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Connections Between Metabolism and Epigenetics in Programming Cellular Differentiation

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Abstract

Researchers are intensifying efforts to understand the mechanisms by which changes in metabolic states influence differentiation programs. An emerging objective is to define how fluctuations in metabolites influence the epigenetic states that contribute to differentiation programs. This is because metabolites such as S-adenosylmethionine, acetyl-CoA, α-ketoglutarate, 2-hydroxyglutarate, and butyrate are donors, substrates, cofactors, and antagonists for the activities of epigenetic-modifying complexes and for epigenetic modifications. We discuss this topic from the perspective of specialized $CD4^+$ T cells as well as effector and memory T cell differentiation programs. We also highlight findings from embryonic stem cells that give mechanistic insight into how nutrients processed through pathways such as glycolysis, glutaminolysis, and one-carbon metabolism regulate metabolite levels to influence epigenetic events and discuss similar mechanistic principles in T cells. Finally, we highlight how dysregulated environments, such as the tumor microenvironment, might alter programming events.

INTRODUCTION

An expanding spectrum of research efforts have substantiated and extended the concept that metabolism and differentiation programs are linked. The ability to measure the activity of metabolic pathways and emerging technologies enabling more precise measurements of individual metabolites have led to the recognition that metabolism dynamically changes during immune cell activation and differentiation (1–5). The study of nutrient-sensitive signaling proteins such as mTOR and AMPK has also connected metabolic changes with signaling cascades that have the capacity to influence activation and differentiation programs within immune cells (6–9). In this review, we focus on a new research area defining how the regulation of metabolites that have roles in epigenetic processes affects differentiation decisions. First, we discuss the mechanistic connections that couple metabolic and differentiation gene programs. The next sections examine specific metabolites and the epigenetic complexes that they influence. This is followed by a discussion of the current understanding of the mechanisms linking nutrients, fluctuations in metabolites, epigenetic events, and gene programming in cellular differentiation decisions. Finally, we briefly highlight how metabolism and epigenetics are emerging as critical concepts in tumor immunology. We focus on findings associated with effector and memory T cell differentiation decisions, with some discussion of $CD4^+$ helper T cell specialization decisions as well. In some instances, we will also highlight how studies in embryonic stem cells have paved the way for defining mechanistically how the regulation of metabolites influence epigenetics and how these concepts are now being identified in T cells. Together, the discussion of these topics points to the importance of expanding our knowledge surrounding the mechanistic connections between metabolism and epigenetic states in immune cell differentiation.

CONNECTIONS BETWEEN METABOLISM AND DIFFERENTIATION GENE PROGRAMS

Researchers oftentimes traditionally think of metabolism as a mostly static process that is involved in cellular homeostasis. Although the expression of genes that encode components in metabolic pathways, and the expression of their protein products, are often similar in terminally differentiated cells, it is now appreciated that the expression of metabolic pathways is dynamically regulated in cells during developmental transitions (10–13). Reflecting this knowledge, our viewpoints have slowly shifted from thinking about metabolism solely as a housekeeping function for cells, to instead viewing metabolism as an active participant in regulating cellular transitions during development.

Metabolism Is Dynamically Regulated During Cellular Differentiation

Studies in T cells highlight that metabolism plays a dynamic role in differentiation (**Figure 1**). Early studies on the activation of naive T cells uncovered a role for glucose metabolism and glycolysis in T cell activation (14, 15). Research examining metabolism in effector versus memory T cells has found that effector T cells display a high rate of aerobic glycolysis that is similar to the Warburg effect observed in cancer cells (1, 16–18). In contrast, memory T cells shift away from glycolysis and instead display increased rates of fatty acid oxidation (2, 11, 19). Numerous studies since have expanded our knowledge of the metabolic states in effector and memory T cells. For example, in addition to enhanced glycolysis in effector T cells, metabolic pathways such as glutaminolysis, lipid biosynthesis, and the pentose phosphate shunt are highly induced during effector T cell differentiation (1, 3, 20–22). Some metabolites in the TCA cycle are also upregulated during effector T cell differentiation (1, 3), but until recently the significance of these metabolites

was not as intensively studied (23, 24). Metabolic profiling studies have now identified changes in metabolism that are associated with the differentiation of specialized $CD4^+$ T cell subtypes, B cells, and macrophages, further solidifying the conclusion that metabolism is a dynamic process and is a component of immune cell differentiation (3, 25–30).

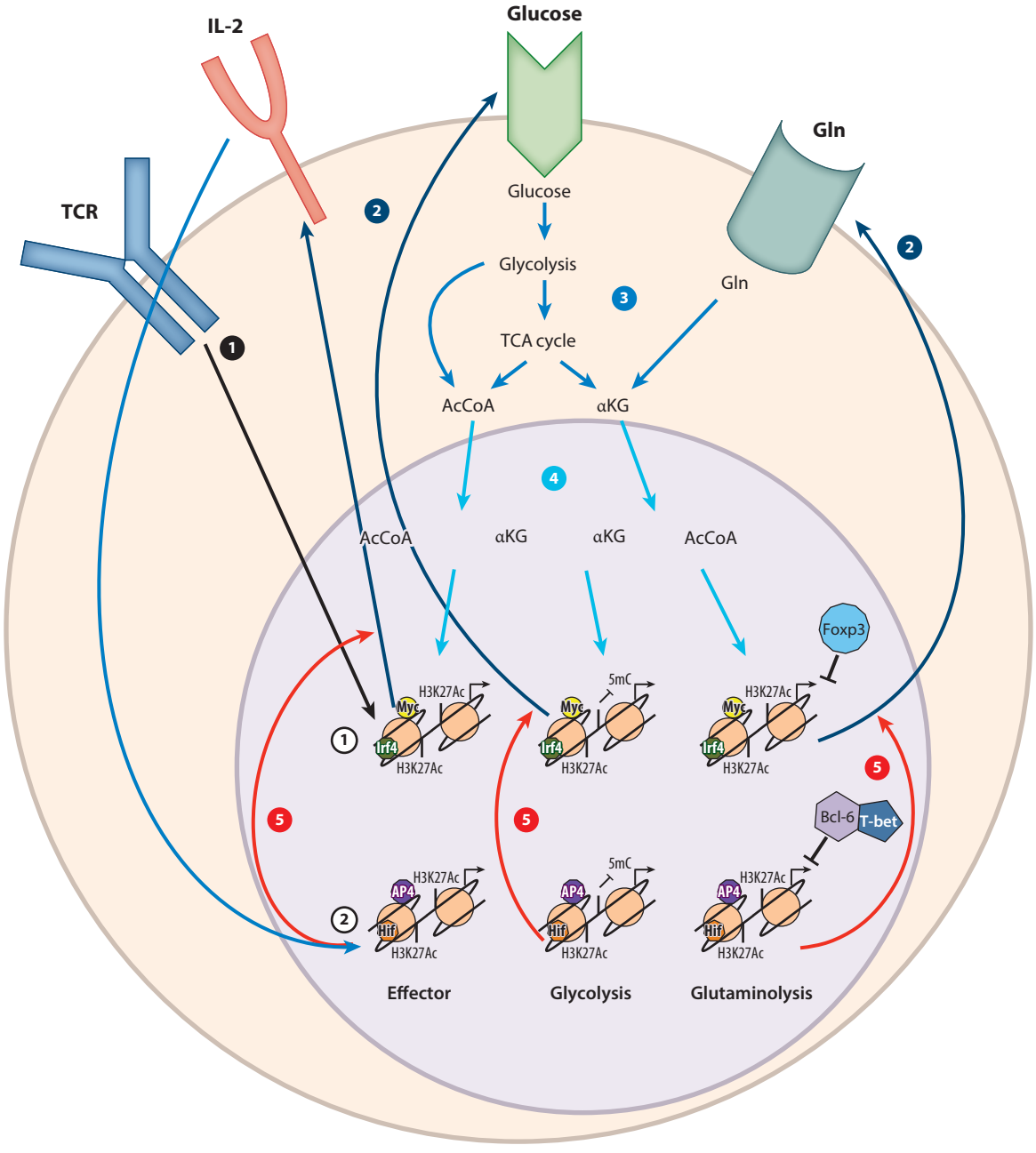
Metabolic Gene Programs Are Dynamically Regulated During Cellular Differentiation

The connection between metabolism and differentiation is in part caused by a similar complement of transcription factors regulating both metabolic and differentiation gene programs. This is evident in T cells, where T cell receptor (TCR) signaling and IL-2 signaling regulate a series of transcription factors that influence both metabolic and T cell differentiation gene programs (1, 20, 22, 31–33). In addition, lineage-specifying transcription factors that control $CD4^+$ T cell specialization decisions also regulate the expression of genes that encode the components of metabolism programs (33, 34). We now briefly highlight the role for transcription factors that are induced by TCR and IL-2 signaling to coordinate metabolism with effector T cell differentiation programs.

The oncogene *Myc* is induced by TCR signaling and encodes for a transcription factor that controls the expression of genes encoding components of metabolic pathways. The early identification of an oncogene in this process drew parallels between the programming of metabolism for a productive proliferative burst in T cells with the dysregulated proliferative state in cancer (1, 9, 35). In both cases, Myc promotes the expression of genes that encode components of glycolysis and other metabolic pathways (1, 9, 36, 37). However, the difference between programming metabolism for a controlled proliferative burst in T cells versus the uncontrolled proliferation in cancer in part stems from the tight regulation of Myc activity in response to TCR signals. In particular, transcription factors such as AP4 precisely control the metabolic gene program required for effector T cell differentiation after the initial rapid induction of this program by Myc (32). Terminating the reliance of the metabolic program on Myc in effector T cell differentiation effectively diminishes the potential for the oncogenic events that occur in the cancer setting, where Myc activity is not downregulated. Thus, it is interesting to note that metabolic programming in T cells and cancerous cells are similar, but T cells retain key balances to regulate metabolic programs that cancerous cells lack.

TCR-inducible transcription factors, such as Myc, interferon regulatory factor 4 (IRF4), and sterol binding proteins (SREBPs), coordinate the regulation of several metabolic gene programs that are needed for effector T cell activation (1, 20, 31). For example, in addition to regulating the expression of genes that encode transporters and enzymes in the glycolysis pathway, Myc also regulates genes in the glutaminolysis pathway and ribosome biogenesis and contributes to generally amplifying gene expression (38, 39). IRF4 also plays a role in regulating these metabolic and cellular processes (31). Importantly, IRF4 induction is sensitive to TCR signal strength, with strong TCR signals causing higher levels of IRF4 expression in comparison to weaker TCR engagement (31, 40–42). One mechanism by which graded IRF4 expression integrates the information about TCR signal strength into the cell is by influencing the levels of IRF4-BATF complexes, with the complex levels then affecting the probability for binding to enhancer elements that have different affinities for the complex (43). The SREBPs regulate the expression of genes that encode components of the lipid biosynthesis pathway (20). The coordination of the lipid biosynthesis pathway with the early stages of T cell activation is critical for generating the material that is required to create new cellular membranes needed for rapid cell division (11, 44). It also contributes to many signaling events by enhancing cholesterol and lipid components utilized in signaling pathways (45).

After initial TCR activation, IL-2 signaling is needed to regulate the activity of transcription factors that are involved in programming metabolism and T cell differentiation decisions (**Figure 1**). For example, the expression and activity of hypoxia-inducible factor (HIF) family members are enhanced by IL-2-dependent events (22). HIF family members are important for sustaining the genes that encode components of metabolic pathways such as glycolysis and glutaminolysis (22, 46, 47). Related to this, HIF protein activity has been shown to be important for



the development of the effector T cell phenotype, and loss of HIF activity severely compromises effector T cell responses to type 1 infections (46). IL-2 signaling also regulates the expression of AP4, which as discussed, contributes to sustaining Myc-sensitive metabolic gene programs (32). Taken together, a series of TCR- and IL-2-sensitive transcription factors are responsible for selecting the metabolic programs that promote effector T cell differentiation and couple metabolism to effector fate decisions (**Figure 1**).

The selection of metabolic programs for T cell differentiation decisions is also mediated by the lineage-specifying transcription factors that direct $CD4^+$ T cell specialization decisions. In regulatory T cells, Foxp3 can inhibit *Myc* expression to indirectly regulate Myc-sensitive metabolic gene programs (34) (**Figure 1**). In Th1 cells, a competitive mechanism between T-bet and Bcl-6 allows T-bet to indirectly promote the expression of genes that encode components of the glycolysis program by inhibiting Bcl-6 from repressing their expression (33) (**Figure 1**). Interestingly, not only does Bcl-6 directly repress genes that encode components of the glycolysis pathway, but two other members of the BTB-ZF transcription factor family, Zbtb7a and Zbtb20, also play similar roles in other developmental settings (48, 49). The conserved role for BTB-ZF transcription factors, as well as the role for Myc, HIF, and SREBP family members in regulating metabolic gene programming decisions, provides mechanistic insight into how metabolism and cellular differentiation are often connected in diverse developmental settings.

The nutrient environment that a cell is exposed to also actively contributes to differentiation decisions. In T cells, the glucose and glutamine in the environment affect differentiation decisions. Specifically, increasing concentrations of glucose and glutamine promote effector T cell programs, while diminished glucose and glutamine concentrations inhibit effector T cell differentiation (17, 50–52). In the case of glutamine, limiting glutamine or glutaminolysis during the early stages of T cell activation can stably affect the differentiation decision both in vitro and in vivo (23, 52). This suggests that at least one role for glutamine-sensitive events relates to a stable epigenetic process that affects the differentiation potential of the T cell. The stability of nutrient effects on cellular differentiation indicates the importance of exploring whether the regulation of metabolism during T cell differentiation decisions affects epigenetic processes.

Dynamic Control of Epigenetics During Differentiation

Research in embryonic stem cells and cancer cells has played a large role in shaping the view that the mechanisms by which metabolism influences fate choices expand beyond the energy needs of a cell. For example, the Warburg effect refers to the observation that cancer cells utilize aerobic glycolysis even though a switch to this metabolic program does not intuitively make sense from the standpoint of energy homeostasis or ATP production (53). Trying to make sense of this phenomenon has

Figure 1

Feed-forward regulation integrates metabolism and differentiation programs. Visual representation of the multilayered steps that connect signaling events, metabolism, and differentiation gene programs in T cells. The engagement of the T cell receptor (TCR) initiates the first cascade of events (*black arrow*, ❶) that upregulate genes associated with effector potential as well as genes associated with metabolic pathways such as glycolysis and glutaminolysis. The upregulation of the proteins encoded by these classes of genes leads to feed-forward mechanisms that initiate a second wave of events that are now dependent on IL-2 signaling (*dark blue arrows*, ❷). The metabolites that are upregulated in this process (*medium blue arrows*, ❸), which include α-ketoglutarate (αKG) and acetyl-CoA (AcCoA), then participate in the regulation of the IL-2-sensitive gene program (*light blue arrows*, ❹). These events then continue to reinforce the program (*red arrows*, ❺). The class of genes on line ① represents the first wave of transcriptional events, whereas line ② represents the same class of genes that is responding to the next series of events. Also displayed is a representation of transcription factors that can participate in the regulation of metabolic programs. Shown are Foxp3, which can inhibit *Myc*, as well as the T-bet–Bcl-6 complex, which can prevent Bcl-6 from repressing glycolysis genes.

led researchers down the path of identifying roles for metabolism and metabolites that are outside of the traditional role in energy homeostasis. This includes understanding signaling pathways; protein synthesis requirements; posttranslational modifications; lipid membrane biogenesis; and the topic of this review, epigenetic processes (13, 37, 54, 55).

Turning to the role of metabolites in epigenetic events, it is important to first briefly discuss how our appreciation for the role of epigenetic states in T cell differentiation programs has evolved. In T cells, early ChIP-seq studies focused on defining changes in histone H3 lysine 4 trimethylation (H3K4me3) and H3K27me3 in specialized $CD4^+$ T cell subtypes (56). H3K4me3 is a generally permissive histone modification associated with active transcription. In contrast, H3K27me3 is generally associated with a nonpermissive or repressive chromatin signature. Interestingly, H3K27me3 found in conjunction with H3K4me3 (often referred to as a bivalent state) is thought to mark genes that are poised for expression (57). In specialized $CD4^+$ T cell subtypes, the promoter regions for genes that encode signature cytokines and lineage-specifying transcription factors are generally contained within permissive H3K4me3 modifications in the subtype where they are expressed (56). In contrast, H3K27me3 modifications localize to the promoters of signature cytokine genes, while the genes encoding the lineage-specifying transcription factors for $CD4^+$ T cell specialization are found in a bivalent configuration, in the alternative subtypes that do not normally express these factors (56). These early data indicated that the expression of $CD4^+$ T cell lineage–specifying transcription factors is not permanently repressed in alternative fate choices; rather, they are subject to dynamic epigenetic control. This has contributed to the appreciation that there is a degree of flexibility in the programming and function of specialized $CD4^+$ T cell populations, and the regulation of epigenetic states contributes to this flexibility (58, 59).

Studies defining epigenetic states across the genome in diverse developmental settings indicate that large enhancers, which have been called super-enhancers, play a key role in regulating the gene programs associated with cellular differentiation (60, 61). In T cells, large regions with histone modifications consistent with super-enhancer elements, which include H3K4me1 and H3K27 acetylation (H3K27Ac), are enriched for transcription factor–binding motifs for factors known to be important in T cell specialization (62). Interestingly, genetic variation associated with diseases is often located within the defined super-enhancer regions (60, 62). Notably, the single nucleotide polymorphisms (SNPs) associated with a disease are enriched in the regions with active enhancer modifications only in the cell types associated with disease pathology. For example, SNPs for autoimmune conditions such as type 1 diabetes, lupus, and rheumatoid arthritis are clustered in genomic regions with active enhancer epigenetic modifications in T or B cells, whereas SNPs for Alzheimer's disease are found within regions with active enhancer epigenetic modifications in brain tissue (60, 62). Taken together, the expanding number of ChIP-seq and ATAC-seq studies characterizing histone modifications and regions of chromatin accessibility in immune cells are providing unprecedented information about the chromatin landscape in developmental and immune cell populations (62–70). However, the mechanisms that regulate these chromatin domains, as well as their functional impact on immune cell programs, are only beginning to be explored.

METABOLITES, EPIGENETIC-MODIFYING COMPLEXES, AND EPIGENETIC STATES

The connection between metabolism and cellular differentiation encompasses more than simply the energetic needs for a cell. Recently, there has been an increase in studies elucidating the role for metabolites in epigenetic processes (see the sidebar titled Diverse Developmental Systems) (23, 71–75). This is in part because metabolites play a direct role serving as substrates, donors, cofactors, and antagonists for epigenetic-modifying complexes and epigenetic modifications, and importantly,

DIVERSE DEVELOPMENTAL SYSTEMS

Research in embryonic stem cells and other developmental systems has played a key role in shaping our mechanistic understanding of the concepts discussed in this review (5, 10, 76). This is important to note because conceptual advances in our understanding of cellular differentiation in early development can help guide the questions that will be important to address in immune cells. Complimentarily, principles uncovered in immune cells can also serve to expand our understanding of cellular differentiation mechanisms in early development. Research examining dysregulated metabolic states in disease can also uncover useful knowledge that can be extrapolated to predict the critical mechanisms that control normal processes (13, 77). Our discussion highlights common principles for metabolites in cellular programming, which can serve as a guide for exploration in diverse contexts of immune cell development and function.

these epigenetic regulatory events might provide a mechanistic link between metabolism and differentiation programs (5, 54, 55) (**Figure 2**). In this section, we discuss metabolites in the context of the epigenetic-modifying complexes that influence differentiation decisions.

S-Adenosylmethionine and Methyltransferases

Both histone methyltransferases and DNA methyltransferases are important in establishing the epigenetic environments that regulate cellular differentiation programs (78, 79). S-adenosylmethionine (SAM) is generated in the one-carbon metabolism pathway and serves as the methyl donor for lysine and arginine histone methyltransferases as well as for DNA methyltransferase (DNMT) complexes (54, 80). One-carbon metabolism encompasses both the folate and methionine cycles. Several amino acids, including threonine, serine, and glycine, can initiate the one-carbon metabolism pathway in the folate cycle to facilitate the generation of SAM in the

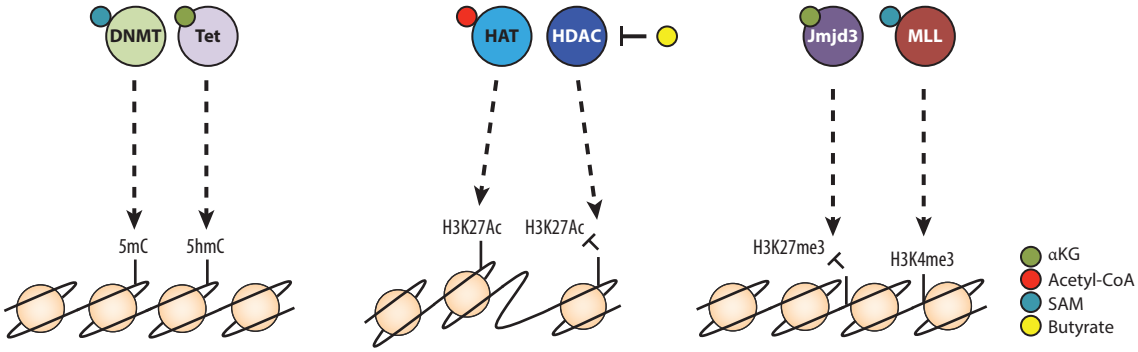


Figure 2

Metabolite and epigenetic-modifying complex pairs. Representation showing the metabolites that serve as donors, substrates, cofactors, and antagonists with the epigenetic-modifying complexes that they influence. Cofactors are shown overlapping with the complex, whereas the donor and substrate metabolites are shown adjacent to the complex. Butyrate, a metabolite that can inhibit HDAC activity, is also shown. Epigenetic-modifying complexes that add or remove a specific type of moiety are shown adjacent to each other in the figure. Dashed arrows indicate that there is an outstanding question in the field as to defining how changes in the metabolites that are associated with epigenetic-modifying complexes are targeted to specific differentiation programs. Abbreviations: αKG, α-ketoglutarate; DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase complex; MLL, mixed lymphocyte leukemia complex; SAM, *S*-adenosylmethionine.

methionine cycle (77, 81). The critical nature of one-carbon metabolism and SAM is highlighted by the observation that mutations in enzymes that are associated with this pathway, along with aberrant methylation states, are found in cancer and developmental abnormalities (82–84). Interestingly, inhibitors that target the one-carbon metabolism pathway and are used to treat cancer have been repurposed to treat autoimmune states such as Crohn's disease, rheumatoid arthritis, and lupus, indicating the importance of one-carbon metabolism in immune cell activation states as well (85).

Histone methyltransferases. All histone methyltransferase complexes require SAM, because it serves as a universal methyl donor. However, it is interesting to note that the H3K4me3 modification appears to be the most sensitive of the histone methylation modifications to changes in the one-carbon metabolism pathway and the concentration of SAM in a cell (72, 86). It is unclear whether there is any gene specificity to the targeting of the SAM-sensitive H3K4me3 changes. Mixed lymphocyte leukemia (MLL) complexes are a family of methyltransferases that can catalyze the H3K4me3 modification. They have been shown to be important in hematopoietic stem cell differentiation as well as the development of diverse lymphoid populations (87, 88). It will be interesting to determine whether MLL activity is regulated by fluctuations in SAM and the one-carbon metabolism pathway.

One of the best-studied methyltransferase complexes in cellular differentiation is the polycomb repressive complex (PRC) (89). EZH2, which contains a SET domain, is the catalytic subunit of PRC2, while EZH1 can also serve as the catalytic subunit in different forms of the complex (89). EZH1 and EZH2 are responsible for the deposition of the H3K27me3 modification. As discussed, H3K27me3 is generally recognized as a repressive histone modification, and the bivalent H3K27me3 and H3K4me3 combination is thought to signify genes that are held in a poised state until their expression pattern is resolved during developmental transitions (57, 90). In development, PRC2 has been shown to deposit H3K27me3 on the X chromosome to promote X chromosome inactivation, and it also deposits H3K27me3 at the genes that encode HOX proteins, T-box proteins, and other developmental transcription factors to tightly regulate their expression during embryonic stem cell differentiation (91–93). Therefore, PRC2 and the H3K27me3 modification are important to defining gene expression states in development.

Recent studies are elucidating a role for PRC2 in T cell differentiation decisions. Studies have shown that EZH2 plays a role in the differentiation of specialized $CD4^+$ T cell subtypes as well as the differentiation decision between effector and memory T cells (64, 94–99). In specialized $CD4^+$ helper T cells, EZH2 regulates the expression of lineage-specifying transcription factors and signature cytokine genes (94, 97). This role for EZH2 is consistent with the epigenetic profiling experiments that found H3K27me3 was deposited at the loci for the signature cytokine genes in naive $CD4^+$ T cells and the alternative subtypes when the genes are repressed (56). Importantly, the loss of EZH2 enhanced the expression of the signature cytokine genes in nonpolarizing or weakly polarizing conditions, and there was greater flexibility in expression when polarization conditions were switched to the alternative subtype (94). EZH2 has also been shown to be important in the generation of $Foxp3^+$ regulatory T cells (96, 100, 101), further solidifying the conclusion that EZH2 is involved in the development of a broad range of specialized $CD4^+$ T cell subtypes.

The role for EZH2 in effector and memory T cell program transitions is complex, and the data indicate that EZH2 is required for a diverse set of events in this process (64, 95, 96). For example, EZH2 is important for inhibiting genes in the apoptosis pathway, and this activity promotes the survival of activated T cells (98). In addition to roles that are related to the initial activation of T cells, recent studies have indicated EZH2 is also involved in regulating the gene programs associated with terminal effector and memory $CD8^+$ T cells (64, 95). Phenotypically, EZH2 is

important for the antigen-specific response of effector T cells during a viral infection, and while EZH2 does not appear to be broadly required for the development of memory cells, it is important for $CD8^+$ memory T cells to mount a proper recall response (64). Collectively the data indicate that EZH2 is important for multiple facets of T cell programming. Importantly, the data also suggest that although EZH2 might be performing similar mechanistic activities in different stages of cellular differentiation, this is interpreted in a context-dependent manner in each cell type.

DNA methyltransferases. The metabolite SAM is also the methyl donor for the DNA methylation modification. DNA methylation is most commonly found on the 5-carbon position of the cytosine residue (5mC) of a CpG dinucleotide. 5mC represents an important epigenetic modification that influences the ability of transcription factors to bind to their DNA-binding element and also recruits methyl-binding domain proteins (79). Three DNMTs have been identified in mammalian cells. DNMT1 is referred to as the maintenance methyltransferase because it copies the DNA methylation state during cellular division. DNMT3A and DNMT3B are both de novo DNA methyltransferases that can make methylation modifications to DNA independent of cell division or the current methylation status of the DNA (79). Importantly, DNA methylation affects cellular differentiation programs, and dysregulated DNA methylation patterns are often found in disease states such as cancer (102, 103).

DNMT complexes affect the development of many different immune cell populations. DNMT1 has been shown to be important in early T cell development, indicating that maintaining DNA methylation states through cell divisions is important for T cell development (104). In addition, the deletion of DNMT1 in activated $CD8^+$ T cells dampened the expansion of antigen-specific cells in response to an acute viral infection (105). In specialized $CD4^+$ T cell subtypes, the loss of the de novo methyltransferase DNMT3A caused the inappropriate expression of the Th1 signature cytokine IFN-γ in the alternative $CD4^+$ T cell subtypes such as Th2, Th17, and Treg cells (106). This indicates that DNMT3A is important for silencing the *Ifng* gene in alternative fates to Th1. In addition to its role in specialized $CD4^+$ T cells, DNMT3A is also important for the proper development of terminal effector and memory precursor $CD8^+$ T cells during an acute infection (107). Together, these studies indicate that DNMTs are important in many facets of T cell development. Their activity, similar to that of histone methyltransferases, is interpreted in a context-dependent manner in different stages of T cell differentiation.

α-Ketoglutarate, αKG Antagonistic Metabolites, and Demethylase Complexes

The metabolite α-ketoglutarate (αKG) is an intermediate of the TCA cycle that is also produced in the glutaminolysis pathway. αKG is a required cofactor for both histone and DNA dioxygenase (commonly referred to as demethylase) complexes (108, 109). The enzymatic steps initiated by αKG-dependent complexes also require oxygen and Fe(II), with vitamin C involved in promoting the reaction as well (55, 108–111). Interestingly, the TCA cycle intermediates succinate and fumarate as well as the by-product of the isocitrate dehydrogenase (IDH) enzymes, 2-hydroxyglutarate (2HG), can act as antagonists to αKG-dependent functions in some circumstances (55, 71, 112). Notably, mutations in the enzymes that produce succinate, fumarate, and 2HG are found in cancers (112–114). Collectively, current data suggest that the balance between αKG with 2HG, succinate, or fumarate in a cell defines the potential to regulate the functional activity of demethylase complexes.

Histone demethylases. The lysine-specific demethylase (LSD) family and the Jumonji C domain (JmjC) family form the two known classes of histone demethylase complexes. The metabolite flavin

adenine dinucleotide (FAD) is a cofactor for the LSD family, whereas the JmjC demethylase family requires metabolites and nutrients such as αKG, Fe(II), and vitamin C for their enzymatic reaction (109, 115). The FAD-dependent LSD1 complex utilizes an amine oxidation reaction to effectively demethylate di- and mono-methylation of H3K4 and H3K9 (115). Interestingly, both members of the LSD demethylase family, LSD1 and LSD2, demethylate di- and monomethylated histone marks, but not the trimethylation modification (116, 117). The JmjC demethylase complexes have been identified with enzymatic specificity for a number of histone methylation modifications, including methylation on H3K27, H3K4, H3K9, and H3K36 (116, 117). Together, the LSD and JmjC families encompass a wide-range of demethylases with diverse specificity, and they are sensitive to the nutrient and metabolite environment.

JMJD3 and UTX are two well-studied members of the JmjC family. The demethylase activity of JMJD3 and UTX is specific for the H3K27-methylation modification (118, 119). Interestingly, JMJD3, UTX, and UTY, the homolog to UTX that is found on the Y chromosome, are all capable of recruiting ATP-dependent chromatin-remodeling complexes to target genes, and this activity is functionally important in development (120, 121). UTY lacks demethylase enzymatic activity but still contributes to embryonic stem cell development (121, 122). Thus, JMJD3, UTX, and UTY all contribute to establishing developmentally appropriate gene expression programs, but the portion of their αKG-dependent demethylase activity and recruitment of ATP-dependent chromatin-remodeling activity are still being elucidated in different developmental settings.

In T cells, JMJD3 and UTX have been shown to play a role in the thymic development of T cells, utilizing the demethylase function to inhibit H3K27me3 at genes such as *Zbtb7b* (encodes Thpok), *S1pr1*, and *Klf2* (123). In $CD4^+$ Th1 cells, JMJD3 is important for expression of STAT4- and T-bet-dependent target genes (120, 124). T-bet-dependent recruitment of JMJD3 correlates with both reduced H3K27me3 and enhancement of general promoter accessibility (120, 125). Therefore, both the αKG-dependent demethylase function and the ability to recruit ATP-dependent remodeling activity are important for the activity of JMJD3 (and potentially UTX) in $CD4^+$ Th1 cells.

TET enzymes. It was long thought that DNA methylation represented a stable modification that could only be removed during cell divisions. However, with the discovery of the DNA dioxygenase activity of the ten-eleven translocation (TET) protein family, it is now appreciated that the DNA methylation modification can be dynamically regulated (108, 126). The enzymatic activity of TET proteins, similar to that of the JmjC demethylase family, requires αKG, Fe(II), and oxygen, and the activity is also enhanced by vitamin C (108, 110). TET proteins oxidize the methylation modification found on the 5-carbon position of cytosine, converting it into a hydroxymethylation modification (5hmC). 5hmC can itself provide information for the recruitment of proteins to DNA; alternatively, the TET enzymes can further oxidize 5hmC into 5-formylcytosine or 5-carboxylcytosine (126, 127). The mechanisms for final conversion to a cytosine without modifications are still being elucidated, but they might include DNA-repair mechanisms (128, 129). Interestingly, 5hmC occurs in the gene bodies of actively expressed genes in many different cell types, including thymocytes (130–132). The localization of 5hmC in gene bodies correlates with the enrichment of RNA polymerase II and H3K36me3, a histone modification associated with elongation, in actively transcribed genes (132). However, as T cells differentiate into more defined subtypes, the correlation with 5hmC enrichment in gene bodies is less prominent, indicating there is some specificity to the context of this modification within developmental stages.

There are three members in the TET family (TET1, TET2, and TET3) that have been shown to play important roles in diverse developmental processes (126). TET2 has been shown to be important for the expression of cytokines in $CD4^+$ and $CD8^+$ T cells (133, 134). However, there

is some discrepancy among findings as to whether it effectively promotes or inhibits cytokine gene expression; it is suggested to be inhibitory in $CD8^+$ T cells but activating in $CD4^+$ T cells. Notably, the experiments examining $CD8^+$ T cells were performed in the presence of TCR stimulation without additional cytokine polarization, whereas the $CD4^+$ T cell experiments were performed in cytokine-polarizing conditions (133, 134). Therefore, aspects of the differentiation conditions or cell type might affect how the TET2-sensitive functions are interpreted. Thus, a common theme for interpreting the effects of epigenetic-modifying complexes is that the context is critical. In addition to the roles for TET2 in T cells, TET2 and TET3 have been shown to be important for the development of invariant natural killer T cells, early development in B cells, and Treg cell development (135–138). Therefore, the TET proteins play important roles in a diverse set of immune cells and the functional consequence of their activity is context-dependent.

Acetyl-CoA and Histone Acetyltransferases

The donor for histone acetyltransferases is acetyl-CoA. Acetyl-CoA is a short-chain fatty acid that is produced from many different metabolic pathways and diverse substrates. For example, the processing of glucose through the glycolysis pathway can lead to acetyl-CoA accumulation, and threonine metabolism can be diverted into acetyl-CoA (72, 73). Reflecting this, several enzymes are involved with the production of acetyl-CoA (5, 55). Acyl-CoA synthetase short-chain (ACSS) family members 1 and 2 can convert acetate to acetyl-CoA. In contrast, the pyruvate dehydrogenase complex catalyzes the conversion of pyruvate into acetyl Co-A, whereas ATP-citrate lyase converts citrate into acetyl-CoA. This illustrates the diverse network of enzymes and substrates that are utilized to produce acetyl-CoA (55). Notably, the expression of the enzymes that produce acetyl-CoA is dynamically regulated in cellular differentiation, and this might play a role in controlling the pool of acetyl-CoA generated from available substrates.

Histone acetyltransferases. Many proteins possess histone acetyltransferase activity. Histone acetylation typically correlates with active gene expression. H3K9Ac and H3K27Ac are two of the most commonly studied acetylation modifications. H3K9Ac typically localizes to promoter regions of genes, whereas H3K27Ac associates with both enhancer and promoter regions (90, 139, 140). In specialized $CD4^+$ helper T cell subtypes, high levels of p300 and H3K27Ac have been used to define the lineage-specific super-enhancers (62, 63). As discussed above, the importance of these enhancer regions is signified by the enrichment of disease-associated sequence variation in these elements (62). In addition, the histone acetyltransferase GCN5 has been shown to play a role in specialized $CD4^+$ T cell programs (141). Below we discuss in more detail the studies implicating how changes in the activity of metabolic pathways that produce acetyl-CoA affect histone acetylation and gene-programming events.

Butyrate, NAD^+, and Histone Deacetylases

Butyrate and β-hydroxybutyrate are metabolites that can inhibit histone deacetylase complex (HDAC) activity. Butyrate is a short-chain fatty acid that is produced by commensal bacteria that ferment fiber in the colon (55). β-Hydroxybutyrate is a ketone produced in the liver during calorie restriction or in the dysregulated state of diabetes (142, 143). Both butyrate and β-hydroxybutyrate can inhibit class I and in some cases class II HDACs (144, 145). The most-studied role for butyrate in the immune system relates to regulatory T cells. Butyrate produced by the microbiota has been shown to promote the development of Treg cells and dampen inflammatory states in the gut (146, 147). This appears to be related in part to butyrate inhibiting HDAC activity at the *Foxp3* locus to promote histone acetylation in the region and *Foxp3* expression (146). In addition to their role

inhibiting HDAC activity, butyrate and β-hydroxybutyrate, like acetyl-CoA, can be found as a modification directly on histones (148–150). Similar to acetylation of histones, this modification neutralizes the positive charge of lysine and is associated with permissive chromatin, but its overall significance is still being explored.

Nicotinamide adenine dinucleotide (NAD^+) serves as an electron acceptor and is involved in redox reactions. NAD^+ is regulated by nutrient states such as fasting and is also produced in the salvage pathway. Related to epigenetics, NAD^+ is needed for the activity of the sirtuin protein family (151). Sirtuins are members of the class III histone deacetylase family. Interestingly, NAD^+ and sirtuin activity can be regulated by the circadian clock, which provides potential connections between nutrient fluctuations, the circadian clock, and epigenetics (143, 151, 152). Notably, sirtuins have been shown to play roles in cellular differentiation, including roles in muscle cell differentiation and specialization of $CD4^+$ T cell subtypes, where SIRT1 expression is regulated by cytokine polarization conditions (153–155). This highlights once again the common theme that protein families with metabolite-sensitive epigenetic-modifying activity, including sirtuins, are linked to diverse differentiation programs.

ATP and ATP-Dependent Chromatin-Remodeling Complexes

ATP is an important metabolite, and it is a basic energy unit. It is involved in the production of many of the metabolites described above that are needed for epigenetic processes. In addition, ATP also plays direct roles in epigenetic complexes. For example, there are several ATP-dependent chromatin-remodeling complexes, including the SWI/SNF family (156). The required role for ATP might imply that the ATP-dependent chromatin-remodeling complexes will be sensitive to metabolically regulated changes in ATP. However, it is thought that these complexes will not be sensitive to metabolic fluctuations in ATP, because cellular ATP levels are saturating relative to the amount that is required for the ATP-dependent remodeling complexes (152). Notably, the protein kinase AMPK has been shown to be a sensor of the ratio of AMP to ATP in the cell (9, 157). Thus, although ATP-dependent chromatin-remodeling complexes are not likely to be sensitive to ATP, gene expression states are still mechanistically regulated by the AMPK cascade, providing a mechanism to sense changes in ATP to promote cellular differentiation programs.

DEFINING MECHANISMS THAT CONNECT METABOLITES TO EPIGENETIC EVENTS AND DIFFERENTIATION GENE PROGRAMS

The information that metabolites are donors, substrates, and cofactors for epigenetic-modifying complexes and epigenetic modifications raises the possibility that changes in metabolism might contribute to the mechanistic regulation of gene programming events. Research in embryonic stem cells has led the way for defining connections between metabolism and epigenetic events (71–73, 110, 143, 158). In this section, we first highlight groundbreaking embryonic stem cell studies that defined connections between fluctuations in metabolites and epigenetics. We then discuss research on T cell differentiation that indicates many similarities in mechanisms between the diverse differentiation systems of embryonic stem cells and T cells. However, the discussion also highlights that the interpretation of these metabolic events can impact gene programming decisions in a cell type–specific manner.

Glutamine and αKG Are Important in Cellular Differentiation

Although demethylase complexes require αKG as a cofactor, until recently, it was not known whether the activity of αKG-dependent complexes was sensitive to fluctuations in nutrient states

and metabolism. Recent research has addressed this question and defined mechanisms whereby glutaminolysis affects αKG levels to influence the pluripotency potential of embryonic stem cells (71). Mechanistically, accumulation of αKG was linked to a decrease in bulk histone methylation related with modifications traditionally associated with repression, such as H3K27me3. In contrast, the permissive H3K4me3 modification was not changed. Furthermore, experiments in embryonic stem cells indicated that the regulatory events associated with the accumulation of αKG are mechanistically linked to the activities of JMJD3 and TET proteins (71). Of note, certain histone modifications and enzymatic activities, such as H3K27me3 modifications and JMJD3 activity, appeared to be more sensitive to fluctuations in metabolites. The mechanisms responsible for this are unknown. Furthermore, it is also worth noting that additional studies in stem cells indicate that the interpretation of αKG-sensitive activities into different programming events can vary depending upon the stage of cellular development (159, 160).

The regulated expression of the metabolic enzymes that control αKG levels have also been connected to embryonic stem cell differentiation gene programs. In embryonic stem cells, the core stem cell transcription factors regulate the transcriptional expression of *Psat1*, the gene that encodes the phosphoserine aminotransferase I (PSAT1) enzyme (160). This is important because PSAT1 regulates αKG accumulation, providing a connection between the activity of the core stem cell transcription factors and regulation of the metabolite αKG. Experimentally, the loss of PSAT1 decreased αKG levels, which promoted DNA hypermethylation and enhanced levels of H3K9me3 and H3K36me3. The data also indicated that αKG levels were important for defining the embryonic stem cell differentiation phenotype (160). Importantly, this study defined a mechanistic pathway connecting the expression of a metabolic enzyme to changes in its metabolite product and subsequent epigenetic regulation of a differentiation gene program. As noted above, dysregulation of several metabolic enzymes can initiate pathogenic differentiation programs (114, 161). Therefore, it will be important to explore the connections that encompass the feed-forward mechanisms that regulate metabolic enzyme expression, the balances of metabolite competitors, and epigenetic events in the context of immune cell differentiation and dysregulated disease states.

Another example connecting nutrient and metabolite regulation to epigenetics is found within tumor microenvironments. Glutamine levels are diminished in tumors relative to normal cells (162, 163). A study in melanomas found that the core of a tumor had lower concentrations of both glutamine and αKG relative to the periphery (162). Consistent with the role for glutamine as one of the major sources for intracellular αKG, the deficiency in αKG levels could be rescued by injecting glutamine into the tumor. The data from this study also suggested a connection between the levels of glutamine and αKG and the state of histone methylation. Specifically, histone methylation, including H3K27me3, H3K9me3, and H3K4me3, was increased in the core of the tumor relative to the periphery (162). Notably, diminished glutamine enhanced H3K27me3 modifications at promoters of genes associated with differentiated states, and this contributed to their repression. Taken together with the studies in embryonic stem cells, these data indicate that the glutamine-sensitive regulation of αKG affects downstream epigenetic events in both normal and tumor cell settings.

In T cells, glutamine- and αKG-sensitive events also affect differentiation gene programs. In particular, glutamine- and αKG-sensitive events regulate aspects of the IL-2-sensitive effector program in $CD4^+$ and $CD8^+$ T cells developing in type I conditions (23). Mechanistically, αKG-sensitive events in part function through regulating H3K27me3 and DNA methylation states. This indicates that, similar to the case of embryonic stem cells, the role for αKG as a required cofactor for DNA and histone demethylases is important for its function in T cells. Importantly, a study in $CD4^+$ Th1 cells also identified a novel mechanism by which αKG-sensitive events influenced CTCF association with a subset of sites (23). At least some of this was mechanistically related

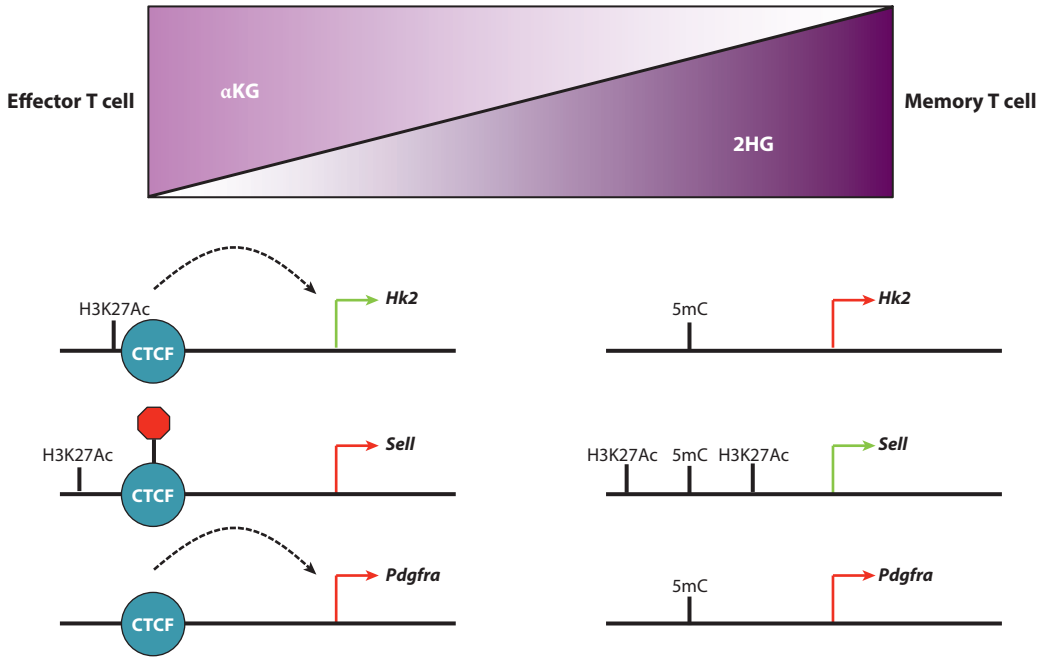


Figure 3

Balance between αKG and 2HG regulates differentiation decisions. The balance between αKG (*light purple*) and 2HG (*dark purple*) influences the activity of TET proteins to affect DNA methylation. One way that this is interpreted is through regulating the association of CTCF with a subset of methylation-sensitive CTCF-binding elements. The αKG/2HG-sensitive regulation of CTCF can be interpreted in a context-dependent manner related to the enhancer landscape, and also potentially depending upon whether CTCF is performing insulator or chromatin-looping activities at an individual gene. Abbreviations: αKG, α-ketoglutarate; TET, ten-eleven translocation; 2HG, 2-hydroxyglutarate.

to αKG-sensitive changes in DNA methylation states influencing CTCF association with these regions (**Figure 3**). Intriguingly, the IL-2- and αKG-sensitive CTCF association was interpreted in a context-dependent manner in part related to the H3K27Ac enhancer landscape in the cell (23). This study provided new mechanistic insight into how changes in the accumulation of metabolites might be targeted to the genome in a similar manner in diverse cellular settings, but this still allows for the overall epigenetic environment to provide the context for how this information will be interpreted in a cell type–specific manner.

Balance Between 2HG and αKG Is Important for Differentiation

2HG is structurally similar to αKG, which allows 2HG to serve as a competitive inhibitor of αKG in demethylase reactions (164) (**Figure 3**). 2HG can be naturally found in two enantiomer forms (R or S). In some cancers, such as subsets of acute myeloid leukemia (AML) and gliomas, mutant IDH proteins overproduce 2HG in the R conformation (113, 161, 165–167). This has been referred to as an oncometabolite, in part because of its role in creating a dysregulated epigenetic environment that contributes to the cancer phenotype (161, 167). Studies have found that cells with IDH mutations display altered histone and DNA methylation patterns, and it is thought that this relates to 2HG antagonizing αKG-dependent JmjC and TET activities (165, 167–169). An analysis of patient primary glioma samples and glioma cell lines found a correlation between IDH

mutation status and a DNA hypermethylation phenotype (170). Intriguingly, CTCF association was disrupted with a select group of CTCF sites that were sensitive to DNA methylation in the IDH mutant cells (170). Importantly, this altered the organization of the genome surrounding the oncogene *PDGFRA*, allowing for an active enhancer to be aberrantly utilized to activate *PDGFRA* transcription. In the $CD4^+$ T cell study just discussed, αKG promoted CTCF association with the *Pdgfra* locus (23). Taken together, the data from the glioma and T cell studies suggest that the competitive balance between the metabolites 2HG and αKG influences CTCF association and genome organization in diverse organisms and cell types.

Further solidifying the role for the balance between 2HG and αKG in defining cellular differentiation decisions, a study found that 2HG is important for $CD8^+$ T cell differentiation (75). In contrast to αKG, 2HG inhibited the effector program in activated $CD8^+$ T cells and promoted the differentiation of the memory program. Notably, 2HG was found to promote an increase in both histone and DNA methylation, including an increase in DNA methylation associated with the region surrounding the *Sell* locus (encodes CD62L) (75). Consistent with the interpretation that the balance between 2HG and αKG is important for defining effector versus memory T cell differentiation decisions, in contrast to the 2HG study just discussed, αKG has been shown to promote a decrease in methylation at an IL-2- and αKG-sensitive CTCF peak in the *Sell* promoter in $CD4^+$ Th1 cells (23). Interestingly, the regulation of CTCF association by DNA methylation may provide insight into why some genes, such as *Sell*, are expressed even when there is methylation associated with the promoter region. That is, 2HG- and αKG-sensitive methylation patterns that affect CTCF might influence either genome organization or insulator barrier functions, which can then be interpreted according to the epigenetic landscape that is brought into proximity of gene regulatory elements (**Figure 3**). Thus, changes in methylation that affect CTCF association would not inherently promote or inhibit gene expression but would instead be interpreted in a context-dependent manner. This is an intriguing hypothesis that will need to be more extensively examined in additional settings and immune cell types.

One-Carbon Metabolism, SAM, and Cellular Differentiation

Several elegant studies in embryonic stem cells have started to define how the processing of amino acids through the one-carbon metabolism pathway causes fluctuations in the universal methyl donor SAM, and how these events influence the regulation of epigenetic processes. For example, threonine metabolism has been shown to be important for regulating the levels of SAM and acetyl-CoA in mouse pluripotent embryonic stem cells (72). Interestingly, SAM levels correlated with global H3K4me3, whereas other histone methylation modification levels were unchanged. H3K4me3 is also sensitive to SAM levels in human pluripotent stem cells, and this is important for maintaining pluripotency (86). Notably, the mechanistic steps and amino acid input into the one-carbon metabolism pathway that generates SAM appear to differ between species. This is because murine embryonic stem cells express an intact threonine dehydrogenase gene, whereas this enzyme is encoded as a pseudogene in humans (72, 86, 171). Therefore, instead of threonine playing a role in SAM levels, methionine is important for SAM levels in human pluripotent stem cells (86). These results illustrate that although there is conservation in the metabolites involved in the epigenetic events, there may be some divergence in the nutrients and pathways that lead to the production of the metabolites. This is an important concept to keep in mind when translating results between species, or between cell types, with the goal of defining which pathways might be advantageous therapeutic targets in human disease.

Recent studies in T cells have indicated that the one-carbon metabolism pathway is also important for T cell differentiation programs. T cells take up serine from the extracellular environment

upon activation (172). This activity is important for appropriate T cell function because dietary restriction of serine and glycine was associated with diminished expansion of effector T cells and reduced IFN-γ-producing cells. It appears that the effect of serine/glycine restriction on T cell differentiation has an epigenetic component, because T cell functions were dampened during a rechallenge response even when concentrations of serine and glycine in the diet were restored to normal levels (172). Although epigenetic events were not monitored in this study, based on research in embryonic stem cells, it might be speculated that in T cells there is also a mechanistic role for one-carbon metabolism in the regulation of SAM levels, which then affects the methylation state of histones and DNA. It is now important to explore this concept in T cell differentiation, guided by mechanistic research in embryonic stem cells, and to determine how fluctuations in metabolites in the one-carbon metabolism pathway regulate epigenetic events that influence T cell differentiation programs.

Glycolysis and Acetyl-CoA in Differentiation Decisions

Studies in embryonic stem cells have identified a role for acetyl-CoA in maintaining the program associated with pluripotency. In particular, during early differentiation of human embryonic stem cells, acetate, which is converted into acetyl-CoA, maintained the pluripotency program even when factors that normally promote differentiation were introduced (73). Mechanistically, it was shown that both H3K27Ac and H3K9Ac were enhanced by acetate and that these acetylation modifications were generally diminished during differentiation. Furthermore, glycolysis was the natural major pathway used to generate acetyl-CoA in this setting to provide a link between glycolysis, acetyl-CoA, and histone acetylation in the regulation of embryonic stem cell differentiation (73).

A recent study in $CD4^+$ Th1 cells has demonstrated that glycolysis is important for the acetyl-CoA levels during T cell differentiation as well (74). In $CD4^+$ Th1 cells, the enzyme LDHA converts pyruvate to lactate to divert the processing of glucose away from the oxidative phosphorylation cycle. This process is thought to effectively promote citrate accumulation in the cytosol to increase the abundance of acetyl-CoA. Similar to findings of studies in embryonic stem cells, ATP-citrate lyase was found to be important for regulating the conversion of citrate to acetyl-CoA in T cells (74, 173). Mechanistically, LDHA activity was important for the production of acetyl-CoA, which then enhanced H3K9Ac and H3K27Ac levels and *Ifng* expression in Th1 cells (74). One critical question that is currently unclear from both the embryonic stem cell and T cell studies is how bulk changes in histone acetylation (or other histone modifications) are specifically targeted to enhance defined differentiation gene programs. Insight into this topic will expand our understanding of the mechanisms that link fluctuations in metabolites to specific differentiation programs. This knowledge should also provide insight into logically designing epigenetic inhibitor strategies to direct differentiation decisions in therapeutic settings.

T CELL METABOLISM, EPIGENETICS, AND TUMOR MICROENVIRONMENT

Integrating information about the metabolic and epigenetic states of tumor-infiltrating lymphocytes (TILs) is a growing emphasis of tumor immunology. One goal for this avenue of research is to better understand how the tumor microenvironment influences TIL metabolism and, in turn, how this might affect the programming of the TILs. Knowledge about these basic processes will likely have significant implications for efforts to improve tumor immunotherapy approaches that are directed at reinvigorating exhausted T cells. Several immunotherapy approaches attempt to rescue functionally exhausted T cells by effectively inhibiting checkpoints, such as the PD-1 pathway

(174, 175). Treatment with PD-1 or PD-L1 antibodies to disengage the checkpoint pathways has been effective in some tumor settings to reactivate the TILs and control tumor burden. However, this treatment is not effective for all patients and is not effective for many tumor types (174, 175). Therefore, to aid in the goal of reversing exhausted T cells to again become functionally competent for clearing cancerous or chronic virally infected cells in a wider population of patients, one focus on the basic research side has been to characterize the mechanisms that contribute to the chronically exhausted state and determine whether new approaches might reverse them.

One area of active research is defining the nutrient environment that TILs are exposed to inside of the tumor microenvironment to understand how this affects the metabolic state and functional activity of TILs. It has long been recognized that the core of solid tumors is hypoxic and nutrient depleted. In particular, studies have shown that the tumor microenvironment is deficient in glucose and glutamine, among other nutrients and amino acids (50, 51, 162). One hypothesis that has emerged from these findings is that part of the dysfunctional state of TILs might result from a lack of glucose availability in the tumor environment. As discussed above, effector T cells utilize the glycolysis pathway to process glucose, and this metabolic activity is important for the development of effector T cells, in part through regulating acetyl-CoA levels and histone acetylation (74). Interestingly, new studies have shown that the depleted glucose levels found in the tumor microenvironment effectively dampen glycolysis in TILs and the decreased glycolysis activity contributes to the hyporesponsive or exhausted state of TILs (50, 51). Importantly, artificially enhancing glycolysis in TILs in the glucose-restricted conditions associated with the tumor microenvironment partially rescues effector potential of exhausted TILs. One mechanism that contributes to the dampened effector phenotype of TILs relates to the reduction in the glycolysis pathway intermediate phosphoenolpyruvate (PEP) (51). PEP inhibits the activity of SERCA, which is an important regulator of calcium stores. Changes in calcium flux regulate activity of NFAT, which is a transcription factor that influences the expression of genes that encode key components of the effector T cell response (176). Importantly, artificially enhancing glycolysis in TILs rescued some of the calcium-dependent enhancement of NFAT activity and contributed to effector T cell functions in tumor models (51). Taken together, studies examining glycolysis in TILs have indicated that TIL metabolism affects gene programming events and the functional activity of the cells, but the role for epigenetic events still needs to be elucidated.

It is interesting to note that glutamine is also depleted in the tumor microenvironment, and although not as well studied in relationship to TILs, it is likely that glutamine availability also affects the programming of TILs. As discussed above, studies examining tumor cells have shown that mechanistically, glutamine levels in the tumor microenvironment control αKG-sensitive pathways that are involved in regulating histone methylation states in tumor cells (162). In this same light, it will be important to examine the role for glutamine restriction in the tumor microenvironment on TIL epigenetic programs. As discussed, glutamine- and αKG-sensitive events influence effector T cell gene programming (23, 52, 157). Therefore, it is likely that the altered glutamine in the tumor microenvironment also affects the metabolic control of epigenetic events in TILs. In this case, it is possible that restoring glutamine will enhance the effector program of the TILs by restoring αKG-sensitive activities. However, studies have indicated that glutamine deprivation during the early stages of T cell activation stably influenced gene programs even when glutamine was restored to the environment (23, 52). In this case, a complete rescue of TIL programming might not be possible. In addition, studies comparing chromatin accessibility profiles of TILs indicated that checkpoint inhibitor treatment does not fully reverse the epigenetic states of exhausted TILs to a functionally competent profile (177). In part this might be explained by the early development of chromatin accessibility changes that define exhausted TILs that rely on antigen-dependent and early activation events that would not be as sensitive to the current state of the tumor environment

(65, 178). This suggests that aspects of T cell programming will be epigenetically stable, based on events that occur at the time of initial antigen engagement. Nevertheless, the roles for glucose and glutamine in regulating gene programming decisions in both tumor cells and diverse immune cell types highlight the importance of understanding the epigenetic and gene programming potential for nutrients and metabolites in both tumor cells and tumor-infiltrating immune cells and how the timing of exposure affects these events. This information will be required to define the possible outcomes for clinical interventions that modulate metabolism. This concept should also raise our appreciation for the importance of defining the commonalities and differences in the mechanisms by which metabolites influence gene-programming events in diverse cell types. This knowledge will extend our ability to logically predict the impact of systemic metabolic interventions and whether they will have utility in the context of the diverse cell types that are present in treatment settings.

FUTURE DIRECTIONS

Research efforts have now made it clear that metabolites with the potential to affect epigenetic events play a role in regulating differentiation gene programs. It will now be important to intensify research investigations to comprehensively define how metabolism influences epigenetic programming in diverse cellular differentiation systems. The mechanistic series of events that need to be defined in diverse cellular settings include how the nutrient environment causes fluctuations in metabolites, how fluctuations in metabolites affect epigenetic-modifying complexes and epigenetic events, and how metabolite-sensitive epigenetic events are translated into specific cellular differentiation gene programs (see the sidebar titled Intertwined Nature of Metabolic Pathways). One area of particular interest for all fields is to determine the mechanisms that target metabolite-sensitive epigenetic events to specific areas of the genome (**Figure 2**). At least part of this will likely involve targeting events through the site-specific recruitment of lineage-specifying transcription factors and their partner epigenetic-modifying complexes. However, at present, it is still unclear how this activity might be functionally integrated in response to changes in metabolites. As highlighted in this review, the embryonic stem cell field has been at the forefront of defining the mechanisms that integrate nutrient signals into fluctuations in metabolites, and how this affects epigenetic and gene programming events. Research in T cells has now made it clear that there is conservation in the principles of these mechanistic events. In designing strategies for

INTERTWINED NATURE OF METABOLIC PATHWAYS

One area of caution when interpreting the connections between metabolic pathways and epigenetic events is that metabolic pathways are interconnected and coordinately regulated. There are also complex feed-forward mechanisms that intertwine metabolic and differentiation gene programs at multiple steps (**Figure 1**). This effectively means that when one metabolic pathway changes in response to an external stimulus, it is highly likely that many other metabolic pathways will change as well. Therefore, fluctuations in the levels of the metabolites involved in diverse epigenetic-modifying complexes and epigenetic modifications will occur together. This complicates the interpretation of the experimental data attempting to establish a definitive order for the series of events that initiate with nutrient intake and proceed through metabolic enzymes, metabolites, epigenetic-modifying complexes, epigenetic events, and finally result in gene programming consequences. Defining the mechanisms that target metabolite-sensitive changes in epigenetic events to specific regions of the genome will help with this topic. However, with the rapid expansion of research in this area, it will be important to be cautious and unbiased when viewing experimental data to prevent overinterpreting the conclusions that can be made from each study.

determining the next series of mechanistic events in this fast-evolving field, immunologists will be aided by the utility of the numerous in vitro primary cell differentiation systems that have been developed for immune cells. These in vitro differentiation systems can be methodically dissected to define key components in metabolite-sensitive epigenetic processes and utilized to uncover the intricate series of events that focus changes in metabolite levels to specific regions of the genome. The information gained with this type of approach, which has been successfully utilized in embryonic stem cells, can then be quickly integrated into testable predications to determine how nutrient and metabolite pathways affect immune cell development and function in normal and pathogenic settings in vivo. Ultimately, coupling these two approaches together, specifically, integrating mechanistic data with information from diverse infectious and disease models in vivo, will expand our capability to logically predict cellular responses to develop therapeutic interventions directed at metabolic pathways.

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