REVIEW

Interplay between epigenetics and metabolism in oncogenesis: mechanisms and therapeutic approaches

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Epigenetic and metabolic alterations in cancer cells are highly intertwined. Oncogene-driven metabolic rewiring modifies the epigenetic landscape via modulating the activities of DNA and histone modification enzymes at the metabolite level. Conversely, epigenetic mechanisms regulate the expression of metabolic genes, thereby altering the metabolome. Epigenetic-metabolomic interplay has a critical role in tumourigenesis by coordinately sustaining cell proliferation, metastasis and pluripotency. Understanding the link between epigenetics and metabolism could unravel novel molecular targets, whose intervention may lead to improvements in cancer treatment. In this review, we summarized the recent discoveries linking epigenetics and metabolism and their underlying roles in tumorigenesis; and highlighted the promising molecular targets, with an update on the development of small molecule or biologic inhibitors against these abnormalities in cancer.

INTRODUCTION

It has been appreciated since the early days of cancer research that the metabolic profiles of tumor cells differ significantly from normal cells. Cancer cells have high metabolic demands and they utilize nutrients with an altered metabolic program to support their high proliferative rates and adapt to the hostile tumor microenvironment. Cancer cells could metabolize glucose via glycolysis to generate lactate, instead of oxidative phosphorylation (OXPHOS), even in the presence of normal oxygen levels.[1–3] Although the process is less efficient compared with OXPHOS, glycolysis has a much higher turnover and provides intermediates for macromolecular biosynthesis and redox homeostasis. Apart from metabolizing glucose, cancer cells are addicted to glutamine. By means of a process known as glutaminolysis, cancer cells could divert a major fraction of glutamine to replenish the tricarboxylic acid (TCA) cycle.[4–6] Hence, glutaminolysis supplies biosynthetic precursors for nucleotides, proteins and glutathione biosynthesis in tumorigenesis.[7,8]

Oncogenic pathways have well-established roles in metabolic rewiring in human cancers. For instance, mutations in KRAS, PIK3CA, PTEN or AKT have been shown to hyperactivate mTOR-AKT pathway, which stimulates glycolysis via upregulation of glucose transporter 1 (GLUT1),[9–11] and the phosphorylation of rate-limiting glycolytic enzymes, including hexokinases (HKs) and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFK2/FBPase2).[12,13] The oncogenic transcription factor MYC mediates the transcription of almost all the genes involved in glycolysis and glutaminolysis,[6,14] and it promotes shuttling of glycolytic intermediates to pentose phosphate pathway to generate large quantities of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and promote macromolecule biosynthesis via the induction of pyruvate kinase isozymes M2 (PKM2).[15] Numerous

metabolic genes have also been identified as driver genes mutated in some cancers, such as isocitrate dehydrogenase 1 and 2 (IDH1/2) in gliomas[16] and acute myeloid leukemia (AML),[17] succinate dehydrogenase (SDH) in paragangliomas[18] and fumarate hydratase (FH) in hereditary leiomyomatosis and renal cell cancer (HLRCC).[19] Metabolic rewiring of cancer cells is considered as one of 10 hallmarks of cancer.[20]

Metabolic rewiring in cancer has profound effects on regulation of gene expression. Although metabolite profiles might have little impact on the genetic level, it appears that they have a fundamental role in epigenetic regulation of gene expression. Epigenetics refers to heritable changes in gene expression, which are not a consequence of alterations in the DNA sequence. Epigenetic regulation of gene expression can be highly plastic and responsive to various environmental clues.[21–23] Epigenetics, which principally involved the chemical modification of DNA and histones, represents an innate mechanism that links nutritional status to gene expression. As such, metabolic rewiring could hijack the epigenome machinery in cancer cells to transmit a mitogenic gene expression profile.[24–26] Reciprocally, epigenetic deregulation in cancer mediates, at least in part, to the altered expression of genes involved in cellular metabolism.

A four-way crosstalk exists between epigenetics and metabolism in cancer (Figure 1). Metabolic rewiring could affect the availability of cofactors required for epigenetic modification enzymes (1) and generate oncometabolites that act as agonists and/or antagonists for epigenetic modification enzymes (2), thus impacting the epigenetic landscape (Figure 2). On the other hand, epigenetic dysfunction modifies metabolism by directly affecting the expression of metabolic enzymes (3) and altering the signal transduction cascades involved in the control of cell metabolism (4) (Figure 3). In this review, we provide a summary of molecular

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Received 20 September 2016; revised 7 November 2016; accepted 7 November 2016; published online 16 January 2017
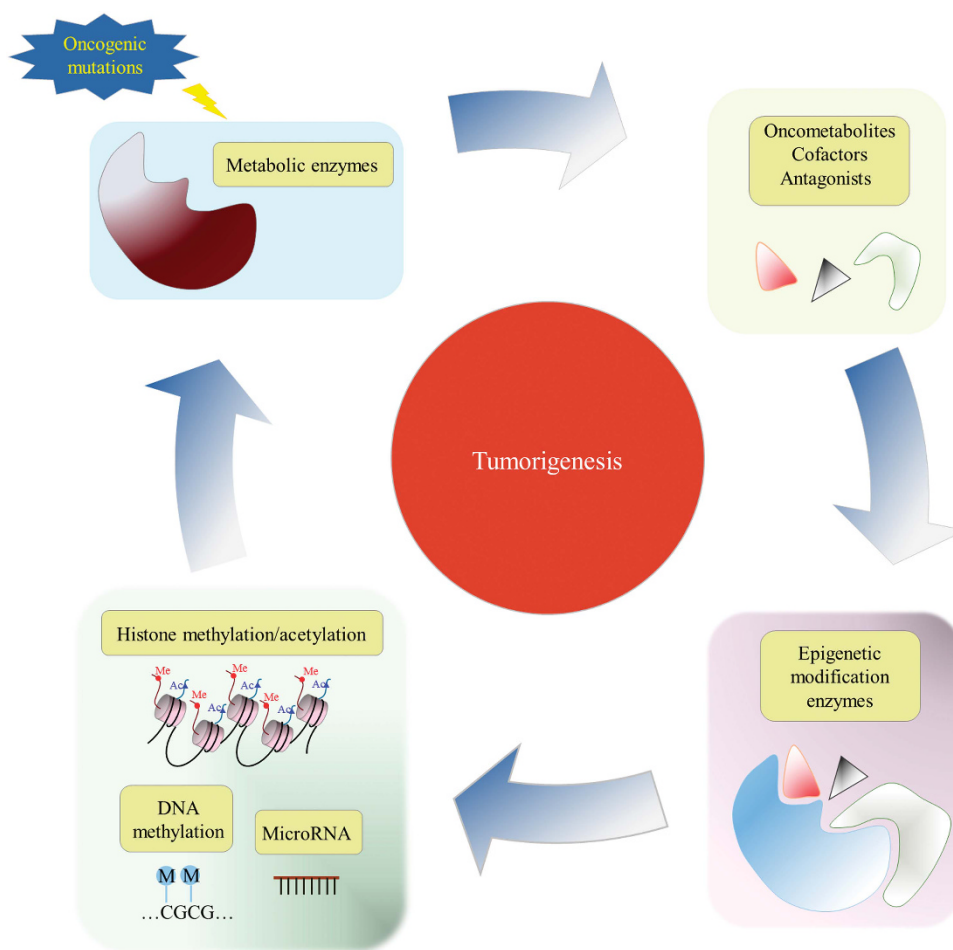
Figure 1. Crosstalks between epigenetics and metabolism in cancer development.

mechanisms linking epigenetics and metabolism; and their underlying roles in tumorigenesis; highlight the potential molecular targets whose inhibition may abrogate these crosstalks and suppress tumorigenesis; and an outline of therapeutics against these potential drug targets.

EFFECT OF METABOLIC REWIRING ON EPIGENETIC MODIFICATION ENZYMES

SAM/SAH ratio regulates DNA and histone methylation

DNA methylation is the most extensively studied epigenetic alteration in cancers. Promoter DNA methylation at CpG sites represses gene expression by impeding access to transcription factors and inhibition of RNA polymerase II.[27–29] In cancer, aberrant DNA methylation is typically observed in the promoter regions of various tumor suppressor genes and microRNAs,[30–38] leading to their transcriptional silence. DNA methylation is mediated by DNA methyltransferases, which catalyze the covalent addition of a methyl group to cytosine to form 5-methylcytosine (5 mC). All DNMT isoforms, DNMT1, DNMT3A and DNMT3B, are overexpressed in cancers.[39] Methylation markers on lysine residues in histone proteins also have a key role in regulating chromatin structure and gene transcription. Multiple lysine residues (H3K4, H3K9, H3K27, H3K36, H3K79 and so on) may be mono-, di- or tri-methylated, giving rise to a very complex histone methylation code.[40] Histone methyltransferases (HMTs) that mediate histone-lysine methylation consist of two enzyme

families, SET-domain (SETD) containing and Dot1-like (DOT1L) proteins.[41]

DNMTs and HMTs utilize a common activated methyl donor for methyltransferase activity: S-adenosylmethionine (SAM). SAM is a product of one-carbon metabolism cycle and is synthesized by methionine adenosyltransferase (MAT) using methionine and ATP as substrates. Donation of methyl group from SAM invariably releases S-adenosyl-homocysteine (SAH) as the product and the latter is a potent inhibitor of methyltransferase such as DNMTs and HMTs. Hence, the SAM/SAH ratio dictates methyltransferase activity in vivo. SAH is physiologically maintained at low levels via hydrolysis to homocysteine, which can be recycled to methionine via the transfer of a methyl group from 5-methyltetrahydrofolate. Alternatively, homocysteine can be catabolized to give amino acids, glutathione and inorganic sulfate. Changes in SAM/SAH ratio and one-carbon cycle will thus modulate the activity of DNMTs and HMTs.[42]

An excess supply of SAM might contribute to DNA hypermethylation at CpG sites and inappropriate gene silencing. Glycine N-methyltransferase (GNMT) deficiency is a rare genetic condition leading to SAM over-production.[43] The genetic knockout of Gnmt in mice increased hepatic SAM by over 40-fold.[43] Moreover, Gnmt knockout mice demonstrated promoter methylation of tumor suppressor genes such as RASSF1 and SOCS2, which led to their transcriptional silencing.[44] As a consequence, Gnmt knockout was associated with activation of oncogenic pathways and an increased incidence of hepatocellular carcinoma.[44] Cancer cells have also been shown to boost SAM availability via promoting
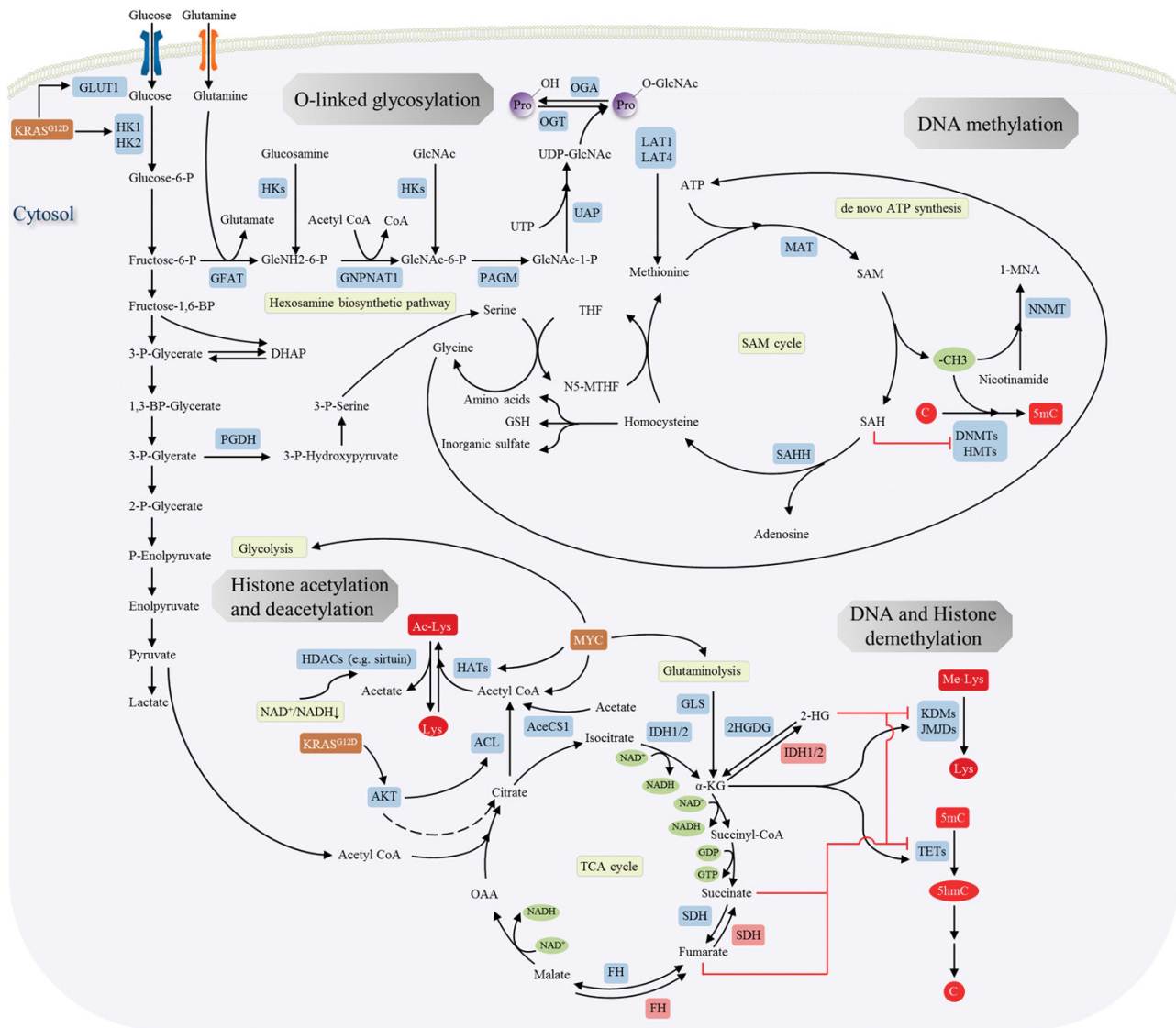
Figure 2. Effect of the tumor metabolome on the epigenetic processes such as histone acetylation, DNA methylation, DNA/histone demethylation, *N*-linked glycosylation in human cancers. An altered epigenetic regulation in turn contributes to deregulation of gene expression.

one-carbon metabolism. Cancer cells could directly increase the uptake of methionine through the overexpression of amino-acid transporters LAT1 and LAT4 (SLC7A5/SLC43A2).[45,46] Alternatively, overexpression of 3-phosphoglycerate dehydrogenase (PGDH) diverts glycolysis intermediates to the serine-glycine biosynthesis pathway.[47,48] Serine participates in one-carbon metabolism through donation of its side chain to tetrahydrofolate to drive the folate cycle, which in turn recycles methionine from homocysteine. Serine also supports SAM synthesis from methionine through *de novo* ATP synthesis, a major contributor to the functional ATP pool in cancer cells.[49]

Alterations in SAM/SAH ratio also profoundly affect aberrant histone methylation in cancers. Nicotinamide *N*-methyl-transferase (NNMT) catalyzes the conversion of nicotinamide to 1-methylnicotinamide (1-MNA) using SAM as methyl donor. NNMT is overexpressed in a variety of cancers, including lung, liver, kidney bladder and colon cancers and exerts an oncogenic effect.[50–53] Recently, NNMT expression was found to be upregulated in human embryonic stem cells, and NNMT is indispensable for the maintenance for pluripotency. NNMT serves as a sink for SAM, severely depleting cellular SAM pool, resulting in >50% reduction in the SAM/SAH ratio and making SAM unavailable for

HMTs.[54] As a consequence, cell lines overexpressing NNMT showed a substantial decrease in histone methylation marks at H3K4, H3K9, H3K27 and H4K20. Altered histone methylation further regulated key signaling pathways associated with acquisition of a more aggressive/pluripotent phenotype. Conversely, knockdown of NNMT increased histone methylation. However, DNA methylation was not affected by NNMT overexpression or knockdown. The apparent discrepancy between DNA and histone methylation may arise from varying K_m values of DNMTs and HMTs for SAM. HMTs possess high K_m values for SAM, thereby conferring a higher sensitivity to changes in SAM levels.

SAM, as an activated methyl donor for DNMTs and HMTs, has a major impact on the epigenomic landscape. Given the myriad of processes that are affected by histone and DNA methylation, and the diversity of the downstream signaling pathways involved, deregulation of SAM levels in cancers likely has a context-dependent effect, and much remains to be explored.

TCA cycle metabolites modulate DNA and histone demethylation
The dynamics of DNA and histone methylation is additionally regulated by the activity of DNA and histone demethylases,
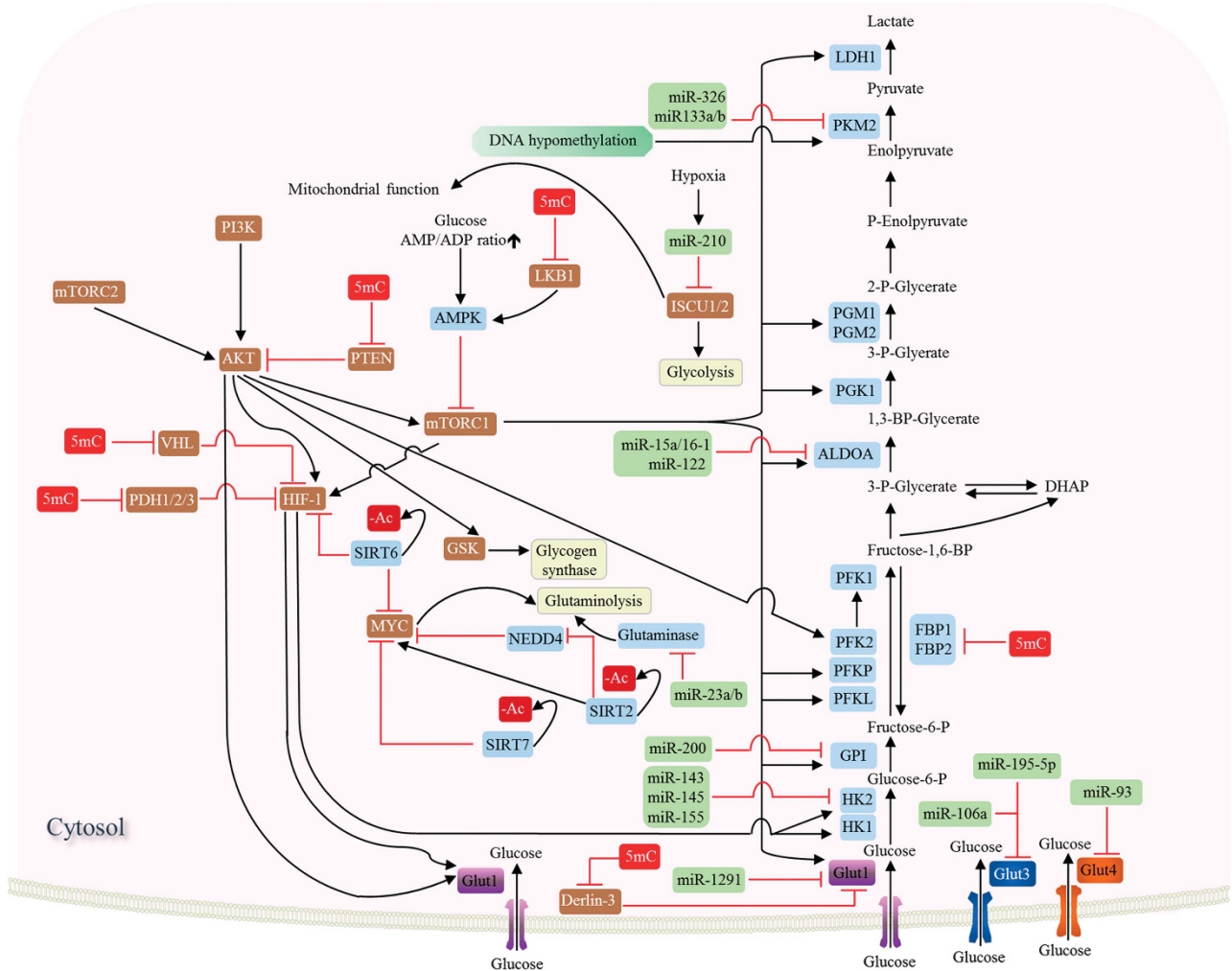
Figure 3. Effect of DNA hypermethylation (5mC), histone deacetylation (-Ac) and microRNA (miRs) on the expression of metabolic enzymes involved in glycolysis and glutaminolysis in cancer.

respectively. Methylated cytosine residues are demethylated in two sequential steps, involving oxidation of 5-methyl-cytosine (5-mC) to 5-hydroxymethyl-cytosine (5-hmC), catalyzed by the 10–11 translocation (TETs) family of proteins,[55–57] followed by reversion to cytosine through oxidation and base excision repair by thymine DNA glycosylase (TDG).[58] TETs are putative tumor suppressors. Frequent inactivating TET2 mutations have been detected in myeloid lineage malignancies[59] and downregulation of TETs have been observed in several human cancers.[60–62] Therefore, hyperactive methylation and deactivated demethylation machinery work in conjunction to induce promoter DNA hypermethylation in cancers. Demethylation of histone lysine marks is mediated by flavin-dependent Histone Lysine Demethylases that consist of lysine-specific protein demethylases (KDM1) family and jumonji C-domain-containing (JMJD) enzymes. The role of histone demethylases in cancer is less clear-cut.[63] In some cases, histone demethylases are downregulated by gene mutations or deletions in cancers, but in others they can be amplified, such as JMJD2C[64] and lysine-specific protein demethylases LSD1.[65] TETs and JMJDs both belong to α-ketoglutarate (α-KG)-dependent dioxygenases that requires α-KG as a cofactor and is competitively inhibited TCA cycle intermediates such as succinate and fumarate.[66] Cancer cells with mutations in metabolic genes may gain the ability to accumulate or synthesize metabolites, such as 2-hydroxylglutarate (2-HG), succinate and fumarate.

2-hydroxyglutarate. Mutations in the metabolic enzymes isocitrate dehydrogenase (IDH) isoforms IDH1 and IDH2 are common in gliomas,[16,67] AML[17,68,69] and angioimmunoblastic T-cell lymphoma.[70] IDH1/2 are Nicotinamide Adenine Dinucleotide Phosphate (NADP)+-dependent metabolic enzymes that participate in the TCA cycle, catalyzing a two-step reaction for oxidative decarboxylation of isocitrate to α-KG.[71] Mutations in IDH1/2 occur at substrate binding sites (IDH1: R132; IDH2: R140/172). Mutant IDH1/2 possess oncogenic properties, and their ectopic expression enhanced cancer cell proliferation, colony formation and inhibits cellular differentiation *in vitro*.[69,72,73] These mutations abrogate the ability of IDH1/2 to synthesize isocitrate from α-KG but are accompanied by the gain-of-function conversion of α-KG to 2-HG.[72] 2-HG is pivotal to the functional effect of mutant IDH1/2. This oncometabolite accumulates to very high levels (5 to 35 mM) in mutant IDH1/2 tumors. 2-HG is structurally similar to α-KG and it acts as a competitive antagonist. Thus, 2-HG inhibits activity of α-KG dependent dioxygenases, such as TETs and JMJDs, which have broad implications for the regulation of epigenome.[74] Apart from tumors with mutant IDH1/2, increased 2-HG have also been reported in breast cancer[75] and renal cancer (L-enantiomer),[76] which is associated with activation of MYC and L-2-hydroxyglutarate dehydrogenase (L2HGDG) deficiency, respectively. The former promotes glutaminolysis and 2-HG production via

wild type IDH2; while inactivation of L2HGDG prevents conversion of 2-HG back to α-KG.[77]

TET1/2-mediated conversion of 5mC to 5hmC is a relevant target of 2-HG.[17,74] *In vitro* enzymatic assays with TET1/2 revealed that 2-HG behaves as a competitive inhibitor.[78,79] Its inhibitory effect was especially pronounced for TET2, with 33% and 83% at 10 and 50 mM, respectively. Either introduction of mutant IDH1/2 or 2-HG abrogated TET1/2-mediated formation of 5-hmC in human cell lines. Moreover, ectopic expression of mutant IDH1[R132H] into primary human astrocytes is sufficient to produce a CpG island methylator phenotype (CIMP) by inducing hyper-methylation in a large number of genes. In human patients, IDH1/2 mutations in glioma or AML define distinct patient subgroups associated with CIMP.[80,81] DNA hypermethylation induced by 2-HG is reversible, and therefore represents a viable therapeutic target in IDH1/2-mutant cancers.[17]

2-HG levels in IDH1/2 mutant tumors also have implications for histone demethylase activity.[78] 2-HG strongly inhibited several histone demethylases (JMJD2A/KDM4A, JMJD2C/KDM4C and JHDM1A/KDM2A) as compared to other dioxygenases. Other studies additionally identified 2-HG as a histone demethylases KDM7A inhibitor[73,79] by binding to catalytic core and competing with α-KG. In U-87MG (human glioma cells), 2-HG or over-expression of mutant IDH1 increased H3K9, H3K27 and H3K79 dimethylation and H3K4 trimethylation.[79] Knockin of IDH1[R132H] in haematopoietic cells was associated with increased dimethylation of H3K79 and trimethylation of H3K4, H3K9, H3K27 and H3K36.[74] Alterations in these methylated histone marks, in particular H3K9 trimethylation, were found to promote pluripotency and inhibit differentiation.[73] Human primary glioma with mutant IDH1 had elevated H3K79 dimethylation levels compared with those with wild type;[73] oligodendroglioma patients with mutations in IDH1 also had higher H3K9me3 compared with those with wild-type IDH1/2.[82] Given a large number of JMJDs enzymes and their diverse substrate specificities, more studies are required to unravel the full spectrum of histone methylation induced by 2-HG and its biological significance.

Succinate and fumarate. Inactivating mutations in TCA cycle enzymes fumarate hydratase (FH) and succinate dehydrogenase (SDH) are driver mutations in a subset of human cancers and they mediate epigenetic reprogramming.[83] SDH mutations are present in gastrointestinal stromal tumors (GISTs), renal cell carcinoma, paraganglioma and pheochromocytoma. [84–88] SDH consists four subunits (SDHA, SDHB, SDHC and SDHD) and it catalyzes oxidation of succinate to fumarate. Mutations in any of the four subunits can inactivate the SDH complex, leading marked accumulation of succinate. Mutations in FH have been detected in HLRCC.[19] Mitochondrial FH mediates the reversible conversion between fumarate and malate, and the loss-of-function mutation of FH resulted in high levels of fumarate.

Recent data have shed new light on the mechanisms of the tumor suppressor effect of FH and SDH. Both succinate and fumarate behave as α-KG competitive antagonists for inhibiting TETs and JMJDs. Both of these metabolites inhibited TETs-catalyzed hydroxylation of 5mC and the activity of histone demethylases KDM2A and KDM4A.[66] Ectopic expression of FH and SDH mutants recapitulated the effect of fumarate and succinate. Furthermore, mouse chromaffin cells with genetic knockout of Sdhb exhibited a methylator phenotype, with an increased 5-mC/5-hmC ratio and enhanced histone methylation at H3K9, H3K27, and H3K27.[89] Epigenetic dysregulation in Sdhb knockout cells triggered a transcriptional program that down-regulated genes associated with the suppression of metastasis, leading to increased cell invasiveness. Consistent with *in vitro* data, a deregulated epigenomic landscape is frequently observed in FH or SDH mutant tumors. Gastrointestinal stoma tumors (GISTs) harboring mutant SDH have genomic DNA methylation an

order of a magnitude greater than c-Kit-mutated GISTs.[90] Genomic hypermethylation was also observed in patients with SDH-mutant hereditary paraganglioma and pheochromocytoma. Moreover, paraganglioma patients with SDH or FH-deficiency associated DNA CIMP had a much worse prognosis compared with other molecular subtypes, indicating that epigenetic dysregulation in SDH or FH-mutant patients contributes to tumor development and progression.[89] Thus, genetic mutations in FH and SDH can lead to accumulation of fumarate and succinate, respectively, which drives tumorigenesis via epigenetic deregulation.

Acetyl-CoA and NAD⁺ influence histone acetylation

Histone acetylation involves the addition of an acetyl group to lysine residues. Histone acetylation is dynamically regulated by opposing actions of histone acetyltransferases (HATs) and histone deacetylases (HDACs) that catalyze the addition and removal of the acetyl group, respectively. HATs are divided into GCN5/PCAF, p300/CBP and MYST (MOZ, Ybf2/Sas3, Sas2, Tip60) families, whereas HDACs are classified into four groups: the zinc-dependent class I, II and IV and NAD⁺-dependent class III HDACs (also known as sirtuins). Histone acetylation decreases the electronic interaction between histones and negatively charged DNA, which is associated with a more open chromatin structure and active gene transcription [91]. HDAC-mediated histone deacty-lation has well-recognized roles in cancers via transcriptional repression of tumor suppressor genes.[92,93] While some HATs are also putative tumor suppressors and inactivating mutations in p300/CBP have been identified in breast, colorectal and gastric cancers,[94,95] several fusion genes that involve HATs, such as MLL-CBP[96] and MOZ-TIF2[97] behaves as oncogenic factors in hematological malignancies.

Acetyl-CoA. Acetyl-CoA is an important molecule in intermediary metabolism. It fuels the TCA cycle and it is at the crossroads of glycolysis, glutaminolysis and β-oxidation of fatty acids in mitochondria. Cytosolic and nuclear acetyl-CoA levels are main-tained by two metabolic pathways, its direct synthesis from acetate and CoA by acetyl-CoA synthetase short-chain family 1 (AceCS1); and the conversion from citrate to acetyl-CoA by ATP citrate lyase (ACL).[98] Acetyl-CoA is utilized extensively as a cofactor for enzymes that catalyze the transfer of an acetyl group, including HATs that utilize the acetyl group of acetyl-CoA to form ε-N-acetyl-lysine. Intracellular concentrations of acetyl-CoA can vary roughly ~ 10-fold under normal physiological conditions and it falls within the K_m range of HATs. Histone acetylation activity is thus dynamically regulated by availability of acetyl CoA.

Both absolute acetyl-CoA and the ratio of acetyl-CoA to coenzyme A have been shown to regulate histone acetylation in cancer.[99,100] Availability of acetyl-CoA for HATs is primarily modulated by 1) ACL expression; and 2) the availability of citrate as a substrate for ACL. ACL protein expression is localized to the nucleus and its activity contributes to nuclear specific acetyl-CoA pool.[100] ACL silencing in HCT116 cells suppressed histone acetylation for all core histones, whereas the knockdown of AceCS1 had no effect. Non-histone protein acetylation was unaltered, suggesting that ACL-derived acetyl-CoA has a specific role in regulating histone acetylation. Cancer cells often over-express ACL[101] and this probably contributes to nuclear acetyl-CoA pool that is necessary for histone acetylation and expression of glycolytic enzymes. Oncogene-driven metabolic reprogram-ming in cancers also promotes a high level of glycolytic flux and mitochondrial production of citrate, which translocates to the cytosol and nucleus. In mouse pancreas, expression of constitutive activated KRAS[G12D] allele resulted in high histone H3 and H4 acetylation in pancreatic adenocarcinoma.[99] KRAS[G12D] dependent activation of AKT promoted nuclear acetyl-CoA accumulation via (1) induction of glycolysis, leading to overproduction of citrate; and

(2) ACL phosphorylation and activation. Indeed, constitutively active AKT induced a rapid and pronounced rise histone acetylation in cancer cells,[99] further confirming its role in mediating histone acetylation.

Another player involved in histone acetylation is MYC. MYC has been shown to upregulate the expression of HAT-GCN5, which induced mono-, di-, tri-, and tetra-acetylation of histone H4 N-terminal.[102] Besides, MYC mediates gene expression of metabolic enzymes linked to acetyl-CoA synthesis, including glycolysis and glutaminolysis. In isogenic rat fibroblasts with $Myc^{-/-}$ or $Myc^{+/+}$, it was demonstrated that Myc increased the mitochondrial export of acetyl-groups and a majority of these acetyl equivalents ended up in histone H4-K16.[103] These data emphasizes the role of Myc in modulating the gene expression of HATs and the availability of acetyl-CoA to support histone acetylation in response to proliferative signals. Hence, multiple oncogenic signals contribute to increased histone acetylation to regulate gene expression.

NAD^+. NAD^+ is essential for the deacetylation activity of sirtuins, a subgroup of HDAC, and changes in $NAD^+/NADH$ ratio is thought to positively regulate activity of sirtuins. $NAD^+/NADH$ ratio is closely associated with energy status in cells. When energy is plentiful, $NAD^+/NADH$ ratio drops; while NAD^+ level can be induced in nutrient deprived conditions. Hence, through sensing of $NAD^+/NADH$ levels, sirtuins serves as a link between energy status and regulation of gene expression.[104] High glycolytic activity in cancers often generates a low $NAD^+/NADH$ ratio that is inhibitory for sirtuins. Repressed sirtuins, together with increased HATs activity induced by acetyl-CoA, may contribute to histone hyperacetylation and aberrant gene transcription. More studies are needed to understand the role of metabolic status and sirtuin activation in cancer.

Hexosamine biosynthetic pathway promotes protein glycosylation
O-GlcNAcylation is one of the most common post-translational modifications in eukaryotic cells, via attachment of O-linked β-D-N-acetylglucosamine (O-GlcNAc) to Ser/Thr residue. O-GlcNAcylation is regulated by competing actions of O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), which, respectively, catalyzes addition and removal of O-GlcNAc from proteins. Recent evidence indicate that modifications of all core histone proteins by O-GlcNAc constitute part of the histone code.[105–108] OGT has been shown to coordinate with TETs to modulate O-GlcNAcylation of histone H2B to activate gene transcription;[109,110] whereas its association with EZH2 in polycomb repressive complex 2 regulates H3K27 me3 to silence tumor suppressor genes.[111] Alternatively, OGT activates gene transcription via O-GlcNAcylation of C-terminal domain of RNA polymerase II.[112] Elevated OGT expression and protein hyper-O-GlcNAcylation is a common feature in human cancers.[113] Cell metabolism has a crucial role in O-GlcNAcylation via modulating the biosynthesis of uridine diphosphate β-D-N-acetylglucosamine (UDP-GlcNAc), the activated substrate for O-GlcNAcylation.

O-GlcNAc.   O-GlcNAc is synthesized via the hexosamine biosynthetic pathway (HBP). In this pathway, glucose entering glycolysis is first metabolized to glucose-6-phosphate (glucose-6-P) and then fructose-6-phosphate (fructose-6-P). About 2–5% of the fructose-6-P will be diverted to fructose-6-P amidotransferase (GFAT), the first and rate limiting enzyme of HBP. GFAT converts fructose-6-P to glucosamine-6-phosphate ($GlcNH_2$-6-P) utilizing an amine group from glutamine. A sequence of reactions then adds an acetyl group from acetyl-CoA and UDP from UTP results in production of UDP-GlcNAc. Hence, HBP integrates substrates from carbohydrate, amino acid, fat and nucleotide metabolism. Glycolysis and supply of glutamine have a pivotal role in UDP-GlcNAc synthesis, as they are obligatory for the rate limiting reaction catalyzed by GFAT. Cancer cells frequently demonstrate

the upregulation of HBP, which is in turn associated with aberrant O-GlcNAcylation and increased malignant behavior.[114–118] The upregulation of HBP in cancer cells is primarily driven by an increased glucose uptake and metabolism. In $KRAS^{G12D}$-driven pancreatic cancer, $KRAS^{G12D}$ promotes glucose utilization via increased expression of glucose transporter 1 (Glut1), hexokinase 1 (Hk1) and hexokinase 1 (Hk2), which induce metabolic flux through GFAT and protein O-GlcNAcylation.[30] $KRAS^{G12D}$ inactivation, in contrast, downregulated global protein glycosylation, suggesting that $KRAS^{G12D}$-mediated glucose utilization via HBP is essential for O-GlcNAcylation. In another study, tumor hypoxia was found to co-ordinately upregulation of glucose and glutamine utilization via HBP, which increased protein O-GlcNAcylation required for tumor survival.[119] Conversely, glucose deprivation suppressed O-GlcNAcylation and cell growth, an effect reversed by addition of N-acetylglucosamine, a HBP substrate. These studies confirm the role of accelerated glucose metabolic in protein O-GlcNAcylation in cancer cells.

EPIGENETIC REGULATION OF METABOLIC GENES EXPRESSION IN CANCERS

DNA methylation
DNA methylation has been shown to modulate the expression of metabolic genes directly by regulating their transcription or indirectly via dysregulation of oncogenic cascades (for example, AKT, AMPK and HIF). DNA methylation mediates silence of fructose-1,6-bisphosphatase 1 (FBP1) and fructose-1,6-bisphosphatase 2 (FBP2) via promoter methylation in breast, gastric, liver and colorectal cancers.[120–123] FBP1 and FBP2 are rate limiting enzymes for gluconeogenesis that antagonize glycolysis, and their decreased expression promotes glycolytic flux for driving macromolecules biosynthesis and ATP production. DNA methylation also mediates overexpression of glucose transporter 1 (GLUT1) by epigenetic loss of Derlin-3, a key gene involved in the proteasomal degradation of GLUT1.[124] Conversely, promoter hypomethylation contributes to upregulation of pyruvate kinase ioszyme 2 (PKM2) in multiple cancer types.[125] PKM2 is a less active isomer that drives glucose flux towards macromolecules biosynthesis and is the predominant isoform in actively proliferating cells. DNA methylation also drives transcriptional silencing of tumor suppressor genes involved in signaling cascades linked to tumor metabolism. PI3K/AKT/mTOR and HIF-1 signaling are central activators of glycolysis and cancer-related metabolism. Multiple tumor suppressors that repress PI3K/AKT/mTOR and HIF-1 signaling are epigenetically silenced by promoter hypermethylation, including PTEN[126–130], LKB1,[131,132] VHL[133–136] and prolyl hydroxylases (PHD1/2/3).[137,138] Hence, differential DNA methylation significantly contributes to glycolytic phenotype in human cancers.

Histone modifications
Among the histone modification enzymes, the role of sirtuins (SIRTs) in regulating cell metabolism has been most extensively investigated. SIRT6 regulates glucose homeostasis by modulating histone acetylation.[139] SIRT6 interacts directly with HIF1 and MYC, and it functions as a co-repressor through histone deacetylation, thereby inhibiting transcription.[139–142] SIRT6 hence acts as a tumor suppressor by repressing HIF-dependent glycolytic switch and MYC-dependent ribosome biogenesis and glutaminolysis. SIRT6 knockout induced a shift towards a 'glycolytic phenotype' and promoted cancer formation and aggressiveness. Consistent with its tumor suppressive role, frequent deletions in SIRT6 have been detected in cancer cell lines and colon, pancreatic and hepatocellular cancers.[143] SIRT7 is another sirtuin that directly interacts with MYC.[144,145] SIRT7 possesses selective catalytic activity towards H3K18Ac. As H3K18 deacetylation is a repressive mark, it is not surprising that SIRT7 opposes MYC- dependent gene

regulation and thus suppresses MYC-mediated metabolic alternations. In contrast to SIRT6/7, SIRT2 promotes deregulated metabolism through indirectly stabilizing MYC.[146] SIRT2 deacetylases histone at H4K16, leading to suppressed expression of ubiquitin-protein ligase NEDD4. NEDD4 is a negative regulator of MYC by targeting it for ubiquitination and degradation. Critically, SIRT2 is itself upregulated by MYC in cancer cell lines; it constitutes a positive-feedback loop that promotes MYC-dependent transcription and oncogenesis. Given the myriad of histone modifications that contributes to gene regulation, much remains to be understood with regards to the role of histone code on metabolic reprogramming in cancer.

miRNA

MicroRNAs (miRNAs) regulate expression of genes involved in diverse cellular functions. Several aspects of cell metabolism are regulated by miRNAs, including glycolysis and mitochondrial TCA cycle, thereby contributing to the Warburg's effect. miRNAs also have a major impact on the signal transduction via PI3K/AKT, HIF1 and Myc that contribute to the metabolic phenotype in human cancers. miRNAs regulates the expression of numerous genes taking part in glucose uptake, including miR-1291 for GLUT1,[147] miR-195-5p and miR-106a for GLUT3,[148,149] and miR-93 for GLUT4.[150] as well as glycolysis, such as miR-143, miR-145 and miR-155 for hexokinase 2 (HK2),[151–156] miR-200 for glucose-6-P isomerase (GPI),[157] miR-15a/16-1 and miR-122 for aldolase A (ALDOA),[158] miR-326 and miR133a/b for PKM2.[159,160] Glutaminolysis is also targeted by miRNAs (miR-23a/b) via glutaminase.[161] Deregulation of aforementioned miRNAs has been reported in cancers and they contribute to increased glycolysis and glutaminolysis in cancers. Moreover, miR-210, by repressing iron–sulfur cluster assembly proteins (ISCU1/2), inhibits mitochondrial function. miR-210 therefore favors a shift towards a glycolytic phenotype and lactate production, which is critical for adaptation to hypoxic tumor microenvironment.[162] miRNAs also have a profound effect on signal transduction. PI3K/AKT/mTOR, LKB1/AMPK, MYC, and HIF1 signaling cascades have all been shown to be regulated by miRNAs. Hence, the dysregulation of metabolic signaling pathways by miRNAs additionally contributes to altered metabolism in cancers.

THERAPEUTIC OPPORTUNITIES TARGETING EPIGENETIC-METABOLISM CROSSTALKS IN CANCER

Reversal of epigenetic dysregulation by targeting cancer metabolism

Glycolysis inhibitors. Accelerated glycolysis in cancer contributes to histone acetylation via citrate and acetyl CoA. Histone acetylation in cells is regulated by glucose flux in a dose-dependent manner[163] and elevated glycolysis in cancer is associated with global histone hyperacetylation.[164] Inhibition of glycolysis holds promise for modulating histone acetylation. 2-Deoxyglucose (2-DG) is a glucose analog that is transported to cells and metabolized by hexokinase (HK) to form 2-DG-P. 2-DG-P cannot be further metabolized by phosphohexose isomerase,[165] leading to the feedback inhibition of hexokinase, a rate-limiting enzyme for glycolysis. Treatment with 2-DG significantly suppresses acetyl-CoA levels, and the acetylation of histone H3, H4, H2A and H2B in multiple cancer cell lines.[164] The reductions in global histone acetylation by 2-DG compromise DNA repair and sensitize cancer cells to DNA-damaging therapeutics. Acetyl-CoA is also required for maintenance of pluripotency through histone acetylation and glycolysis inhibition by 2-DG or 3-bromopyruvate (BrPA, a GAPDH inhibitor), which was found to induce differentiation in embryonic stem cells.[166] Hence, glycolysis represents a viable target for modulating histone acetylation.

Glutaminolysis inhibitors. Glutaminolysis is frequently elevated in cancer, and accumulating evidence indicates that its inhibition is effective for targeting glutamine-addicted cancers.[5] Glutaminase (GLS), which catalyzes the deamination of glutamine to glutamate, is the most extensively studied drug target in this pathway. Several inhibitors, such as bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide (BPTES),[167] compound 968[168] and CB-839[167] have been characterized, and CB-839 is currently undergoing Phase I dose escalation trials in solid and hematological malignancies (Table 1). Glutaminolysis generates α-KG, TCA cycle intermediates and acetyl-CoA, which in turn influence epigenetic status. Indeed, treatment of breast cancer cells with compound 968 significantly altered histone H4K16 acetylation and histone H3K4 methylation, leading to downregulation of numerous cancer-related genes.[169,170] Using an unbiased small molecule screen, Elhammali *et al.*[171] unravelled Zaprinast, a phosphodiesterase 5 inhibitor, as a potent inhibitor of mutant IDH1[R132C]-mediated 2-HG biosynthesis in HT1080 cells. Surprisingly, Zaprinast did not target mutant IDH1, but instead it suppressed GLS. GLS-mediated glutaminolysis is essential for maintaining supply of α-KG, upstream of IDH1/2 metabolism of α-KG to 2-HG. As a consequence, Zaprinast treatment resulted in a marked reduction in histone H3K9me2/3 methylation. These studies highlight potential utility of GLS inhibitors in the reversal of epigenetic dysregulation in cancer, especially in the context of IDH1/2 mutations.

IDH1/2 inhibitors. Inhibition of IDH1/2 has been pursued as a strategy to suppress the production of oncometabolite 2-HG. Rohle *et al.*[172] described the first selective inhibitor (AGI-5198) of mutant IDH1,[R132H] which selectively inhibited mutant IDH1 ($IC_{50} = 70$ nM), but not wild-type IDH1 or any of the IDH2 isoforms ($IC_{50} > 100$ μM). In IDH1-mutant glioma cells, AGI-5198 inhibited 2-HG production and cell growth *in vitro* and *in vivo*. AGI-5198 induced demethylation of H3K9me3 and H3K27me3, whilst it had no effect on DNA methylation. AGI-5198 also demonstrated anticancer activity in human chondrosarcoma cells harboring IDH1 mutations.[173] Subsequently, novel mutant IDH1[R132H] inhibitors has been reported, such as AG-120, AG-881, ML309,[174] 2-(3-trifluoromethylphenyl)isothioazol-3(2H)-one,[175] bis imidazole phenol (cpd 1),[176] 1-hydroxypyridin-2-one compounds[177] and GSK321 and GSK864[178] (Table 1). These drugs inhibited mutant IDH1[R132H] in nanomolar range and exhibited promising selectivity over wild type IDH1.

AG-221 is a first-in-class inhibitor of mutant IDH2[179] (Table 1). AG-221 was found to suppress 2-hydroxyglutarate (2-HG) levels in hematopoietic cells expressing mutant IDH2[R140Q], and in murine models of IDH2-mutant leukemia. Consistent with its role in epigenetic dysregulation, mutant IDH2 inhibition with AG-221 reversed DNA hypermethylation in LSK stem cells from mice expressing mutant IDH2. Notably, AG-211 induced cell differentiation in leukemia cells from IDH2-mutant expressing mice and it synergized with Flt3 inhibition to reduce leukemic cell burden *in vivo*. AG-211 also demonstrated a survival benefit in primary human IDH2 mutant AML xenografts.[180,181] AG-211 has since been introduced into Phase I clinical trials and a Phase III trial has been initiated in 2015. Interim results presented thus far suggest that AG-211 is highly effective in decreasing plasma and bone marrow 2-HG levels and achieved durable remission in some patients with IDH2 mutant advanced hematologic malignancies.[182] AGI-6780 is another selective inhibitor towards mutant IDH2[R140Q].[183] AGI-6780 treatment in IDH2 mutant cells resulted in histone and DNA demethylation,[184] and reversed gene signatures caused by epigenetic dysregulation. These proof-of-concept studies indicate that targeting of mutant IDH1/IDH2 has potential clinical applications as a differentiation therapy in cancers bearing mutant forms of these proteins.

Table 1. Reversal of epigenetic dysregulation by targeting cancer metabolism

Inhibitor	Target enzyme	Mode of action	Ongoing clinical use/trials	Ref
Glycolysis inhibitors				
2-Deoxyglucose (2-DG)	Hexokinases	2-DG inhibits hexokinase, a rate limiting enzyme for glycolysis. 2-DG suppressed acetyl-CoA levels, and reduced the acetylation of histone H3, H4, H2A and H2B in multiple cancer cell lines	2-Deoxyglucose (Phase I/II)	164,165
3-Bromopyruvate (BrPA)	Glyceraldehyde 3-phosphate dehydrogenase	BrPA inhibits GAPDH and acetyl-CoA production BrPA suppressed histone acetylation and induced differentiation of embryonic stem cells	NA	166
Glutaminolysis inhibitors				
Bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl) ethyl sulfide (BPTES), CB-839, Compound 968, Zaprinast	Glutaminase (GLS)	GLS inhibitors suppress acetyl CoA and 2-HG production Compound 968 reduced histone H3K4me3 in breast cancer cells. Zaprinast decreased H3K9me2/3 in IDH1-mutant cancer cells	CB-839 (Phase I)	167–171
IDH1 inhibitors				
AG-120, AG-881, AGI-5198, BAY1436032, bis imidazole phenol, FT-2102, GSK321, GSK864, 1-Hydroxypyridin-2-one compounds, IDH305, ML309, 2-(3-Trifluoromethylphenyl)isothioazol-3(2H)-one	Mutant IDH1	IDH1 inhibitors suppressed 2-HG production in IDH1 mutant cells. AGI-5198 induced demethylation of H3K9me3 and H3K27me3 in IDH1-mutant chondrosarcoma cells. GSK321 induced genome-wide DNA hypomethylation in IDH1 mutant acute myeloid leukemia cells	AG-120 ± Azacitidine (Phase I/II), AG-881 (Phase I), BAY1436032 (Phase I), FT-2102 ± Azacitidine (Phase I), IDH305 (Phase I)	172–178
IDH2 inhibitors				
AG-221, AG-881, AGI-6780	Mutant IDH2	IDH2 inhibitors suppressed 2-HG production in IDH2-mutant cells. Both AG-221 and AGI-6780 induced demethylation of DNA and histone marks in IDH2-mutant leukemia cell lines	AG-221 ± Azacitidine (Phase I/II), AG-881 (Phase I)	179–184
SAM cycle inhibitors				
DZNep (3-deazaneplanocin A), adenosine dialdehyde	SAH hydrolase	DZNep and adenosine dialdehyde increased the SAH-to-SAM ratio and inhibited DNA and histone methylation in cancer cell lines	NA	185–190
NNMT inhibitor				
N-Methylnicotinamide	Nicotinamide N-methyl-transferase (NNMT)	N-methylnicotinamide increased SAM level and histone methylation in NNMT-overexpressing cancer cells	NA	191
Hexosamine biosynthesis pathway inhibitors				
O-Diazoacetyl-L-serine (azaserine), 6-Diazo-5-oxo-L-norleucine (DON)	Glutamine-Fructose-6-Phosphate Transaminase	Azaserine and DON decreased protein O-GlcNAcylation levels	NA	192

Abbreviation: NA, not applicable.

SAM cycle inhibitors: As the availability of SAM is critical for the activities of DNMTs and HMTs, SAM cycle blockade will likely affect DNA and histone methylation. *S*-adenosylhomocysteine hydrolase (SAH hydrolase) participates in the activated methyl cycle through catalyzing the hydrolysis of SAH into adenosine and homocysteine. SAH hydrolase is essential for the maintenance of methylation homeostasis, as SAH caused the byproduct inhibition of DNMTs and HMTs. DZNep (3-deazaneplanocin A) was first identified as a SAH hydrolase inhibitor,[185] and subsequent studies showed that DZNep treatment in cancer cell lines globally inhibited DNA and histone methylation, an effect that is non-selective.[186] Another SAH hydrolase inhibitor (adenosine dialdehyde) also had the similar impact on DNA and histone methylation. EZH2, an oncogenic HMT that methylates histone H3K27 and facilitates transcriptional repression, is indirectly targeted by DZNep in cancer cells via SAH hydrolase inhibition. DZNep treatment reactivates a subset of developmental genes, but it is ineffective towards genes silenced by dense promoter methylation. Hence, combination of DZNep with 5-aza-2′-deoxycytidine (5-Aza), a DNMT inhibitor, has shown synergistic anticancer activity in leukemia and colorectal cancer,[187–190] by activating genes that are aberrantly silenced by histone and DNA methylation.

NNMT inhibitor: As NNMT overexpression induced SAM depletion and histone hypomethylation, it might be a potential drug target in NNMT-overexpressing cancer cells. *N*-methyl-nicotinamide, a reaction side product of NNMT, is a specific and potent inhibitor towards NNMT. Indeed, NNMT inhibition *in vivo* using *N*-methylnicotinamide is able to increase histone methylation at H3K4 and increased methylated H3K4 occupancy at gene promoters.[191] Thus, inhibition of NNMT is a viable approach for modification of histone methylation.

Hexosamine biosynthesis pathway inhibitors: UDP-GlcNAc, the substrate for protein *O*-GlcNAcylation, is synthesized via HBP. GFAT is the rate-limiting enzyme in HBP that can be targeted by well characterized inhibitors, *O*-diazoacetyl-L-serine (azaserine) and 6-diazo-5-oxo-L-norleucine (DON).[192] Cancer cells cultured under high glucose exhibited increased HBP pathway flux and protein *O*-GlcNAcylation, which mediates transcriptional activation of β-catenin, thereby promoting the Wnt/β-catenin signaling and cell proliferation.[193–195] Treatment with either azaserine or DON decreased protein *O*-GlcNAcylation level, reduced β-catenin expression and reversed glucose-mediated cell proliferation.[193,194] Protein *O*-GlcNAcylation has also been shown to be upregulated in CD133$^+$ cancer stem cells.[196] Inhibition of HBP using azaserine reduced the CD133$^+$ subpopulation and CD133 expression; whereas treatment with *N*- *N*-acetylglucosamine (GlcNAc, which promotes HBP) had a reverse effect. It will be of great interest to investigate whether intervention of HBP will impact epigenetic regulators and histone modifications.

Modulation of cancer metabolism using epigenetic drugs

DNMT inhibitors. DNA methylation can be therapeutically targeted using DNMT inhibitors. Two inhibitors of DNMT, 5-azacytidine and 5-aza-2′-deoxycytidine, have been clinically approved by FDA for treatment of myelodysplastic syndrome, and the latter has been approved for acute myeloid leukemia (AML). Clinical trials (Phase I–III) have also been conducted in several solid malignancies (Table 2). These are cytosine analogues that non-selectively inactivate DNMT1, DNMT3A and DNMT3B.[197] It is largely unknown whether DNMT inhibitors can have a metabolic effect on cancer. Given the non-specific nature of these DNA methylation inhibitors and their widespread effect on gene expression, it will be important to elucidate their roles in cancer metabolism. DNMT inhibitors may be useful in reversing DNA methylation induced by metabolic alteration. IDH1/2-mutant cancers, which exhibit DNA hypermethylation, are sensitive to 5-azacytidine and 5-aza-2′-deoxycytidine. 5-Azacytidine induced tumor regression in a patient-derived IDH1 mutant glioma xenograft model;[198] while 5-aza-2′-deoxycytidine effectively suppressed growth in IDH-mutant glioma cells *in vitro* and *in vivo*.[199] DNMT inhibitors reversed the hypermethylator phenotype and resulted in cell differentiation and slowed growth. In the latter study, 5-aza-2′-deoxycytidine was actually shown to be more effective than IDH inhibitors in inducing the differentiation of IDH-mutant glioma cells. Hence, targeting the methylome may be a complementary approach to counteract the effect of oncometabolites in cancers.

HDAC inhibitors. HDAC inhibitors represent a diverse class of compounds that inhibit HDACs activity. Two HDAC inhibitors Vorinostat and Romidepsin have been approved for cutaneous T-cell lymphoma, and their use in solid tumors is an area of active investigation (Table 2). HDAC inhibitors induce histone acetylation and reverse gene silencing by HDACs in human cancers. Emerging evidence suggests that inhibition of HDACs may impact cancer metabolism. In HT29 colorectal cancer cells, treatment with HDAC inhibitors butyrate or trichostatin A was associated with a significant reduction in glucose uptake, glycolysis flux and lactate production.[200] In multiple myeloma cells, Vorinostat or valproic acid treatment suppressed GLUT1 expression and inhibited hexokinase I activity.[201] In H460 lung cancer cells, butyrate or trichostatin A treatment suppressed glycolysis and triggered a shift in metabolism away from glycolysis by activating mitochondrial metabolism.[202] Butyrate also attenuated glycolysis in breast cancer cells.[203] These results suggest that inhibition of HDAC may promote the reversion of glycolytic phenotype in cancer cells.

Sirtuin activators and inhibitors. Despite their importance in metabolic regulation in cancers, limited attention has been paid to the potential use of sirtuin activators and inhibitors to influence cancer metabolism. Sirtuin 6 (SIRT6), a tumor suppressor that opposes glycolysis, has been shown to be activated by free fatty acids (myristic, oleic and linoleic acids) up to ⩽ 35-fold.[204] Discovery of small molecule activators of SIRT6 may unveil a novel approach to target tumor metabolism. On the other hand, several inhibitors that block oncogenic Sirtuin 2 (SIRT2) has been described.[205] Further investigations are required to define the effect of sirtuin activators and inhibitors on cancer metabolism and their role in cancer management.

miRNA. Modulation of miRNAs holds promise as therapeutic targets, given its regulatory roles in the dysregulation of metabolism in carcinogenesis. Currently, there are two approaches to target miRNAs. Aberrantly silenced miRNAs can be restored using synthetic miRNA mimics; although overexpressed miRNAs can be silenced using miRNA sponges or antisense oligonucleotides.[206–208] Both approaches have been utilized to manipulate metabolic genes in cancer. As an example, the re-expression of miR-143, which targets hexokinase II 3′-untranslated region, overturned the glycolytic phenotype and inhibited cancer growth.[154] On the contrary, oncogenic miRNAs that target LKB1/ AMPK or PTEN tumor suppressive pathways may represent attractive targets for the design of therapeutic anti-miRNAs. For instance, anti-miR-21-mediated inhibition (which targets PTEN tumor suppressor), restored PTEN expression in hepatocellular cancer and contributed to treatment.[209] One major challenge facing miRNA-targeting focuses on the safe and efficient delivery of miRNA mimics and anti-miRNAs.[210] Advances in delivery technology will accelerate realization of miRNA-based therapeutics in the clinical practice.

Table 2. Reversal of cancer metabolism using epigenetic drugs

Inhibitor	Target enzyme	Mode of Action	Ongoing clinical use/trials	Ref
DNMT inhibitors				
5-Azacytidine, 5-Aza-2'-deoxycytidine	DNA methyltransferases	Both drugs non-selectively inactivate DNMT1, DNMT3A and DNMT3B DNMT inhibitors reversed the hypermethylator phenotype in IDH1-mutant glioma cells	Azacitidine and 5-Aza-2'-deoxycytidine (Approved for myelodysplastic syndrome and acute myeloid leukemia, Phase I-III for other malignancies)	198,199
HDAC inhibitors				
Butyrate, Romidepsin, Trichostatin A, Valproic acid, Vorinostat	Histone deacetylases (HDACs)	HDAC inhibitors induce histone acetylation and reverse aberrant gene expression caused by HDACs. Treatment of cancer cells with HDAC inhibitors was associated with the reduction in glucose uptake, glycolytic flux and lactate metabolism	Romidepsin and Vorinostat (Approved for cutaneous T cell lymphoma, Phase I-III for other malignancies), Valproic acid (Phase I-III)	200–203
Sirtuin activators and inhibitors				
Linoleic acid, Myristic acid, Oleic acid	Sirtuin 6 (SIRT6)	Free fatty acids activate SIRT6, which functions as a tumor suppressor to inhibit glycolysis	NA	204
miRNA modulators				
Synthetic miRNA mimics	miRNAs	miRNA mimics restores silenced miRNA function. For example, re-expression of miR-143, which targets hexokinase II 3'-UTR, suppressed glycolysis	NA	154
miRNA sponges, Antisense oligonucleotides	miRNAs	Anti-miRs silences overexpressed miRNA. For example, anti-miR-21 restored PTEN expression	NA	209

Abbreviations: HDAC, histone deacetylase; miRNA, microRNA; NA, not applicable; 3'-UTR, 3'-untranslated region.

Impact of tumor microenvironment and metabolism on epigenetic therapy

Epigenetic drugs, including DNMT and HDAC inhibitors, have been approved by the FDA for use in hematological malignancies. However, the use of these drugs has been met with limited success in solid tumors thus far. Several Phase I clinical trials that examined the pharmacodynamics of DNA demethylation drugs or histone deacetylase inhibitors indicated the reversal of epigenetic abnormalities in solid tumors following drug treatment.[211–213] However, the therapeutic effects of epigenetic therapies towards solid tumors has been disappointing. Solid tumors, unlike hematological malignancies, are characterized by regions of hypoxia (low oxygen), which has a key role in tumor progression, aggressiveness and drug resistance.[214,215] Hypoxic tumor cells display epigenetic abnormalities.[216–220] Hypoxia is associated with DNA hypomethylation. Hypoxia has been shown to downregulate the expression of DNMT1, DNMT3A and DNMT3B in human colorectal cancer cells.[217] On the contrary, hypoxia results in HIF-dependent transcriptional activation of TET1.[219,220] Given that a hypoxic tumor microenvironment promotes DNA hypomethylation, efficacy of DNA demethylation agents, such as modulators of DNMTs/TETs, are likely suppressed in solid tumors as compared to hematological malignancies. On the other hand, hypoxia induces histone hypoacetylation that may be potentially targeted by inhibitors of HDAC.[221] Indeed, HDAC inhibitors such as Vorinostat and valproic acid have shown promising results in solid tumors, especially given in combination with chemotherapeutic agents.[222–225] Therefore, approaches to target epigenetic mechanisms should take into consideration of the potential impact of the tumor microenvironment and metabolism.

CONCLUSION AND 5-YEAR VIEW

Crosstalks between epigenetics and metabolism are fundamental aspects of cellular adaptation to nutrition status. The human epigenome is dynamically regulated by the metabolome. Alterations in either the epigenome or metabolome arising from genetic mutation may therefore coordinately drive aberrant gene expression, which in turn, contributes to tumor development and progression. Here we have outlined potential strategies that target crosstalks between epigenome and metabolome that might be exploited to selectively inhibit tumorigenesis. At present, much of our knowledge has been gathered in simplistic in vitro cell culture systems that might poorly reflect the complex interaction of epigenome and metabolome in vivo. In the future, preclinical studies need to better define their crosstalk in context of the tumor microenvironment that consists of stromal and immune components and to validate potential targets using appropriate in vivo models, which ultimately will contribute to development of novel therapeutic targets for intervention.

With numerous drugs targeting metabolism in the drug development pipeline, in the next 5 years we will be able to effectively target these abnormalities in cancer. Novel drugs targeting mutant IDH1/2, for example, are already undergoing phase II/III trials with treatment of advanced leukemia harboring these mutations. With the promising preliminary data, IDH1/2 inhibition represents a highly specific therapy for this subset of cancers.[183,184] Metabolic reprogramming in cancer cells might also be targeted by epigenetic drugs such as DNMT and HDAC inhibitors. However, targeting epigenetic machinery likely has a broad impact on gene expression, and more studies are needed to define their specific effects on tumor metabolism. A caveat of targeted therapies, as exemplified by the development of tyrosine kinase inhibitors, is that they are useful only when their target(s) are the main drivers of carcinogenesis. To fully realize the potential of metabolic/epigenetic modulators, future clinical trials should incorporate analysis of biomarkers to unravel epigenomic

(DNA methylation and histone lysine acetylation) and metabolomic (metabolites) markers that allow the selection of subsets of patients that may benefit most from these treatments. Given the extensive crosstalk between epigenetics and metabolism, perhaps it is the development of combinatorial approaches involving metabolism inhibitors and epigenetic modulators might achieve synergistic tumor inhibition. Notably, mutant IDH1/2 inhibitors are being evaluated in Phase I/II clinical trials with inhibitors of DNMTs (Table 1), with the rationale being that they might promote active DNA demethylation and suppress DNA methylation, respectively, to reverse DNA methylation in IDH1/2-mutant cancers. Development of rationale drug combinations involving metabolism inhibitors, epigenetic modulators and traditional chemotherapeutics will likely have the greatest impact on future cancer management.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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