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# A chromatin perspective on metabolic and genotoxic impacts on hematopoietic stem and progenitor cells

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## Abstract

The fate determination in self-renewal and differentiation of hematopoietic stem and progenitor cells (HSCs and HPCs) is ultimately controlled by gene expression, which is profoundly influenced by the global and local chromatin state. Cellular metabolism directly influences the chromatin state through the dynamic regulation of the enzymatic activities that modify DNA and histones, but also generates genotoxic metabolites that can damage DNA and thus pose threat to the genome integrity. On the other hand, mechanisms modulating the chromatin state impact metabolism by regulating the expression and activities of key metabolic enzymes. Moreover, through regulating either DNA damage response directly or expression of genes involved in this process, chromatin modulators play active and crucial roles in guarding the genome integrity, breaching of which result in defective HSPC function. Therefore, HSPC function is regulated by the dynamic and two-way interactions between metabolism and chromatin. Here, we review recent advances that provide a chromatin perspective on the major impacts the metabolic and genotoxic factors can have on HSPC function and fate determination.

### Keywords

Chromatin; Histone modifications; Demethylation; Hematopoietic stem cells; Metabolism; Genome integrity; DNA damage response

## Introduction

The mission of somatic stem cells is to maintain tissue homeostasis throughout the lifespan of an organism. To accomplish this mission, stem cells have two defining properties, self-renewal to replenish themselves and differentiation to give arise to cells of all lineages in the specific tissue. Hematopoietic stem cells (HSCs) provide a continuous supply of blood cells



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by strictly regulating self-renewal and balanced lineage differentiation through generating hematopoietic progenitor cells (HPCs) and further downstream cells. Blood disease can arise as a result of dysregulated activities of HSCs and/or HPCs (HSPCs), as seen in bone marrow failure or hematologic malignancy [1]. Fate determination is thus probably the most pertinent question in stem cell biology. Chromatin is at the forefront of stem cell biology for two main reasons: (1) chromatin is the physiological substrate of transcription reaction and profoundly regulates gene expression including transcription and RNA splicing, and it is the gene expression profile of a cell that ultimately determines the cell to be still a stem cell or a daughter cell of a particular lineage. (2) Chromatin constitutes the physical carrier of genetic information, whose integrity is influenced by chromatin state and directly linked to how stem cells decide to choose the path forward.

Chromatin in eukaryotic cells consists of nucleosomes with genomic DNA wrapping around histone proteins, and can be packaged at different levels. Two copies each of H2A, H2B, H3, and H4 form the histone octamer in the core nucleosome particle, and histone H1 sits at the base of the nucleosome and binds to the linker DNA. In general, chromatin represents a barrier to all DNA-based processes including transcription, replication, and DNA repair. However, chromatin provides an important regulatory platform for these processes by integrating signals from many aspects of cellular state, through epigenetic mechanisms to regulate how compact and accessible the local chromatin is [2]. These epigenetic mechanisms include histone and DNA modifications, chromatin remodeling, and high-order chromatin structure formation. Histones are subject to a wide range of post-translational modifications including acetylation, methylation, phosphorylation, ubiquitylation, succinylation, formylation, hydroxylation, O-GlcNAcylation, and ADP ribosylation, among many others [2, 3]. DNA is also subject to chemical modifications, mainly cytosine methylation and subsequent oxidation including 5-hydroxymethylcytosine (5hmC) [4]. These modifications are dynamically controlled by a balanced act of “writers” (enzymes that add these modifications) and “erasers” (enzymes that remove these modifications) [3]. The functions of these modifications can be through a direct physical impact on the compact level of chromatin, through an impact on charge, or, more commonly, mediated by “readers” that selectively bind to the modified chromatin [2, 3]. Aberrant chromatin modifications and remodeling are frequently associated with human diseases including cancer, and drugs targeting chromatin regulators are being developed to treat cancers including blood cancer [2, 3, 5–7].

Adult HSCs reside in a hypoxic bone marrow environment with limited nutrients. To adapt to this condition, HSCs remain in a quiescent state and mainly utilize glycolysis for energy production, but switch to mitochondrial oxidative phosphorylation upon activation, such as acute blood loss or viral infection [8–10]. Mutations of metabolic enzymes and dysregulation of metabolic pathways are commonly associated with human disease [11], including disorders and cancer in the blood system [12–14]. Like all cellular activities, chromatin-based processes depend on and are regulated by metabolism. Metabolism provides the basic building blocks of nucleic acids and histones, as well as energy that is required for the chromatin-based processes. Moreover, many small molecules used or produced in metabolism are essential co-substrates or co-factors in reactions that add or remove modifications of histones and DNA. In addition, certain metabolites can influence

chromatin activity via covalently or allosterically modulating the activities of the proteins that modify histones and DNA [15–17]. Metabolism is also reciprocally regulated by these chromatin-based processes, mostly through control of the expression level and activities of key enzymes involved in metabolic reactions (Fig. 1A). Certain core metabolic enzymes can translocate to nucleus and modulate gene expression either by modifying chromatin or chromatin modulators or by forming complexes with transcription and chromatin modulators [17], but this is not strict “metabolic regulation” of chromatin and thus not reviewed here.

All cells constantly face genotoxic insults induced by both environmental agents and cellular processes, which can cause various types of DNA damage and catastrophic consequences to the function of cells and organism. Many metabolic pathways produce these genotoxic molecules, including reactive oxygen species (ROS) and aldehydes. Due to the special role of stem cells in tissue homeostasis, maintaining genetic stability is particularly crucial for them to ensure the propagation of correct genetic information throughout the daughter cells in the tissue [18]. Following the detection of DNA lesions, the intricate network of DNA damage response (DDR) orchestrates a signal transduction pathway that activates multiple layers of decisions, including cell cycle arrest, apoptosis, senescence, and DNA repair [19]. DDR profoundly influences both normal hematopoiesis and hematologic malignancies [20], as demonstrated by human blood disease such as Fanconi anemia that results from mutations in DNA repair genes [21], and HSC defects in mice deficient of active components in either recognition (such as ATM [22]) or repair [23, 24] of DNA lesions in the DDR pathways. As DDR occurs in the context of chromatin, it is governed by epigenetic mechanisms regulating chromatin structure and accessibility [25, 26].

Many excellent reviews have covered the biochemical relationships between metabolism and chromatin [15–17, 27–29], and between DNA damage and chromatin [25, 26, 30]. Others have covered how HSCs are regulated by epigenetics [31, 32], metabolism [10, 33–35], and DNA damage [18, 20, 36]. It is possible that most metabolic impacts on stem cells fate determination ultimately cause changes in global or local chromatin states and transcription, but many of these changes are probably indirect. Here, we focus on our current understanding of how metabolism and chromatin modifications mutually and directly regulate each other and impact genome integrity, to fulfill their roles in normal as well as malignant hematopoietic cells (Fig. 1). Many biochemical studies and studies from other cellular systems are formulating conceptual frameworks that have not yet been tested or shown in HSPCs. Therefore, in addition to reviewing published works that provide evidence of these cellular processes in HSPCs, we also try to briefly include these findings here to prompt interest in testing them in hematopoiesis and thus lead to better understanding of normal and diseased hematopoiesis.

## Metabolic regulation of chromatin in HSPCs

### Regulation of chromatin enzyme activities by metabolic molecules

It is increasingly evident that metabolism is dynamically regulated during cellular differentiation, and alteration of the metabolites direct impacts chromatin modifications, thereby influencing fate determination of embryonic stem cells and somatic cells in different lineages, including muscle and immune cell lineages [15, 16, 37]. Histone modifications

have been reported to be affected by metabolites such as s-adenosylmethionine (SAM), alpha-ketoglutarate (α-KG), 2-hydroxyglutarate (2-HG), acetyl-coA, butyrate, and $NAD^+$ [15, 16, 37] (some examples are shown in Fig. 1B). Moreover, through direct impact on chromatin modifications, metabolites can influence spatial interactions of chromatin regions and higher order chromatin structure, thereby influencing gene expression for T-cell fate determination [38] and cancer [39]. Below, we will mainly review the direct metabolism–chromatin connections in HSPCs, focusing on the metabolic regulation of α-ketoglutarate (α-KG)-dependent dioxygenases, a paradigm for metabolic factors regulating chromatin and cell fate. A large variety of enzymes in the α-KG-dependent dioxygenases family directly or indirectly regulate chromatin, employ Fe(II), oxygen, and α-KG as cofactors or co-substrates, and are stimulated by ascorbate (vitamin C, VC) [40]. These small molecules are all intrinsically involved in metabolism, thus offering rich connections between chromatin and the cellular metabolic states (Fig. 2).

**Oxygen—**Oxygen has profound impact on cell fate determination through both indirect and direct effects on chromatin and gene expression. The indirect effects are mainly through the intricate network around the HIF (hypoxia-inducible factor) transcription factors, which induce target genes for hypoxia adaptation. Oxygen sensing is accomplished by the oxygen- and α-KG-dependent dioxygenase family members that prolyl hydroxylate HIFα, causing its ubiquitination and degradation under normoxic conditions [41]. The HIF network, partly by promoting the expression of glycolytic enzymes and key enzymes that suppress tricarboxylic acid cycle, plays an important role in keeping HSCs in a quiescent state mainly engaged in anaerobic glycolysis instead of the TCA cycle and mitochondrial respiration in the hypoxic bone marrow niche [10].

A more direct regulation of chromatin by oxygen is mediated by Jmjc domain-containing histone lysine demethylases (KDMs) and the 10–11 translocation (TET) proteins, which are α-KG-dependent dioxygenase family members that carry out demethylation of histones and DNA, respectively [40]. The intracellular oxygen level can impact activities of some of these enzymes that have oxygen affinity commensurate with physiological oxygen concentration [42]. The activity of KDM5A as a H3K4 demethylase is inhibited by low oxygen level, and is important for chromatin and transcriptional response to hypoxia in cells [43, 44]. KDM6A, a histone H3K27 demethylase, has the lowest oxygen affinity of all tested KDMs and is thus highly sensitive to oxygen levels. Hypoxia promotes the persistence of H3K27me3 by directly inactivating KDM6A and inhibits transcriptional reprogramming and muscle cell fate transition [45]. Profound hypoxia found in tumors can inhibit TETs and lead to DNA hyper-methylation, which contributes to tumor progression under hypoxic conditions [46]. It remains to be tested if the hypoxic condition in bone marrow contributes to the maintenance of HSCs in an undifferentiated state partly by suppressing the activity of these oxygen-sensitive chromatin enzymes and thus keeping differentiation genes from aberrant activation. On the other hand, it is possible that as HPCs move into microenvironments with higher oxygen levels, the increased activities of these enzymes may promote lineage differentiation by removal of suppressive marks from the lineage-specific genes. Obviously, different KDMs have different impact on the chromatin and transcription

states, and their activities need to be coordinated to respond to oxygen levels to govern stem cell fate.

**Vitamin C—**Recent studies have shown a crucial role of VC, an essential vitamin for human, in stem cells and leukemogenesis through directly influencing chromatin modifications. VC can enhance the activity of JmjC domain-containing KDMs, TET enzymes, and various other FeII- and α-ketoglutarate-dependent dioxygenases [47]. VC treatment of mouse embryonic stem (ES) cells induces a blastocyst-like state through promoting TET-mediated DNA demethylation [48], and maternal VC is important for the development of female fetal germ cells likely via regulating TET-dependent DNA demethylation [49]. Moreover, VC can enhance the reprograming efficiency of mouse embryonic fibroblasts to a pluripotent state [50], through the activation of KDMs for H3K36 and H3K9 [51, 52], and also by elevating DNA 5hmC levels in a TET-dependent manner [53].

VC is enriched in human and mouse HSCs and multipotent progenitor cells (MPPs) compared to the hematopoietic progenitors and downstream committed blood cells [54]. Such a high VC level limits the frequency and function of HSCs, and also inhibits leukemogenesis, in part through promoting the activities of TET2 in DNA demethylation [54]. Depletion or loss-of-function mutations of TET2 are frequently found in hematologic malignancies, and its deficiency leads to skewed differentiation and enhanced repopulating capacity of HSCs [55–59]. Treatment of *TET2*-mutated HSCs with VC blocks their aberrant self-renewal activity, mimicking the effect of TET2 restoration [56]. Incubation of VC with human leukemia cells impairs their colony formation, and administration of high dose of VC in vivo increases 5hmC level and inhibits disease progression in leukemia xenograft models [56], suggesting a therapeutic potential of VC in leukemia treatment.

VC is also required for HPC generation from human pluripotent stem cell differentiation in vitro and also during zebrafish development [60]. In the absence of VC, endothelial cells fail to undergo endothelial-to-hematopoietic transition to generate functional hemogenic endothelial cells, likely because of failure to open up chromatin regions important for hematopoiesis. Deletion of VC-dependent H3K27 demethylases, KDM6A (UTX) or KDM6B (JMJD3), or DNA demethylase TET1 impaired HPC generation in the presence of VC. These results demonstrate a critical role of VC in HPC generation through regulation of key histone and DNA demethylase activities [60].

**α-KG—**Intracellular α-KG is mainly generated by catalyzing the oxidative decarboxylation of isocitrate by isocitrate dehydrogenase 1/2 (IDH1/2), which are frequently mutated in human cancers including acute myeloid leukemia. IDH1/2 mutants convert α-KG to 2-hydroxyglutarate (2-HG), which competitively inhibit a wide range of α-KG-dependent dioxygenases including TETs and KDMs [61]. However, the effects of *IDH* mutations on cancer phenotypes may differ greatly for different stages and types of cancer, due to the different enzymes that are affected (Fig. 2). In contrast to the oncogenic role of 2-HG in *IDH* mutant cancers largely through inhibiting DNA and histone demethylation (Fig. 2A), 2-HG also has anti-leukemic activity by inhibiting FTO, an α-KG-dependent dioxygenase that functions as an RNA N6-methyladenosine (m6A) demethylase, and the subsequent

destabilization of *MYC* transcript [62] (Fig. 2B). *IDH* mutations also impact DNA repair either indirectly through influencing histone methylation state and expression of DDR gene [63] (Fig. 2A) or directly through inhibiting ALKBH proteins that are α-KG-dependent dioxygenases responsible for repairing DNA alkylation lesions [64] (Fig. 2C). The latter mechanism sensitizes *IDH*-mutated cancer cells to alkylating agents [64]. 2-HG also inhibits prolyl hydroxylases that control the stability of HIF-1α, but the role of this regulation in cancer and cell fate determination remains controversial [61].

**$NAD^+$—**Mammalian sirtuins are $NAD^+$-dependent deacetylases for both histones and numerous non-histone proteins, and regulate a large number of cellular pathways [65]. As different sirtuins have different subcellular localization, probably only the nuclear SIRTs (SIRT1, 6, and 7) can impact stem cell functions by directly regulating chromatin. SIRT1 is the best studied sirtuin in HSC function [66]. SIRT1-deficient HSPCs show aberrant expansion under stress [67], and myeloid bias in differentiation and aging-like phenotype [68]. SIRT1 binds to the *Hoxa9* gene, erases its promoter H4K16 acetylation, and helps to maintain the repressive H3K27me3 mark [67]. In line with such a direct role of SIRT1 in repressing HSC maintenance genes, another study shows that the deacetylation activity of SIRT1 counteracts the non-catalytic activity of MLL1 in recruiting histone acetyltransferase to HSC maintenance genes [69]. SIRT1 can also indirectly impact chromatin by directly deacetylating lysines of TET2 in its catalytic domain and enhancing TET2 activity, which is known to restrict HSPC transformation. By doing so, SIRT1 activation can inhibit the aberrant HSPC functions in Myelodysplastic syndrome, a type of hematologic malignancy [70]. SIRT1 and other sirtuins also regulate normal hematopoietic and leukemic stem cells through mechanisms with indirect or unclear involvement of chromatin [65, 71]. The NAD requirement indicates an intrinsic link of metabolism and sirtuins' activities. Indeed, muscle stem cells undergo a metabolic shift leading to reduction of $NAD^+$ and SIRT1 activity, with increase in H4K16 acetylation and altered gene expression during activation [72]. However, it remains to be demonstrated that NAD-related metabolism directly affects HSPC function via impacting the sirtuins' activities.

**The mitochondria–chromatin connections—**As a central player in metabolism, mitochondria are reviewed here on their connections with chromatin in HSPC function through their impacts on metabolites. Mitochondria are responsible for major energy production, and also play many important roles including the generation of redox molecules and metabolites, regulation of cell signaling, cell death, and biosynthetic metabolism [73]. Relative inactive in quiescent HSCs, mitochondria are rapidly activated to fulfill the metabolic demand upon differentiation [10]. Perturbations to increase [74] or disrupt [75] mitochondrial oxidative phosphorylation aberrantly activate HSCs or block their differentiation, respectively.

The mitochondrial activity can have major impact on cell fate determination through affecting chromatin. Short-term opening of the mitochondrial permeability transition pore enhances reprogramming of mouse embryonic fibroblasts, as the transient release of mitochondrial ROS upregulates a KDM to remove the H3K9 and H3K27 methylation marks from pluripotency gene promoters [76]. Loss of the mitochondrial complex III subunit

Rieske iron–sulfur protein (RISP) in mouse fetal HSCs impairs their differentiation with little effect on proliferation. RISP-deficient adult HSCs also have multiple defects, including impaired differentiation, loss of quiescence, and increased DNA damage and apoptosis [77]. RISP-deficient HPCs have altered global gene expression and histone modifications, including increase in methylation of H3K4, H3K9, and H3K79. As the authors also detected relative increases in L(S)-2-HG, succinate, and fumarate, all known inhibitors of DNA and histone demethylases, the altered gene expression, and histone modifications are attributed to direct impact of the metabolic changes following mitochondrial inactivation [77]. Mitochondrial inactivation by depletion of mitochondrial transcription factor A in erythroid progenitors causes defective erythropoiesis, partly due to histone hyperacetylation and dysregulated gene expression following increased levels of β-hydroxybutyrate, a histone deacetylase inhibitor [77]. These studies are thus consistent with the notion that mitochondrial activities and metabolites directly control chromatin activity to govern HSPC fate and function.

While the aforementioned studies show the importance of sufficient mitochondrial activity in maintaining HSPC function through epigenetic mechanisms, another study reveals the other side of the coin for a balanced mitochondrial activity in chromatin control for HSC stemness. In this study, autophagy is shown to be important in maintaining HSC quiescence by clearing metabolically active mitochondria [78]. Genetic disruption of autophagy in the hematopoietic system results in premature blood aging phenotypes associated with metabolic activation, mitochondria accumulation, and accelerated myeloid differentiation of HSCs. Autophagy-deficient HSCs exhibit altered (mainly reduced) DNA methylation and an increasing trend in α-KG level. Moreover, treatment of differentiating HSCs in vitro with metabolites (SAM and α-KG) or metabolism-affecting agent impacts HSC differentiation. This study thus suggests that the uncleared active mitochondria alter HSC fate determination by directly affecting chromatin modifications [78]. On a similar line, treatment of an $NAD^+$-boosting agent in mice potently enhances hematopoiesis and HSC functionality through promoting mitochondrial clearance and asymmetric HSC division, although it is unclear if chromatin is directly affected by the $NAD^+$-mediated metabolic changes [79]. Therefore, these studies underscore the tight control of mitochondrial activity in maintaining the normal function of HSCs.

## Metabolic impacts on genome integrity

While metabolic reactions provide energy and molecules for chromatin and chromatin-based processes, they also generate molecules that can seriously damage DNA. In addition, stress associated with DNA replication in proliferative cells also causes DNA lesions. Although the hypoxic condition and low metabolic and replicative state in quiescent HSCs help to reduce intracellular oxidative and genotoxic stress, quiescent HSCs also have limited choice (error-prone non-homologous end joining) [80] and capacity in DNA repair [81]. Consequently, quiescent HSCs actually accumulate DNA damage that can be repaired upon entry into cell cycle when cells upgrade their capacity and mode (high-fidelity homologous recombination) to repair DNA [81]. In response to physiologic stress (infection or chronic blood loss), however, activation out of the quiescent state expose HSCs to greater endogenous DNA

damage from the increased metabolic and replicative activities, and contributes to HSC attrition and aging phenotypes over time [82].

**ROS—**ROS are the best-known metabolites that can impair genome integrity. Intracellular ROS are generated mainly from metabolic reactions, such as oxidative phosphorylation in mitochondria and long-chain fatty acid oxidation in peroxisomes [83], and can also be induced by exposure to environmental toxins [80, 84]. HSC cells in the hypoxic niche mainly utilize glycolysis to produce energy, consequently making less ROS [10]. HIF proteins play a crucial role in controlling ROS production [10]. Keeping ROS levels low is important for maintaining genomic stability and self-renewal capacity of HSCs [85]. Metabolic switch to oxidative phosphorylation upon HSC activation results in increase in ROS, which further promote differentiation and maturation [10].

Low or moderate levels of ROS appear to play an important role in stem cell proliferation, differentiation, mobilization, and maintenance [10, 86]. Physiological levels of ROS are required to activate DNA repair pathway and maintain genomic stability in stem cells [87]. However, excessive amount of ROS in HSCs leads to oxidative DNA damage and, consequently, bone marrow failure. FOXO family transcription factors induce the production of many ROS-detoxifying proteins, and their deficiency results in high ROS levels and loss of self-renewal of HSCs [88]. In vitro or in vivo addition of anti-oxidative agent such as N-acetyl-l-cysteine (NAC) and catalase often greatly restores the HSC functions, suggesting a functional role of ROS in bone marrow dysfunction [22, 88, 89]. In contrast to the bulk of the highly proliferative cancer cells, the majority of leukemia stem cells (LSCs) have relatively low ROS levels associated with a quiescent cell cycle status and a low rate of energy metabolism, which may contribute to their resistance to the commonly used chemotherapy strategies that involve the induction of oxidative stress [90].

**Aldehydes—**Aldehydes, a common class of highly reactive metabolites, constitute a serious threat to genomic integrity that is counteracted by aldehyde-catabolizing enzymes and the Fanconi anemia (FA) DNA repair pathway [91–94]. FA is a rare disorder with congenital abnormalities including bone marrow failure and increased cancer risks. FA arises from mutations in a group of genes that regulate response to genotoxic stresses, especially DNA interstrand crosslinks. Mice double deficient in aldehyde catabolism and FA pathway show developmental defects, predisposition to acute leukemia, and increased bone marrow failure upon consumption of ethanol, which is metabolized to acetaldehyde in cells [91, 92]. Endogenous and alcohol-derived aldehydes in these mice result in persistent DNA damage in HSPCs that lead to dramatic loss of functional HSC pool [92–94]. FA human patients with mutations in *ALDH2* (acetaldehyde dehydrogenase 2) show accelerated bone marrow failure [95]. Therefore, HSCs have a coordinated pathway in response to genotoxic stresses induced by reactive aldehydes. The aldehyde-detoxifying enzymes provide the primary protection, and if this protection is compromised (by genetic mutations) or saturated (e.g., over-drinking), the potential genotoxic damage is counteracted by DDR including the FA pathway, non-homologous end joining, and homologous recombination [94]. These findings have important public health implications linking genetic mutations in metabolite-detoxifying genes and DNA repair genes, alcohol consumption, and blood diseases.

**Replicative stress—**Replicative stress refers to problems in DNA replication that causes the replication fork to stall, and is an important endogenous source of genome instability and a feature of cancer cells [96]. HSPCs are particularly sensitive to replicative stress as shown by impaired functionality of HSCs and erythrocyte precursors with limited replication origin licensing, a mechanism relieving replication stress [97]. While HSCs expand during aging, their functional activity declines over time. Persistence of replication stress in old HSCs is associated with cell cycle defects and chromosome breaks, and is an important driver of functional decline in aging HSCs [98].

## Chromatin regulation of metabolic enzymes in hematopoietic cells

Chromatin does not just get passively impacted by metabolism and DNA damage, but plays an active role in regulating metabolism and response to DNA damage (Fig. 1) [29]. Metabolism is controlled by the expression level and activities of numerous enzymes, especially those catalyzing the rate-limiting reactions for the entire pathway. Similarly, expressions of proteins in DDR are also subject to chromatin regulation. Therefore, chromatin regulation of expression of genes in metabolism and DDR plays an important role in stem cell fate determination.

Dpy30, a common core subunit of the SET1/MLL complexes that catalyze H3K4 methylation, is critical in sustaining long-term HSC self-renewal and also in enabling their differentiation [99, 100]. Loss of DPY30 in mouse results in defective HSC function associated with impaired energy metabolism in both glycolytic and mitochondrial activities. DPY30 directly regulates the expression of several genes encoding rate-limiting enzymes in glycolysis [101]. Overexpression of one of these enzymes, *Pklr*, could transiently and modestly rescue HSC differentiation [101], suggesting that DPY30 supports HSPC function partially through regulation of glycolysis. As DPY30-deficient cells are in an aberrant quiescent state of cell cycle, DPY30 also functions to enable HSC activation by fueling metabolic reprogramming at the chromatin level [101].

KDM4C/JMJD2C removes the repressive H3K9 methyl mark at target genes including those encoding key enzymes in the serine metabolic pathway and transcription factors in amino acid deprivation response, thereby activating amino acid biosynthesis and transportation and promoting cancer cell proliferation [102]. Loss of KDM3C/JMJD1C, another histone H3K9 demethylase, greatly impairs the self-renewal of LSC, but has minor effect on normal hematopoiesis [103, 104]. JMJD1C overexpression promotes LSC proliferation partially by reprogramming energy metabolism-related gene expression, including upregulation of genes that promote glycolytic and oxidative pathways, with consequent increase in energy production in LSCs [105]. Interestingly, the thyroid-hormone receptor-binding domain, but not the demethylase-related domains, of JMJD1C is required for this regulation [105]. Another study used CRISPR/Cas-mediated domain screening approach to show that endogenous JMJD1C requires its demethylase activity and zinc-finger domain for supporting *MLL*-rearranged leukemia [104]. The apparent discrepancy in domain requirement between these studies may arise from the different approaches of overexpression [105] versus deletion of endogenous gene domains [104], the former needing cautious interpretation in the absence of evidence for JMJD1C overexpression in leukemia.

This is an important question as a requirement of the demethylation activity would support a rationale in targeting this enzymatic region. Further studies on identification of the direct target genes and deeper biochemical dissection of JMJD1C will help to tease out the specific contribution of different molecular activities of this chromatin modulator in connecting metabolism and LSC activity.

## Role of chromatin modulators in guarding HSPC genome integrity

Much like its impact on transcription, chromatin organization also represents a major barrier to DDR, as both the sensing and repair factors need to gain access to the damaged DNA that is associated with the packed chromatin. Therefore, the cooperation of DDR factors and chromatin modulators is crucial for efficient recognition and repair of DNA lesions [106, 107]. In addition to the direct involvement in DDR, chromatin modulators also indirectly control DDR by regulating expression of DDR genes (Fig. 1). Consequently, aberrant chromatin modifications can result in impaired DDR, increased genomic instability, and defective HSPC function, as demonstrated by a number of studies targeting various chromatin modulators in hematopoietic cells (Table 1).

## DNA modifications

DNA methylation is thought to have an important role in maintaining genome integrity against the extensive retrotransposons in our genome [108, 109]. The dynamic removal of DNA methylation is also important for genome integrity. TET1 deficiency in mouse results in hematologic malignancy mainly in the B-cell lineage, increase in DNA damage, and downregulation of DNA repair genes in LSK, MPP, and pro-B cells [110]. Loss of both TET2 and TET3 results in aberrant HSPC differentiation with myeloid bias at the expense of erythropoiesis and lymphopoiesis, and induces the development of myeloid leukemia. The double-deficient bone marrow cells, mainly the late HPCs and mature myeloid cells, show accumulation of phospho-H2AX, impaired DNA repair after irradiation, and altered expression of DNA repair genes [111]. These studies support a role of the TET enzymes in guarding genome integrity through regulating DDR genes.

A direct role of 5hmC in DDR is suggested by its enriched localization to sites of DNA damage and repair [112]. Local 5hmC deposition is increased by DNA damaging agents and requires TET enzymes (especially TET2), and loss of TET compromises genome integrity in mouse ES cells during replication stress. As *BRCA2*, a key DNA repair gene, is downregulated by TET deficiency, the contribution from regulation of DDR gene expression cannot be excluded, but the overall data suggest a direct role of TET enzymes in DDR [112]. Another study shows that, in response to DNA damage, TET3 protein is upregulated and activated (via ATR-dependent phosphorylation), and plays an important role in DNA damage signaling and DNA repair [113]. Therefore, it is possible that DNA modifications can impact DDR through both direct (DDR per se) and indirect (expression of DDR genes) mechanisms to ultimately impact the activities of stem cells including HSPCs.

On the other hand, overexpression of catalytically active, but not inactive, TET2 in mouse pro-B cell line results in increased level of 5hmC and chromosomal instabilities, and can lead to increased mutagenesis if the subsequent modifications cannot be efficiently

eliminated in the context of deficiency of the base excision repair pathway [114]. When taken together with the role of TETs and 5hmC in guarding genome integrity shown in the loss-of-function studies, these findings suggest that the balanced activity of TET2 and the proper levels of different DNA modifications are important for genome integrity.

## Histone modifications/chromatin remodeling

**KMT2 family proteins and complexes—**The KMT2 family proteins include KMT2A/MLL1/MLL, KMT2B/MLL2 (also known as MLL4), KMT2C/MLL3, KMT2D/MLL4 (also known as MLL2), KMT2E/MLL5, KMT2F/SET1A, and KMT2G/SET1B. With the exception of MLL5, they are the major H3K4 methyltransferases in mammals by individually acting as the catalytic subunit of the SET1/MLL complexes, which also contain all of WDR5, RBBP5, ASH2L, and DPY30 as the common core subunits that are important for the catalytic activity of the complexes [115, 116]. In general, SET1A is responsible for the bulk H3K4me3 at promoters, and MLL1 and MLL2 catalyze promoter H3K4me3 at regulated developmental stages, whereas MLL3 and MLL4 mainly catalyze H3K4me1 at gene enhancers [117]. It is worth mentioning that these proteins have physiologically relevant activities independent of H3K4 methylation [116]. Many of these complex components are known to play important roles in HSPC function, and are extensively associated with human cancers [116, 118].

**KMT2A/MLL1/MLL:** MLL1 plays a critical role in normal hematopoiesis and hematologic malignancy [119, 120]. Chromosomal fusion of *MLL1* with a large number of partners drives mixed lineage leukemia. MLL1 is essential for hematopoiesis and self-renewal of HSCs. MLL1 participates in multiple cellular activities, including transcriptional regulation of key targets (e.g., *HOXA9*) through H3K4 methylation-dependent and -independent mechanisms [119], and integration of cell cycle control and DDR [121]. In response to genotoxic stress in S phase of the cell cycle, MLL1 is phosphorylated by ATR and, consequently, escapes Skp2-mediated degradation, and the accumulated MLL1 methylates H3K4 at late replication and delays DNA replication. MLL1 is thus directly involved in DDR and acts as a key effector of S phase checkpoint. It is likely that the unrestrained HSC proliferation in MLL1 deficiency [120] is mediated by its roles in executing S-phase checkpoint and in transcriptional control of target genes involved in cell proliferation [122]. Importantly, MLL fusions act as dominant negative mutants that interfere with the ATR-regulated stabilization of MLL1 [121]. These studies suggest that dysregulation of the cell cycle control and DDR incurred by MLL translocations may contribute to leukemogenesis [121].

**KMT2C/MLL3 and KMT2D/MLL4:** *MLL3* and *MLL4* are highly mutated in a wide range of human cancers including blood cancer, and have established roles as important tumor suppressors [118, 123]. The detailed tumor suppression mechanisms are not very clear [123], and may be in part related to their emerging role in maintaining genome stability. MLL3 suppression impairs HSPC function in differentiation [124]. Depletion of MLL3 in bladder cancer cells results in the downregulation of genes encoding key components of DDR (ATM and ATR) and the homologous recombination-mediated DNA

repair pathway, thereby substantially increasing DNA damage and genomic instability in cells [125].

Loss of MLL4 results in increased DDR, genome instability, and transcription stress (referring to RNA polymerase stalling, pausing, arrest, and/or backtracking), especially at the early replicating fragile sites. MLL4-mediated co-transcriptional H3K4 methylation is proposed to help resolve the transcription stress and/or impact replication [126]. The latter is supported by a very recent work in yeast, showing that H3K4 methylation relieves transcription–replication conflicts by acting as speed bumps to slow down replication machinery [127]. The feasibility of directly mutating histone residue in yeast [127] (not feasible in mammalian cells) greatly helps to establish the involvement of the histone modification in these processes. MLL4-mediated H3K4 methylation and PTIP (an MLL3/4 complex subunit) are also important for degradation of stalled and compromised replication fork (in the absence of BRCA2) by facilitating recruitment of the MRE11 nuclease [128]. These studies together support the important role of MLL4 and its H3K4 methylation activity in the maintenance of genome integrity facing the challenge of transcription and replication stresses.

In another report, MLL4 is shown to be required for normal HSC function and *MLL*-rearranged leukemia as its loss leads to defects in HSC function and induces myeloid differentiation of leukemic blasts [129]. MLL4 deficiency causes increase in ROS and DNA damage and dysregulation of transcriptional programs involved in the antioxidant response [129]. Treatment of ROS scavengers mitigates DNA damage and keeps the MLL4-deficient leukemia from differentiation, and artificial induction of DNA damage by homing endonuclease induces leukemia differentiation without affecting ROS levels, indicating a causal role of ROS and DNA damage in leukemia differentiation and probably disruption of HSC function. Therefore, MLL4 both directly participates in pathways regulating genome integrity and also transcriptionally controls genes that influence genome stability, consistent with its very frequent mutations in cancers.

**KMT2E/MLL5:** MLL5, a distinct KMT2 protein [130], is essential for hematopoiesis and normal HSPC function [131–133]. MLL5 deficiency in HSPCs impairs DNA repair and leads to accumulation of DNA damage, which triggers type 1 interferon response and mitochondrial accumulation of BID protein followed by increase in intracellular ROS levels. Interestingly, interferon inactivation or ROS quenching markedly restores HSPC function without alleviating DNA damage or improving DNA repair. The findings in this report suggest that (1) there is a two-way impact between DNA damage and ROS levels (Fig. 1A), as also seen by ROS increase upon loss of ATM [22], and (2) increased DNA damage itself, but not ROS, is compatible with normal HSPC function [134].

What is far unclear is how MLL5 deficiency impairs DNA repair and increases DNA damage. MLL5 lacks methyltransferase activity, but an MLL5 isoform can reorganize chromatin structure and affect local chromatin openness and accessibility (biased toward a more compact state), and promote tumor cell self-renewal via repressing H3 variant H3.3 expression and reducing global H3K4me3 level [135]. The altered chromatin state may directly impair the accessibility to DNA repair factors. Moreover, MLL5 helps to maintain

genomic stability by regulating chromosome spatial organization and spindle bipolarity [130]. MLL5 may thus represent a good example of chromatin state influencing HSPC function by directly impacting DNA damage and ROS levels.

**KMT2F/SET1A:** SET1A deficiency in mouse HSCs disrupt hematopoiesis and impairs HSC proliferation and repopulation [136]. While ROS levels are reduced, SET1A-deficient HSCs accumulate DNA damage as a result of impaired DDR gene expression and DNA repair capacity. SET1A protects DNA damage-induced HSC attrition following inflammation-induced activation. As energy metabolism and oxidative homeostasis genes are significantly enriched in genes upregulated in SET1A-deficient HSPCs, SET1A may also regulate metabolism [136]. SET1A supports the survival of *MLL*-rearranged leukemia cells by promoting expression of DNA repair genes (including *FANCD2*) through interaction with Cyclin K, rather than its H3K4 methylation activity [137]. H3K4 methylation by SET1A protects stalled replication forks from deleterious resection in replication stress by enhancing FANCD2-dependent histone chaperone activity, thereby maintaining genome stability [138]. The seemingly inconsistent effects of SET1A- [138] and MLL4- [128] mediated H3K4 methylation at replication fork actually suggest the coordinated activities of these KMT2s in the divergent cellular and/or genomic context, e.g., SET1A has a constitutive role in protecting fork degradation, whereas MLL4 acts to remove the fork whose stability is already compromised (in the absence of BRCA2). The close corporation of SET1A and DDR is also reflected by its regulation of FANCD2 at both transcription [137] and protein levels [138]. A direct involvement of SET1A in DDR is also suggested by its physical and functional association with DNA repair protein RAD18, apparently independent of the core subunits of the SET1A complex, thus independent of its H3K4 methyltransferase activity [139].

**DPY30:** The DPY30 and WDR5 core subunits of SET1/MLL complexes have also been shown to play a role in maintaining the genome integrity [116]. In addition to the impaired energy metabolism mentioned above, DPY30-deficient HSPCs show increase in DDR, DNA damage, and impaired DNA repair ability. The colony formation ability of DPY30-deficient HSPCs can be rescued by treatment of DDR inhibitors, and the hematopoietic functionality in vivo can be partially rescued by inactivation of CDK inhibitor p21 [101], suggesting that DPY30 regulates hematopoiesis partially through protecting genome integrity. Moreover, it seems that response to DNA damage, rather than the damage per se, has a major impact in HSPC function, echoing conclusions from the other studies [89, 134]. However, it is likely that the preserved HSPCs with unrepaired genome will have higher potential to give rise to malignancy later on.

Therefore, the common increase in DNA damage upon loss of the SET1/MLL complex subunits suggests an important role of H3K4 methylation in regulating genome integrity in HSPCs, while also compatible with the important contribution from the non-catalytic chromatin-regulatory activities of at least SET1A and MLL5. The SET1/MLL complexes regulate the expression of DNA repair genes [101, 136, 137] or genes in redox homeostasis [129]. Moreover, SET1/MLL complex-mediated H3K4 methylation may directly influence the generation of DNA damage in transcription and replication stress, and also regulate DNA

repair at the damaged sites including stressed replication sites. The genome-protection role of H3K4 methylation is conserved throughout evolution as evident in yeast [140], where the H3K4 methylation enzyme and the mark itself are found at newly created DNA breaks and *C. elegans* [141]. However, the direct influence of H3K4 methylation on chromatin integrity is likely to be complicated, as it may facilitate DNA repair by promoting the accessibility of DNA repair proteins, but also predispose chromatin for DNA double-stranded breaks through decondensing local chromatin [142].

**Other histone modifications and chromatin remodelers—**Studies of *IDH* mutations are a great example of how metabolic dysregulation results in HSC defects through epigenetic impact on DDR pathway. A mouse model-bearing endogenous *IDH1* mutation shows HSC attrition and impaired self-renewal capacity [63]. The *IDH1* mutation causes downregulation of *Atm*, a key DDR gene, and accumulation of DNA damage and impaired DNA repair in HSCs. The authors show that this regulation is independent of TET2, but likely via direct inhibition of KDM4 that demethylates H3K9, as they detected increase in H3K9 methylation globally and also at *Atm* promoter (Fig. 2A) [63].

BMI1, a chromatin modulator in the Polycomb family, is essential for the maintenance of HSCs, in part through repressing *p16Ink4a* and *p19Arf* [143]. BMI1-deficient HSCs have impaired mitochondrial oxidative respiration capacity, elevated ROS levels, and increased DNA damage and DDR [89]. The functional importance of ROS and DDR is shown by the mitigation of many hematopoietic phenotypes of the BMI1-deficient mice by either treatment of antioxidant or genetic inactivation of *Chk2*, a cell cycle checkpoint in response to DNA damage. However, the long-term repopulation defect cannot be rescued by DDR inactivation. Regarding the mechanisms underlying the increase in ROS levels, the authors show that it is a result of the impaired electron transport chain function. In addition, the dysregulation of many redox regulatory genes and the derepression of *p16Ink4a* and *p19Arf* [134] in BMI1-deficient cells may all contribute to ROS-level alteration. These results demonstrate a crucial role of BMI1 in maintaining stem cell function by coordinating energy metabolism, redox homeostasis, and protection of genome integrity, likely through chromatin regulation of key genes for mitochondrial function and redox homeostasis [89].

Depletion of DMAP1, a core component of the NuA4/Tip60 histone acetyltransferase complex, induces DNA damage and severely impairs proliferation in vitro and long-term repopulating of mouse HSCs [144]. Loss of SIRT1, an $NAD^+$-dependent deacetylase, in adult HSPCs results in aberrant HSPC fate determination, associated with increase in ROS, DNA damage, and dysregulation of antioxidant gene expression [67, 68]. These studies suggest the importance of the dynamic regulation of histone acetylation in protecting genome integrity for HSPC function.

Ubiquitination of histones H2A and H2B is prominent chromatin modifications enriched at damaged genomic sites, and plays an important role in efficient repair of DNA double-strand breaks [30]. Loss of Mysm1, one of the H2A-deubiquitinase, results in defective differentiation of hematopoietic cell lineages and impaired HSC function [145]. Mysm1-deficient HSPCs have increased DNA damage and ROS, indicating an important role of Mysm1 in maintaining genome integrity and oxidative stress. However, the molecular

mechanisms underlying such regulation are unclear, and may include the regulation of expression of DDR genes and direct regulation of DDR through H2A ubiquitination control at the sites of DNA damage [145].

SETD2-mediated H3K36 tri-methylation is a chromatin modification associated with active gene expression, and also plays a direct role in DNA repair including mismatch repair [146] and homologous recombination repair [147, 148] by facilitating the recruitment of repair factors to DNA damage sites. *SETD2* is recurrently mutated in a number of human cancers including blood cancer. Perturbation of SETD2 in vitro or in an animal model of leukemia leads to increased resistance to DNA-damaging agents as a result of impaired local DDR including inefficient DNA damage repair and increased survival, and pharmacologic inhibition of H3K9/36 demethylase can restore the sensitivity to the chemotherapy agents [149]. However, severe depletion of SETD2 in *MLL*-rearranged leukemia leads to growth arrest and myeloid differentiation, accompanied by increased DNA damage, DDR, and DNA damage-associated gene expression [150]. This is in line with leukemia differentiation upon experimentally induced DNA damage [129].

The role of many chromatin modulators in HSPC function is usually explained based on their impact on transcription. Given the fundamental role of chromatin conformation in DDR, it is likely that the HSPC phenotypes following perturbation of many chromatin modulators arise from dysregulated DDR. For instance, the requirement of PRC2 proteins (including EZH2) for HSC function is largely considered via epigenetic repression of target expression [151, 152]. It is likely that some of the phenotypes are mediated by the direct role of EZH2 in DDR [153, 154]. In particular, EZH2-mediated H3K27 methylation at stalled replication forks helps to recruit MUS81 nuclease to promote fork degradation [155]. This regulation thus joins the MLL4–H3K4 methylation–MRE11 [128] and the SET1A–H3K4 methylation–FANCD2 [138] pathways in a direct and coordinated control of fork stability by histone modifications that have been well established for transcription regulation.

## Conclusions and perspectives

Chromatin represents the central stage where signals and impacts from different cellular processes, including metabolism, are integrated and relayed to the DNA-based activities, which ultimately influence cell function. As underscored in this review, chromatin does not merely take the impacts from metabolic and genotoxic molecules, but actively and crucially regulate pathways in metabolism and DDR. These cellular processes constitute a major target of chromatin regulation to maintain the normal function and fate determination of HSPCs, as in part reflected by a profound control of HSPC fate determination by both the Polycomb (BMI1 [89]) and Trithorax (DPY30 [101]) families of chromatin modulators [156] via regulating energy metabolism and genome integrity. Considering the important roles played by many chromatin modulators in hematologic malignancies [6, 7], it will be of great interest to study if and how their roles in blood cancer are at least partially linked to regulation of cellular metabolism and genome integrity. These studies will likely provide combinatorial strategies for cancer treatment, given the high druggability of many enzymes involved in metabolism, chromatin modifications, and DDR.

Many challenges remain. Although many reports show concomitant alterations of metabolism, chromatin state, and HSPC functions, it is difficult to establish a precise causality for a particular molecular process in generating a biological outcome, due to the ubiquitous influence of both metabolism and chromatin on cellular physiology. For instance, dysregulated mitochondrial activities are often accompanied by altered expression of oxidative homeostasis genes, making it difficult to discern their causal versus a compensatory role in ROS-level regulation. Moreover, the functional interplay among the metabolic regulation, ROS levels, DNA damage, DDR, and the chromatin state in controlling HSPC activities clearly requires further studies. Apparently inconsistent or paradoxical conclusions are not uncommon regarding which process(es) lead to the others. For instance, while high levels of ROS are widely believed to cause (thus upstream of) DNA damage, the reverse also seems true [22, 134]. Despite their close connections, ROS and DNA damage are separable and can impact HSPC function independently of each other [129, 134]. Many studies suggest that active DDR protects, and accrued DNA damage disrupts, HSPC functionality, whereas others suggest that ongoing DDR, rather than the damage itself, results in defective HSPCs [89, 101]. While these observations are not contradictory to each other and probably reflect the interconnectivity of these processes, they clearly indicate a need of further work to tease out the cause and consequence of each molecular event.

Another challenging question is whether and how metabolism specifically affects gene expression through regulating the chromatin state, considering the universal usage of certain metabolites as co-factors for many chromatin modifications that can have very different impacts on the chromatin-associated processes. For example, the dynamic regulation of chromatin methylation by methyl writers and erasers is directly influenced by levels of SAM and α-KG (and related metabolites), respectively [17]. Perturbations to affect these metabolite levels, such as methionine restriction [157] or mitochondrial inactivation [77], alter several histone methyl marks including those on H3K4 and H3K9, as well as gene expression. As H3K4 and H3K9 methylation have opposite effects on gene expression, the extent of effect on these marks can lead to different outcome in gene expression. The biochemical basis for a selective impact is the different kinetic properties of the chromatin enzymes in relationship with the metabolite concentrations [16]. However, it may not be easy to accurately determine these enzymatic properties in cells. For instance, while the catalytic domains of some KMT2 family H3K4 methyltransferases have been kinetically characterized in vitro [158], the properties of the full-length enzymes in complexes assembled at higher order in cells [116] and, thus, their responsiveness to the SAM level may differ significantly. Moreover, we do not know well how the metabolites directly affect chromatin modifications at specific regions given the rapidly diffusive nature of metabolites. One mechanism is through the local supply of metabolites provided by the chromatin-bound metabolic enzymes, typically through interaction with transcription factors [17, 159, 160]. Finally, we know very little about the specific gene regulation by metabolism in the context of HSPC functions.

Aberrant chromatin modifications are commonly associated with genomic instability which is represented as increase in DDR. Many studies merely use gamma-H2AX as a readout of increased DDR, which can be a result of increased DNA damage, increased susceptibility or

response to the same amount of damage, and/or impaired DNA repair. Chromatin modifications can influence DDR either indirectly by affecting expression of DNA repair genes, or directly by regulating DNA damage sensing and repair at the site of modifications. More studies are needed to better distinguish these mechanisms in cells, including identification of the precise genomic locations of DNA damage and the chromatin modulators and modifications, and biochemical characterizations of how the chromatin modulators and modifications directly link to DDR.

Looking forward, it is highly likely that new connections between metabolism and chromatin will be discovered to regulate HSPC functions. New connections may come from newly identified chromatin modifications. For instance, the newly identified lactate-derived histone lactylation responds to metabolic lactate level and directly stimulates chromatin transcription [161], but its physiological role in HSCs has not been studied. HSCs are more reliant on anaerobic glycolysis and thus produce more lactate from glucose normalized to cellular ATP, but the lactate level is lower than in the other hematopoietic cells due to the overall low level of metabolism in HSCs [8].

New connections may also come from some other fundamentally new chromatin-regulatory mechanisms. As a biopolymer, chromatin's activity is subject to regulation by factors that affect many physical properties of a polymer. Chromatin and its modulators have been recently shown to adopt the form of phase-separated biomolecular condensates [162–169], which are now recognized as a fundamental principle in organizing cellular space and biochemistry [170–172]. These condensates are driven by weak and multivalent interactions and display a continuum of material properties and molecular dynamics, which profoundly impact the activities of the molecules in the condensates. Importantly, their properties are critically affected by intrinsic factors including histone modifications and extrinsic factors including ionic strength, pH, hydrotropes, $Mg^{2+}$ concentration, temperature, etc. [173], many of which are influenced by metabolic activities in cells. Of particular relevance, ATP at physiological concentrations (~mM levels) acts as a biological hydrotrope and has widespread influence on protein solubility, phase separation, and stability [174, 175]. This offers a completely different mechanism by which metabolites may affect chromatin activities, since most ATPases only require ATP concentrations roughly thousands of times lower than the cellular levels and are thus insensitive to metabolic changes of ATP levels [16]. More studies will likely provide unprecedented understanding of how chromatin is influenced by and responds to metabolic pathways for HSPC function.

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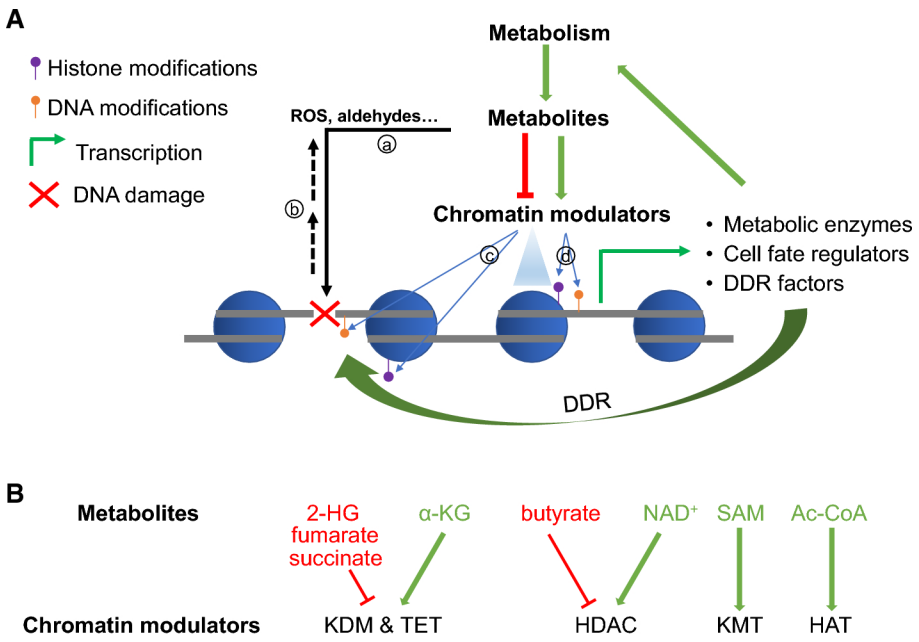


**Figure 1. Cross-regulation of metabolism and chromatin that control HSPC function.**

**(A)** Metabolic reactions use precursors and produce metabolites, some of which are (a) genotoxic and damage DNA, and some of which can directly regulate (enhance or inhibit) the activities of chromatin modulators in modifying DNA and/or histones and remodeling nucleosomes. (b) DNA damage is also suggested to cause increased ROS. (c) Changes in the chromatin state at the damaged DNA sites can directly influence the sensing and repair of the damage. (d) Chromatin changes also activate or repress transcription and expression of genes encoding key metabolic enzymes, cell fate regulators, and DDR factors that participate in DDR, which together influence the function and fate determination of cells in different lineages, including HSPCs.

**(B)** Examples of metabolites that inhibit (in red) or promote (in green) the corresponding chromatin enzymes.

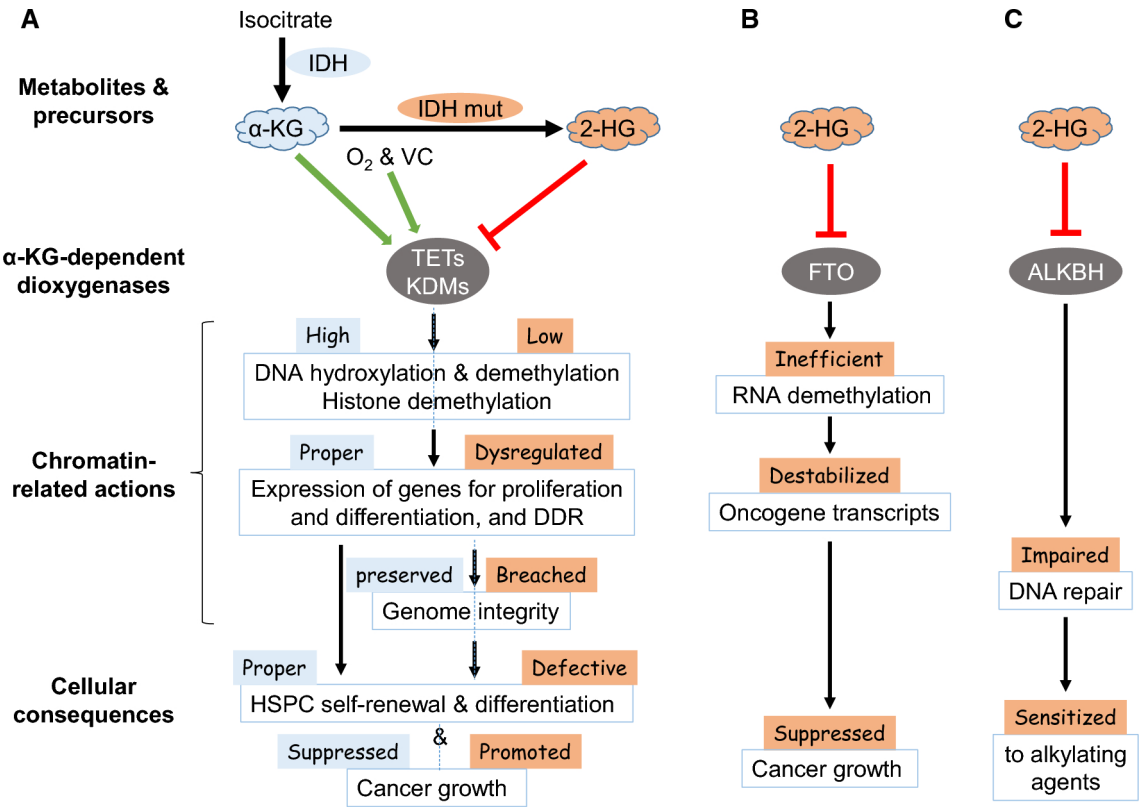


**Figure 2. Different chromatin regulation of cell functions by the metabolites from Isocitrate.**

α-KG is generated from isocitrate by IDH, and serves as an essential cofactor for a large number of α-KG-dependent dioxygenases including those shown here. These enzymes exert different molecular activities on chromatin (A), RNA modifications (B), and DNA repair proteins (C), which affect gene expression and DDR to ensure proper gene expression and preserve genome integrity for normal hematopoietic cell fate determination and function. In contrast, 2-HG is generated from α-KG by IDH mutants frequently found in cancer, and inhibits these α-KG-dependent dioxygenases and affect the different activities on chromatin (A), RNA (B), and DNA repair (C), and eventually lead to aberrant hematopoietic fate determination and different outcome of cancer growth.

**Table 1.**

Targeting chromatin modulators showing a role in maintaining genome integrity and function of hematopoietic cells.

| Protein | Molecular Function | DNA damage | DDR, repair | ROS | Possible mechanism | | Ref. |
|---|---|---|---|---|---|---|---|
| | | | | | Direct | Indirect | |
| TET2&3 | DNA hydroxylation & demethylation | | DDR↑ Repair ↓ in mature cells | | Accumulate at damage sites | Regulating repair gene expression | [111] |
| KMT2A/ MLL1 | H3K4 methylation and more | | | | S-phase checkpoint execution | | [121] |
| KMT2D/ MLL4 | H3K4 methylation (and more?) | ↑ | DDR↑ | ↑ | Relieving transcription stress; Promoting replication fork degradation | Regulating redox gene expression and ROS | [126, 129, 135] |
| KMT2E/ MLL5 | Organizing chromatin | ↑ | DDR↑ Repair ↓ | ↑ | | | [134] |
| KMT2F/ SET1A | H3K4 methylation and more | ↑ | DDR↑ Repair ↓ | ↓ | Protecting replication fork degradation | Regulating repair gene expression | [136–138] |
| DPY30 | H3K4 methylation (and more?) | ↑ | DDR↑ Repair ↓ | ↓ | | Regulating repair gene expression | [101] |
| BMI1 | | ↑ | DDR↑ | ↑ | | Metabolically increased ROS | [89] |
| MYSM1 | H2A deubiquitination | | DDR↑ | ↑ | | | [145] |
| SETD2 | H3K36 methylation | ↑ | DDR↑ Repair ↓ | | Facilitating repair | | [149, 150] |
| SIRT1 | Deacetylation of histones and other proteins | ↑ | DDR↑ | ↑ | | Regulating redox gene expression | [67, 68] |