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Circles of Life: linking metabolic and epigenetic cycles to immunity

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Summary

Metabolites are the essential substrates for epigenetic modification enzymes to write or erase the epigenetic blueprint in cells. Hence, the availability of nutrients and activity of metabolic pathways strongly influence the enzymatic function. Recent studies have shed light on the choreography between metabolome and epigenome in the control of immune cell differentiation and function, with a major focus on histone modifications. Yet, despite its importance in gene regulation, DNA methylation and its relationship with metabolism is relatively unclear. In this review, we will describe how the metabolic flux can influence epigenetic networks in innate and adaptive immune cells, with a focus on the DNA methylation cycle and the metabolites *S*-adenosylmethionine and α-ketoglutarate. Future directions will be discussed for this rapidly emerging field.

Keywords: 5-hydroxymethylcytosine; B cells; DNA methylation; DNA methyltransferases; epigenetics; immunometabolism; Krebs cycle; macrophages; mitochondria; one-carbon metabolism; T cells; ten–eleven translocation.

Introduction

Metabolism is the constellation of chemical reactions in cells. These reactions are catalyzed by specific metabolic enzymes to support and maintain cellular and tissue homeostasis. In immune cells, cell differentiation, effector function, and cell proliferation are accompanied by a meticulous metabolic reprogramming. Therefore, the imbalance of intrinsic and/or extrinsic metabolites can strongly influence the differentiation and function of immune cells and could attribute to immune-related diseases.[1,2]

Epigenetics describes the inheritable traits without changes in the DNA sequence. In 1957, Conrad Waddington introduced the concept of the 'Epigenetics Landscape' to describe the decision-making process during cellular development.[3] In the model, developing cells are marbles on top of a hill among the landscape of choices. Despite having the same genetic material, each cell can 'roll down' and adopt one of the genetically pre-defined paths and differentiate into various cell lineages. Many factors can influence the decision-making and impact the outcome of cells, and studies have implicated cell-intrinsic metabolic activity

Abbreviations: 2-HG, 2-hydroxyglutarate; 2OGDD, 2OG-dependent dioxygenases; 5hmC, 5-hydroxymethylcytosine; 5mC, 5-methylcytosine; αKG, α-ketoglutarate; αKGDH, α-ketoglutarate dehydrogenase; CoA, coenzyme A; DNMT, DNA methyltransferases; EAE, experimental autoimmune encephalomyelitis; IDH, isocitrate dehydrogenase; IFN, interferon; IL-1β, interleukin-1β; ImmGen, Immunological Genome Project; JmjC, Jumonji C; JMJD, lysine (and/or arginine) demethylases; LCMV, lymphocytic choriomeningitis virus; NADH, nicotinamide adenine dinucleotide; oxi-MC, oxidized methylcytosine; PD-1, programmed cell death protein 1; PHD, prolyl-hydroxylase domain; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; TET, Ten-Eleven Translocation; Th1, T helper type 1; Treg, regulatory T

and cell-extrinsic nutrient availability. Epigenetic processes are known to contribute significantly to immune cell development, activation, and function.[4-6] Increasing evidence has suggested that epigenetic modification enzymes can modulate the epigenetic patterns by integrating metabolic cues from metabolic pathways, including one-carbon metabolism and the Krebs cycle. More specifically, the level of intermediary metabolites strongly affects the activity of most epigenetic enzymes,[7] which ultimately influences gene expression in immune cells in response to tissue homeostasis and inflammation.

In this review article, we first introduce the metabolism of *S*-adenosylmethionine (SAM) and α-ketoglutarate (αKG), followed by the roles of the DNA-modifying enzymes DNA methyltransferases (DNMT) and Ten–Eleven Translocation (TET) in controlling immune cell fate. We will discuss the interconnection between metabolism, epigenome, and immunity.

One-carbon metabolism and SAM

One-carbon metabolism provides one-carbon units required for the synthesis of essential metabolites, including amino acids, nucleotides, nicotinamide adenine dinucleotide (NADH), and SAM.[8] It is a crucial and complex metabolic network involving the folate and methionine cycles (Fig. 1). Notably, the one-carbon metabolic process is largely derived from the non-essential amino acids serine and glycine, which can be acquired from the extracellular environment or by *de novo* synthesis. The *de novo* serine biosynthesis starts from the 3-phosphoglycerate of glycolysis, followed by serial conversions through phosphoglycerate dehydrogenase, phosphoserine aminotransferase 1, and phosphoserine phosphatase (Fig. 1). Deprivation of serine was showed to induce cell cycle arrest in proliferating lymphocytes[9,10] and tumor cells.[11]

One-carbon metabolism is essential for immune function. For instance, T-cell expansion requires serine to fuel the *de novo* nucleotide biosynthesis.[10,12] Whereas most serine is acquired extracellularly,[10] cell-intrinsic serine synthesis is also required as effector T-cell proliferation was impaired when the rate-limiting enzyme phosphoglycerate dehydrogenase was inhibited.[13] In addition, compared with young mice, naive CD4 T cells from aged mice were decreased in percentage and showed a proliferation defect that was linked to impaired mitochondrial respiration and one-carbon metabolism.[14] In myeloid cells, lipopolysaccharide stimulation enhanced serine one-carbon metabolism to support the production of interleukin-1β (IL-1β) in inflammatory macrophages.[15,16] Concordantly, inhibition of *de novo* serine synthesis protected mice from endotoxemia.[15] However, in a mouse model of *Pasteurella multocida* infection, exogenous administration of serine abated macrophage pro-inflammatory response, decreased bacterial colonization, and increased animal survival.[17] While serine one-carbon metabolism is clearly essential for T cells, its role in regulating myeloid function remains to be determined.

Methionine is an intermediate of the methionine cycle in one-carbon metabolism (Fig. 1) and can be adenylated by methionine adenosyltransferase to serve as the substrate for the production of SAM, the primary methyl donor in cellular methylation.[18] It has been shown that the activity of methionine adenosyltransferase for SAM synthesis is essential for the function of T cells[19] and macrophages.[16] During methyl transfer, SAM is catalyzed by cytosolic or nuclear methyltransferases to form the methylated substrate and *S*-adenosylhomocysteine (SAH). SAH is hydrolyzed back to homocysteine and adenosine through a reversible reaction catalyzed by *S*-adenosylhomocysteine hydrolase to complete the methionine cycle (Fig. 1). As the increased level of homocysteine can have a negative effect on the activity of methyltransferases, it is efficiently remethylated back to methionine.[18]

Methionine metabolic regulation is crucial for the differentiation and function of CD4 T cells and B cells.[20-22] Restriction of methionine availability in activated T cells decreased the intracellular levels of SAM and histone methylation (active histone mark H3K4me3), impaired cell proliferation and cytokine production, and reduced the severity of experimental autoimmune encephalomyelitis (EAE) in mice.[21] In human B cells, extracellular methionine is required for BLIMP1-dependent plasmablast differentiation.[22] BACH2 represses the expression of *PRDM1*, which encodes BLIMP1. Methionine induced the H3K27 methyltransferase EZH2, which catalyzed the repressive mark H3K27me3 at the *BACH2* locus and decreased the gene expression.[22] Whether methionine affects SAM level was not addressed. In myeloid cells, Toll-like-receptor-4-stimulated macrophages require both exogenous serine and methionine to sustain the methionine cycle for generating SAM, which supports methylation reactions including the H3K36 trimethylation at the gene body of *Il1b*.[16]. However, oral SAM supplementation inhibited inflammatory response and fibrosis in a chronic asthma model.[23] As in the case of dietary serine supplementation, the discrepancy between *in vitro* and *in vivo* data is likely due to the pleiotropic effect of amino acids on whole animals.

Krebs cycle and αKG

Krebs cycle, also known as the tricarboxylic acid cycle, is the central hub of the metabolic network and provides essential metabolites and substrates in the mitochondrial matrix to maintain cellular homeostasis and function (Fig. 1).[24] This cycle contains a series of reactions to convert intermediates such as citrate, isocitrate, αKG, succinate, fumarate, malate, and oxaloacetate. Primarily, the Krebs cycle is supplied with new substrate in the form of acetyl-CoA, which is generated from glucose-derived

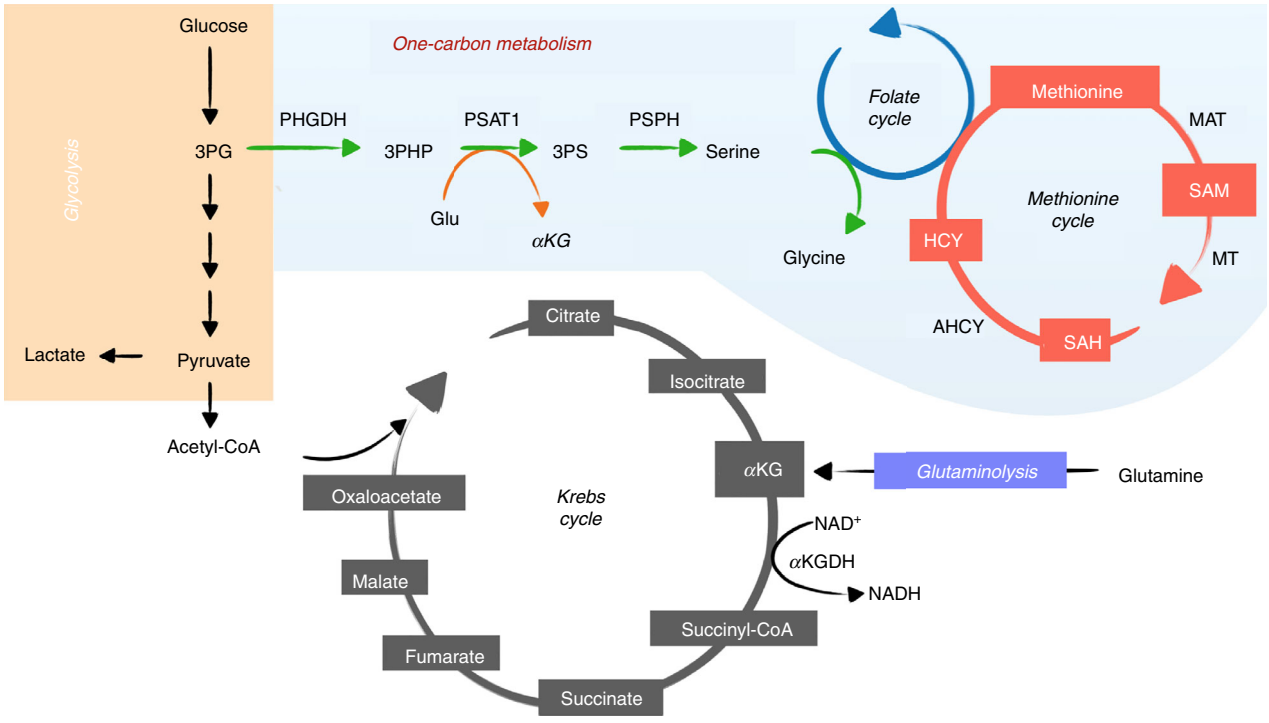

Figure 1. Metabolic pathways that provide metabolic substrates for epigenetic cycle. Glycolysis (orange), involves the enzymatic catabolism of glucose to pyruvate and lactate in the cytoplasm. Pyruvate can be converted to acetyl-CoA in mitochondria and shuttled through several enzymatic reactions of the Krebs cycle (gray) to generate metabolic intermediates. Glutamine through glutaminolysis (purple) can be metabolized to α-ketoglutarate in the Krebs cycle. Intermediates from glucose catabolism during glycolysis can branch out through the serine one-carbon metabolism (blue) to generate amino acids of serine and glycine fueling into the folate and methionine cycle to generate *S*-adenosyl-methionine. 3PG, 3-phosphoglycerate; 3PHP, 3-phosphohydroxypyruvate; 3PS, 3-phosphoserine; PHGDH, phosphoglycerate dehydrogenase; PSAT1, phosphoserine aminotransferase 1; PSPH, phosphoserine phosphatase; MAT, methionine adenosyltransferase; SAM, *S*-adenosyl-methionine; MT, methyltransferase; SAH, *S*-adenosylhomocysteine; HCY, homocysteine; AHCY, *S*-adenosylhomocysteine hydrolase; αKG, α-ketoglutarate; KGDH, α-ketoglutarate dehydrogenase

pyruvate, fatty acid oxidation, or amino acid catabolism. Additionally, glutamine-derived glutamate can be catabolized by glutamate dehydrogenase to generate αKG, sending the carbon backbone towards the cycle anaplerosis. The completion of the Krebs cycle produces NADH and flavin adenine dinucleotide that fuel into the complex I and complex II of the electron transport chain to generate electrons to support oxidative phosphorylation for cellular energy production in the mitochondria.

α-Ketoglutarate dehydrogenase (αKGDH) is a rate-limiting metabolic enzyme in the flux of the Krebs cycle.[25] αKGDH is a multiprotein complex that reacts with acetyl-coenzyme A (CoA) and NAD^+ to decarboxylate αKG to succinyl-CoA, which is further metabolized to become succinate. An increase of cytosolic calcium leads to rapid mitochondrial acidification and promotes αKGDH activity, thereby boosting NADH production and oxidative metabolism.[26,27] In addition, αKGDH is responsive and sensitive to the levels of reactive oxygen species, and oxidative stress-induced reactive oxygen species impair αKGDH function.[28] It has been reported that αKGDH reaction is essential for neuronal viability, and deficiency in the αKGDH activity appears to be associated with the chronic aberrant inflammation of neurodegenerative disorders, including Alzheimer's disease.[29,30] Moreover, Toll-like receptor 4 stimulation in pro-inflammatory macrophages, but not in the alternatively activated macrophage, markedly induced the expression of αKGDH, which promotes the conversion of αKG to succinate and limits the production of anti-inflammatory cytokine IL-10.[31]

Chemical modifications of nucleosome and DNA play a central role in the development and effector function of immune cells.[4-6] The epigenetic enzymes responsible for these modifications often require the intermediary metabolites from the Krebs cycle. Among metabolites, αKG (also known as 2-oxoglutarate or 2OG), is a crucial molecule involved in multiple metabolic and cellular pathways. αKG is the essential co-factor for the reaction of 2OG-dependent dioxygenases (2OGDD), a diverse superfamily of Fe(II)-dependent, oxygen-consuming enzymes that are crucial for cell development and diseases.[32] The 2OGDD family includes the prolyl-hydroxylase domain (PHD) -containing proteins, Jumonji C (JmjC) domain-containing proteins, and TET proteins.

These enzymes use αKG and O_2 to hydroxylate various types of substrates (including proteins, nucleic acids, and lipids) and produce succinate and CO_2. Therefore, the activities of 2OGDD positively correlate with the intracellular ratio of αKG to succinate and fumarate, another Krebs cycle downstream metabolite; elevated levels of succinate or fumarate will therefore inhibit 2OGDD function. In addition, 2OGDD can be inhibited by 2-hydroxyglutarate (2-HG), a metabolite structurally similar to αKG (discussed below).[33]

One branch of the 2OGDD family, the PHD proteins, is critical for hypoxia response. In normoxia (sufficient oxygen level), PHDs hydroxylate the prolines of the transcription factor hypoxia inducible factor-1α (HIF) that becomes the target for proteasome degradation. Under hypoxia, or reduced αKG level, the activity of PHDs is abolished, resulting in the stabilization and activation of hypoxia inducible factor-1α, which in turn induces gene expression related to metabolism and modulates immune cell function.[24,34] The other two branches of the 2OGDD family, JmjC and TET proteins, are essential epigenetic erasers, the activity of which is similarly regulated by αKG/succinate/fumarate.[35] Most JmjC proteins are lysine (and/or arginine) demethylases (JMJD) that target histones and other proteins. In macrophages, the increased αKG from glutaminolysis contributed the alternative activation of macrophages upon IL-4 stimulation. αKG suppresses IκB kinase/nuclear factor-κB-dependent pro-inflammatory effects, and modulates the activity of the histone demethylase JMJD3, thus favoring the acquisition of an anti-inflammatory phenotype.[31]

DNA methyltransferases

DNA methylation is the earliest known epigenetic modification.[36] Although DNA methylation was once thought to be a static repressive mark, recent studies have shown that DNA methylation is a dynamically regulated process and could have various functions depending on the protein binders and genomic locations.[37] In mammals, most DNA methylation occurs at the cytosines of CG motifs and is catalyzed by one of the DNA methyltransferases: DNMT1, DNMT3A, and DNMT3B. DNMT transfers the methyl group from SAM to the fifth carbon of cytosine on DNA, producing 5-methylcytosine and SAH (Figs 2, 3). The *de novo* methyltransferases DNMT3A and DNMT3B methylate the unmodified cytosine to establish the methylation pattern. During DNA replication, methylation patterns at CG motifs are replicated onto the newly synthesized DNA by the maintenance methyltransferase DNMT1 complex. Since the discovery of TET enzymes, it is now known that cytosine can go through the methylation cycle, which describes the intermediates between methylation and demethylation (see below and Fig. 2).

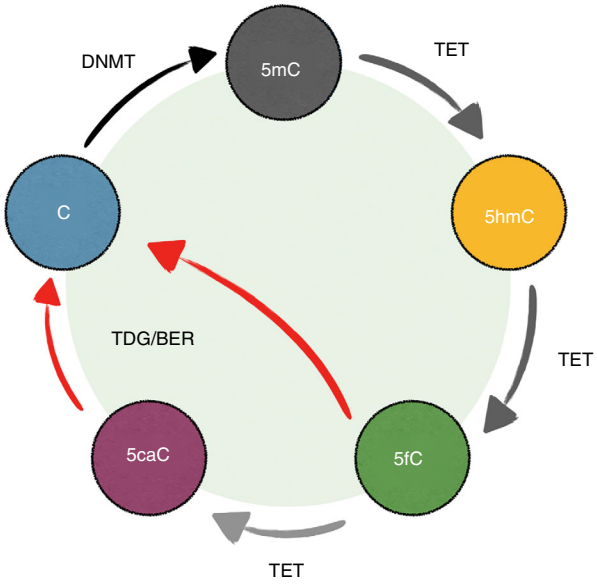

Figure 2. The DNA methylation cycle. In the mammalian genome, the majority of the cytosines at CG motifs are methylated. DNA methyltransferases (DNMTs) catalyze the addition of a methyl group to the fifth carbon of cytosine (C), generating 5-methylcytosine (5mC). TETs then convert 5mC into oxidized methylcytosines (oxi-mCs): 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC). While TETs are capable of the complete oxidization of 5mC to 5caC *in vitro*, the majority of oxi-mCs in the cells are 5hmC. 5hmC is stable and a potential epigenetic mark. 5fC and 5caC are unstable and are removed by TDG (thymine DNA glycosylase) with the base-excision repair. The base removal process (red arrows) constitutes 'active DNA demethylation'. During DNA replication, the pairing between newly synthesized DNA with the original modified CpG motif creates the hemi-modified CpG. The maintenance DNA methyltransferase complex DNMT1/UHRF1 recognizes the hemi-methylated CpG and methylates the unmodified cytosine on the new DNA. However, DNMT1/UHRF1 cannot recognize the hemi-methylated CpG containing oxi-mCs, preventing the methylation of the newly synthesized DNA. Therefore, the methylation pattern will be erased after rounds of DNA replication

Maintenance methyltransferase DNMT1

In the immune system, DNMT1 is highly expressed in $CD4^-$ $CD8^-$ double-negative and Pro-B cell stages in developing T and B cells, respectively (Immunological Genome Project; ImmGen). Consistently, conditional deletions of *Dnmt1* using *Lck-Cre* in early T cells[38] and *Mb1-Cre* in early B cells[39] resulted in the corresponding developmental blockades. Whereas T cells were able to develop when *Dnmt1* was deleted at the $CD4^+$ $CD8^+$ double-positive stage using *Cd4-Cre*,[38] studies have shown that DNMT1 is required to repress the ectopic expression of IL-4[40] and Foxp3[41] in CD4 and CD8 T cells.

Foxp3 is the essential transcription factor for regulatory T (Treg) cells.[83] Although loss of DNMT1 de-represses *Foxp3*, DNMT1 is required to maintain the lineage

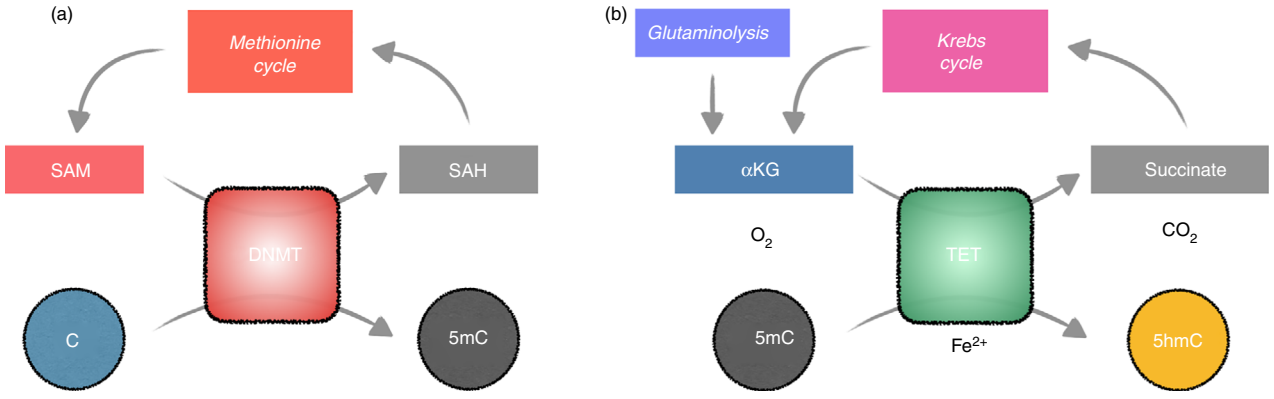

Figure 3. The intersection between metabolic cycles and DNA methylation cycle. (a) DNMT and methionine cycle. To methylate cytosine, DNMT uses *S*-adenosyl methionine (SAM) as the methyl group donor, producing *S*-adenosylhomocysteine (SAH) as a result. SAH is then recycled back to the methionine cycle (Fig. 1), regenerating SAM for additional methylation. (b) TET and Krebs cycle. With reduced iron (Fe^{2+}) as a co-factor, TET converts the substrates 5mC, α-ketoglutarate (αKG), and oxygen into the products 5hmC, succinate, and carbon dioxide. TET can further oxidize 5hmC into 5fC and 5caC (not depicted). Succinate can be shuttled back to the Krebs cycle and regenerating αKG. Additional αKG can be derived from glutamine via glutaminolysis (Fig. 1).

stability of Treg cells and prevents autoimmune diseases as shown by *Foxp3-Cre Dnmt1*-deficient mice.[42] In CD8 T cells, when *Dnmt1* is conditionally deleted with granzymeB (*GzmB*)-*Cre*, antigen-specific CD8 T cells failed to differentiate into effector and memory cells.[43] Similarly, peripheral B cells from a mouse expressing a DNMT1 hypomorphic mutant failed to differentiate into germinal center B cells in response to immunization.[44] In myeloid cells, the deletion of *Dnmt1* in macrophage enhanced the alternative polarization (M2) and increased M2 adipose tissue macrophages.[45]. Similarly, treatment with the DNMT inhibitor 5-Aza-2′-deoxycytidine potentiated macrophage M2 polarization in a dose-dependent manner.[45] As DNMT1 is essential for the maintenance of DNA methylation during DNA replication, pharmacological or metabolic inhibition (low SAM/SAH ratio) of DNMT will likely result in a global loss of DNA methylation and lead to global ectopic gene expression and genomic instability.[46]

De novo methyltransferases DNMT3A and DNMT3B

During acute infection with lymphocytic choriomeningitis virus (LCMV), antigen-specific CD8 T cells remodel their methylome when differentiating from naive to effector or memory cells.[47] In the absence of DNMT3A, CD8 T cells preferentially differentiate into memory cells.[48,49] TCF-1 (encoded by *Tcf7*) is the transcription factor that is important for CD8 memory cell differentiation. In early effector cells, DNMT3A methylates the regulatory elements of *Tcf7*, repressing TCF-1 expression and memory differentiation.[48,49] Therefore, DNMT3A promotes terminal effector differentiation and restricts memory precursor in CD8 T cells. Besides effector differentiation, *de novo* DNA methylation is required for terminal T-cell exhaustion, a dysfunctional state describing the loss of T-cell effector function.[50] The inhibitory receptor programmed cell death protein-1 (PD-1; encoded by *Pdcd1*) is essential for T-cell exhaustion and antibody blockage of PD-1 has been the key to recent success in cancer immunotherapy. In CD8 T cells, inhibition of *de novo* DNA methylation, either by deletion of *Dnmt3a* or by pharmacological inhibition, potentiated the effect of anti-PD-1-mediated reversal of exhaustion that is induced by chronic LCMV infection and tumor.[50] Consistent with the acute LCMV infection models,[48,49] DNMT3A methylates the elements at *Tcf7* and *Ifng*, both of which contribute to the tumor clearance. Intriguingly, in mouse CD8 T cells, two conserved regions of *Pdcd1* are methylated in naive, demethylated in effector, and remethylated in memory CD8 T cells.[51] In exhausted CD8 T cells, these regions were fully demethylated and correlated with PD-1 expression. The role of DNMT3 and TET enzymes in PD-1 regulation remains to be established. In summary, dependent on the context, *de novo* DNA methylation facilitates effector differentiation and exhaustion while limiting memory formation in CD8 T cells.

De novo DNA methylation is important for lineage restriction in CD4 T cells. Although only wild-type T helper 1 (Th1) cells express *Ifng*, CD4 T cells from *Cd4-Cre Dnmt3a*$^{fl/fl}$ mice fail to silence *Ifng* after differentiation into Th2, Th17, and induced Treg cells.[52,53] Although DNMT1 is required for Treg lineage stability, the deletion of *Dnmt3a* with *Foxp3-Cre* has no effect in the steady state.[42] Interestingly, in an EAE model, most Treg cells express BLIMP1 at the sites of tissue inflammation. BLIMP1 protects the lineage stability by inhibiting the expression of *Dnmt3a*, which would otherwise be induced by IL-6. In the absence of BLIMP1, DNMT3A

methylates *Foxp3 CNS2*, decreasing *Foxp3* expression and compromising Treg cell identity.[54]

In B cells, DNMT3A and DNMT3B are required for the B-lineage commitment from hematopoietic stem cells *in vitro*.[55] Once the B-cell progenitor has committed, *de novo* DNA methylation is not required for the major checkpoints during B-cell development as demonstrated in the *Mb1-Cre Dnmt3a/b*-deficient mice.[56,57] However, the use of light-chain Vκ genes was skewed,[56] potentially due to altered CTCF binding and long-range chromatin interactions.[58] In the periphery, antigen-stimulated B cells remodel their methylome during differentiation into germinal center B cells, plasma, and memory cells.[59,60] Similar to the *Mb1-Cre* model, the deletion of *Dnmt3a/b* with *Cd19-Cre* has no discernable phenotype in bone marrow development. However, *Dnmt3a/b* deficiency in B cells increased germinal center B cells and plasma cells in response to immunization.[57] Consistent with its opposite role as an eraser for DNA methylation, *Tet2* deficiency inhibits plasma cell differentiation, potentially through a lack of demethylation at *Prdm1*/BLIMP1, a transcription factor essential for plasma cells.[61] However, both TET2 and DNMT3A/B function to limit the expansion of germinal center B cells, a similar overlapping function was also observed in hematopoietic malignancies.[62] How these enzymes with seemingly opposite functions have a similar role in repressing cell proliferation remains to be addressed.

In macrophages, DNMT3B represses M2 differentiation, and the deficiency of *Dnmt3b* increased the expression of IL-4-induced M2 genes in bone-marrow-derived mouse macrophage.[63] Mechanistically, DNMT3B methylates the promoter of peroxisome proliferator-activated receptor-γ (PPAR), a key transcription factor for promoting macrophage alternative M2 polarization.[63] In addition, peritoneal macrophages from *Lyz2-Cre Dnmt3a*-deficient mice were defective in the production of type I interferon (IFN-I), and animals were more susceptible to the infection of vesicular stomatitis virus.[64] DNMT3A regulates interferon induction indirectly by maintaining the expression of HDAC9, which in turns deacetylates TBK1, the key kinase for innate immune sensing.[64] Therefore, in macrophages, both DNMT1 and DNMT3B are required for the restricting alternative M2 activation, while DNMT3A is important for IFN-I production.

TET methylcytosine oxidases

TET enzymes (TET1, TET2, TET3) are 2OGDD that are essential for cell differentiation and functions.[65,66] The function of TET is to oxidize and demethylate cytosine on DNA to regulate gene expression and other undefined processes. Similar to other 2OGDD, TETs use αKG and oxygen to hydroxylate 5-methylcytosine (5mC) into 5-hydroxymethylcytosine (5hmC), a stable epigenetic mark and one of the oxidized methylcytosines (Fig. 2). Further reaction can produce the other two oxidized methylcytosines: 5-carboxylcytosine and 5-formylcytosine, both of which are removed by base-excision DNA repair and are about 10-fold and 100-fold lower in abundance compared with 5hmC, respectively. These oxidized methylcytosines serve as the key intermediates for passive (replication-dependent) or active (replication-independent) DNA demethylation.[65] The three TET family members regulate distinct regions in the genome: TET1 preferentially regulates promoters, while TET2 and TET3 regulate enhancers. Therefore, 5hmC is enriched at enhancers, promoters, and gene bodies, with the level of enhancer 5hmC positively correlating with enhancer activity (marked by H3K27Ac) and gene body 5hmC with transcriptional activity. The current model suggests that TET enzymes (especially TET2 and TET3) regulate the activity of lineage-specific super-enhancers,[67] in part by facilitating the enhancer accessibility.[68-70] As 5hmC is stable on the DNA, the pleiotropic phenotypes after TET deletion are often observed at delayed times and are usually manifested after rounds of cell proliferation. Mice with germline deletion of *Tet1* and *Tet2* are largely viable,[71-73] but the *Tet3* homozygous mutation results in embryonic or perinatal lethality.[73,74]

In humans, *TET2* is one of the most recurring loss-of-function mutations in hematopoietic malignancies, implying a role of TET in immune cell differentiation and function (TET in hematopoietic cancers was recently reviewed[75]). In mouse blood cells, TET1 is preferentially expressed in hematopoietic stem cells, developing B and T lymphocytes, and naive T cells (data from ImmGen); TET2 and TET3 are expressed ubiquitously. The genome-wide 5hmC undergoes dynamic changes often around the key lineage genes during T-cell and B-cell differentiation.[67,76-78] As TET2 and TET3 function redundantly, no significant B-cell or T-cell developmental phenotype was observed in either germline or conditional *Tet2*-single-deficient models.[68-71,79,80] However, when both *Tet2* and *Tet3* (*Tet2/3*-double knockout) were both deleted in developing B cells using *Mb1-Cre*, bone marrow B-cell development was blocked at the pro-B to pre-B cell stage transition due to a deficiency in the rearrangement of immunoglobulin light chain.[68,70] In T cells, while conventional CD4 and CD8 T cells were able to develop, *Cd4-Cre Tet2/3*-double knockout developed a massive lymphoproliferation caused by the expansion of self-reactive RORγt$^+$ IL-17$^+$ natural killer T cells.[69]

TET enzymes regulate a diverse array of immune cell functions. In CD4 T cells, TET2 is required for the production of IFN-γ and IL-17 by Th1 and Th17 *in vitro*, respecitvely.[79] However, *Tet2* deficiency in T cells exacerbated the IL-17-dependent pathology in two EAE models, potentially due to decreased IL-10 production.[79] Unlike CD4 T cells, CD8 T cells from the *Cd4-Cre Tet2$^{fl/fl}$* mice

produced more IFN-γ in response to the acute LCMV infection, with increased differentiation of memory precursor cells and decreased short-lived effector cells.[81] Consistent with mouse CD8 T cells, *TET2* deficiency in human CD8 CAR-T cells resulted in the differentiation of central memory cells with enhanced tumor clearance.[82] Hence, although promoting pro-inflammatory cytokines *in vitro*, TET enzymes function to suppress T cell immune response *in vivo*.

TET2 and TET3 are required for the demethylation of *Foxp3 CNS2*, an intronic enhancer known to be demethylated in Treg cells. Deletion of *Tet2* and *Tet3* either using *Cd4-Cre* or *Foxp3-Cre* increased the DNA methylation at CNS2, resulting in decreased *Foxp3* expression, lineage instability, and the unleashing of the effector potentials of these Treg cells.[84-86] Interestingly, Treg-specific deletion of *Uqcrsf1*, the gene encoding the essential mitochondrial complex III protein Rieske iron-sulfur protein, resulted in an autoimmune phenotype caused by impaired suppressive function of Treg cells. Loss of complex III resulted in increased levels of the 2-HG and succinate, both of which are TET inhibitors, and so destabilized Treg cell lineage identity.[87] Therefore, existing data suggest that TET enzymes are required for Treg cell function.

In B cells, loss of TET enzymes resulted in impaired class switch recombination, a process by which antibody switch from IgM to other isotypes.[61,67,88] Mechanistically, TET2 and TET3 cooperate with transcription factor Basic leucine zipper transcription factor (BATF) to promote the expression of activation-induced cytidine deaminase, the key enzyme for antibody maturation.[67] *Tet2* deficiency resulted in increased germinal center B cells and decreased plasma cell differentiation after immunization.[61] Therefore, TET enzymes have both positive and negative roles in B-cell responses.

In myeloid cells, one of the functions of TET2 is to repress pro-inflammatory cytokines, including IL-1β[89,90] and IL-6.[91,92] Consistent with the anti-inflammatory role of TET2, loss of *Tet2* facilitates atherosclerosis in mice,[90] a phenotype reminiscent of humans with clonal hematopoiesis caused by *TET2* mutation.[93] Most importantly, *Tet2*-deficient macrophages (*Lyz2-Cre*) exhibited a pro-inflammatory phenotype in the immunosuppressive tumor microenvironment and delayed melanoma growth *in vivo*.[94]

Links between metabolism, epigenome, and immune function

Epigenetic modifications are essentially biochemical reactions catalyzed by enzymes. Therefore, the concentrations of metabolic substrates, co-factors, and products would dictate the reaction rate. For instance, 2OGDDs including TET and JmjC histone demethylases require αKG, the availability of which directly affects the epigenome. Indeed, in activated T cells, the IL-2-sensitive differentiation programs depend on the level of αKG and glutamine.[95] High levels of IL-2 favor effector differentiation; whereas low levels of IL-2 favor memory or follicular T helper cells. High IL-2 induces the accumulation of glycolysis and glutaminolysis metabolites including αKG. The level of αKG appears to be instructive in gene expression: the addition of cell-permeable αKG can mimic a high-IL-2-like gene expression profile even when cultured in low IL-2. Mechanistically, αKG likely promotes the enzymatic reactions by JmjC proteins and TETs to promote histone and DNA demethylation, respectively. DNA demethylation at CG-containing CTCF motifs permits CTCF binding, facilitating the genome reorganization and gene expression.[95] Another example of metabolism affecting the epigenome is a study of methionine metabolism in T cells (discussed above). *In vitro* activated T helper cells actively uptake extracellular methionine, from which the majority of the SAM pool is derived. Activated CD8 T cells cultured in methionine-restricted conditions had dramatically decreased the SAM level and global H3K4me3 level, whereas the global H3K4me3 level was slightly decreased in Th1 and Th17 cells. Nonetheless, Th17 cells cultured in low methionine had decreased expression of *Il17a*, *Batf*, *Cd5l*, and cell cycle-related genes accompanied by decreased H3K4me3 at the corresponding promoters. A low-methionine diet also ameliorates the severity of the Th17-driven disease EAE, at least in part by limiting the proliferation of Th17 cells.

2-HG was first identified as the 'oncometabolite' produced by the isocitrate dehydrogenase 1 and 2 (IDH1/2) mutant in glioma cells.[33] Structurally similar to αKG, 2-HG can inhibit the activity of 2OGDD enzymes. 2-HG exists as two enantiomers that differ in their ability to inhibit 2OGDD enzymes; with (S)-2HG (also known as L-2HG) being the more potent inhibitor than (R)-2HG (or known as D-2HG). Notably, it has been reported that 2-HG can be generated endogenously in normal cells. For instance, in CD8 T cells, T-cell receptor stimulation induces the generation of L-2HG as early as 2 days post-activation.[96] Similar to *Tet2* deficiency, treatment with L-2HG induced higher levels of Eomes and CD62L, resembling central memory cells. Interestingly, OT-I T-cell-receptor-transgenic CD8 T cells treated with L-2HG *in vitro* appear to have increased survival and tumor clearance after *in vivo* transfer to recipients bearing tumors expressing ovalbumin,[96] suggesting that the effect of L-2HG is likely via epigenome. Therefore, these recent examples showcased the link between metabolism and epigenome and their effect on immune responses.

Concluding remarks

The DNA methylation program mediated by DNMT and TET is essential for regulating cell homeostasis and effector function in immune cells. Most studies of the

metabolome–epigenome focus on the global level of histone modifications, but the effect of metabolites on the DNA methylome should warrant more attention. The perturbation of epigenetic enzymes may not affect the abundance of a given epigenetic mark globally,[21,67] thus genome-wide epigenome profiling will likely be required to pinpoint the local changes underlying the phenotype. In addition, recent advances in single-cell technologies in epigenome sequencing[97] and mass spectrometry[98] will greatly improve the capacity of analysis in the often small immune populations isolated *in vivo*, including tumor-infiltrating and tissue-resident immune cells.

The studies discussed above have demonstrated promising therapeutic interventions to achieve the desired immune response by modulating the cellular metabolism and/or epigenome. However, it has yet to explore the broad effects of metabolite levels on global epigenetic landscapes of immune cells. Similar to the ImmGen consortium for genomics and epigenomics, it would be beneficial to expand the scope to include metabolomic profiles for individual immune cell types. Understanding the differential metabolic circuits in each cell type may allow cell-specific rewiring of the metabolome and potentially the epigenome.

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Disclosures

The authors declare no conflict of interests.

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