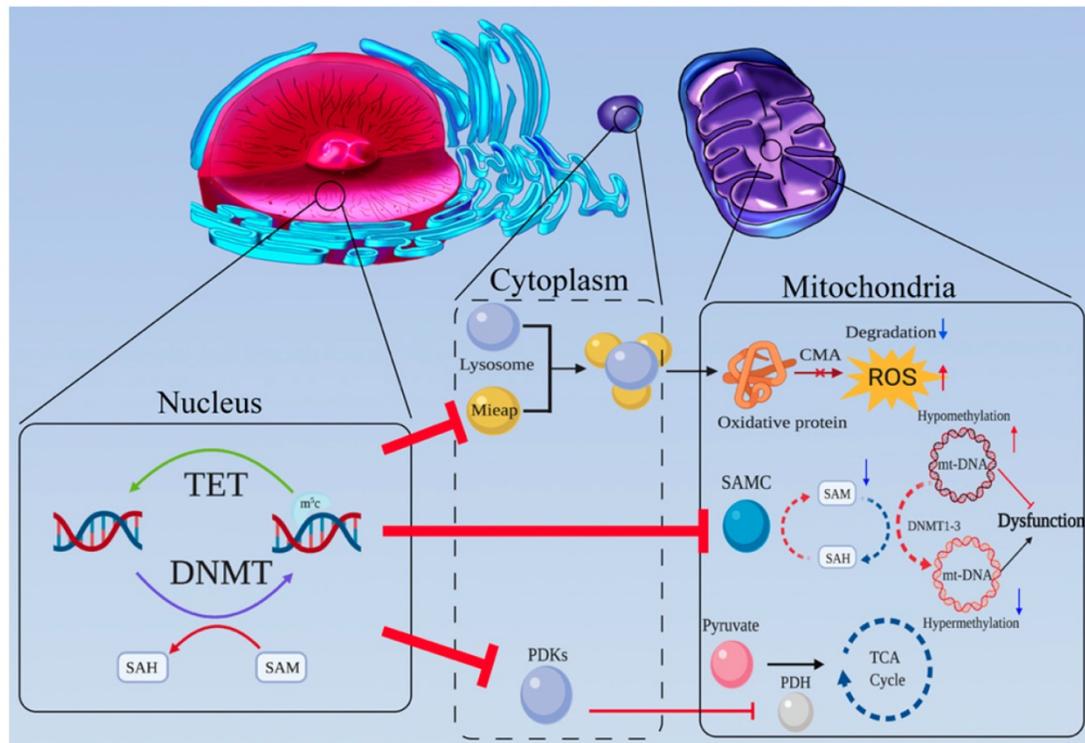


Figure 2. Mitochondrial dysfunction caused by DNA methylation is a potential trigger for aerobic glycolysis. **(a)** MtDNA is crucial for mitochondrial function. Once mtDNA is methylated by mt-DNMTs, OXPHOS is inhibited. Due to promoter hypermethylation, SAMC is downregulated, and mitochondrial SAM levels decrease. Hence, the methylation status of mtDNA may be downregulated. **(b)** Mieap-mediated, lysosomal involved oxidative protein clearance is an important pathway for the maintenance of normal mitochondrial function. Hypermethylation of Mieap causes mitochondria to become severely dysfunctional. **(c)** The activity of PDH can be inhibited by PDKs, which are modulated by DNA methylation.

mitochondrial proteins in mitochondria [70,71]. It dramatically induces the accumulation of lysosomal proteins in mitochondria without destroying the mitochondrial structure, leading to increased ATP synthesis and decreased ROS generation. [72]. An existing model for the mechanism by which Mieap induces lysosome-like organelles enter into mitochondria is chaperon-mediated autophagy (CMA) way, one of the three main pathways of autophagy. In this mechanism, lysosomes can specifically absorb oxidized proteins containing a common motif through the interaction of lysosome-associated membrane protein 2A (LAMP2A) and heat shock protein 70 (HSP70) [73]. If entire structure of lysosomes or lysosomal like organelles can enter and reside in the mitochondria, these organelles may act through a CAM-like mechanism in the mitochondria to eliminate and degrade oxidative proteins. The exact mechanism by which Mieap helps lysosomes enter the mitochondria without damaging normal mitochondrial structures remains unclear. However, in many kinds of tumor cell lines, Mieap expression is often inhibited by promoter methylation, leading to ROS accumulation, protein oxidation and subsequent mitochondrial destruction [74].

Abnormalities in key TCA cycle enzymes

The TCA cycle is the first reaction cycle undergone by glycolysis products in the mitochondria. Abnormalities in TCA enzymes lead to mitochondrial dysfunction and are potential triggers of aerobic glycolysis. PDH mainly mediates the entry of pyruvate into the TCA cycle, while the family of PDH kinases (PDKs) inhibits PDH activity to promote aerobic glycolysis and tumor progression [75,76]. Leclerc et al. observed that PDH kinase 4 (PDK4) is distinctly upregulated by hypomethylation of its promoter [77]. In addition, 2-hydroxyglutarate (2-HG) produced by IDH1/2 mutation [32], as well as the accumulation of succinate and fumarate caused by mutations in fumarate hydratase (FH) [78] and succinate dehydrogenase (SDH), can not only promote the Warburg effect by inhibiting the degradation of HIF [79], but also enhance the methylation level of the whole genome by inhibiting TET enzymes [80].

DNA methylation participates in the glycolysis bypass pathways

Tumor cells are rapidly and malignantly proliferating cells, that require many raw materials to support their continuous proliferation. Hence, Heiden

et al. proposed another explanation for the Warburg effect: proliferating cells have important metabolic requirements that extend beyond ATP [81]. The PPP, a glycolysis bypass route, plays an important role in the production of raw material production, such as nucleic acid and amino acid sugar phosphate precursors. Moreover, the Warburg effect is also affected by changes in the expression levels of PPP enzymes in the nonoxidative pathway.

Transketolase (TKT) like-1 (TKTL1) is an essential gene that encodes an enzyme responsible for the TKT reaction in nonoxidized portion of PPP. There is considerable evidence that tumor cells can upregulate the activity of TKT [82] and induce the expression of TKTL1, whose expression has been shown to be increased due to promoter hypomethylation [83]. Wenyue et al. showed that high expression of TKTL1 in tumor cells significantly increases the production of lactate and pyruvate, which are hallmarks of the Warburg effect [84]. In addition, they demonstrated that TKTL1 promotes the stability and accumulation of HIF1- α , which is a key molecule for DNA methylation and aerobic glycolysis, that will be discussed later.

Nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2) is a master transcriptional activator of cyto-protective genes [85,86]. Its main function is protecting cells from the effects of allogeneic organisms and oxidative stress. However, high Nrf2 expression in tumor cells is often associated with poor prognosis [87,88]. Mitsuishi et al. analyzed Nrf2 from the perspective of its effects on tumor metabolism [89]. Nrf2 directly activates glucose 6-phosphate dehydrogenase (G6PDH), PGD, TKT, transaldolase and IDH, which are key enzymes of PPP, through well-conserved antioxidant response elements (AREs). Thus, Nrf2 has been shown to promote NADPH and nucleotide production in tumor cells [90]. Under normal conditions, Nrf2 is continuously degraded in a Keap1-dependent manner through the ubiquitin-proteasome pathway [91,92]. Upon the increased methylation in the promoter region, the expression level of Keap1 is significantly decreased, which activates Nrf2 and PPP enzymes, as has been confirmed in lung cancer cells and glioma cells [93,94].

Most studies tend to the catabolism of glucose, ignoring the role of anabolism in aerobic glycolysis. Gluconeogenesis, a process that converts a variety of non-sugar substances into glucose or glycogen, is less investigated than glycolysis but may play an equally important role in switching the metabolism of tumor cells to aerobic glycolysis. Because glycolysis involves a three-step irreversible reaction, gluconeogenesis is not a simple reversal of glycolysis. Notably, the gluconeogenesis pathway can be modulated by DNA

methylation. Cai et al. reported that betaine, a methyl donor, can significantly change the methylation statuses of CpGs in the promoters of gluconeogenesis-associated genes [95].

Fructose-1,6-bisphosphatase (FBP) plays a crucial role in the process of gluconeogenesis which catalyzes the hydrolysis of fructose-1,6-bisphosphate into fructose-6-phosphate and inorganic phosphate. There are two isoforms of FBP in humans: FBP1 and FBP2. FBP1 is widely reported to be downregulated, due to abnormal methylation of its promoter sequence in non-small cell lung cancer (NSCLC) [96], HCC [97], basal-like breast tumor [98], gastric cancer [99], small intestinal neuroendocrine tumor [100] and colon cancer [101]. Although studies on FBP2 have rarely been reported, hypermethylation in the FBP1 promoter region has been shown to occur in gastric cancer cells [102].

In addition, FBP1 has been reported to be a tumor suppressor that regulates tumor glucose metabolism and inhibits aerobic glycolysis, such as by increasing glucose uptake and macromolecules biosynthesis [98,103]. FBP1 has also been demonstrated to be involved in posttranslational modification of PKM2 which is a crucial glycolytic enzyme [104-106]. Koeck et al. found that upregulation of FBP1 significantly increases mitochondrial complex I activity. In contrast, loss of FBP1 inhibits oxygen consumption and ROS production [107]. Li et al. reported that ectopic FBP1 expression in renal cell carcinoma reduces lactate secretion, NADPH level, PPP flux and glycolysis derived TCA cycle intermediates levels [108]. These data provide mechanistic insights that loss of FBP caused by DNA methylation may result in glycolytic flux, glucose uptake, ATP production maintenance [98,109], and OXPHOS functional inhibition [98], all of which are features of the Warburg effect.

FBP, a key enzyme of gluconeogenesis pathway, mainly converts lactate and pyruvate into glucose for reuse, which is beneficial to cells. However, in tumor cells, FBP acts as a tumor suppressor and inhibits aerobic glycolysis. We hypothesize that FBP can promote glycolytic flux into the gluconeogenesis pathway and destroy the acidic environment. Moreover, gluconeogenesis, the reverse pathway of glycolysis, requires much more energy than glycolysis since it must overcome the three-step irreversible glycolysis reaction [110,111], which is not efficient for smart tumor cells.

Oxygen-sensing pathway connects DNA methylation and aerobic glycolysis.

As the central protein of the hypoxia regulatory pathway, HIF has been demonstrated to be associated

with the Warburg effect. HIF consists of oxygen-related α -subunits (HIF1- α and HIF2- α) and a constitutively expressed β -subunit (HIF1- β) [112]. Among the subunits, HIF1- α is believed to be the one most related to tumor glycolysis [113]. Under conditions of normal oxygen, α -ketoglutarate-dependent prolyl-hydroxylases (PHDs) promotes the hydroxylation of HIF- α proline residues [114-116], which becomes the optimal recognition sites for the von Hippel-Lindau (VHL) tumor suppressor protein. VHL binding activates ubiquitination pathways to degrade HIF [117-119]. Hydroxylation requires oxygen and α -ketoglutarate, and produces carbon dioxide and succinate. In the absence of oxygen, PHD activity is inhibited, and the HIF- α subunit is no longer degraded; rather it binds to the constitutively expressed HIF- β subunit to form a HIF dimer. HIF dimer then enters the nucleus and binds to hypoxia response elements (HREs) (Figure 3) [120-122].

The presence of hypoxic regions is characteristic of the microenvironment of solid tumors, such as liver cancers [123]. HIF regulates many genes in multiple cell types, including those related to glycolysis [124]. This regulation allows tumor cells to transfer the center of energy metabolism from OXPHOS to

glycolysis under hypoxic condition [125]. Many studies have demonstrated that the expression levels of glycolysis related genes such as 6-phosphofructo-1-kinase (PFK-1), PKM2 and 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase genes (PFKF B1-4), are increased in tumor cells under hypoxia condition[126,127]. Keith B et al. reported that elevated HIF activity stimulates the expression of glycolysis-related genes, such as LDHA, phosphoglycerate kinase 1 (PGK1), and activated PDH kinase 1(PDK1) to inhibit glycolytic flux into the TCA cycle in clear cell renal cell carcinoma (ccRCC) [128]. Bo Li et al. found that FBP1 suppresses HIF activity and eventually reduces the expression of HIF target genes (PDK1, LDHA and GLUT1) [103].

Hu et al. first demonstrated that HIF1- α and HIF2- α have unique targets, and that HIF1- α (not HIF2- α) stimulates glycolysis-related gene expression. Swati Dabral et al. found that Ras association domain family 1A (RASSF1A) can bind to HIF1- α , block its degradation in the PHD-VHL-lysosome pathway, and thus enhance the activation of the glycolytic switch in lung cancer cells [129]. HIF-1 α can also inhibit mitochondrial function and thus promote aerobic glycolysis. After entering the mitochondria, pyruvate dehydrogenase (PDH) catalyzes the conversion of

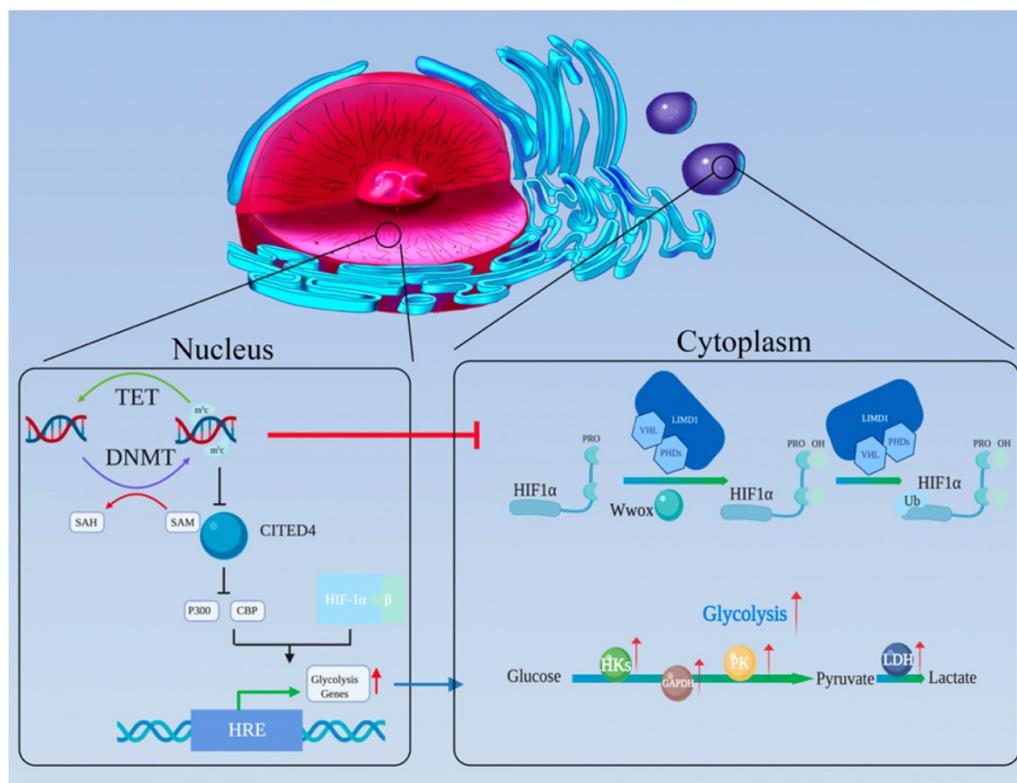


Figure 3. The oxygen-sensing pathway connects DNA methylation and aerobic glycolysis. (a) The HIF complex can recruit P300 and CBP and then stimulate the HREs in the promoters of its target genes, promoting glycolysis-related gene expression. (b) CITED4 can competitively bind P300 and CBP, thereby inhibiting HIF's function. However, CITED4 is regulated by DNA methylation. (c) Wwox, which is regulated by DNA methylation, could disrupt HIF1- α 's stability by affecting the PHD pathway. (d) LIMD1 acts as a scaffold to bind PHDs and VHL, which are key molecules for the degradation of HIF1- α . LIMD1 and VHL can be inhibited by DNA methylation.

pyruvate into acetyl coenzyme A and enters the TCA cycle. HIF-1 α can activate PDK1 (phosphatidylinositol-dependent protein kinase 1), which can then phosphorylate and inhibit pyruvate dehydrogenase (PDH) E1 α [130], thus inhibiting acetyl coenzyme A synthesis, and blocking the TCA cycle and thereby freeing pyruvate from mitochondrial OXPHOS. Therefore, we can conclude that HIF is closely related to glycolysis.

The finding described above reveal that HIF plays an important role in aerobic glycolysis. In addition, there is considerable evidence that HIF can be regulated by DNA methylation (Figure 3). M Koslowski et al. first revealed the relationship between tumor-associated CpG demethylation and HIF-1 α . In colon cancer cell lines, treatment with the DNA-demethylating agent 5-azacitidine significantly enhances the expression of HIF-1 α its target genes. The potential mechanism involves positive autoregulation of HIF-1 α is governed by a methylation-sensitive HRE in its promoter [131].

Furthermore, DNA methylation is involved in regulating the functional pathways of HIF. After HIF-1 α and HIF- β combine to form a stable dimer and translocate to the nucleus, the HIF dimer needs to recruit the transcriptional adapter/histone acetyltransferase protein, P300 and CREB-binding protein (CBP), to the promoter of its target genes for transcription stimulating [132,133]. Carboxy-terminal domain 4 (CITED4) could competitive bind P300/CBP and inhibit the HIF complex. Due to hypermethylation of its promoter, the expression of CITED4 is inhibited in breast cancer, while that of HIF and its target genes is significantly increased [134].

In addition, DNA methylation regulates HIF degradation pathways. As a tumor suppressor, WW-domain containing oxidoreductase (Wwox) has been reported to modulate glucose metabolism [135]. Abu-Remaileh M et al. found under aerobic conditions, Wwox loss activates glycolysis-related gene expression and inhibits pyruvate entry into the TCA cycle which are features of the Warburg effect [136]. The authors explained that these effects happen because Wwox disrupts HIF1- α 's stability via affecting the PHD pathway and inhibiting its transcriptional activity [137]. Ekizoglu S. et al. found that the expression level of Wwox and the methylation of its promoter are inversely correlated. In other words, Wwox expression is regulated by DNA methylation [138]. The tumor suppressor protein LIM domain containing protein (LIMD1) has been demonstrated to act as a scaffold to bind PHDs and VHL, which are responsible for HIF degradation [139]. And Panda CK et al. showed that upregulation of HIF1- α and its target genes was due to high

methylation status of LIMD1 and VHL in cervical carcinoma [140].

Although HIF1- α is often thought to be associated with glycolysis, HIF-2 α has also been showed to regulate GLUT-1, which could enhance aerobic glycolysis and glucose transport into renal carcinoma cells [141]. Some research has shown that a target gene of HIF2- α , endothelial PAS domain-containing protein 1 (EPAS1), is modulated by DNA methylation. Blockade of the transcription of EPAS1 by DNMT3a can inhibit tumor proliferation in renal cell carcinoma and glioblastoma cell lines [142,143].

Abnormal conditions related to DNA methylation

The methyl donor SAM is a key factor for DNA methylation. The main principle of DNA methylation is the addition of a methyl group contributed by SAM to CpG dinucleotides to create m5c. Folate is an important source for SAM synthesis. After absorption in the small intestine, folate is converted into its active form, tetrahydrofolate (THF), which then combines with one carbon unit to form methylene THF. It is a raw material for DNA and RNA synthesis. Reductase converts methylene THF into 5-methyl-THF, which is combined with homocysteine (Hcy) to form methionine; methionine then is converted to SAM [144].

Low-folate nutritional status (LF) has been reported to play a roles in lung cancer [145] and colon cancer [146]. LF leads to low SAM expression, which reduces the methylation levels in tumor cells, thereby changing the functions of tumor cells. In addition, Jin Fan et al. first demonstrated that folate metabolism is a crucial method for NADPH production; these authors knocked down the folate-dependent enzymes, methylenetetrahydrofolate dehydrogenase (MTHFD), which significantly reduced NADPH/NADP+ ratios [147]. In addition, MTHFD expression has been found to be related to the progression of cancers [148]. It has even been shown that the folate pathway produces more NADPH than the PPP. Roland Nilson et al. reported that MTHFD2 is overexpressed in 12 tumor types [149]. Under LF, NSCLCs have been reported to undergo metabolic reprogramming, which includes elevation in lactate release, acidification of the microenvironment, the change in the NADH/NAD+ redox status and NADPH/NADP+ ratios. Changes in these characteristics will inhibit aerobic glycolysis. And The LF-induced aerobic glycolysis phenotype of NSCLC can be reversed by the DNA methylation inhibitor 5-azacitidine. This result suggests that the LF status promotes the Warburg effect by reducing methylation levels in tumor cells [150]. The methionine salvage

pathway is another crucial pathway for SAM production [151,152]. Serine metabolism was thought to promote the methionine salvage pathway, a bypass pathway for glycolysis, through a range of anabolic processes, including NADPH, methylene THF and nucleotides [153]. These metabolites are all important intermediates for the methionine salvage pathway to promote SAM and DNA methylation.

Future perspectives

The synergistic effect of The Warburg effect and DNA methylation is worthy of further study, which is conducive to further revealing the mechanism of tumor development and tumor therapy.

The synergistic effects for tumor immunity

Aerobic glycolysis and DNA methylation not only work together to regulate tumor cells directly, but also may influence the development of tumors by regulating the function of the immune system. Aerobic glycolysis is considered to be a metabolic hallmark of activated T cells. Pearce EL et al. suggested that aerobic glycolysis augments effector T cell responses, including expression of the proinflammatory cytokine interferon (IFN)- γ via 3' untranslated region (3'UTR)-mediated mechanisms [154]. Li et al. found that aerobic glycolysis can promote effector T cell differentiation [155]. While GA et al. reported that DNMT3a can also regulate T cell development and suppress T-cell acute lymphoblastic leukemia transformation [156]. Hence, further studying DNA methylation and aerobic glycolysis in immune cells could be of great significance for enhancing understanding of tumor immune tolerance.

The synergistic effects for tumor therapy

Both DNA methylation and the Warburg effect are important mechanisms of tumor development and provide us with new strategies for tumor treatment. The Warburg effect provides advantages for the growth of tumor cells; therefore, some drugs can alleviate mitochondrial OXPHOS defects and inhibit glycolysis by regulating the energy acquisition pathways of cells. For example, 2-deoxy-d-glucose (2-DG), a glucose analog, affects glucose metabolism, depleting cancer cells of energy and eliciting antitumor effects [157]. Dichloroacetic acid (DCA) can reverse the Warburg effect by inhibiting PDK1 to switch cytoplasmic glucose metabolism to OXPHOS, providing a new approach to antitumor therapy [158]. 3-Bromopyruvate (3-BP) is also a widely recognized inhibitor of glycolysis [159]. As a trigger of the Warburg effect, ROS are produced mainly through mitochondria. Strategies for mitochondrial

metabolism have been reported in many clinical studies [160]. In addition to limitation of aerobic glycolysis, inhibition of DNA methylation is also considered an important approach for cancer therapy. The DNA methylation inhibitors decitabine (5-aza-2'-deoxycytidine) and 5-azacitidine have been widely used in the treatment of leukemia [161].

Many studies have shown that the regulation of ROS and DNA methylation play a synergistic role in the treatment of tumors. Poly (adp-ribose) polymerase (PARP) inhibitors (PARPi) are an effective anti-tumor drug for breast and ovarian cancer. And DNA methylation inhibitor decitabine can mediate the activation of PARP by increasing the accumulation of ROS, and promote the sensitivity of PARPi to tumors through the cAMP/PKA pathway, so as to play a more effective anti-tumor effect in collaboration with PARPi[162]. Zhou et.al proposed that Live-attenuated measles virus vaccine as a potential oncotherapeutic agent, confers cell contact loss and apoptosis of ovarian cancer cells via ROS-induced silencing of E-cadherin by DNA methylation [163]. Deepika et.al reported a special hypoxia-selective epigenetic agent RRx-001, which induces reactive oxygen species and nitrogen (RON), and in turn induces oxidative and nitrogen narrative stress, leading to cell death in myeloma. RRx-001 also inhibits DNA methylation by down-regulating DNA methyltransferase (DNMTs) and induces tumor cell apoptosis [164]. These suggest that combining multiple mechanisms to treat tumors may yield better results.

Elucidating the relationship between various important tumorigenesis mechanisms, finding out the root cause of dysfunction, and developing new combination treatment will be the development direction of tumor therapy.

Conclusion

DNA methylation, a type of epigenetic modification, plays an important role in both normal and tumor cells. The Warburg effect, a characteristic marker of abnormal metabolism in tumor cells, warrants further study. In this review, we have summarized the correlations between DNA methylation and the Warburg effect, and have discussed the mechanism by which DNA methylation may contributed to the Warburg effect. DNA methylation can regulate glycolysis related enzymes, inhibit mitochondrial functions and glyconeogenesis-related enzymes, promote aerobic glycolysis, enable the rapid energy needs of tumor cells to be met and reduce ROS damage. The PPP, which produces NADPH for redox equilibrium and raw material production, can also be modulated by DNA methylation. HIF has been widely reported to

promote aerobic glycolysis and is closely associated with DNA methylation. We have also discussed how abnormal DNA methylation conditions can affect aerobic glycolysis, further supporting the important role of DNA methylation in the occurrence and development of Warburg effect. However, as Otis W. Brawley once said, "One cancer cell is smarter than 100 brilliant cancer scientists". Thus, there is still much to be learned about the association between DNA methylation and the Warburg effect.

Abbreviations

BORIS: brother of regulator of imprinted sites; CITED4: carboxy-terminal domain 4; CGIs: CpG islands; CAV-1: caveolin-1; CARM1: coactivator-associated arginine methyltransferase 1; CMA: chaperon-mediated autophagy; DNMTs: DNA methyltransferases; DERL3: derlin-3; EPAS1: endothelial PAS domain-containing protein 1; FBP: fructose-1,6-bisphosphatase; GLUT: glucose transporter; G6P: glucose-6-phosphate; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; G3P: glyceraldehyde-3-phosphate; HIF: hypoxia-inducible factor; hm⁵c: 5-hydroxymethylcytosine; HMGA: high mobility group protein A; HREs: hypoxia response elements; HKs: hexokinases; IDH: isocitrate dehydrogenase; LDH: lactic dehydrogenase; LF: low-folate nutritional status; LIMD1: LIM domain containing protein; MTHFD: methylenetetrahydrofolate dehydrogenase; m⁵c: 5-methylcytosine; Mieap: mitochondrial quality control protein; NF-E2: nuclear factor erythroid 2; Nrf2: NF-E2-related factor 2; NADPH: reduced nicotinamide adenine dinucleotide phosphate; PPP: pentose phosphate pathway; PDK1: phosphatidylinositol-dependent protein kinase 1; PGK1: phosphoglycerate kinase 1; PFKFB : 6-phosphofructo-2-kinase /fructose 2,6-bisphosphatase; PFK-1: 6-phosphofructo-1-kinase; PHD: prolyl-hydroxylases; PDH: pyruvate dehydrogenase; PK: pyruvate kinase; ROS: reactive oxygen species; SAM: S-adenosylmethionine; TCA: tricarboxylic acid cycle; TET: ten-eleven translocation methylcytosine dioxygenases; TKTL1: transketolase like-1; THF: tetrahydrofolate; OXPHOS: oxidative phosphorylation; VHL: von Hippel-Lindau; Wwox: WW-domain containing oxidoreductase; 1,3-BPG: 1,3-bisphosphoglycerate.

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Authors Contributions

PS and SZ designed the study. XZ drafted the manuscript. ZX, JC, and ZL critically revised the

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Competing Interests

The authors have declared that no competing interest exists.

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