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SPOTLIGHT REVIEW

Chromatin modifiers and the promise of epigenetic therapy in acute leukemia

SM Greenblatt and SD Nimer

Hematopoiesis is a tightly regulated process involving the control of gene expression that directs the transition from hematopoietic stem and progenitor cells to terminally differentiated blood cells. In leukemia, the processes directing self-renewal, differentiation and progenitor cell expansion are disrupted, leading to the accumulation of immature, non-functioning malignant cells. Insights into these processes have come in stages, based on technological advances in genetic analyses, bioinformatics and biological sciences. The first cytogenetic studies of leukemic cells identified chromosomal translocations that generate oncogenic fusion proteins and most commonly affect regulators of transcription. This was followed by the discovery of recurrent somatic mutations in genes encoding regulators of the signal transduction pathways that control cell proliferation and survival. Recently, studies of global changes in methylation and gene expression have led to the understanding that the output of transcriptional regulators and the proliferative signaling pathways are ultimately influenced by chromatin structure. Candidate gene, whole-genome and whole-exome sequencing studies have identified recurrent somatic mutations in genes encoding epigenetic modifiers in both acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL). In contrast to the two-hit model of leukemogenesis, emerging evidence suggests that these epigenetic modifiers represent a class of mutations that are critical to the development of leukemia and affect the regulation of various other oncogenic pathways. In this review, we discuss the range of recurrent, somatic mutations in epigenetic modifiers found in leukemia and how these modifiers relate to the classical leukemogenic pathways that lead to impaired cell differentiation and aberrant self-renewal and proliferation.

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INTRODUCTION

Leukemia research has largely focused on regulators of signaling and cellular differentiation for the past 15 years. Although the classical model of leukemogenesis has suggested that a mutation in a gene encoding a regulator of signaling/proliferation is a requirement for transformation, recent characterization of *de novo* acute myeloid leukemia (AML) has suggested that over 40% of patients do not have an identifiable mutation in a signaling gene.[1] Similarly, mutations in myeloid transcription factors occur in around 20% of AML patients and gene fusion events occur in less than half of AML patients. Taken together, these findings fail to completely explain the impaired differentiation that is a defining characteristic of leukemia. Similarly, genetic characterization of pediatric T-cell acute lymphoblastic leukemia (T-ALL) has indicated that somatic mutations in genes involved in development or signaling are not found in 20% of early T-cell precursor -ALL and 69% of non-early T-cell precursor-ALL.[2] Mutations in epigenetic modifiers are emerging as a large class of mutations that is critical in the development of both AML and subtypes of ALL. In contrast to the previous view that this class of mutations are rare, analysis of 200 cases of *de novo* AML by a combination of whole-exome and whole-genome sequencing showed that over 70% of patients had at least one non-synonymous mutation in a DNA methylation-related gene or another epigenetic modifier.[1] Adding to this importance, many mutations classically defined for their role in proliferation and differentiation are now understood to have important roles in regulating chromatin structure.

SOMATIC MUTATIONS AND ALTERATIONS IN CHROMATIN-MODIFYING ENZYMES

Chromatin modifiers are enzymes that catalyze the chemical conversion of cytosine residues in DNA, or lysine, arginine, tyrosine and serine residues in histone proteins. The importance of epigenetic modifiers in leukemia was first suggested by the identification of recurrent translocations in histone acetyltransferase and methyltransferase genes (for example, *CBP*, *P300*, *NSD1*, *MLL* and *MOZ*). In recent years, somatic mutations have also been identified in genes that encode the proteins controlling DNA cytosine modifications (for example, *DNMT3A* and *TET2*). Figure 1 depicts the epigenetic regulation of methylation and acetylation, and their potential targeting in leukemia by 'epigenetic-modifying therapy'.

Cytosine modifications

DNA methylation has an important role in myeloid and lymphoid commitment, as well as hematopoietic stem cell (HSC) self-renewal.[3–5] Methylation profiling of mouse multipotent progenitor cells has indicated that the promoters of several transcription factors become methylated during the cellular differentiation toward common myeloid progenitors and common lymphoid progenitors.[6] Furthermore, methylation profiling has been used to classify subtypes of leukemia, with prognostic significance.[7–9] Genome-wide cytosine methylation profiling, combined with copy number and gene expression analysis in childhood ALL,

Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, USA. Correspondence: Dr SD Nimer, Sylvester Comprehensive Cancer Center, University of Miami, Clinical Research Building, 6th Floor, Suite 660, 1120 NW 14th Street, (C241) Miami, FL 33136-1000, USA.
E-mail: SNimer@med.miami.edu
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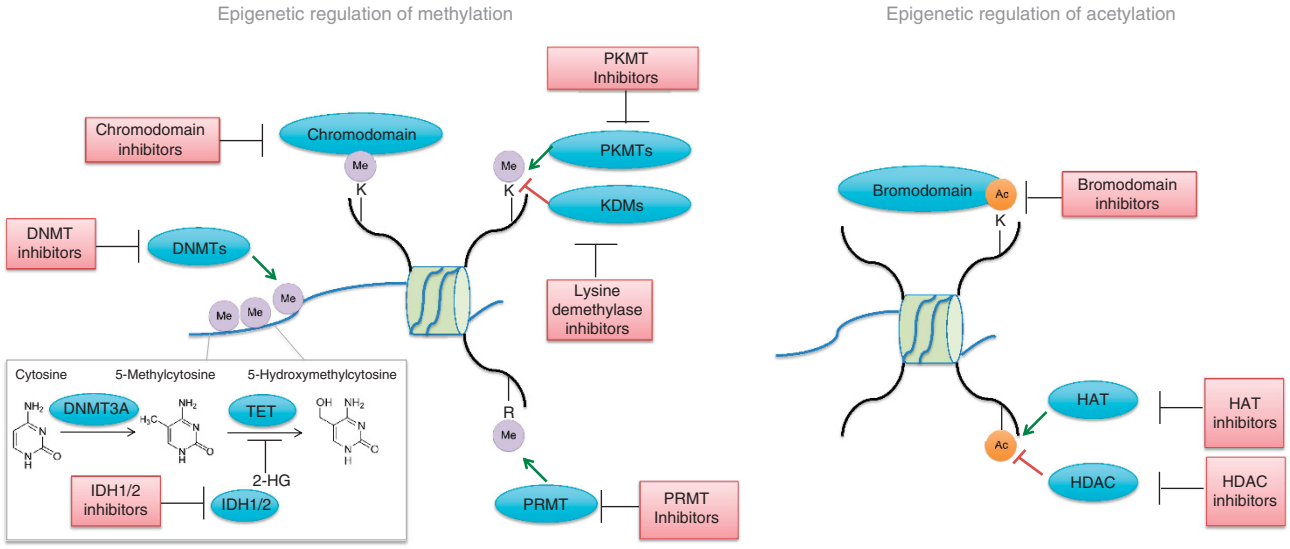

Figure 1. Regulation of methylation and acetylation in leukemia and their therapeutic potential. The figure shows a selection of proteins that add, remove and recognize chromatin modifications, as well as the the proteins that regulate DNA methylation. The genes encoding these proteins can be altered through mutation, deletion or altered expression in leukemia. Ac, acetylation; DNMT, DNA methyltransferase; HAT, histone acetyltransferase; HDAC, histone deacetylase; KDM, lysine demethylase; Me, methylation; PKMT, lysine methyltransferase; PRMT, arginine methyltransferase.

has suggested that there is an aberrant epigenetic signature that is common to all cases, regardless of disease subtype. This suggests that a common set of epigenetically deregulated genes may be required for the initiation or maintenance of hematopoietic transformation. However, DNA methylation patterns clearly associate with specific chromosomal rearrangements. Indeed, oncogenic translocations involving transcription factors such as *ETV6-RUNX1* have prognostic value (favorable in this case) and are associated with specific alterations in methylation.[10] Overexpression of *EVI1* has been associated with an aberrant hypermethylation signature and poor prognosis in AML.[11] Finally, DNA methylation profiling in myelodysplastic syndrome (MDS)/AML suggests that aberrant methylation may be the primary mechanism of tumor suppressor gene silencing and clonal evolution to acute leukemia.[12]

DNMT3a is an enzyme required for *de novo* methylation and a frequent target of somatic mutations, occurring in over 30% of cytogenetically normal-AML patients and 16% of T-ALL.[13–17] Approximately 60% of the mutations in *DNMT3A* result in the heterozygous substitution of arginine 882 in the catalytic domain of the enzyme, leading to decreased methyltransferase activity *in vitro*.[18] Interestingly, the wild-type *DNMT3A* allele is still expressed and recent data suggest that the DNMT3A mutant proteins exert a dominant negative effect through interactions with wild-type DNMT3A and DNMT3B.[19] DNMT3A-deficient mouse HSCs display altered patterns of cytosine methylation including both hypomethylated and hypermethylated regions.[13,18,20] DNMT3A appears to be required for the normal self-renewal capacity of HSCs in adult mice and for maintaining the differentiation potential of serially transplanted HSCs in wild-type recipients.[3] DNMT1 also appears to be critical for leukemia stem cell function, as haploinsufficiency of *Dnmt1* in an MLL-AF9-induced mouse model resulted in reduced DNA methylation and bivalent chromatin marks associated with tumor suppressor gene de-repression.[21]

The ten-eleven translocation (TET) family of proteins has recently been shown to contribute to the regulation of DNA methylation through the conversion of 5-methylcytosine (5-mc) to 5-hydroxymethyl cytosine (5hmC).[22] This modification is thought to block the binding of proteins that mediate transcriptional silencing by recognizing methylated DNA; thus, it is found in regulatory regions of genes that are actively transcribed.[23,24] 5hmC is also thought to be a critical step on the path to DNA demethylation.[25] *TET2* mutations occur in 7–23% of AML and 49% of chronic myelomonocytic leukemia and are associated with poor prognosis in cytogenetically normal-AML.[26–29] Deletion of *Tet2* in mice leads to increased self-renewal, expansion of the hematopoietic stem and progenitor cell compartment and altered cell differentiation toward the monocytic/granulocytic lineages.[30–32] *TET2* mutations in myeloid malignancies are generally associated with low 5hmC levels and both DNA hypermethylation and hypomethylation at CpG sites in AML.[33] *TET2* mutations are mutually exclusive with gain of function mutations in the isocitrate dehydrogenase 1 and 2 enzymes (IDH1/2) that are found in 15–33% of AML.[26,34–38] In general, *IDH1/2* mutations are associated with poor prognosis, but outcome may vary somewhat based on the location of the *IDH1/2* mutation.[39] The reason for this mutual exclusivity was rapidly identified; IDH1/2 regulates the conversion of isocitrate to α-ketoglutarate (α-KG), and mutations in the arginine residues of IDH1/2 alter its enzymatic function, leading to the aberrant accumulation of a 2-hydroglutarate (2-HG), an 'oncometabolite' that impairs the function of TET proteins and the activity of the jumonji (JmJ) family of histone demethylases, which also require α-KG.[40,41] Thus, *IDH1* mutations impair histone demethylation, and, biologically, appear to inhibit differentiation.[42] In a bone marrow transplantation model, IDH1 mutations cooperated with HOXA9 to accelerate the development of an MPD-like disorder.[43] Knock-in mice that express the *IDH1* (R132H) mutation have increased (that is, detectable) 2-HG serum levels and expansion of the multipotent progenitor population.[44] The same increase in 2-HG is seen in patients with *IDH1/2* mutant AML.[45]

Polycomb group proteins

Hematopoiesis requires the proper temporal and lineage-specific regulation of gene expression, such as the homeotic genes, whose expression is reciprocally controlled by large protein complexes containing the polycomb group (PcG) proteins or trithorax group (Trx) proteins. The balance between these complexes is crucial for the normal regulation of embryonic development and cell differentiation, with alterations in *HoxA* and *HoxB* cluster gene

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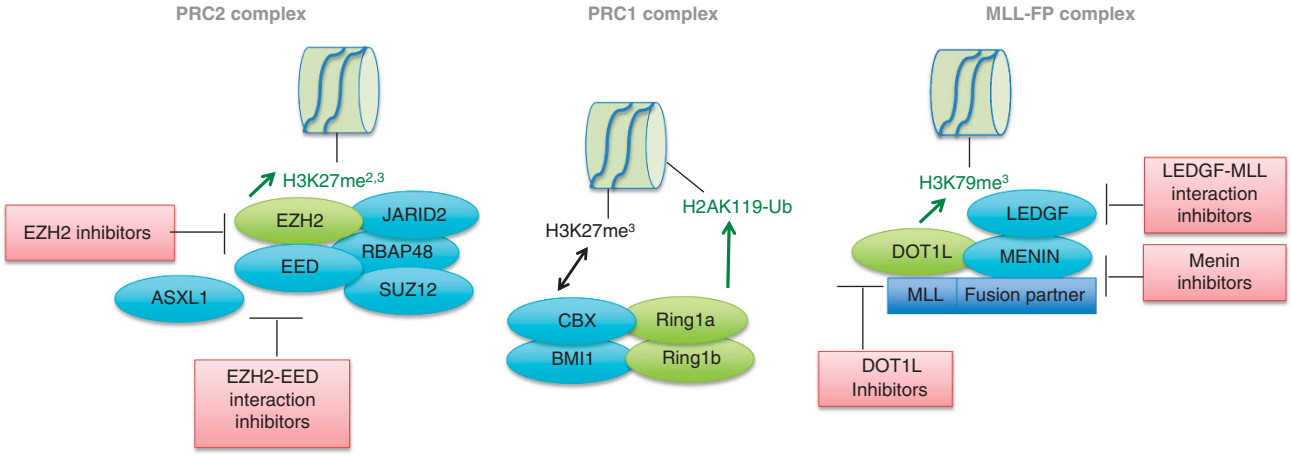

Figure 2. Polycomb repressive complexes and MLL-fusion complexes in leukemia and their therapeutic potential The PcG protein complexes, known as PRC1 and PRC2, maintain transcriptional silencing. EZH2 contains the methyltransferase activity for PRC2 that catalyzes the di-and trimethylation of H3K27. Recurrent deletions and sequence mutations in EZH2, SUZ12 and EED are found in T-ALL. ASXL1 mutations promote transformation by decreasing PRC2 recruitment and contributing to loss of transcriptional repression. Another PRC2-interacting protein, JAR1D2, is involved in the recruitment of the complex to target loci and is deleted in the progression of chronic phase myeloid malignancies to acute leukemia. PRC1 complex recognizes H3K27me3 via the chromodomain-containing CBX proteins and is involved in the maintenance of gene repression through histone H2A ubiquitination and the recruitment of DNA methyltransferases. PRC1 contains several proteins linked to cancer including Bmi-1, a protein associated with HSC self-renewal, and the ubiquitin ligases Ring1A and Ring1B. Several MLL fusion proteins can aberrantly recruit the DOT1L methyltranferase, leading to methylation of H3K79 and the activation of genes driving cellular transformation. MLL fusion proteins are also dependent on Menin, a component of the MLL-SET1-like histone methyltransferase complex and an adaptor to the chromatin-associated protein LEDGF.

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expression being a characteristic of many hematologic malignancies.[46] Figure 2 depicts the normal PcG complexes, the leukemia-associated *MLL* fusion complexes and their relevance to the epigenetic therapy of leukemia. The major PcG protein complexes, known as polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2), maintain transcriptional silencing. The PRC2 complex consists of four core members, EZH1/2, EED, SUZ12 and RBAP48. EZH2 contains the methyltransferase activity that catalyzes the di- and trimethylation of H3K27, which is generally a repressive chromatin mark.[47] *EZH2* is the most frequently mutated PRC2 component in cancer and it is also upregulated in many solid tumors, often serving as an indicator of aggressive disease. While heterozygous gain-of-function mutations of *EZH2* have been identified in diffuse large B-cell lymphomas, loss-of-function missense, nonsense and frameshift mutations are typically observed in myeloid malignancies, especially MDS.[48–50] *EZH2* mutations are rare in most acute leukemias (1–2% of *de novo* AML), but they are found in 16–19% of T-ALL.[51,52] Recurrent deletions or somatic mutations in *SUZ12* (21%) and *EED* (15%) were also identified in early T-cell precursor-ALL.[2]

The site-specific recruitment of PRC complexes to chromatin is an important step in the regulation of histone methylation. As PRC complexes do not contain DNA sequence specific binding activity, they are subject to interaction with other proteins, such as ASXL1 (addition of sex combs like 1). Deletions and point mutations in *ASXL1* occur in 6–30% of AML and 43% of chronic myelomonocytic leukemia.[28,53] These mutations promote transformation by decreasing PRC2 recruitment and thereby reducing H3K27 methylation, leading to loss of the transcriptional repression of genes whose expression can promote leukemogenesis, including *HOXA9*.[54] Loss-of-function *Asxl1* mutations showed a mild phenotype in mice, with defects in myeloid and lymphoid progenitors, but no evidence of myelodysplasia or leukemic progression, while conditional knockout of *Asxl1* in the hematopoietic compartment resulted in myelodysplasia.[54,55] Another PRC2 interacting protein, JARID2, is also involved in recruiting the complex to target loci.[56] JARID2 inhibits the lysine methyltransferase activity of PRC2, and it is deleted during the progression of some chronic phase myeloid malignancies to acute leukemia.[57]

The PRC1 complex recognizes H3K27me3 via its chromodomain-containing CBX proteins and is involved in the maintenance of gene repression through histone H2A ubiquitination and the recruitment of DNA methyltransferases.[58] The diverse forms of the PRC1 complex consist of a core containing BMI1 and the ubiquitin ligases RING1A and RING1B, but also CBX proteins, Ph homologs (PHC 1–3) and other RING-finger domain containing proteins. PRC1 contains several proteins linked to cancer including BMI1, a protein associated with HSC self-renewal and the leukemic reprogramming of myeloid progenitors.[59,60] RING1A and RING1B contain important histone ubiquitin ligase activity, and both BMI1 and RING1 components have been shown to be overexpressed in myeloid malignancies and have a critical role in hematopoiesis.[61] *Bmi1*-deficient granulocyte/macrophage progenitors (GMPs) transformed with MLL-AF9 showed impaired leukemia stem activity and increased differentiation potential. Conditional inactivation of Ring1B resulted in a reduction in total bone marrow cell numbers, while the proliferation of myeloid progenitors and percentage of lineage negative cells increased.[62] Other fusion proteins such as PLZF/RARA have been shown to interact with BMI1 and PRC1 complexes, recruiting the complex to retinoic acid response elements.[63] Taken together, these studies suggest that oncogenic fusion genes require PRC1 protein activity in order to establish the leukemic reprogramming of myeloid progenitors, including the block in their differentiation.

The recurrent loss-of-function mutations, copy-number alternations or overexpression of PRC components in lymphoid leukemia suggest that these complexes may act as epigenetic tumor suppressors. Supporting this notion is the finding that overexpression of PRC2 components can inhibit the function of the complex by altering the subunit composition, thereby leading to aberrant targeting of the complex.[64] The recruitment of DNA methyltransferases by PRC2 is another critical step in transcriptional repression and it too may be altered by somatic mutations in *DNMT3A*.[65] All these changes may lead to aberrant

activation of PRC target genes or the inappropriate recruitment of the complex to other gene targets, leading to aberrant transcriptional repression.

MLL proteins

Mutations involving mammalian versions of trithorax genes are found in leukemia (*MLL1*) and in solid tumors as well (*MLL 2,3,4,5*). The MLL genes encode histone methyltransferases whose mutations can alter chromatin structure. MLL proteins are members of the SET domain containing protein lysine methyltransferase family; they methylate H3K4, generating a mark of transcriptional activation. Translocations involving *MLL* and its in-frame fusion partners have been observed in 5–10% of AML and greater than 70% of infant ALL; they are generally associated with poor prognosis.[66–70] In-frame partial tandem duplications of MLL occur in 5–7% of *de novo* AML and they too are associated with an unfavorable prognosis.[71] The direct binding targets of MLL fusion proteins include *HOXA* cluster genes and *MEIS1*.[72] Enforced expression of MLL-AF9, or a combination of HOXA9 and MEIS1, induces the leukemic transformation of hematopoietic stem and progenitor cells in mouse models.[73] *MLL* rearrangement leads to acquisition of H3K79 methyltransferase activity at MLL target sites due to the recruitment of the methyltransferase DOT1L.[74–76] MLL fusion proteins are also dependent on Menin, a component of the MLL-SET1-like histone methyltransferase complex, that serves as a link to the chromatin-associated protein LEDGF.[77,78] Genetic loss of Menin induces differentiation and reverses aberrant *HOX* gene expression in leukemic blasts, while disrupting the Menin interaction domain of MLL downregulates MEIS1 and inhibits cell proliferation.[79,80] Peptides that directly disrupt the LEDGF-MLL interface have shown efficacy in MLL-AF9-induced AML, and it appears that numerous MLL-interacting proteins must remain fully functional for MLL-FP-driven leukemias to persist.[81]

Other lysine methyltransferases and demethylases

NSD1 encodes another protein lysine methyltransferase involved in leukemia through its fusion with the nucleoporin 98 gene (*NUP98*), generating the *NUP98-NSD1* fusion protein that is associated with poor prognosis in patients.[82–84] Histone lysine methylation can be reversed by the amine oxidase type lysine-specific demethylases, including LSD1 and LSD2, which are generally referred to as 'erasers' because they remove histone marks. LSD1 is of particular interest as it exhibits specificity for H3K4 and H3K9 methylation, is critical for erythroid differentiation and is highly expressed in AML.[85] Global H3K4 methylation levels can also be altered by mutations or gene expression changes in the Jumanji C (JmjC) family of lysine demethylases. These genetic abnormalities include translocations involving the *JARID1* family of histone H3K4 demethylases and overexpression of KDM2B, an H3K36me2-specific demethylase that is required for initiation and maintenance of AML.[86] Fusion of the *NUP98* and *JARID1A* genes (also known as *KDM5A*) occurs in 10% of acute megakaryoblastic leukemia.[87,88] This translocation creates haploinsufficiency for Nup98 and JAR1D1A, and it leads to alteration of JARID1 function, possibly due to recruitment of p300/CBP by the fusion protein.[89] *UTX*, another member of the JmjC family of lysine demethylases, is altered through inactivating mutations in AML and ALL.[90–92]

Protein arginine methyltransferases

Protein arginine methyltransferases (PRMTs) catalyze the mono- or dimethylation of arginine residues in histones, and other non-histone substrates, including transcription factors. Asymmetric dimethylation of histones is generally associated with gene activation, whereas symmetric dimethyl-arginine is associated with gene repression. PRMTs appear to have some role in acute leukemia, as several members of the PRMT family are overexpressed in AML including PRMT4 and PRMT5.[93–95] Our lab has recently reported that PRMT4 can block myeloid differentiation, at least in part by promoting the assembly of a repressive RUNX1 complex. Knockdown of PRMT4 in several human leukemia cell lines, and in human $CD34^+$ cells, promotes myeloid differentiation.[96] PRMT1 appears to be a critical member of the MLL transcriptional complex, while PRMT6 has been shown to inhibit H3K4 methylation by MLL.[97,98]

Histone acetyltransferases

Histone acetyltransferases (HATs) catalyze the transfer of an acetyl group to lysine residues, neutralizing the positive charge and promoting a less compact chromatin state that is associated with increased gene transcription. Mutations in the HAT *CBP* have been identified in 18% of relapsed acute lymphoblastic leukemia, resulting in impaired histone acetylation and aberrant transcriptional regulation of CBP targets.[51,99,100] MOZ, a member of the MYST family of acetyltransferases, is a critical regulator of HSC maintenance and global Hox gene expression, through its effects on histone H3K9 acetylation at Hox loci.[101–103] *MOZ* is involved in several chromosomal translocations in leukemia including fusion to *P300*, *CBP* and *TIF2*.[104–106] TIP60 is another HAT thought to have a tumor suppressive role in leukemia through the recognition of H3K9me3.[107] TIP60 protein levels are reduced in AML patients and TIP60 has been shown to interact with ETV6, a frequent fusion partner in B-precursor ALL and a frequent site of deletions or mutations in acute leukemia.[108]

Cross-talk between chromatin regulatory complexes

Transcriptional activation requires both the addition of activating post-translational modifications and the removal of the repressive modifications, such as H3K27 methylation. Therefore, it is not surprising that leukemia cells often display changes in both PcG and Trx group proteins concomitantly. The first connection between PcG proteins and MLL leukemia-associated factors emerged from the observation that MLL-AF9-expressing leukemic stem cells achieve transcriptional activation and overcome senescence through interactions between the PRC1 components BMI1 and CBX8.[59,109]

The cross-talk between normal or oncogenic epigenetic modifiers and other oncogenes may have a potential for therapeutic intervention. A prime example of this is the efficacy of inhibiting PRC2 activity in *MLL*-rearranged leukemias.[110–112] Conditional deletion of *Ezh2* in GMPs expressing MLL-AF9 reduced their proliferation in culture and attenuated the progression to AML.[113] Although genetic loss of *Ezh2* resulted in a mild phenotype in the MLL-AF9 mouse model, loss of PRC2 function through deletion of *Eed* significantly inhibited leukemia cell growth.[110] Inhibition of PRC2 components has shown pre-clinical efficacy in acute promyelocytic leukemia as well, a disease driven by the PML-RARα fusion, also known as the (15;17) translocation. PML-RARα was shown to complex with the PRC2 components SUZ12, EZH2 and EED and recruit them to specific promoters. This study showed a link between PML-RARα and PRC2-driven H3K27 methylation and DNA methylation. Inhibition of PRC2 induced demethylation of PML-RARα target genes, reactivating the promoters and driving granulocytic differentiation.[62,114]

The ability of oncogenic fusion proteins to interact with proteins, which their wild-type constituents may not, can be used to develop new therapeutics. For example, MLL fusion interact with the disruptor of telomere silencing 1-like (DOT1L) protein, a histone methyltransferase that catalyzes the methylation of H3K79, while wild-type MLL does not.[115] This association leads to aberrant recruitment of DOT1L and enhanced H3K79 methylation at MLL fusion protein-directed loci.[74] Loss of DOT1L in MLL-rearranged leukemia cells promotes differentiation and apoptosis as well as the decreased expression of MLL fusion

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targets. Finally, the development and maintenance of *MLL*-rearranged leukemia appears to be dependent on DOT1L *in vivo*, so that DOT1L inhibitors have potential therapeutic promise in this disease.[75,116–118]

One of the challenges in developing new epigenetic therapies is to understand how these pathways act in concert to regulate transcription. Integrating large amounts of genetic data may mean letting go of preconceived notions of mutation classification and protein function. One of the findings of The Cancer Genome Atlas (TCGA) recent characterization of *de novo* AML was the discovery of recurrent mutations in genes that encode components of cohesion and the spliceosome complexes.[1] Mutations in the cohesion complex were found in 13% of *de novo* AML and were predicted to occur in the leukemic initiating clone.[119] Recent work has shown the association of DNMT3B with this complex, possibly providing a link between chromatin condensation and cytosine methylation.[120] Similarly, mutations in genes encoding the spliceosome complex have been found in many myeloid and lymphoid malignancies including 14% of *de novo* AML, 10–15% of CLL and 38% of MDS.[121,122] Interestingly, in MDS patients, these mutations were more likely to co-occur with mutations in epigenetic modifiers, suggesting a possible cross-talk between these two pathways.[123]

Significance of chromatin modifiers in leukemia

An important question is whether these mutations in epigenetic modifiers are truly leukemic drivers and therefore appropriate therapeutic targets. MLL fusion proteins may be sufficient to drive leukemogenesis, as MLL-rearranged leukemias have the fewest number of mutations of any of the known cancers for which TCGA data exist.[1] The identification of *TET2* and *DNMT3A* mutations in a normal elderly population suggests that these mutations may be involved in clonal selection and clonal fitness over time, but they can exist without malignant transformation.[124,125] This enhanced capacity for self-renewal, clonal expansion and skewing toward the myeloid lineage may make individual cells more susceptible to malignant transformation by other genetic alterations. The rate of these mutations in pre-leukemic disorders supports a role for epigenetic modifiers in the early stages of leukemic development. *ASXL1* mutations have been identified in over 30% of patients with refractory anemia with excess blasts and in a similar percentage of AML that evolved from MDS.[126] Interestingly, chronic myelomonocytic leukemia, the disease with the highest rate of epigenetic modifier gene mutations, is a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) rather than an acute leukemia.[28,127,128]

Numerous studies show that introducing epigenetic modifier gene mutations into mouse hematopoietic stem/progenitor cells often confers increased self-renewal, and myeloproliferation with extramedullary hematopoiesis, but not transformation to acute leukemia. Genetic knock out of these genes in mouse models have shown that they are critical for driving HSC self-renewal and differentiation; thus, they contribute to but are not themselves sufficient to cause acute leukemia. Given the remarkable demonstrations of clonal heterogeneity in cancer, the early occurrence of epigenetic mutations suggests that they may be the most relevant therapeutic targets, as they are present in a greater number of leukemic clones than those mutations that occur in the final stages of transformation.

PROGNOSTIC IMPORTANCE OF CHROMATIN MODIFIERS AND THERAPEUTIC INTERVENTION

The discovery of recurrent mutations in chromatin modifiers has led to efforts to correlate these genetic changes with clinical characteristics, and several such mutations do have prognostic significance (Table 1). *TET2* mutations have been associated with adverse overall survival in intermediate risk AML.[29] Similarly, multiple studies have shown that *DNMT3A* mutations confer

Table 1. Clinical significance of chromatin modifiers in leukemia

Mutation	*Clinical association*	*Role in chromatin biology*	*References*
TET2	Mutations found in 7–25% of AML. Mutations and copy-number changes associated with worse prognosis in CN-AML.	Regulates demethylation through the conversion of 5-methylcytosine to 5-hydroxymethyl-cytosine. Mutations result in global DNA hypermethylation.	22,26,27,29,33
IDH1/2	Missense mutations in the active site of the enzyme seen in 6–30% of adult AML. Adverse survival in CN-AML.	Altered enzyme activity leading to the accumulation of 2-HG; associated with aberrant locus-specific hypermethylation.	33–45
DNMT3A	Mutations occur in up to 36% of CN-AML and 16% of adult ETP-ALL. Associated with worse overall survival.	Enzyme involved in *de novo* DNA methylation. Mutations associated with gene expression changes in HoxB cluster.	13–20
ASXL1	Deletions and point mutations in 6–30% of AML patients.	Mutations result in decreased H3K27 methylation and decreased recruitment of PRC2 to target loci.	28,53–55
EZH2	Mutations identified in 2% *de novo* AML.	Important H3K27 methyltransferase that is the enzymatic component of PRC2.	48–52
MLL-FP	MLL-rearrangement occurs in 5–10% of AML. MLL rearrangements observed in 70% of infant ALL. Associated with poor prognosis.	Acquisition of H3K79 methyltransferase activity due to recruitment of Dot1L. Regulates HoxA cluster genes and Meis1 gene expression.	67–73
CBP	Mutations and deletions identified in 18% of relapsed ALL.	Transcriptional co-activator that has histone and non-histone protein acetyltransferase activity.	99,100
NUP98-NSD1	Detected in 5% of pediatric AML patients. Poor prognosis in children and adults.	Thought to upregulate HoxA cluster genes and Meis1 expression through recruitment of CBP/p300 and maintenance of H3K36 methylation.	82–84
ETV6-RUNX1	Translocations occur in 25% of B-precursor ALL. Associated with poor prognosis.	Associated with dominant negative RUNX1 and ETV6 functions, as well as aberrant DNA hypomethylation.	152,153

Abbreviations: ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CN, cytogenetically normal; ETP, early T-cell precursor; PRC, polycomb repressive complex.

adverse risk to intermediate risk AML patients, although this appears to be restricted to the subset of patients with FLT3-ITD mutations.[13,14] *ASXL1* mutations are associated with adverse overall survival in cytogenetically normal-AML or intermediate risk AML, whereas specific *IDH1* mutations have been shown to be associated with favorable outcomes.[29,39] When combined with current cytogenetic and mutations testing, these markers may be useful in risk stratification or treatment selection. For example, genetic profiling of AML patients suggests that those with *DNMT3A* mutations have improved outcome when treated with high dose vs lower dose daunorubicin.[52]

Epigenetic therapeutics

We now know that epigenetic (DNA and chromatin) modifications are in fact, generally reversible, allowing for lineage specific changes in gene expression during differentiation, cell division and DNA repair. Indeed, it is the inherent plasticity of epigenetic modifications that makes them susceptible to pharmacological intervention. Thus, the discovery of recurrent mutations in chromatin modifiers has provided additional insights into the pathogenesis of leukemia, as well as the development of new highly potent and directed epigenetic therapies. A summary of current epigenetic therapies and their relevance to leukemia can be found in Table 2.

DNA methyltransferase inhibitors and histone deacetylase inhibitors were the first epigenetically targeted inhibitors to be FDA approved for the treatment of cancer in the United States. Azacitidine and decitabine are nucleoside analogs and inhibitors of the DNA methyltransferase enzymes DMNT1 and DMNT3. They are thought to act through the gene hypomethylation (for example, of tumor suppressor genes); however, this has never been formally shown. Combinations of these inhibitors are currently being evaluated in clinical trials for AML and MDS. histone deacetylase inhibitors demonstrated modest therapeutic potential in early clinical trials for B-cell lymphoma, myeloma,

Table 2. Epigenetic targeted therapy in leukemia

New and notable epigenetic therapies in leukemia

Class of epigenetic target	*Target of therapeutic*	*Mechanism and biological support*	*References*
Histone acetytransferases (HATs)	p300/CBP	Inhibits cellular proliferation, reduces colony formation and induces apoptosis in AML1-ETO positive AML cell lines and primary blasts. Small-molecule inhibition of CBP/catenin interactions eliminate drug-resistant clones in ALL.	154,155
	MYST family (TIP60, MOZ)	Small molecule inhibition of histone acetyltransferase activity. Knockdown of Tip60 in a CML cell line results in loss of transcriptional repression at c-myb targets.	101–106
Readers of lysine acetylation	Bromodomain-containing proteins (BRD4)	Small molecule inhibitors targeting the acetyl-lysine binding pocket. Efficacy against MLL fusion leukemia cell lines and mouse models through the induction of early cell cycle arrest and apoptosis. In Phase I clinical trials for patients with acute leukemia.	136–139
Histone lysine demethylases	LSD1 (KDM1A)	Disruption of histone demethylase activity. Inhibitors have been shown to induce differentiation in MLL-rearranged leukemias. Inhibitors may be efficacious when combined with ATRA in non-APL patients.	131,132
	JmjC-containing demethylases (UTX, JMJD3, JARID1, KDM2B)	Small molecular inhibitors are competitive for 2-oxoglutarate. A JMJD3/UTX inhibitor reduces proinflammatory cytokine production by macrophages. Depletion of Kdm2b in hematopoietic progenitors impairs Hoxa9/Meis1-induced leukemic transformation.	86,158,159
Metabolic modulators of methylation	IDH1/2	IDH1/2 inhibitors decrease the production of 2-HG, induce demethylation of histone H3K9me3 and increase expression of genes associated with differentiation.	40–45
Histone methyltransferases and associated proteins	Menin/LEDGF	Small molecule inhibitors that target the Menin–MLL interaction developed for MLL rearranged leukemias. Inhibitor induces growth arrest and inhibits transformation in MLL-transduced bone marrow cells. Small peptides disrupting the LEDGF-MLL interaction show increased disease latency in an MLL-AF9 leukemia model.	77–81
	Dot1L	Selective for MLL-rearranged acute leukemia cell lines. Inhibited H3K79 methylation and MLL-fusion target gene expression.	74–76,115–118
	EZH2	Small molecules that disrupt the methyltransferase activity of PRC2. Peptides have been developed that disrupt the EZH2–EED protein interactions. MLL-AF9 leukemia cells treated with inhibitor undergo growth arrest and myeloid differentiation.	133–135
	G9a	Small molecular inhibitors targeting the histone peptide binding pocket G9a inhibition resulted in repression of JAK2 in a CML cell line.	160,161
	Arginine methyltransferses (PRMTs)	Knockdown of PRMT1 suppresses the self-renewal capability of AE9a cells. Downregulation of PRMT4 promotes myeloid differentiation in leukemia cells and prolongs survival in a leukemia transplantation model.	162–164

Abbreviations: ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; ATRA, all trans-retinoic acid.

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myelodysplastic syndrome and AML. They are currently FDA approved only for the treatment of cutaneous T-cell lymphoma. Despite extensive research, we still do not fully understand the mechanism of action of these therapeutics.

The discovery of recurrent IDH1/2 mutations has led to the development of small molecule inhibitors that aim to target the mutant enzymes. These agents are being evaluated in preclinical studies for their use in patients with glioma or AML. In cell lines, a selective IDH1-R132H inhibitor decreased the production of R-2-hydroxyglutarate (R-2HG), induced demethylation of histone H3K9me3 and increased expression of genes associated with differentiation.[129] A small molecule inhibitor specific for IDH2-R140Q induced the differentiation of an erythroleukemia cell line and also human acute leukemia cells.[130] Similarly, inhibition of mutant IDH1 decreased 2-HG production, induced apoptosis in murine cells and inhibited the proliferation of progenitor cells obtained from AML patients with *IDH1* mutations.[43]

The high frequency of mutations in components (or regulators) of the PRC2 complex in myeloid and lymphoid malignancies, and the resulting changes in the level of H3K27me3, has sparked interest in the lysine demethylases as therapeutic targets. Small molecule catalytic site inhibitors are being developed for this family of proteins and show selectivity for these enzymes. Inhibition of LSD1, by RNAi or small molecules, has recently been shown to induce the differentiation of *MLL*-rearranged leukemias. In addition, there is evidence that LSD1 inhibitors may be efficacious in non-APL patients when combined with all trans-retinoic acid (ATRA).[131] This could make a currently FDA-approved therapy (ATRA) more applicable to this larger number of patients.[132] Potent inhibitors of the JmjC family of histone demethylases, which includes UTX, JMJD3 and JARID1, are being developed for use in cancer, and potent and selective inhibitors of EZH2 have been developed for use in lymphoma and possibly leukemia as well.[133,134] Another strategy has been used to develop peptides, which disrupt EZH2–EED protein interactions, and these show efficacy in MLL-AF9-expressing leukemia cells.[135]

Another level of transcriptional regulation is provided by the proteins that recognize specific histone residues based on post-translational modifications, known as chromatin 'readers'. The bromodomain-containing proteins are responsible for recognizing acetylated lysine residues on histone tails. Quite unexpectedly, MLL fusion protein-driven AML is sensitive to JQ1, a BRD4 inhibitor, and MYC-driven malignancies are also quite sensitive to such bromodomain inhibitors *in vitro* and in animal models.[136,137] Several bromodomain-containing proteins are amenable to small molecule inhibition and demonstrate therapeutic efficacy in preclinical models of AML.[138] Bromodomain inhibitor treatment of B-ALL cell lines decreased their viability and induced the loss of BRD4 at the *MYC* promoter, causing downregulation of *MYC* transcription and the reduced expression of c-Myc target genes.[139] A Phase I study of a BRD2/3/4 inhibitor in patients with hematologic malignancy is ongoing (NCT01713582). Inhibitors of other 'epigenetic readers' such as the chromodomain-containing proteins that recognize methyl-lysine are also being explored.[140]

Challenges for epigenetic-targeted therapy

A major challenge in the development of more effective epigenetic therapies is the lack of biomarkers to evaluate efficacy in a clinical setting. In contrast to the pharmacodynamics of receptor tyrosine inhibitors or chemotherapeutics, the effects of epigenetic therapies often take a long time to observe. Clinical trials using hypomethylating agents have suggested that maximal DNA hypomethylation may occur more than a week after their administration, while effects on proliferation, differentiation and cell survival may not occur for weeks.[141–143] Therefore, established measures of clinical response may be unhelpful in evaluating the mechanism of action of these compounds. There has been an effort to correlate response with the methylation status of specific genes, changes in gene expression or levels of microRNA; however, the connection between these biomarkers and efficacy is generally weak.[144–146] Although methylation of tumor suppressor genes decrease in some patients, global methylation changes are not consistently associated with changes in gene expression or clinical responses. Also, there is a need to understand why certain subsets of patients fail to respond to treatment.[147]

There is also a need to understand more about the biology of these large multiprotein chromatin-modifying complexes and the mechanism of action of the inhibitors. The function of these complexes may be cell context dependent, hinging on the underlying genetic landscape of the cell and the available interacting proteins. Mutations in the methyltransferase EZH2 demonstrate how both gain-of-function and loss-of-function mutations can lead to lead to aberrant transcription and proliferation, depending on the cellular context. Chromatin-modifying enzymes may have much more diverse roles than previously thought, as many histone methyltransferases are now known to act on non-histone substrates. Despite these limitations in our understanding, chromatin-modifying protein inhibitors will be an important tool to help define these functions and develop more effective therapeutic strategies.

Although leukemia cells have relatively few mutations compared with solid tumors, it is rare for cancer to be driven by a single genetic or epigenetic mutation. In solid tumors, targeting of an oncogenic driver often leads to activation of the same or distant oncogenic pathways that allow the tumor to escape treatment. To date, most preclinical studies of chromatin modifier inhibitors have focused on *MLL*-rearranged leukemia, which is highly dependent on fusion protein-dependent effects for survival. To make 'epigenetic-modifying therapy' more applicable to patients, we will need to consider how chromatin-modifying mutations fit into the larger genetic landscape, which include changes in signaling pathways. Epigenetic therapy might lead to resistance through epigenetic or non-epigenetic mechanisms. For example, clinical trials of DNMT inhibitors in MDS and CML have shown that cells present at the time of relapse have less methylation than those present before treatment, suggesting methylation-independent clonal evolution. Hopefully, there will be successes in combining epigenetic modifying therapies with other classes of inhibitors. Histone deacetylase inhibitors have shown promising preclinical activity in combination with chemotherapeutic drugs, inhibitors of heat-shock proteins, proteasome proteins and tyrosine kinases.[148–150] It is also encouraging that the first studies of the inhibitors of epigenetic modifiers have demonstrated unexpected effects on previously identified leukemic drivers. For example, the ability of bromodomain inhibitors to target critical oncogenes, such as c-Myc or Bcl-2, is promising and somewhat unexpected.[151–154] Although the biology of epigenetic regulation is complex, novel epigenetic therapies show tremendous potential for improving our therapy of acute leukemia and other related diseases.[155–157]

SUMMARY

The recent completion of the Cancer Genome Atlas Research Network's analysis of adult *de novo* AML has highlighted the importance of mutations in chromatin modifiers. Although individual mutations in chromatin modifiers do not appear to be sufficient to induce leukemia in mouse models, the changes in self-renewal and differentiation they mediate are critical steps in the transformation of hematopoietic stem/progenitor cells. Moreover, it is becoming increasingly clear that the interaction of transcription factors with epigenetic modifiers is critical to their oncogenic activity. When these interactions are inhibited in mouse models, leukemic precursors lose the ability to differentiate,

self-renew and propagate in recipient mice. The challenge for the future will be to translate this knowledge into the development of new, combination therapies, targeting leukemic driver mutations and their dependence on chromatin-modifying enzymes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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