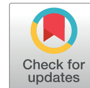

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Metabolic Signaling to Epigenetic Alterations in Cancer

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Abstract

Cancer cells reprogram cellular metabolism to support the malignant features of tumors, such as rapid growth and proliferation. The cancer promoting effects of metabolic reprogramming are found in many aspects: generating additional energy, providing more anabolic molecules for biosynthesis, and rebalancing cellular redox states in cancer cells. Metabolic pathways are considered the pipelines to supply metabolic cofactors of epigenetic modifiers. In this regard, cancer metabolism, whereby cellular metabolite levels are greatly altered compared to normal levels, is closely associated with cancer epigenetics, which is implicated in many stages of tumorigenesis. In this review, we provide an overview of cancer metabolism and its involvement in epigenetic modifications and suggest that the metabolic adaptation leading to epigenetic changes in cancer cells is an important non-genetic factor for tumor progression, which cooperates with genetic causes. Understanding the interaction of metabolic reprogramming with epigenetics in cancers may help to develop novel or highly improved therapeutic strategies that target cancer metabolism.

Key Words: Cancer, Metabolism, Epigenetics, Acetylation, Methylation, Metastasis

INTRODUCTION

Metabolic reprogramming, one of the emerging hallmarks of cancer, has been recognized for decades since the first observation of “aerobic glycolysis” in cancer cells by Otto Warburg (Warburg, 1956). In terms of energy metabolism, such as ATP production, the advantage of cancer metabolism represented by the upregulation of aerobic glycolysis seems elusive, as cancer cells retain the capacity for mitochondrial oxidative phosphorylation, which is ~18-fold more efficient than glycolysis (Vander Heiden and DeBerardinis, 2009). Rather, the significance of cancer metabolism has been found in providing anabolic building blocks and regulating the cellular redox state (Vander Heiden and DeBerardinis, 2017). More recently, metabolism has drawn much interest as it is intimately related to epigenetic regulation by supplying intermediary metabolites as the cofactors for epigenetic enzymes. Thus, the altered metabolism in cancer cells may cause distinct epigenetic changes that can contribute to cancer development and progression.

In fact, epigenetic dysregulation is tightly involved in tumorigenesis (Feinberg *et al*., 2016). In some cases, genetic mutations on chromatin modifiers cause aberrant epigenetic modifications in cancer. However, many epigenetic variations related to differential clinical outcomes cannot be explained solely by genetic reasons. Metabolic reprogramming in cancer is considered one of the non-genetic factors to alter the epigenetic landscape. Epigenetic regulators use different metabolites as co-substrates to modify chromatin structure. In addition, several metabolites inhibit the catalytic activity of epigenetic modifiers. There are at least three different mechanisms by which cancer metabolism affects epigenetics: (1) alteration of metabolite levels by reprogramming metabolic pathways, (2) nuclear production of metabolites by the metabolic enzymes translocated to the nucleus, and (3) generation of oncometabolites, whose accumulation drives cancer progression, to regulate the activity of epigenetic enzymes.

In this article, to expand the current understandings of the pathogenic roles of altered metabolism in cancer cells, we review the current knowledge on how metabolic reprogramming affects the epigenetic landscape, directing the fate of cancer cells. Further, given that cancer progression, such as the development of metastasis and anti-cancer drug resistance, can

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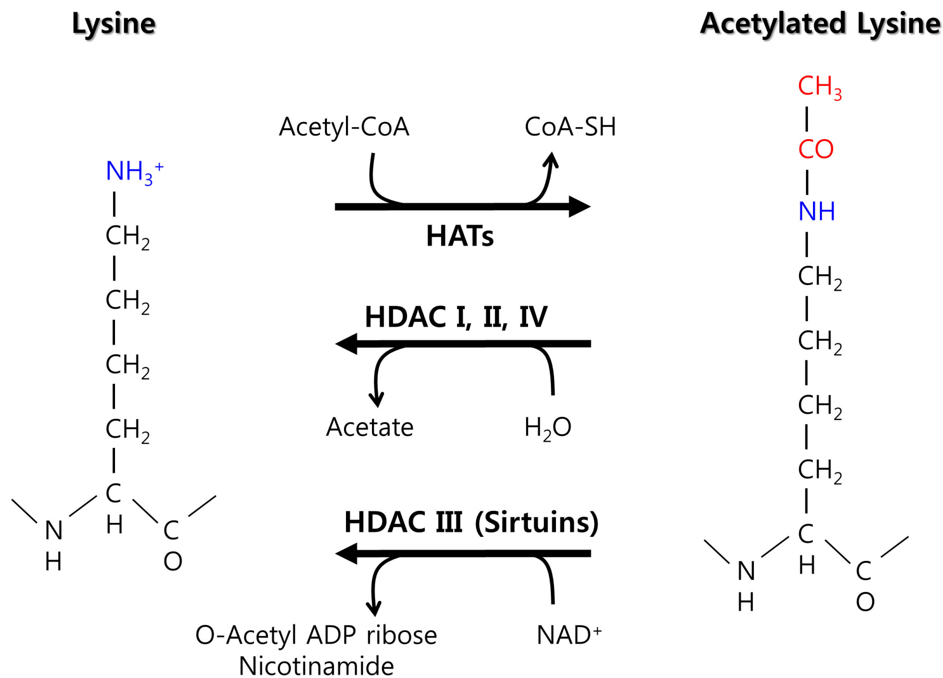

Fig. 1. Histone acetylation and deacetylation. The enzymes involved in the pathways are in bold. HATs: histone acetyltransferases, HDACs: histone deacetylases.

be mediated by epigenetic plasticity and metabolic adaptation (Valastyan and Weinberg, 2011; Brown *et al*., 2014), we pay special attention to the role of metabolic signaling in the regulation of epigenetic changes that drive aggressive cancer development, hoping to provide mechanistic insights into developing potential anti-cancer therapeutic strategies (Kim, 2015).

EPIGENETIC MODIFICATIONS RELATED TO TUMORIGENESIS

Modifications of DNA and histones constituting nucleosomes are the most extensively studied epigenetic alterations related to cancer. Among different types of nucleosomal modifications, we focus here on the histone acetylation and DNA/histone methylation events that have crucial implications in tumorigenesis.

DNA methylation

Methylation of cytosine in CpG islands, which mostly reside at promoter regions, is strongly implicated in transcriptional silencing. In normal cells, CpG islands are largely unmethylated, whereas CG-poor regions within gene bodies tend to be highly methylated. However, in various cancers, aberrant DNA methylation linked to pathological gene expressions has been widely profiled (Easwaran *et al*., 2014). In many cases, cancer cells display distinct shifts in DNA methylation patterns toward hypermethylation at CpG islands and hypomethylation within the gene bodies (Ehrlich, 2009). Specifically, DNA methylation-mediated silencing of tumor suppressor genes, such as CDKN2A (Cyclin-dependent kinase inhibitor 2A) and SFRPs (Secreted frizzled-related proteins), has been identified as a driver for the progression of lung carcinoma and colorectal cancer, respectively (Belinsky *et al*., 1998; Suzuki *et al*., 2014). More recent genome-wide epigenetic profiling analyses involving whole-genome bisulfite sequencing reported that high levels of DNA methylation at insulator regions can relieve the transcriptional suppression of oncogenes, such as PDGFRA (Platelet-derived growth factor receptor alpha; Flavahan *et al*., 2016). This new finding expands the cancer driving function of DNA methylation to the upregulation of oncogenes.

Histone acetylation

The acetylation of histone lysine residues facilitates gene transcription either by loosening chromatin compaction or by enhancing the recruitment of transcriptional activators. Consistently, genome-wide analyses showed the strong enrichment of histone acetylation at promoters and enhancers of active genes (Wang *et al*., 2008; Agrawal-Singh *et al*., 2012). Acetylation on lysine 27 of histone H3 (H3K27) has been used as one of the distinctive histone marks to define active promoters and enhancers (Djebali *et al*., 2012). In some cancer cells, the pathological activation of tumorigenic enhancers has been associated with the aberrant accumulation of H3K27 acetylation at the region (Hnisz *et al*., 2013; Roe *et al*., 2017). Understanding the underlying mechanism by which oncogenic enhancers are activated, specifically in a cancer context, will help to develop clinical strategies for cancer treatment. Small molecules disrupting the interaction between histone acetylation and other transcription machinery, such as JQ1, have shown a promising effect as anti-cancer drugs (Delmore *et al*., 2011; Lockwood *et al*., 2012). In addition, some drugs to downregulate histone acetylation have been approved by the FDA, underscoring the target value of histone acetylation (Jones *et al*., 2016).

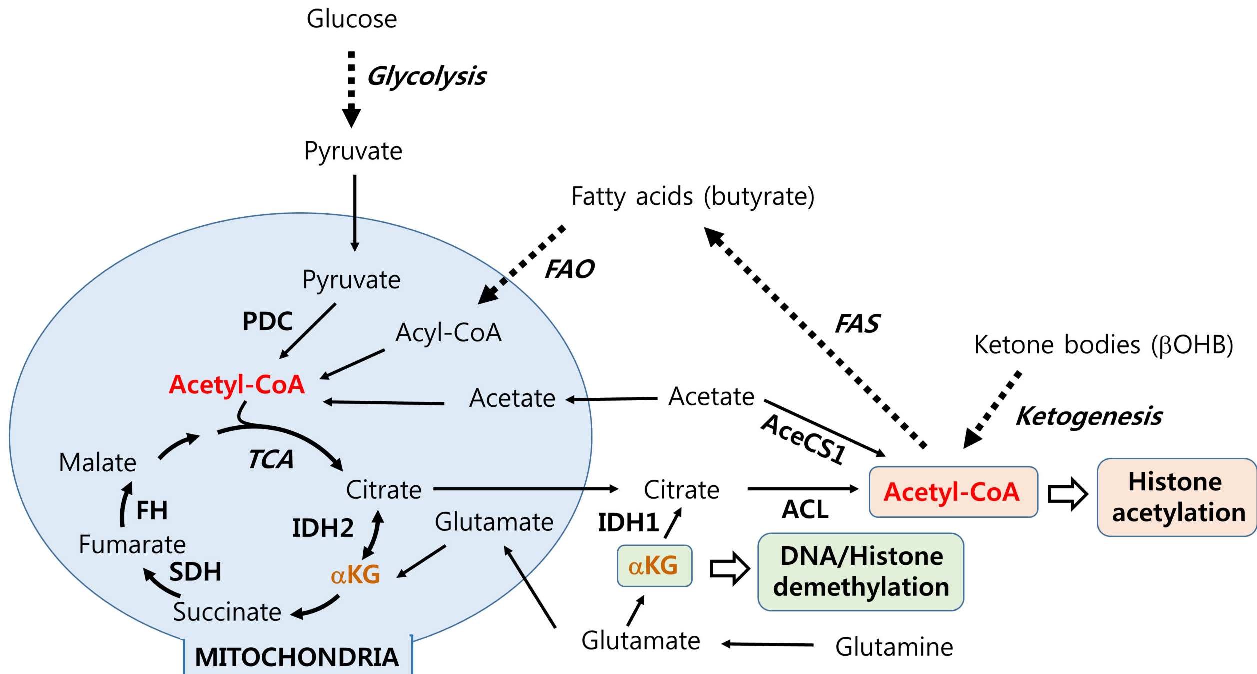

Fig. 2. Metabolic pathways to generate acetyl-CoA and αKG. Acetyl-CoA is produced by multiple metabolic pathways. The enzymes involved in the pathways are in bold. TCA: tricarboxylic acid cycle, FAO: fatty acid oxidation, FAS: fatty acid synthesis, αKG: α-ketoglutarate, βOHB: beta-hydroxybutyrate, PDC: pyruvate dehydrogenase complex, IDH1/2: isocitrate dehydrogenase 1/2, SDH: succinate dehydrogenase, FH: fumarate hydratase, AceCS1: Acetyl-CoA synthetase 1, ACL: and ATP citrate lyase.

Histone methylation

The methylation of histones can occur at two different amino acid resides, lysine and arginine. We focus our discussion on histone lysine methylation in this review. Unlike histone acetylation, the effect of histone methylation on gene expression is methylation site-specific and chromatin context-dependent. For example, methylation on lysine 4 residue of histone H3 (H3K4) is strongly correlated with active transcription, whereas methylation on lysine 27 of H3 (H3K27) is involved in transcriptional suppression. Concurrent methylations of H3K4 and H3K27 have been reported in multiple chromatin regions, so-called bivalent domains, which exhibit poised transcriptional activities (Bernstein *et al*., 2006). Gene expression within the bivalent regions is largely suppressed, although in response to different cues, their transcriptional states can be robustly reset to either active or inactive states. Bivalent domains have been reported in several cancer cells and suggested to promote the plasticity and adaptation of tumors in different environments (Harikumar and Meshorer, 2015). The methylation of the lysine 9 residue on H3 silences the transcription of repetitive elements, such as LINEs (long interspersed nuclear elements) and SINEs (short interspersed nuclear elements). Genetic mutations on the enzymes responsible for the dynamics of histone methylation are recurrent in many cancers, implying that aberrant histone methylation is tumorigenic (Easwaran *et al*., 2014). Accordingly, there have been a number of efforts to target histone methylation as anti-cancer strategies, some of which are quite promising to some extent (Hojfeldt *et al*., 2013; Song *et al*., 2016).

METABOLITES FOR EPIGENETIC MODIFICATIONS

Acetyl-CoA for histone acetyltransferases

Histone acetylation is catalyzed by various histone acetyltransferases (HATs; Shi and Tu, 2015), which transfer the acetyl moiety from acetyl-CoA to the ε-amino group of lysine residues (Fig. 1). Acetyl-CoA is a key metabolite for energy generation via oxidative phosphorylation and for anabolic pathways, such as lipid biogenesis (Pietrocola *et al*., 2015). Acetyl-CoA is produced in both mitochondria and cytoplasm (Fig. 2). In mitochondria, pyruvate generated from glycolysis as well as fatty acid β-oxidation is converted to acetyl-CoA. Acetate entered into mitochondria is also used for acetyl-CoA production by mitochondrial acetyl-CoA synthetase (AceCS2). Acetyl-CoA is not permeable through the mitochondrial membrane. Instead, citrate converted from mitochondrial acetyl-CoA is transported to the cytoplasm and subsequently converted to generate cytoplasmic acetyl-CoA by ATP-citrate lyase (ACL). In addition, acetate present in the cytoplasm can be converted to acetyl-CoA by the cytosolic form of acetyl-CoA synthetase (AceCS1).

In cultured human cells, the concentration of acetyl-CoA ranges from 2 to 20 μM, while the Km values of HATs are 0.5-7 μM (Fan *et al*., 2015). Depending on the cell type and environmental condition, the intracellular amount of acetyl-CoA can physiologically fluctuate. Early studies with yeast have shown that glycolytic activities dynamically regulate acetyl-CoA levels in cells, and the fluctuation of cellular acetyl-CoA levels is tightly linked to HATs-mediated histone acetylation (Friis *et al*., 2009; Cai *et al*., 2011). Consistent results were also observed in mammalian cells; glucose availability regulates

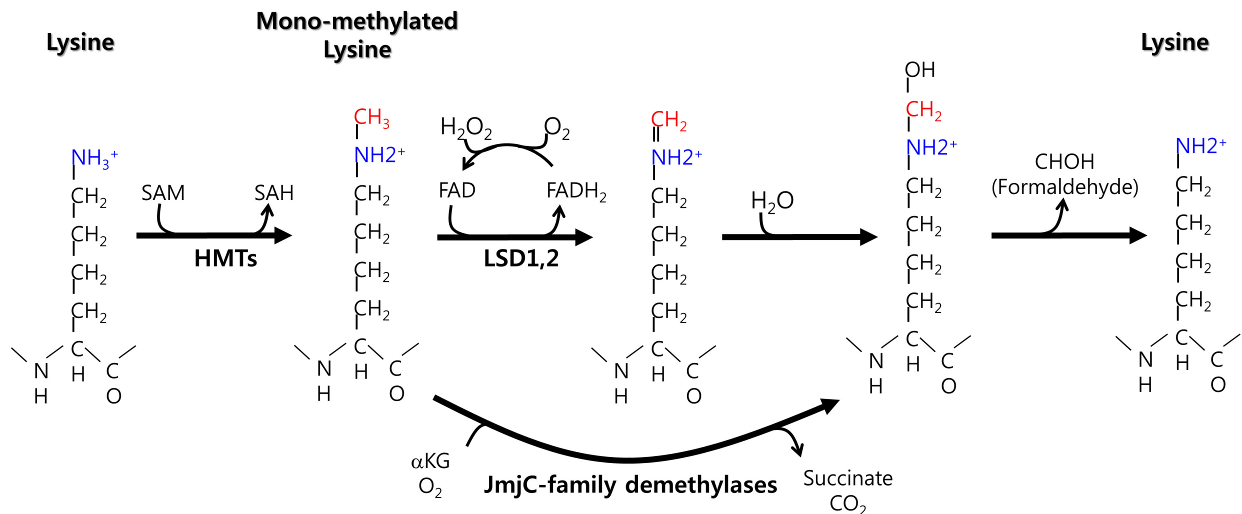

Fig. 3. Histone lysine methylation and demethylation. The enzymes involved in the pathways are indicated in bold. HMTs; histone methyltransferases, LSD1/2: lysine demethylase 1/2, JmjC: Jumonji-C, αKG: and α-ketoglutarate.

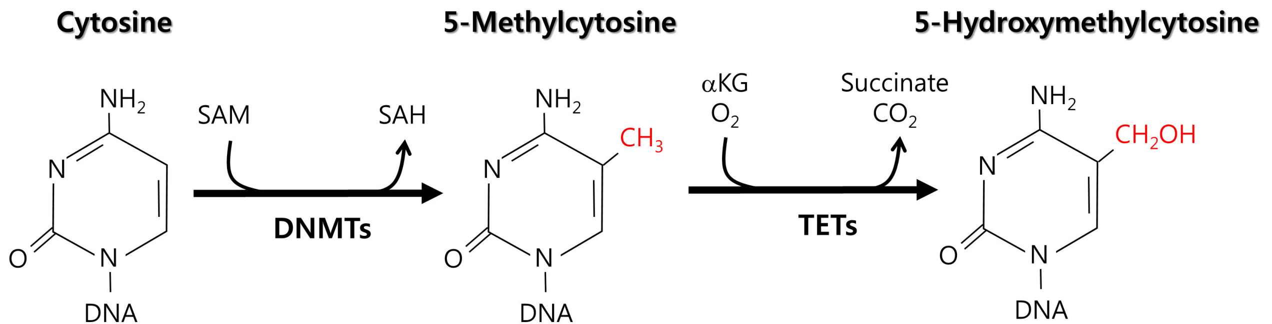

Fig. 4. DNA methylation and demethylation. DNMTs: DNA methyltransferases, TETs: ten-eleven translocations, SAM: S-adenocylmethionine, SAH: S-adenosylhomocysteine, and aKG: α-ketoglutarate.

cellular acetyl-CoA levels and subsequently modulates HATs-mediated histone acetylation (Lee *et al*., 2014). These findings highlight the functional connection of acetyl-CoA metabolism to the dynamics of histone acetylation.

Antagonizing histone deacetylases by different metabolites; butyrate, β-hydroxybutyrate, and lactate

Mammalian histone deacetylases (HDACs) are categorized into four different classes: I, II (IIa and IIb), III and IV. Classes I, II and IV are dependent on Zn^{2+} for their activity and generally inhibited by trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) (Seto and Yoshida, 2014). The activity of these HDACs can be antagonized by different cellular metabolites. Butyrate, a form of short fatty acid, is produced by colonial bacteria, which ferments dietary fiber. In addition to being utilized as an energy source for colon cell growth, butyrate shows the potent inhibition of the activity of HDACs I, II, and IV (Candido *et al*., 1978; Fan *et al*., 2015). β-hydroxybutyrate (βOHB), an endogenous metabolite generated by ketogenesis and used for energy production in some tissues, such as brain (Newman and Verdin, 2014), is capable of suppressing the activity of class I and IIa HDACs (Shimazu *et al*., 2013). Further, lactate, the final product of glycolytic metabolism, inhibits HDACs in cells with similar specificities with TSA and butyrate (Latham *et al*., 2012). The metabolic dynamics affecting the accumulation of the metabolic inhibitors of HDACs in cells could regulate histone acetylation status.

NAD^+ regulates HDAC III (Sirtuins)-mediated histone deacetylation

Unlike the other classes of HDACs, the HDAC III family (a.k.a., Sirtuins) requires NAD^+ as a catalytic cofactor. NAD^+ is an important electron transporter and serves as a powerful redox cofactor in cells. The metabolic response to caloric restriction (CR) is a good example to show the functional connection of NAD^+ metabolism to the HDAC III activity. When catabolic metabolism is suppressed by CR, such as glucose depletion, AMPK, which is activated in response to ATP depletion, induces NAD^+ synthesis by the transcriptional upregulation of NAMPT, a rate-limiting enzyme for NAD^+ salvage pathway (Canto *et al*., 2009). In addition, the ratio of NAD^+/NADH is elevated in glucose-depleted cells due to a reduction in TCA cycle activity. Consequently, CR enhances the activity of NAD^+-dependent SIRT1, a member of HDAC III. Increased consumption of NAD^+ can also affect the SIRT1 activity. The inactivation of DNA damage-responsive PARP1, which uses

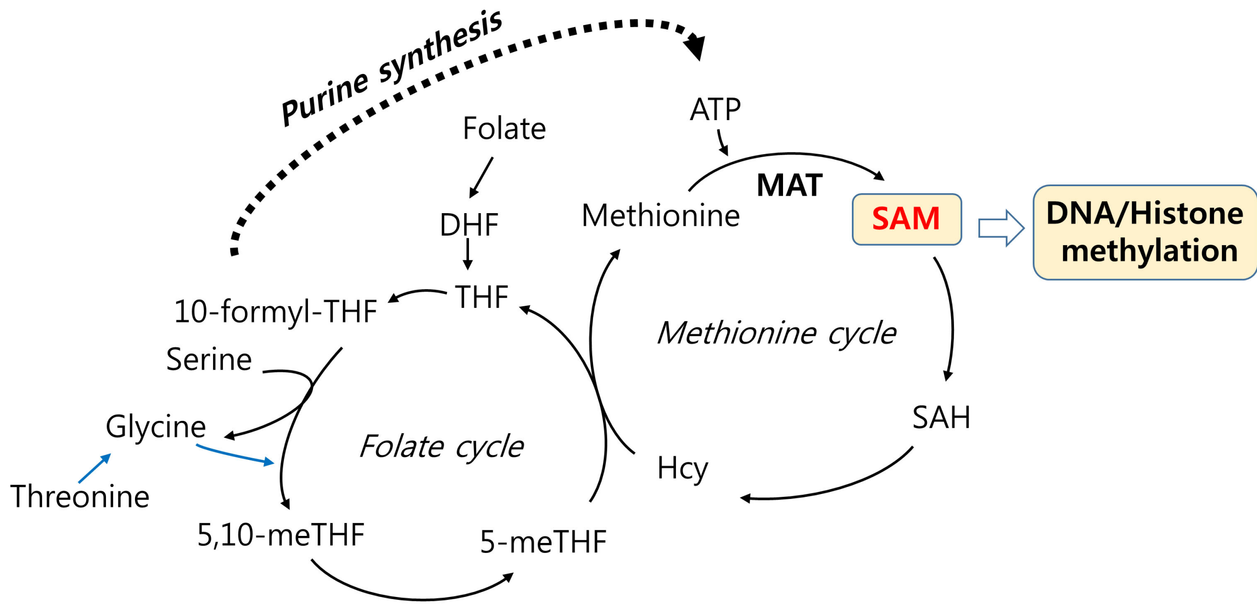

Fig. 5. One-carbon metabolism to produce SAM. The enzymes involved in the pathways are indicated in bold. THF: tetrahydrofolate, DHF: dihydrofolate, 5,10-meTFH: 5,10-methyl-tetrahydrofolate, 5-meTHF: 5-methyl-tetrohydrofolate, SAH: S-adenosylhomocysteine, aKG: α-ketoglutarate, MATs: Methionine adenosyltransferases.

NAD⁺ for poly-ADP ribosylation, downregulates the histone deacetylation activity of SIRT1 by decreasing cellular NAD⁺ levels (Bai *et al*., 2011). Collectively, these findings suggest that the activity of HDAC III is regulated by sensing intracellular levels of NAD⁺, the metabolism of which can be affected by multiple signaling pathways.

SAM for DNA/histone methylation

Methylation of DNA and histones requires S-adenosylmethionine (SAM) as the active methyl donor (Fig. 3, 4). The methylation reaction is catalyzed by three different isoforms of DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B) and multiple histone methyltransferases (HMTs). Km values of the methyltransferases for SAM are close to the physiological concentration of SAM (10-100 μM), which varies depending on the type of tissues (Fan *et al*., 2015). Thus, changes in the cellular SAM level potentially affect the methylation status of DNA and/or histones. S-adenosyl-homocysteine (SAH), which is generated from SAM as a product of the methylation reaction, is inhibitory to methyltransferases. Thus, the cellular ratio of SAM/SAH, like that of NAD⁺/NADH, is a potent determinant in the regulation of chromatin methylation.

SAM is produced via one-carbon metabolism coupling the folate and the methionine cycles (Fig. 5). Recent studies have revealed that one-carbon metabolism is functionally connected to histone methylation. In mouse embryonic stem cells (mESCs), threonine is converted to glycine to enter one-carbon metabolism. When threonine uptake is limited, mESCs displayed a reduction in SAM levels, leading to a decrease in H3K4 methylation (Shyh-Chang *et al*., 2013). The effect of threonine restriction was overcome by the addition of glycine and pyruvate in the culture medium to restore SAM production. Consistent with the mouse study, defective SAM production in human cells cultured in methionine-depleted medium led to a marked decrease in H3K4 methylation (Mentch *et al*., 2015). Additionally, an intimate relationship between methionine and folate diets, which can fuel the one-carbon cycle in cells, and histone and DNA methylation was observed (Anderson *et al*., 2012). These dietary effects on chromatin structure reinforce the physiological significance of metabolic signaling for the epigenetic landscape.

TCA cycle-intermediary metabolites affect DNA/histone demethylation

The TCA cycle generates several intermediary metabolites, a few of which are involved in DNA/histone demethylase activities. Histone lysine demethylases are categorized into two families: the JmjC-containing family and the LSD family (LSD1 and LSD2). The catalytic activity of the latter enzymes is dependent on FAD⁺, one of the cellular redox cofactors. The LSD family demethylates lysine via amine-oxidation reaction converting FAD⁺ to FADH (Fig. 3). Decreasing the cellular FAD level by inactivating the FAD biosynthesis pathway suppresses LSD1 activities (Hino *et al*., 2012; Yang *et al*., 2017). These findings suggest that the dynamics of the cellular FAD level are sensed by LSD1.

In contrast, the activity of the JmjC family is dependent on α-ketoglutarate (αKG), which serves as a catalytic cofactor for hydroxylation-mediated lysine demethylation. The αKG-dependent demethylation reaction subsequently releases succinate as a reaction product. The catalytic chemistry of TETs, which oxidize methyl-cytosine to hydroxymethyl-cytosine and thereby mediate DNA demethylation, is also similar to that of the JmjC family (Fig. 4). The activities of both TETs and the JmjC family are antagonized by succinate as well as fumarate converted from succinate (Xiao *et al*., 2012). Notably, αKG, succinate and fumarate are all intermediary metabolites of TCA cycle. Thus, both DNA and histone demethylation can be affected by the activity of the TCA cycle (Fig. 2). Acetyl-CoA derived from glucose or fatty acids is the key carbon fuel for

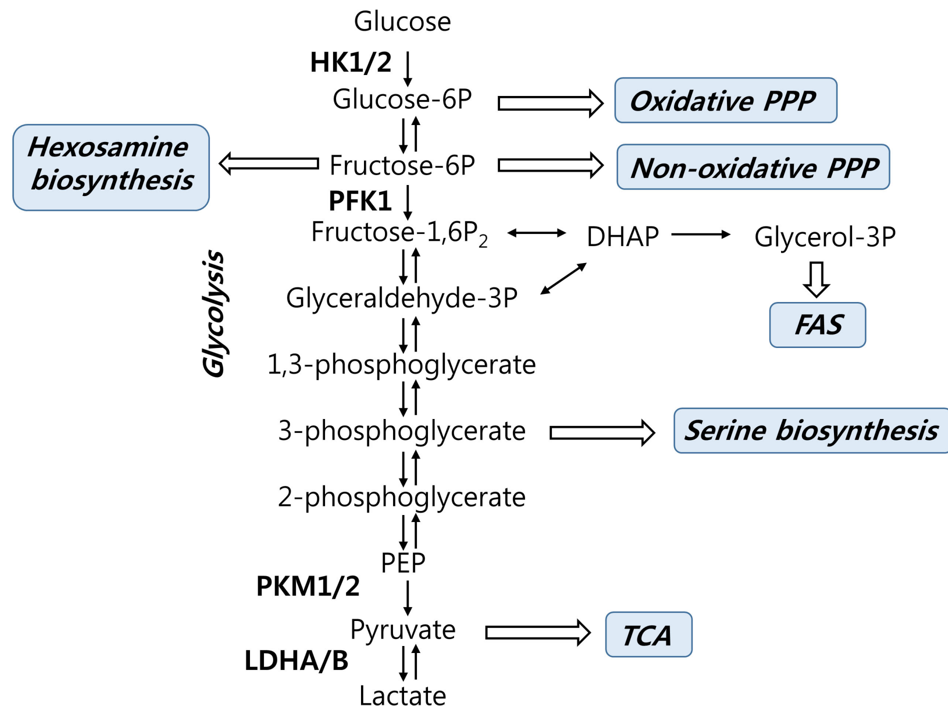

Fig. 6. Glycolysis and its branching pathways. The enzymes involved in the pathways are in bold. Glycolysis and its branching metabolic pathways are in italics. PPP: pentose phosphate pathway, FAS: fatty acid synthesis, TCA: tricarboxylic acid cycle, HK1/2: hexose kinase 1/2, PFK1: phophofructokinase 1, PKM1/2: pyruvate kinase 1/2, LDHA/B: lactate dehydrogenase A/B, Glucose-6P: glucose-6-phophate, Fructose-6P: fructose-6-phosphate, Fructose-1,6P2: fructose-1,6-bisphosphate, DHAP: dihydroxyacetone phosphate, Glycerol-3P: glycerol-3-phosphate, Glyceraldehyde-3P: glyceraldehyde-3-phosphate, and PEP: phosphoenolpyruvate.

the normal operation of the TCA cycle, generating αKG. However, when the TCA cycle is suppressed due to the blockade of acetyl-CoA production under stress conditions, such as hypoxia, glutamine can supply αKG via a reductive carboxylation in mitochondria (Wise *et al*., 2011). In addition, glutamine-mediated αKG production can occur by the action of a cytoplasmic isoform of isocitrate dehydrogenase, IDH1 (Metallo *et al*., 2011). The existence of multiple routes to producing αKG suggests that homeostasis of αKG is highly important for maintaining cellular activity. Indeed, the manipulation of the αKG/succinate ratio dramatically affects the identity of mouse and human pluripotent stem cells by regulating the TET and Jmj-C enzyme activities (Carey *et al*., 2015; Hwang *et al*., 2016; TeSlaa *et al*., 2016). This result implies that cellular metabolism, by regulating DNA and histone demethylation reactions, can function to determine cell fates, providing critical driving forces for cancer progression.

METABOLIC REPROGRAMMING IN CANCER

One of the most distinctive features of cancer metabolism is the increased uptake of glucose and amino acids compared to that in quiescent normal cells (Pavlova and Thompson, 2016). Glucose in cancer cells is then predominantly metabolized by aerobic glycolysis rather than by oxidative metabolism. The significance of upregulated glycolysis in cancer cells has been highlighted as a critical metabolic hub to interconnect with other metabolic pathways (Fig. 6), including (1) the pentose phosphate pathway (PPP) to generate pentose phosphates for ribonucleotide synthesis and NADPH, (2) the hexosamine pathway to synthesize UDP-N-acetylglucosamine for protein glycosylation, and (3) the serine biosynthesis pathway coupled with the one-carbon cycle to produce SAM and NADPH (Hay, 2016). Thus, cells with upregulated glucose uptake increase the flux to these branching pathways and thereby modulate the cellular levels of their intermediary metabolites.

The increased flux of glycolysis may exceed the requirement of the branching pathway activities required for cancer cell proliferation and generate an excess amount of pyruvate to enter the mitochondrial TCA cycle. Electron transport chains overloaded by excess supply of pyruvate can be harmful when they exceed the capacity of ATP synthase and result in the formation of reactive oxygen species (ROS). In the last step of glycolysis, lactate dehydrogenase converts cytosolic pyruvate to lactate, avoiding the mitochondrial import of pyruvate and the subsequent ROS generation. In addition, LDH-mediated lactate production coupled with NADH to NAD^+ conversion plays an important role in maintaining NAD^+ homeostasis in highly glycolytic cells (Fan *et al*., 2011).

Among different amino acids, glutamine is the most rapidly consumed amino acid in cancer cells (Pavlova and Thompson, 2016). Glutamine serves as the nitrogen donor for anabolic pathways to synthesize nucleotides, amino acids, and hexosamine in cells. Further, glutamine is an important carbon source for anaplerosis by the TCA cycle. Given that cytosolic acetyl-CoA is a precursor of fatty acid synthesis, glutamine uptake and the following reductive carboxylation cycle seems supportive of lipogenesis for tumor growth and progression (Mullen *et al*., 2011). In addition, intermediary metabolites of

TCA cycles can be used for synthesizing amino acids, such as aspartate and asparagine. Therefore, many tumors rely on glutaminolysis, which catabolizes glutamine as a carbon donor, not only for ATP energy generation but also for biosynthesis.

Oncogenic signals that drive tumorigenesis facilitate metabolic reprogramming in cancer cells. Expression of glucose and/or glutamine transporters is upregulated by oncogenes, such as c-MYC (Osthus *et al*., 2000; Wise *et al*., 2008). Further, the catalytic activity of metabolic enzymes such as PKM2 can be modulated by oncogenic signaling (Hitosugi *et al*., 2009). However, changes in the activities of metabolic pathways themselves enable the transmission of signals for cancer progression. Below, we further discuss the significance of the availability of metabolic cofactors of epigenetic modifiers as signals affecting cancer cell activities.

CANCER METABOLISM TO AFFECT EPIGENETIC MODIFICATIONS

Elevated acetyl-CoA production in cancer cells upregulates histone acetylation

Oncogenic singling either from K-RAS or c-MYC is known to enhance the flux from glucose to acetyl-CoA (Yun *et al*., 2009; Miller *et al*., 2012; Ying *et al*., 2012). c-MYC induces the production of mitochondrial acetyl-CoA, which can be subsequently converted to cytosolic acetyl-CoA via citrate shuttle (Morrish *et al*., 2010). In contrast, K-RAS upregulates the activity of ACL, the key enzyme to convert cytosolic citrate to acetyl-CoA, in an Akt-dependent manner (Lee *et al*., 2014). The overexpression of ACL has been observed in different cancer cells (Migita *et al*., 2008). Elevated acetyl-CoA levels enhance histone acetylation in cancer cells as described above. Given that histone acetylation is involved in cell cycle regulation and facilitates transcriptional activation, the supply of acetyl-CoA as the substrate of histone acetyltransferases is critical for rapidly proliferating cancer cells. In addition to glucose, cells can utilize glutamine and fatty acids as alternative carbon sources for acetyl-CoA production (Le *et al*., 2012; McDonnell *et al*., 2016). While the involvement of glutamine-derived acetyl-CoA in histone acetylation has little evidence thus far, metabolic tracing experiments showed that acetyl-CoA produced from fatty acids contribute to histone acetylation (McDonnell *et al*., 2016). Moreover, acetate is critical to the generation of acetyl-CoA in hypoxic cancer cells (Mashimo *et al*., 2014). Intriguingly, in this condition, the upregulation of histone acetylation predominantly occurs in specific target genes related to hypoxic metabolic adaptation. Although the mechanism of target specificity in response to acetate-derived acetyl-CoA remains unknown, this observation suggests that metabolic response to an environmental stress governs cellular adaptation by controlling specific regions of chromatin.

Accumulation of metabolic inhibitors of HDACs in cancer cells

Metabolic reprogramming can affect histone acetylation by accumulating metabolites inhibitory to histone deacetylases in some cancers. Colon cancer cells as well as normal colon cells uptake the butyrate produced by colonial bacteria. In normal colon cells, butyrate is converted to acetyl-CoA for energy generation and HAT activities, and thereby supports the growth of colon cells. In contrast, enhanced aerobic glycolysis activity in colon cancer cells results in the accumulation of butyrate by suppressing its conversion to acetyl-CoA (Donohoe *et al*., 2012). Consequently, the increased availability of butyrate as an inhibitor of HDACs causes the suppression of colon cancer cell proliferation by altering chromatin structure. When aerobic glycolysis was inhibited, butyrate treatment facilitated the growth of cancer cells, where butyrate was metabolized for acetyl-CoA production. These observations imply that metabolic reprogramming can instruct cancer cells to utilize metabolites distinctively from normal cells to mediate differential epigenetic modifications.

A similar example was also observed in brain tumors. While βOHB generated from ketogenesis is used as an energy source in normal brain cells, as it is metabolized to acetyl-CoA, upregulated glycolytic activity suppresses the conversion to acetyl-CoA in brain tumor cells, resulting in the accumulation of endogenous βOHB, which has a high potential to inhibit histone deacetylation (Newman and Verdin, 2014). In this regard, induced ketogenesis may even have therapeutic effects against the growth of brain tumor cells.

Enhanced glycolytic activity in cancer cells generates a large amount of lactates, which are exported out to extracellular medium. Despite its robust release from cells, the lactate level in cancer cells is usually higher than that in normal cells. Thus, it is possible that a change in cellular lactate level may control HDAC activities and consequent gene expression in cancer cells. Interestingly, the treatment of ketones or lactate to breast cancer cells induced distinctive gene expression signatures related to cancer stemness (Martinez-Outschoorn *et al*., 2011). Thus, it is possible that HDAC inhibitor activity of ketones or lactate might be involved in changing the fate of cancer cells by altering their epigenetic landscapes in favor of aggressive progression.

Metabolic adaptation controls the supply of αKG and SAM for the regulation of DNA/histone methylation in cancer cells

Glutamine is the most significantly deficient amino acid in the core of melanoma tumors with poorly organized vasculature (Pan *et al*., 2016). This finding reinforces the idea that cancer cells consume a large amount of glutamine for rapid growth. The depletion of glutamine led to a dramatic histone hypermethylation due to reduced αKG levels in the melanoma cells. Interestingly, among different types of histone methylations, H3K27 methylation is the most prominently upregulated in response to decreased αKG levels in the tumor core. This finding indicates that the H3K27 demethylase UTX6 is the most responsive to the availability of αKG in melanoma cells. The increase in H3K27 methylation was dramatic at the genes associated with dedifferentiation of cancer cells. Thus, the glutamine deficiency-induced dedifferentiation rendered the melanoma cells resistant to anti-cancer treatments, such as BRAF inhibitor, in an H3K27 methylation-dependent manner.

Another amino acid highly demanded by cancer cells is serine, which is a key molecule for the one-carbon cycle. In colorectal cancer cells, serine is critical for maintaining the methionine cycle via one-carbon metabolism, which generates SAM (Maddocks *et al*., 2016). The limitation of serine dramatically reduced de novo synthesis of ATP, which is involved in transferring a methyl group from methionine to SAM. Consequently, DNA methylation was impeded in the cells

starved of serine. It may be an interesting to examine whether the epigenetic structure in cancer cells is altered under serine-depleted conditions, where SAM is limited for DNA and histone methylation.

Nuclear production of metabolites to provide epigenetic cofactors in cancer cells

Recent studies suggest that metabolic enzymes involved in supplying the epigenetic cofactors are present in the nucleus. A substantial amount of ACL and AceCS1 has been detected in glioma and colon cancer cell lines (Wellen *et al*., 2009). In addition, translocation of AceCS2 to the nucleus in cancer cells is induced by oxygen and serum limitation (Bulusu *et al*., 2017). These nuclear localized enzymes supply acetyl-CoA for histone acetylation by metabolizing citrate and acetate, which are imported to the nucleus. Nuclear acetate can be released from chromatin by HDAC I-, II-, and III-dependent histone deacetylation reactions. Acetate released from histone deacetylation is recaptured by AceCS2 to supply acetyl-CoA for histone acetylation. Local supply of acetyl-CoA in the nucleus of cancer cells may have the advantage of retaining histone acetylation, even in nutrition- and oxygen- depleted microenvironments. This mechanism may represent a strategy for cancer cells to sustain growth, even in conditions where energy-generating metabolism is suppressed.

The nuclear translocation of pyruvate dehydrogenase complex (PDC) is another mechanism to produce nuclear acetyl-CoA. In response to growth promoting signals, such as serum and EGF, PDC translocates from the mitochondria to the nucleus in different cancer cell lines (Sutendra *et al*., 2014). The nuclear translocation of PDC is also induced during S phase. Nuclear PDC is fully functional to generate acetyl-CoA from pyruvate and contributes to histone acetylation involved in S phase entry and cell cycle progression. However, it remains unclear how pyruvate is supplied to the nucleus. It is likely that growth factors such as EGF coordinate the nuclear translocation of PDC along with PKM2, which is capable of converting PEP to pyruvate. Consistently, nuclear PKM2 was shown to enhance tumor growth via histone acetylation (Yang *et al*., 2012).

The nuclear production of the methyl donor SAM has also been observed in cancer cells. An isoform of methionine adenosyltransferase (MAT), MATIIα, was detected in the nucleus of murine cancer cell lines. MATIIα physically associates with the transcription repressor MafK, which is involved in the oncogenic differentiation of erythroid (Katoh *et al*., 2011). The enzymatic activity of MATIIα to produce SAM is critical for MafK-mediated transcriptional expression. This raises an intriguing possibility that metabolic enzymes can serve as local metabolite generators to regulate specific chromatin regions. The physical association of H3K9 methyltransferase SETDB1 with MATIIα (Kera *et al*., 2013) reinforces the possibility that supplying metabolic cofactors to epigenetic modifiers is a local event leading to epigenetic modification at target sites. There may be additional interaction pairs between nuclear metabolic enzymes and epigenetic regulators, which are specific in certain types of cancer cells.

Genetic mutation promotes oncometabolite production to affect DNA/histone methylation in cancer cells

Oncogenic mutations in isocitrate dehydrogenases IDH1 and IDH2 are frequently found in gliomas and various blood cancers (Parsons *et al*., 2008; Ward *et al*., 2010). Gain-of-function mutations on IDH1 and IDH2 occur at the substrate binding residues (R132 of IDH1 and R140/172 of IDH2), which irreversibly convert αKG to 2-hydroxyglutarate (2-HG) instead of mediating reversible conversion between αKG and isocitrate (Ward *et al*., 2010). With its structural similarity to αKG, 2-HG functions as a competitive inhibitor of DNA and histone demethylase, TETs and Jmj-C families (Figueroa *et al*., 2010; Lu *et al*., 2012). Therefore, cancer cells carrying IDH1/2 mutations display hypermethylation of DNA and histones. Given the significant impact on epigenetics in cancer, 2-HG has been regarded as a prominent oncometabolite. Interestingly, 2-HG can also be generated by promiscuous activities of wild-type IDH2 and removed by L-2-hydroxyglutarate dehydrogenase (L2HGDG) (Shim *et al*., 2014). In certain renal cancers with defective L2HGDH, the reconversion of 2-HG to αKG is impaired, causing the accumulation of 2-HG.

Inactivating mutations in succinate dehydrogenase (SDH) and fumarate hydratase (FH), which are components of TCA cycle, have also been observed in some cancers (Gaude and Frezza, 2014). The oncogenic function of these mutations account for the accumulation of succinate and fumarate to inhibit the activities of TET and Jmj-C family enzymes (Xiao *et al*., 2012). Consistently, DNA hypermethylation was observed in paragangliomas harboring the SDH mutation (Letouze *et al*., 2013). More specifically, the knockout of SDH in cancer cells downregulated metastasis suppressor genes by increasing H3K9 and H3K27 methylation at target genes. In clinical samples, cancer patients with SDH mutations displayed DNA hypermethylation patterns and had poor prognosis (Bardella *et al*., 2011). These findings imply that the unbalanced levels of TCA intermediary metabolites, succinate and fumarate mediate the dysregulation of DNA and histone methylation involved in cancer progression to advanced stages.

METABOLIC SIGNALING FOR THE EPIGENETIC REGULATION OF CANCER PROGRESSION

Tumor heterogeneity has been implicated in driving metastasis, although the underlying mechanisms remain largely elusive. To determine a mechanistic link between the genetic heterogeneity of tumors and metastatic progression, extensive genome-sequencing projects have been conducted. Many of these attempts were not successful in finding a prominent genetic cause of metastasis. Instead, these studies show that the vast majority of mutations in metastatic cancers were the same as those in primary cancer cells or passengers arisen in the course of cancer progression (Yachida *et al*., 2010; Brannon *et al*., 2014). These findings suggest that non-genetic events, rather than genetic mutations, may function as the critical drivers of metastasis. Recently, global epigenetic reprogramming was detected in metastatic pancreatic ductal adenocarcinomas (PDACs) compared to primary PDACs (McDonald *et al*., 2017). Cancer cells taken from widespread distant metastatic tumors displayed extensive loss of H3K9 methylation, which is associated with large heterochromatin domains. A number of genes within the reorganized chromatin domain were differentially expressed, many of which were involved in malignant traits of metastasis. Strikingly, distant metastatic tumor cells carrying reprogrammed epigenomes displayed highly elevated activity of the oxidative branch of the pentose phosphate pathway (oxPPP). Further, inhibition

of oxPPP reversed epigenetic reprogramming and the related malignant gene expression programs. These findings suggest that metabolic reprogramming is coupled to epigenetic alterations that promote tumorigenesis, and targeting the metabolic pathway linked to epigenetic reprogramming could be an effective therapeutic strategy to treat metastatic PDACs.

Rapid tumor growth is often accompanied by a hypoxic environment due to poor vasculature. As oxygen is required for the catalytic reaction by DNA/histone demethylases, oxygen depletion is capable of directly affecting chromatin modification. For example, hypoxia promotes stem cell phenotypes by suppressing the activities of KDM6A/B, which catalyze H3K27 methylation in an oxygen-dependent manner (van den Beucken *et al*., 2014), indicating that hypoxia-induced epigenetic changes mediate the development of aggressive cancer. Cellular metabolism is also greatly reprogrammed in response to hypoxia. Glycolysis is elevated, while oxidative phosphorylation by the TCA cycle is downregulated. Reductive carboxylation produces acetyl-CoA as a substrate for lipid biosynthesis by using glutamine instead of glucose in oxygen-depleted cells (Metallo *et al*., 2011). Further, under hypoxic conditions, LDHA generates the oncometabolite 2-HG by promiscuous substrate usage of αKG (Intlekofer *et al*., 2015). The expression of HIFs, which are critical for the hypoxia-induced metabolic reprograming (Semenza, 2013), has been associated with an increased incidence of metastasis (Rankin and Giaccia, 2016). Altogether, these findings suggest that the altered metabolite levels by hypoxia-induced metabolic reprogramming, including the accumulation of 2-HG, are potent enough to significantly modify epigenetic landscapes. We expect that identifying direct connections between hypoxic metabolism and epigenetic alterations will provide significant insight into the understanding of hypoxia as one of the non-genetic driving forces for metastasis.

CONCLUSIONS AND PERSPECTIVES

We reviewed the current understandings of the links between cancer metabolism and epigenetic regulation. However, despite considerable progress in this field, there are several key questions that remain poorly answered. First, how is the specificity of the metabolite-driven epigenetic regulation on particular targets obtained? Changes in acetyl-CoA level by glucose availability preferentially control histone acetylation associated with the genes for cell proliferation (Lee *et al*., 2014). However, in cells exposed to octanoate, a form of fatty acid, acetyl-CoA derived from this fatty acid affects the histone acetylation of genes involved in lipid biosynthesis (McDonnell *et al*., 2016). What determines the target chromatin regions to be differentially affected by the same metabolite? One possibility is that the activity of different HATs varies in a given metabolic status, and thus, the target genes are selected by the HATs modulated by the metabolic condition. Another possibility is that a specific transcription factor is activated by the signaling events triggered in the metabolic condition and thereby recruits its interacting HATs to the target genes. In this case, the target specificity is achieved by the cooperation of signals from altered metabolite levels and from the transcription factor activities. Thus, identifying transcription factors activated in different metabolic challenges will help to understand the underlying mechanism by which metabolic signaling are integrated into chromatin. Second, although the list of metabolic enzymes found in the nucleus is fast growing (Boukouris *et al*., 2016), the contributions of these enzymes to the epigenetic landscape remains largely unclear. Further, whether these enzymes remain catalytically functional in the nucleus should be determined. To address these questions, technical challenges in measuring the amount of metabolites in different cellular compartments should be overcome. Additionally, signals to trigger nuclear localization of the metabolic enzymes should be increasingly elucidated in the future. By addressing these remaining questions, the current understanding of the role of metabolic signaling in epigenomic regulation in cancers will be greatly expanded to shed light towards the development of more powerful and sophisticated therapeutic means for the war on cancers.

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