Epigenetics in the hematologic malignancies

Chun Yew Fong,[1,2] Jessica Morison,[1] and Mark A. Dawson[1,2]

[1]Cancer Epigenetics Laboratory, Peter MacCallum Cancer Centre, East Melbourne; and [2]The Sir Peter MacCallum Department of Oncology, University of Melbourne, Parkville, Australia

ABSTRACT

A wealth of genomic and epigenomic data has identified abnormal regulation of epigenetic processes as a prominent theme in hematologic malignancies. Recurrent somatic alterations in myeloid malignancies of key proteins involved in DNA methylation, post-translational histone modification and chromatin remodeling have highlighted the importance of epigenetic regulation of gene expression in the initiation and maintenance of various malignancies. The rational use of targeted epigenetic therapies requires a thorough understanding of the underlying mechanisms of malignant transformation driven by aberrant epigenetic regulators. In this review we provide an overview of the major protagonists in epigenetic regulation, their aberrant role in myeloid malignancies, prognostic significance and potential for therapeutic targeting.

Introduction

Our expanding knowledge of the genome and epigenome in cancer cells has highlighted the central role that aberrant epigenetic regulation plays in the pathogenesis of many hematological malignancies.[1-4] The unparalleled view of the epigenetic landscape provided by new technologies affords us the opportunity to gain insights into key pathways and nodes of epigenetic regulation, further enhancing our ability to deliver effective novel compounds to clinical practice. In this review, we provide an overview of the major pathways involved in epigenetic regulation, their aberrant role in myeloid malignancies, prognostic significance and potential for therapeutic targeting.

DNA methylation

The regulation and maintenance of DNA methylation is essential for appropriate embryonic development, cellular differentiation and genome stability. In eukaryotes, the catalytic activity of a family of enzymes known as DNA methyltransferases (DNMTs) results in the addition of a methyl group to the five-carbon position of cytosine bases in CpG dinucleotides, yielding 5-methylcytosine (5mC).

DNA methylation has traditionally been thought to mediate transcriptional silencing and the formation of repressive chromatin states in addition to maintaining gene expression patterns through mitotic cell division.[5,6] These functions are achieved through a variety of mechanisms including the direct obstruction of transcriptional activators from their cognate promoters and the recognition of 5mC and consequent recruitment of co-repressor complexes by methyl CpG binding proteins (MBP).

Disruption of methylation profiles and genome wide loss of epigenetic stability is observed in malignant transformation. Thus far, research delineating focal methylation changes in malignancy has centered upon hypermethylation of short stretches (0.5-4 kb) of CpG rich DNA termed CpG islands.[7,8] Although aberrant hypermethylation and silencing of tumor suppressor genes has been found in almost all forms of cancer, both hypomethylation and hypermethylation of promoter CpG islands can affect the expression of protein coding genes and non-coding RNAs resulting in tumorigenesis.[9-12] These changes are highly disease specific with distinctive methylation patterns able to distinguish between hematologic malignancies and even subtypes of these malignancies.[13]

Whilst hypermethylation of CpG islands is one of the best studied epigenetic features in malignant cells, it is becoming increasingly apparent that alteration in DNA methylation outside the context of CpG islands may have an equal if not more important role in the initiation and/or maintenance of the malignant phenotype. Intriguingly, the recent identification of tissue-specific differentially methylated regions (DMRs) outside of CpG islands, which control differentiation and pluripotency, has focused attention on the role of methylation in CpG island shores and exon bodies.[14-16] Although poorly understood, DMRs in intragenic regions are postulated to control gene expression via a number of mechanisms including regulation of transcriptional elongation efficiency,[17] determination of alternative polyA sites,[18] tissue-specific selection of alternative promoters[19] and regulation of pre-mRNA splicing.[20] In particular, CpG island shores (regions 2kb either side of a CpG island) in malignant cells demonstrate striking variation in DNA methylation and loss of the normally sharp demarcation of methylation state between CpG islands and shores.[21] This hypervariability is associated with aberrant gene expression and potentially gain of epigenetic plasticity that imparts a survival advantage to malignant cells.[22]

DNA methyltransferase enzymes

The establishment and maintenance of DNA methylation is mediated by three main DNMT enzymes. *DNMT1*, a maintenance methyltrasferase, recognizes hemimethylated CpG sites and restores symmetry to newly synthesized nucleotides following DNA replication.[23] Whilst also capable of mainte-

 doi:10.3324/haematol.2013.092007
Manuscript received on April 7, 2014. Manuscript accepted on September 3, 2014.
Correspondence: Mark.Dawson@petermac.org

nance,[24] *DNMT3A* and *DMNT3B* function primarily in *de novo* methylation during embryogenesis.[25] Initial investigations demonstrated abnormalities in the expression of DNMTs in a range of solid organ malignancies.[26,27] However, it is the finding of *DNMT3A* mutations in acute myeloid leukemia (AML) that has generated significant interest in the hematology community.

Using emerging high throughput DNA sequencing techniques, recurrent *DNMT3A* mutations were identified in approximately 20% of patients with AML.[28-30] *DNMT3A* mutations are enriched in cytogenetically normal, intermediate risk AML and commonly co-occur with mutations in Fms-Related Tyrosine Kinase 3 (*FLT3*), Nucleophosmin 1 (*NPM1*) and isocitrate dehydrogenase (*IDH*) 1/2. Although associated with poorer outcomes overall, modulation of this association through tailoring of conventional chemotherapeutic regimens with the addition of high-dose daunorubicin results in improvement of overall survival (OS).[3,31,32] The impact of *DNMT3A* mutations in AML on sensitivity to hypomethylating agents is unclear. Retrospective examination of small cohorts treated with varying regimens suggests that hypomethylating agents abrogate the negative impact of *DNMT3A* mutations but further elucidation in prospective clinical trials is warranted.[33,34]

Somatic mutations either cause premature truncation of the protein or affect a single amino acid, R882, resulting in attenuation of enzymatic activity. Intriguingly, heterozygous mutations are most common with recent data demonstrating that R882 *DNMT3A* mutations have a dominant negative effect through inhibition of DNMT3A oligomerization.[35-37] *DNMT3A* mutations have also been identified in patients with myelodysplastic syndromes (MDS)[38] and myeloproliferative neoplasms (MPN),[39] and are associated with increased likelihood of progression to AML. Indeed, in some studies, the same *DNMT3A* mutation as the antecedent hematologic disorder is identified in secondary AML, suggesting that these mutations may be an early event in malignant clonal evolution.[40] This is consistent with findings in mice where loss of DNMT3A activity in hematopoietic stem cells leads to a block in differentiation and an expansion of the stem cell pool without overt leukemia.[41] These observations are further reinforced by recent findings demonstrating that recurrent *DNMT3A* mutations are frequently present in a pool of clonal pre-leukemic hematopoietic stem cells (HSCs) from which AML develops.[42] These HSCs have a competitive multi-lineage repopulation advantage over wild-type HSCs, and furthermore, are demonstrated to persist following chemotherapy thereby acting as a reservoir for therapeutic resistance.[42] However, the role of *DNMT3A* in malignant transformation is yet to be fully elucidated. Analysis of global methylation levels by liquid chromatography-tandem mass spectrometry (LC-MS) does not demonstrate a significant difference in *DNMT3A* mutant leukemic cells. In addition, although differential methylation of key promoter CpG islands is observed, there is a lack of correlation between methylation changes and differential gene expression.[28,30]

DNA methylation as a therapeutic target in myeloid malignancies

Emerging therapeutic strategies targeting epigenetic mechanisms of disease have shown significant promise with the establishment of DNMT inhibitors as a cornerstone of management in MDS.[43-45] DNMT inhibitors such as 5-azacitadine and 5-aza-2'-deoxycytidine are nucleoside analogs that covalently trap DNMT1 following incorporation into DNA resulting in genome-wide hypomethylation through passive dilution of 5mC.

The hypomethylating effects of these agents are at non-cytotoxic dose ranges limiting the severity of side effects. Interestingly, the effectiveness of these agents is not reliant in attaining a complete remission, with improved survival, transfusion independence and reduced hospitalization observed despite persistent disease.[46] Further development of the treatment paradigm has suggested that less toxic regimens (lower doses with more frequent dosing) and the use of maintenance DMNT inhibitors as adjunct therapy or in combination with other novel therapies such as lenalidomide may be effective in subsets of patients with high-risk MDS/AML.[47-49]

DNA hydroxy-methylation and the TET enzymes

Though DNA methylation was initially believed to be a relatively stable DNA modification, genome-wide high resolution mapping of 5mC during cellular differentiation and the recent identification of the Ten-Eleven-Translocation (TET) enzymes has revealed a more dynamic state of affairs.[15,50,51] The three TET enzymes (TET1-3) are α-ketoglutarate (α-KG) and Fe^{2+}-dependent dioxygenase enzymes, which catalyze the successive oxidation of 5mC to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine and 5-carboxycytosine.[50-53]

Although the exact function of the 5mC derivatives is yet to be fully established, it is evident that they play an important role in transcriptional regulation. They have been shown to act as essential intermediates in both active and passive DNA demethylation, to modulate the binding and recruitment of chromatin regulators including the polycomb repressive complexes (PRC), and are involved in in the reversal of transcriptional silencing.[54] Additionally, mapping of 5hmC in mouse embryonic stem cells has highlighted its role in the establishment and maintenance of pluripotency through context-dependent promoter hypomethylation of pluripotency factors or modulation of PRC recruitment.[55]

Mutations of *TET2* in myeloid malignancies were first described in MDS and MPN through single nucleotide polymorphism (SNP) arrays identifying a minimally deleted region on chromosome 4q24.[56,57] Subsequently, *TET2* has been shown to be mutated in myeloid malignancies including AML, MDS and MPN with a high proportion of patients with MDS and chronic myelomonocytic leukemia (CMML) harboring mutations.[58,59] *TET2* mutations are enriched in patients presenting with a normal karyotype, is associated with poorer OS in AML and CMML but is not predictive regarding clinical outcome in MDS and MPN.[2,59-61] Although *TET2* mutations do not have a strong predictive correlation with clinical outcome in MDS, *TET2* mutations may independently act as a biomarker for response to hypomethylating agents.[62]

α-ketoglutarate and the link between metabolism and epigenetics

Recurrent somatic mutation of the cytosolic enzyme *IDH1*, or its mitochondrial homolog *IDH2*, have been identified in approximately 20% of AML genomes and less commonly in other hematologic malignancies.[1,63] These abnormalities, in a core cellular metabolic pathway, are

associated with specific epigenetic signatures.[64] IDH1 and IDH2 normally catalyze the conversion of isocitrate to α-KG. However, the most common *IDH1* (R132) and *IDH2* (R140, R172) mutations result in acquisition of neomorphic enzymatic activity that generates high intracellular concentrations of the aberrant oncometabolite 2-hydroxyglutate (2-HG).[65,66] 2-HG, a structural analog of α-KG, results in competitive inhibition of Fe^{2+} and α-KG dependent demethylases including the TET enzymes and JmjC-domain containing lysine demethylases (KDMs).[67] Inhibition results in aberrant DNA and histone methylation, altered gene expression and impaired lineage specific differentiation.[67,68] Consistent with a common role in AML pathogenesis, *IDH1/2* and *TET2* mutations are mutually exclusive but associated with overlapping specific hypermethylation signatures.[64] Mice expressing the *IDH2* mutations demonstrate an expansion of hematopoietic stem and progenitor cells. Interestingly, these models were also used to show co-operation with clinically relevant mutations such as *FLT3-ITD* in the development of AML.[69,70]

The overall effect of *IDH1/2* mutations on clinical outcome in AML is still unclear, as the prognostic impact appears to be dependent on the mutant allele present in the context of other co-existing molecular abnormalities. Multiple studies have identified *NPM1* mutations and an intermediate karyotype as significant associations.[71-75] No consistent independent association with overall or event-free survival is observed.[71,72] However, *IDH1/2* mutations are demonstrated to modulate the outcome of patients defined as molecular low-risk (*NPM1*-mutant/*FLT3-ITD* negative) where *IDH1-R132* and *IDH2-R172* mutant alleles are associated with impaired outcome and *IDH2-R140* mutations are associated with favorable outcomes.[73-75] Recently, a number of novel small molecule inhibitors targeting the aberrant gain-of-function consequent to mutant IDH alleles have demonstrated promising specific *in vitro* potency through induction of differentiation and apoptosis in IDH mutant leukemia cell lines.[76,77] This has led to the initiation of early phase clinical trials targeting specific IDH1/2 mutants (Table 1).

Histone modifications

The post-translational modification of histone tails by chromatin modifying enzymes has significant impact on intra- and inter-nucleosomal interactions. A considerable number of histone residues can be modified and the diversity of modifications result in highly complex and orchestrated chromatin environments that are dynamically altered in specific cellular contexts. These modifications not only have the ability to regulate the binding of effector molecules essential to DNA processes including transcription, repair and replication, but also the ability to regulate higher order chromatin structure and stability.[78] Therefore it is not surprising that many chromatin modifying enzymes are deranged during malignant transformation.

Chromatin-modifying enzymes are often found in multi-protein complexes, which serve to modulate substrate specificity, enzyme activity and recruitment to target loci. Further layers of complexity and control are introduced through crosstalk between different histone and DNA modifications. In this situation, one modification may influence the deposition, removal or interpretation of another chromatin modification on a separate site. This may occur through the obstruction of binding to target substrates by the presence of an adjacent modification, competitive antagonism of modification pathways for the same substrate, dependence of a chromatin-modifying enzyme on the presence of another modification, or co-operation between modifications to recruit specific factors.[79]

Critical protein-protein interactions and essential co-factors for enzymatic activity have been identified as viable therapeutic targets and demonstrate significant promise in the treatment of malignancies arising from abnormalities in epigenetic regulation.[80] Although much progress has been made in demystifying the 'epigenetic landscape', the mechanisms by which histone modifications and chromatin-modifying enzymes exert their influence still has to be fully elucidated. We present a summary of the key histone modifications and the chromatin-modifying enzymes responsible for writing, reading and erasing them (Figure 1).

Acetylation

Histone acetylation, one of the best studied histone modifications, is dynamically controlled by two opposing families of enzymes: lysine acetyltransferases (KATs) and histone deacetylases (HDACs).[81]

The catalytic activity of KATs result in transfer of an acetyl group from the common co-factor acetyl CoA to the ε-amino group of lysine side chains in histones.[82] Consequent neutralization of the positive charge weakens interactions between histones and negatively-charged DNA. This results in open chromatin conformations thereby facilitating access of chromatin-associated proteins and is functionally consistent with the identification of KATs as transcriptional co-activators. KATs are subdivided on the basis of intracellular localization into predominantly nuclear (type A) or cytoplasmic (type B) subtypes. Enzymes found in the CBP/p300, MYST and GNAT families are type A KATs.

Recurrent mutations in *CBP* and *p300* are noted in a range of hematologic malignancies, especially the lymphoid neo-

Table 1. Current development of targeted epigenetic therapies.

	Target enzyme	Disease type	Current stage of development
Writers			
Acetylation	CBP/p300	AML, Ovarian, Colon, Melanoma	Pre-clinical
	PCAF	Ovarian, Colon	Pre-clinical
Methylation	DOT1L	MLL-r leukaemia	Clinical
	EZH2	NHL, advanced solid tumors	Clinical
Phosphorylation	JAK2	MPN	FDA approved
	Aurora kinase	NHL, CML, ALL	Clinical
Erasers			
Acetylation	HDACi	CTCL	FDA approved
Methylation	LSD1/KDM1A	AML	Clinical
	UTX/JMJD3	Inflammatory response	Pre-clinical
Readers			
Acetylation	BET	Haematological malignancies, NUT midline carcinoma	Clinical
DNA Methylation			
	DNMT	MDS	FDA approved
	IDH inhibitors	AML, glioblastoma	Clinical

MLL-r: mixed lineage leukemia rearranged; NHL: non-Hodgkin lymphoma; MPN: myeloproliferative neoplasms; CML: chronic myeloid leukemia; ALL: acute lymphoblastic leukemia; CTCL: cutaneous T-cell lymphoma; AML: acute myeloid leukemia; MDS: myelodysplastic syndrome.

plasms.[83,84] Similarly, chromosomal translocations involving KATs (e.g. *MLL-CBP*[85,86] and *MOZ-TIF2*[87]) are found in myeloid malignancies. In particular, the KAT domain and bromodomain of CBP were demonstrated to be essential for leukemic transformation following an initial myeloproliferative phase in murine models of MLL-CBP leukemia.[88] Similarly, the MOZ-TIF2 fusion protein is sufficient for leukemic transformation through its ability to bind nucleosomes and recruit CBP to aberrant sites, resulting in the activation of a self-renewal program and the acquisition of stem cell properties.[89,90]

The acetyltransferase activity of KATs is not limited to histone substrates and can regulate protein-protein interactions and the activity of target non-histone proteins. For example, acetylation of the leukemic fusion protein AML1-ETO by KAT3B (p300) has been demonstrated to be essential for conferring self-renewal ability and leukemogenicity. Pharmacological inhibition of KAT3B leads to improved survival in a murine AML1-ETO model.[91]

In general, therapeutic targeting of KATs has thus far been hampered by their low substrate specificity and broad involvement in multi-protein complexes that define their molecular activity. Interestingly, a recent structure based *in-silico* approach has identified a commercially available, small molecule p300/CBP inhibitor, C646.[92] C646 resulted in selective *in vitro* inhibition of primary human AML bearing the AML1-ETO translocation through cell cycle arrest and apoptosis. This was associated with a dose-dependent reduction in global histone H3 acetylation and decreased expression of c-kit and bcl-2.[93]

HDACs reverse lysine acetylation restoring the positive charge and, consistent with their predominant role as transcriptional repressors, result in the stabilization of local chromatin architecture.[94] Eighteen human iso-enzymes of HDACs have been identified and are grouped into four classes on the basis of sequence homology. Similar to KATs, HDACs can target both histone and non-histone proteins with substrate specificity determined by the members of component protein complexes.[79]

Notably, recurrent mutations of HDAC's are not observed in cancer genomes yet HDAC inhibitors have broadly been trialed in a range of malignancies. This is primarily because they are aberrantly recruited by various oncoproteins to inappropriately initiate or maintain malignant gene expression programs. For instance, the leukemic fusion proteins PML-RARα and PZLF-RARα have been shown to recruit HDAC containing repressor complexes resulting in aberrant gene silencing.[95-98] In murine models of APML, the use of HDAC inhibitors (HDACi) is effective in potentiating or restoring the retinoid-induced differentia-

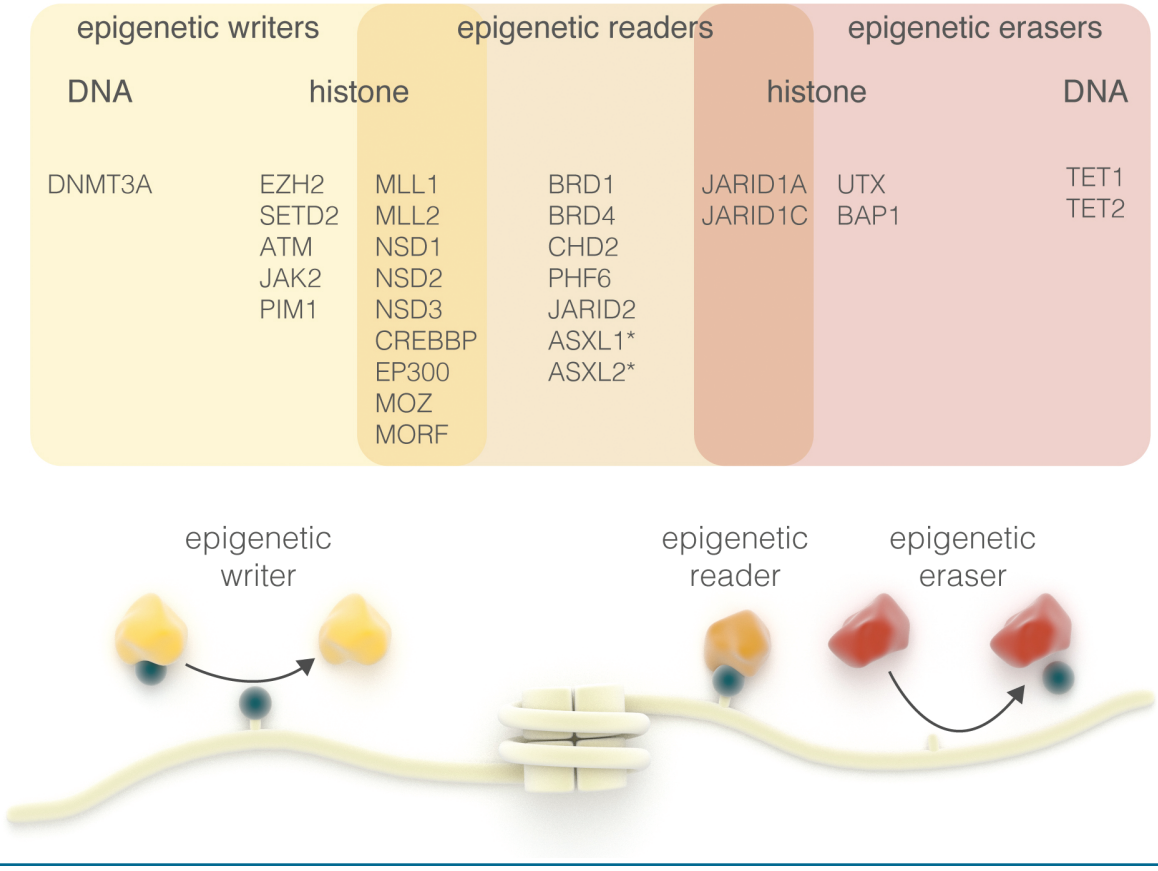

Figure 1. Epigenetic writers, readers and erasers mutated or translocated in hematologic malignancies. Epigenetic writers catalyze the chemical modifications of amino acids on histones or the cytosine base of DNA. Epigenetic erasers catalyze the removal of these modifications and epigenetic readers recognize these modifications and recruit larger macromolecular complexes to the chromatin template. A number of epigenetic writer and erasers also have domains that allow them to function as epigenetic readers (highlighted in the overlap shaded areas). *ASXL1 and ASXL2 have a PHD domain that may allow them to function as epigenetic readers; however, there is still no conclusive evidence for this.

tion of retinoic acid sensitive and resistant tumors resulting in improved survival.[99]

The efficacy of HDACi in the treatment of cutaneous T-cell lymphoma has been established. However, the broader application of this class of therapies in other hematologic malignancies is yet to be clinically proven.[94] HDACi predominantly function by specifically blocking the entry of required co-factors to the active site.[100] A myriad of cellular responses, including modulation of pathways involved in cell cycle progression, differentiation, angiogenesis, immune function and apoptosis, result in malignant cell death. Although initially regarded as straightforward activators of transcription through direct histone hyperacetylation, a greater appreciation of the non-histone effects of HDACi on proteins such as p53 and key members of the proteasome/aggresome pathways, HSP90 and tubulin have emerged.[101] Indeed, recent mechanistic insight into the anti-leukemic activity of HDACi in t(8;21) AML demonstrates the induction of terminal myeloid differentiation following HDACi mediated proteasomal degradation of the AML1/ETO9a fusion protein.[102]

Acetylation of lysine residues is primarily recognized by protein-binding motifs named bromodomains. Over 40 bromodomain containing proteins in eight subfamilies with functionally diverse roles such as chromatin remodeling, post-translational histone modification and transcriptional co-activation have been identified. The activity of bromodomain containing proteins is not limited to histone targets with binding to non-histone targets such as the NF-κB subunit RelA and GATA1 described.[103,104] Whilst critical residues required for the recognition of acetylated lysines within the hydrophobic binding pocket of bromodomains are highly conserved, considerable variation of residues at the opening of the pocket allows for variability in the specificity of individual bromodomains. This also provides the opportunity to develop specific small molecule inhibitors targeting certain families of bromodomains.

For example, highly specific small molecule inhibitors targeting the protein-protein interactions of the Bromodomain and Extra Terminal (BET) proteins (BRD2, BRD3, BRD4 and BRDt) have emerged as promising therapeutic avenues in inflammation and cancer.[80,105,106] BET proteins are a family of chromatin readers containing tandem N-terminus bromodomains and an extra-terminal domain at their C-terminus. Whilst the BET proteins do not possess enzymatic activity at chromatin, following bromodomain-mediated localization to acetylated histones, the extra-terminal domain acts as a scaffold for the recruitment of general transcription factors or chromatin-modifying enzymes.

Indeed, determination of the complete BET protein interactome, utilizing a novel tripartite proteomic approach, prominently identifies components of core transcriptional regulatory machinery, in particular, the polymerase-associated factor complex (PAFc) and super elongation complex (SEC),[80] which are essential to the pathogenesis of the most common mixed lineage leukemia (MLL) translocated leukemias (*see below*). Pharmacological BET inhibition shows remarkable efficacy *in vitro* and *in vivo* against MLL fusion leukemia through rapid induction of cell cycle arrest and apoptosis.[80,107]

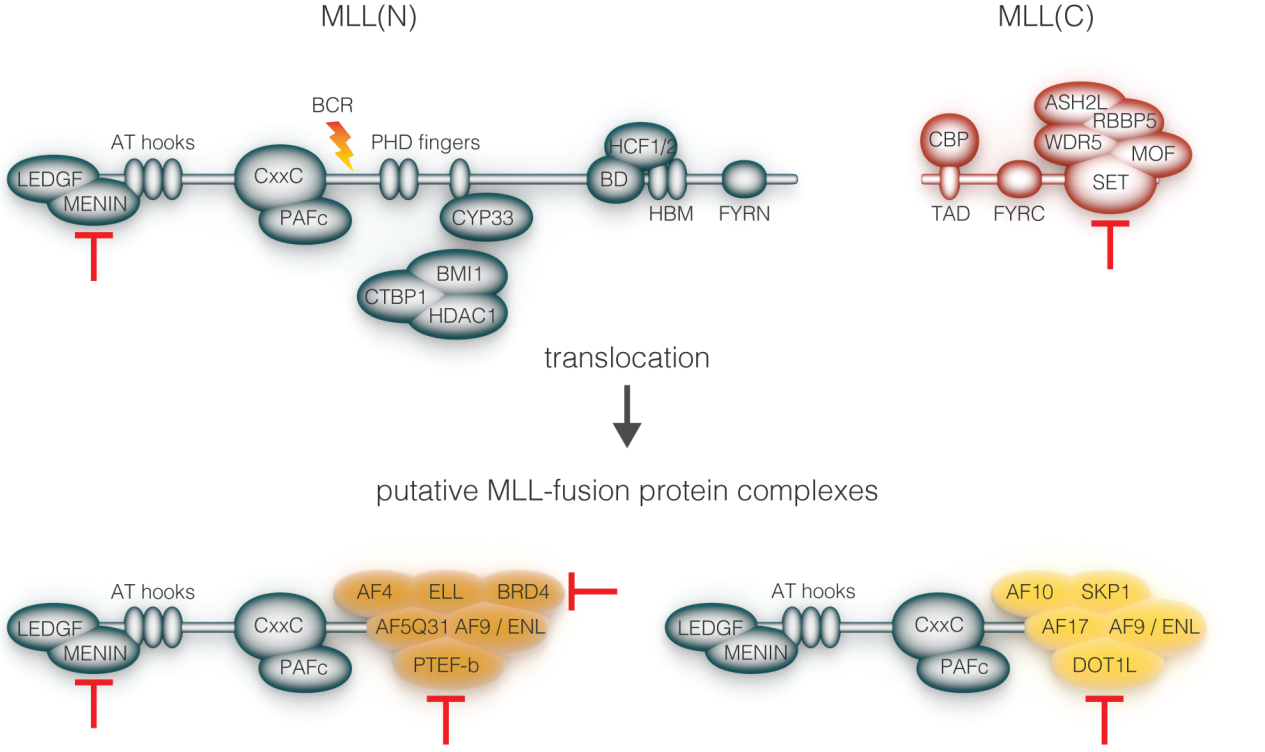

Figure 2. MLL fusion proteins as targets for small molecule inhibition. Schematic diagram of wild-type MLL illustrating the various specialized domains and the protein-protein interactions mediated by them. Also illustrated are the purported MLL-fusion protein complexes. Following translocation, a fragment of the N-terminal portion of MLL is fused in frame with a translocation partner leading to the formation of novel MLL-fusion protein complexes including the SEC and DOT1L complex. It is unclear whether these are separate entities or co-exist as one large complex. Highlighted are various small molecules that have been developed to target the leukemogenic capacity of either wild-type MLL or MLL-fusion proteins. BCR: breakpoint cluster region; HBM: host cell factor binding motif; TAD: transactivation domain.

Broader extension of pharmacological BET inhibition to other genetically distinct AML subgroups results in the identification of a core transcriptional program including critical oncogenic targets such as *BCL2* and *C-MYC*. This suggests a role for BET proteins as a common terminal effector of malignant transcription and is supported by the efficacy of BET inhibition in NPM1c mutant leukemia.[108] Originally identified in the BET interactome, wild-type NPM1 is demonstrated to exert a repressive effect on BRD4 binding to target loci resulting in decreased transcription. Loss of inhibition resulting from mislocalization of NPM1c, consequent to gain of an aberrant NPM1 nuclear export signal, results in release of BRD4 repression and activation of aberrant transcription.[108]

Downregulation of the core transcriptional program underlies sensitivity to BET inhibition in AML and may serve as biomarkers of response to BET inhibitors. Interestingly, many of the genes identified are associated with super-enhancers, large enhancer regions containing high levels of BRD4 and mediator that are exquisitely sensitive to BET inhibition.[109]

The efficacy of BET inhibition has been replicated in a broad range of hematologic malignancies including multiple myeloma,[110] non-Hodgkin lymphoma[111] and ALL.[112] These serve as proof of principle for epigenetic targeted therapies directed against protein-protein interactions, and have formed the basis for the initiation of early phase clinical trials.

Methylation

Histone methylation occurs predominantly on lysine and arginine residues and is mediated by lysine methyltransferases (KMTs) and protein arginine methyltransferases (PRMTs).[113] Lysine residues can be mono-, di- or tri- methylated whereas arginine can be mono-, symmetrically or asymmetrically di-methylated. Histone methylation does not alter the charge on histone tails but influences the affinity of reader proteins to methylated residues. KMTs and PRMTs are highly substrate specific and transfer methyl groups from S-adenosyl methionine (SAM) to target amino acid residues.

The vast majority of KMTs contain a conserved SET catalytic domain, a sequence of approximately 130 amino acids initially characterized as a common motif in drosophila Suppressor of position-effect variegation [Su(var)], Enhancer of Zeste [E(z)] and Trithorax genes. The only exception is the catalytic domain of the H3K79 methyltransferase, KMT4/DOT1L (disruptor of telomeric silencing 1-like), which more closely resembles that of PRMTs. The degree of lysine modification is determined by key residues within the SET domain.[114] In addition to the SET domain, KMTs have I-SET, pre-SET and post-SET domains which vary in sequence and are present in different combinations. These domains serve as a scaffold for substrate and co-factor interaction and determine substrate specificity.[115]

The functional impact of histone methylation is contextual and can lead to both transcriptional activation and repression. The best-characterized sites of histone lysine methylation include H3K4, H3K9, H3K27, H3K36, H3K79 and H4K20. These modifications are associated with both actively transcribed genes in euchromatin (H3K4, H3K36 and H3K79) and silenced genes in heterochromatin (H3K9, H3K27 and H4K20).[116] Adding to the complexity, the methylation state of individual histone residues also influences functional relevance. For example, monomethylation of H3K9 is associated with active transcription whereas trimethylation is associated with repression[116] and, whilst H3K4me2/3 is associated with TSS of active genes,[117] H3K4me1 is associated with active enhancers.[118] Furthermore, although H3K79me has been predominantly associated with actively transcribed genes, the functional role of this modification in negative regulatory contexts has been highlighted.[119]

Aberrant methyltransferase activity resulting in alterations to the location and amplitude of histone methylation can play a critical role in malignant transformation. Key examples in hematologic malignancies include abnormalities in the *MLL* and enhancer of zeste homolog 2 (*EZH2*) genes. Abnormalities in both result in the misappropriation of key components of gene regulatory machinery.

MLL leukemia as a model for therapeutic targeting of disordered epigenetic regulation

Wild-type MLL (WT-MLL) plays an integral role in normal embryogenesis and hematopoiesis.[120] It is a 430 kDa protein post-translationally cleaved into N-terminal and C-terminal fragments which re-associate to form the MLL complex.[121] The C-terminal fragment contains a SET domain, which methylates H3K4. WT-MLL also has 3 HMG-like AT hooks that bind AT rich DNA; a CxxC domain, four Plant Homeo-Domain (PHD) fingers, a bromodomain, host cell factor binding motif and transactivation domain mediate interactions with several protein complexes (Figure 2A).

Translocations involving this essential epigenetic regulator account for the vast majority of infantile and approximately 10% of adult leukemias.[122] MLL leukemias follow an aggressive clinical course with poor response to conventional chemotherapy and frequent early relapse. The breakpoint cluster region in virtually all *MLL* translocations is located between the CxxC domain and the PHD fingers resulting in fusion proteins that lack the SET domain.[123] More than 70 MLL translocation partners have been identified, many of which are members of multi-subunit protein complexes that alter the structure and function of chromatin.[124] The 5 most common MLL fusion partners (*AFF1/AF4, MLLT3/AF9, MLLT1/ENL, MLLT10/AF10* and *ELL*, accounting for approx. 80% of MLL rearrangements) are components of the SEC or DOT1L complex (Figure 2B). These complexes, in association with the PAFc, play a central role in the regulation of transcriptional elongation.[125,126]

The functional integrity of the SEC and DOT1Lc are critical for MLL-FP mediated malignant transformation and offers a rational target for epigenetic therapies with compounds directed against various components of these complexes.[127-129] In addition to the BET proteins as described earlier, attention has focused upon targeting KMT4 (DOT1L) and the menin-MLL interaction (Figure 2C). The direct or indirect recruitment of KMT4 (DOT1L) is frequently linked with leukemogenic *MLL* translocations.[130-133]

DOT1L, the only human H3K79 methyltransferase, plays a central role in normal hematopoiesis[134-136] and has been reported to be involved in a variety of cellular processes including telomeric silencing, cell cycle progression, DNA repair and replication and transcriptional control.[126] Much of the emphasis in studying the role of DOT1L in leukemia has centered on understanding the transcriptional programs controlled by this methyltransferase. Misdirected H3K79 methylation has been shown to sustain the expression of key pro-leukemic genes such as the *HOXA* genes and

MEIS1.[137,138] Moreover, the disruption of DOT1L function by genetic means or a selective small molecule inhibitor blocks cellular H3K79 methylation, abrogates malignant gene expression signatures and has *in vivo* efficacy in MLL xenograft models. This pre-clinical data has led to the initiation of early phase clinical trials with DOT1L inhibitors.[139,140]

Other therapeutic avenues currently being explored in MLL-FP leukemia includes the disruption of the menin-MLL interaction.[141] Menin, which directly binds the N-terminal fragment of MLL retained in all MLL-FP, is an essential oncogenic co-factor required for the leukemogenic activity of MLL-FP.[142,143] Novel small molecules identified using high throughput screening and structure-based design has led to a refinement of selectivity in targeting critical residues in the large binding site. Interruption of the menin-MLL interaction results in selective induction of apoptosis and differentiation, a block in proliferation and reversal of malignant gene expression signatures in cell lines bearing MLL-FP.[141,144] Finally, whilst a selective inhibitor to the catalytic activity of MLL1 has been developed,[145] the role of the SET domain of wild-type MLL1 in the initiation and maintenance of leukemia is not fully resolved.[146]

The role of the polycomb group proteins in hematological malignancy

Polycomb group (PcG) proteins are transcriptional repressors, which are crucial for the regulation of genes involved in cell fate decisions. Two distinct complexes, PRC1 and PRC2, work in concert to establish specific post-translational histone modifications resulting in the initiation and stable maintenance of transcriptional silencing. PRC2 consists of the core components EZH1/2, EED and SUZ12. EZH2, and the closely related EZH1, are H3K27 methyltransferases, which form the enzymatic core of PRC2. Subsequent recognition of H3K27 methylation by PRC1 occurs through component chromobox (Cbx) family members that target the complex to specific loci.[147] PRC1-mediated H2AK119 ubiquitylation and chromatin compaction follows, resulting in transcriptional silencing.[148,149]

EZH2 is the most frequent PcG member implicated in the pathogenesis of malignancy. Enzymatic hyperactivity of EZH2 has been linked to aberrant repression of tumor suppressor genes in diverse cancers, including germinal center B-cell lymphomas.[150,151] In particular, recurrent mono-allelic somatic mutations observed in lymphoma at Y641 of the SET domain confers enhanced catalytic activity and a preference for di- and tri-methylation of H3K27.[152] Selective small molecule inhibition of EZH2 is effective in inhibiting the proliferation of EZH2 mutant lymphoma cell lines and mouse xenografts.[153]

Intriguingly, loss of function mutations of *EZH2* predominate in myeloid malignancies.[154,155] Prognostically, these mutations portend a poorer OS in CMML, MDS and primary myelofibrosis.[2,156-158] The biological implications of inactivating *EZH2* mutations in hematopoiesis are unclear. Inactivating mutations of other core PRC2 components in myeloid malignancies are less common suggesting that EZH2 plays an important non-redundant role in hematopoiesis.[159,160] Nonetheless, the dichotomous role played by EZH2 as both an oncogene and tumor suppressor in the development of malignancies highlights the tissue-specific role of H3K27 methylation. Recent data have also linked inactivating *ASXL1* mutations to loss of PRC2-mediated H3K27 methylation.[161] Mutations have been identified in a wide range of myeloid malignancies, most commonly in patients with CMML, MDS or MPN, and are biomarkers of adverse outcome.[2,3,162] Although *ASX*, the ortholog of human *ASXL1* in *D. Melanogaster*, has been demonstrated to function as part of the polycomb-repressive deubiquitylase complex, no significant changes in H2AK119 ubiquitylation are observed in human ASXL1 mutant cells.[161,163] Instead, *ASXL1* mutations resulted in global decrease of H3K27 methylation and upregulation of transcriptionally poised genes normally bivalently marked with H3K27me3 and H3K4me3. This, coupled with identification of a direct interaction between ASXL1 and EZH2 through co-immunoprecipitation assays and the loss of EZH2 occupancy at *HOXA* genes highlight the specific role of ASXL1 in epigenetic regulation of gene expression by facilitating the recruitment/stabilization of PRC2 at target loci.[161]

Other PcG proteins that have also been demonstrated to play important roles in hematopoiesis are members of PRC1 and include the cbx family and BMI-1. Target selectivity of the PRC1 complex is dependent upon the sole constituent cbx family member. In HSCs, cbx family members cbx7 and cbx8 have been demonstrated to mediate the balance between self-renewal and differentiation through co-regulation of a set of common genes.[147] Cbx7 overexpression in murine models results in enhanced self-renewal and induction of leukemia whereas cbx8 overexpression is associated with lineage commitment and HSC exhaustion. Similarly, BMI-1 is critical for both hematopoietic and leukemic stem cell self renewal.[164,165] It is an interchangeable subunit of PRC1 which is specifically expressed in immature hematopoietic cells and enhances the H2AK119 ubiquitin ligase activity of the core members, RING1A and RING1B.[166,167] Increased expression of *BMI-1* is associated with impaired survival in CML,[168] MDS[169] and AML[170] and may be a useful prognostic marker in myeloid malignancies.

Demethylation

Analogous to DNA methylation, the discovery of enzymes capable of reversing lysine methylation has highlighted the dynamic nature of histone modifications. Two classes of KDMs have been identified. Class one enzymes are amine oxidases consisting of only two members including the first identified KDM, lysine-specific demethylase 1 (LSD1) or KDM1A.[171] The second, more expansive class of KDMs, contain a Jumonji domain (JmjC) which functions as a Fe^{2+} and α-KG dependent dioxygenase.

Aberrant regulation of KDMs has been linked to malignant progression; however, compared to the extensively studied KMTs, very little is known about how histone demethylation results in abnormal gene expression patterns. *UTX/KDM6A* was the first mutated KDM to be linked to malignant transformation.[172] Deletions or loss-of-function point mutations occurring within the JmjC domain of *UTX* inactivate the H3K27 demethylase activity and have been identified in a wide variety of cancers, including multiple myeloma and acute lymphoblastic leukemia.[172,173] The development of specific UTX/JMJD3 inhibitors through rational, structure-guided and chemoproteomic approaches, has served to highlight the critical role of KDM6 family members as determinants of pro-inflammatory gene activation in macrophages.[174] However, the potential application of these small molecule inhibitors as potential anti-cancer therapy is yet to be established.

Two recent publications have demonstrated the impor-

tance of KDMs in facilitating malignant gene expression in AML and highlighted the potential role of KDM1A inhibition as a therapeutic strategy.[175,176] KDM1A has a dual role in normal cells as both a transcriptional activator and repressor through interactions with multiple protein complexes. High expression of KDM1A is observed in patients with AML[177] and is thought to perturb this balance.[178] In MLL-FP models, KDM1A is required for leukemia stem cell function with pharmacological inhibition resulting in induction of differentiation and loss of colony forming ability in both murine and primary human MLL-FP cells.[175] In cell line models of other subtypes of AML, pharmacological inhibition of KDM1A in combination with ATRA results in reactivation of ATRA-dependent differentiation pathways.[176] These effects were associated with gene-specific, selective increases in H3K4me2 and were respectively associated with downregulation of genes bound by MLL-FP and upregulation of genes associated with myeloid differentiation.

Methyl-binding proteins and readers of histone methylation

More distinct recognition motifs are able to recognize lysine methylation than any other modification and are broadly divided into two families, the Royal Family [Tudor domains, chromo domains and malignant brain tumor (MBT) domains] and plant homeo domain (PHD) fingers. Akin to abnormalities in methyllysine writers and erasers, aberrant function of methyllysine readers are causally linked to the pathogenesis of hematologic malignancy.

For example, leukemogenesis of a subset of NUP98 translocated AML is dependent on the retained function of H3K4me3 reader PHD finger located in the C-terminal portion of translocation partners JARID1A and PHF23. The aberrant function of these fusion proteins results in upregulation of many critical oncogenes such as *HOXA9* and *MEIS1* through the blockade of PcG-mediated H3K27me3 deposition.[179] Although functional compensation through the substitution of other PHD fingers is possible, these recognition motifs specifically require H3K4me3 binding ability. The specificity of this protein-protein interaction resulting in malignant transformation makes methyllysine readers an attractive therapeutic target.

Phosphorylation

Kinases and phosphatases control the addition and removal of phosphate groups on serine, threonine and tyrosine residues of component histone proteins. Transfer of a phosphate group from ATP to the hydroxyl group of target amino acids results in the addition of a significant negative charge. Histone phosphorylation results in gross changes in chromatin structure and has been implicated in the regulation of gene transcription, DNA repair and chromatin condensation.

Aberrant kinase activity is one of the most commonly observed processes in malignant transformation.[180] Whilst attention has focused upon the cytoplasmic role of these master regulators of intracellular signal transduction, it has recently been recognized that some kinases may also have critical nuclear functions including histone phosphorylation.[181]

Constitutive activation of JAK2, a non-receptor tyrosine kinase crucial for cytokine signaling in normal hematopoiesis, commonly occurs in MPN. JAK2 is demonstrated to specifically phosphorylate H3Y41 within the nucleus, resulting in the exclusion of transcriptional repressor HP1α from chromatin and the activation of hematopoietic oncogenes such as *LMO2*.[182] Jak2 nuclear activity is closely correlated with levels of H3Y41ph. Interestingly, genomic profiling of H3Y41ph demonstrates that only a small subset of genes are uniquely heavily blanketed with this histone modification.[183] Several genes marked with H3Y41ph are also bound by members of the STAT family suggesting that the functional interaction with JAK kinases and STAT family members may not be confined to the cytoplasm but may extend all the way to the chromatin interface.

Aberrant JAK2 function also has indirect effects at chromatin. The most common mutation, JAK2 V617F, interacts with PRMT5 in the cytoplasm and nucleus of hematopoietic cells. This interaction results in a novel gain of function whereby JAK2 phosphorylates PRMT5.[184] Abrogation of histone methyltransferase activity ensues with global decrease of H2/H4 R3 methylation and altered gene expression. Inhibition of PRMT5 activity results in promotion of progenitor cell proliferation and erythrocytosis.

The identification of multiple pathogenic consequences of aberrant signaling kinase activity at chromatin broadens the therapeutic scope of kinase inhibitors currently in clinical development. Several kinase inhibitors result in global reduction of histone modification laid down by target enzymes (e.g. JAK2 and Aurora kinase inhibitors) and thus can be considered as potential epigenetic therapies.

Conclusions

The increasing availability of high-throughput genomic technologies in clinical settings allows for more accurate diagnostic and prognostic information, which may in turn guide therapeutic choices and make personalized medicine a reality. However, taking full advantage of these advances requires clarification of molecular mechanisms underlying malignant transformation. Although highly heterogeneous in nature, aberrant regulation of epigenetic processes has emerged as a prominent unifying theme in hematologic malignancies. Thus, the hematologic malignancies serve as effective models to investigate key epigenetic pathways and nodes of regulation in the ongoing quest for more effective therapies.

Already, the incorporation of genomic mutational analysis into prognostic algorithms is able to stratify outcomes and allows for tailoring of existing conventional chemotherapeutic regimens.[2-4] Somatic alterations of epigenetic regulators such as *DNMT3A*,[28] *TET2*,[56] *IDH2*,[185] *MLL*,[3] *EZH2* and *ASXL1*[186] have prospective prognostic value in AML and MDS and can also modulate the outcomes consequent to mutations found in cell signaling pathways such as *FLT3-ITD*.[3] Therapies directed against epigenetic mechanisms of disease have also entered widespread clinical practice with resultant improvement in clinical outcomes. Furthermore, targeted epigenetic therapies are taking shape as effective therapies in advanced pre-clinical and early clinical development.

Epigenetic regulators provide an attractive target for directed small molecule inhibition. Proteins involved in epigenetic regulation often depend on critical protein-protein interactions within macromolecular complexes for appropriate function, and also require essential co-factors for enzymatic activity. Nevertheless, achieving inhibitor specificity is challenging. Ubiquitously expressed epigenetic reg-

ulators participate in both normal and malignant processes. Moreover, histone-modifying enzymes also have non-histone targets. Therefore, the rational use of targeted epigenetic therapies will require a thorough understanding of the underlying mechanisms and key interactions resulting in malignant transformation driven by aberrant epigenetic regulators. This knowledge will also allow us to mitigate the pervasive issues of drug resistance and adverse side effects by developing effective drug combinations either with existing conventional therapies or even dual-targeted epigenetic therapies.

Authorship and Disclosures

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

References

1. Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K, et al. Recurring mutations found by sequencing an acute myeloid leukemia genome. N Engl J Med. 2009;361(11):1058-66.
2. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, et al. Clinical effect of point mutations in myelodysplastic syndromes. N Engl J Med. 2011;364(26):2496-506.
3. Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079-89.
4. Haferlach T, Nagata Y, Grossmann V, Okuno Y, Bacher U, Nagae G, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. Leukemia. 2014;28(2):241-7.
5. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. Trends Biochem Sci. 2006;31(2):89-97.
6. Bird A. DNA methylation patterns and epigenetic memory. Genes Dev. 2002;16 (1):6-21.
7. Bird AP. CpG-rich islands and the function of DNA methylation. Nature. 1986;321 (6067):209-13.
8. Illingworth RS, Bird AP. CpG islands--'a rough guide'. FEBS Lett. 2009;583(11): 1713-20.
9. Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer. 2011;11(10):726-34.
10. Akalin A, Garrett-Bakelman FE, Kormaksson M, Busuttil J, Zhang L, Khrebtukova I, et al. Base-pair resolution DNA methylation sequencing reveals profoundly divergent epigenetic landscapes in acute myeloid leukemia. PLoS Genet. 2012;8(6):e1002781.
11. Jones PA, Baylin SB. The fundamental role of epigenetic events in cancer. Nat Rev Genet. 2002;3(6):415-28.
12. Esteller M. Relevance of DNA methylation in the management of cancer. Lancet Oncol. 2003;4(6):351-8.
13. Saied MH, Marzec J, Khalid S, Smith P, Down TA, Rakyan VK, et al. Genome wide analysis of acute myeloid leukemia reveal leukemia specific methylome and subtype specific hypomethylation of repeats. PloS One. 2012;7(3):e33213.
14. Doi A, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. Nat Genet. 2009;41(12): 1350-3.
15. Liang P, Song F, Ghosh S, Morien E, Qin M, Mahmood S, et al. Genome-wide survey reveals dynamic widespread tissue-specific changes in DNA methylation during development. BMC Genomics. 2011;12(1):231.
16. Meissner A, Mikkelsen TS, Gu H, Wernig M, Hanna J, Sivachenko A, et al. Genome-scale DNA methylation maps of pluripotent and differentiated cells. Nature. 2008;454 (7205):766-70.
17. Lorincz MC, Dickerson DR, Schmitt M, Groudine M. Intragenic DNA methylation alters chromatin structure and elongation efficiency in mammalian cells. Nat Struct Mol Biol. 2004;11(11):1068-75.
18. Wood AJ, Schulz R, Woodfine K, Koltowska K, Beechey CV, Peters J, et al. Regulation of alternative polyadenylation by genomic imprinting. Genes Dev. 2008;22(9):1141-6.
19. Maunakea AK, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, et al. Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature. 2010;466(7303):253-7.
20. Maunakea AK, Chepelev I, Cui K, Zhao K. Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. Cell Res. 2013;23(11):1256-69.
21. Hansen KD, Timp W, Bravo HC, Sabunciyan S, Langmead B, McDonald OG, et al. Increased methylation variation in epigenetic domains across cancer types. Nat Genet. 2011;43(8):768-75.
22. Timp W, Feinberg AP. Cancer as a dysregulated epigenome allowing cellular growth advantage at the expense of the host. Nat Rev Cancer. 2013;13(7):497-510.
23. Stein R, Gruenbaum Y, Pollack Y, Razin A, Cedar H. Clonal inheritance of the pattern of DNA methylation in mouse cells. Proc Natl Acad Sci U S A. 1982;79(1):61-5.
24. Jones PA, Liang G. Rethinking how DNA methylation patterns are maintained. Nature Rev Genet. 2009;10(11):805-11.
25. Okano M, Xie S, Li E. Cloning and characterization of a family of novel mammalian DNA (cytosine-5) methyltransferases. Nat Genet. 1998;19(3):219-20.
26. el-Deiry WS, Nelkin BD, Celano P, Yen RW, Falco JP, Hamilton SR, et al. High expression of the DNA methyltransferase gene characterizes human neoplastic cells and progression stages of colon cancer. Proc Natl Acad Sci USA. 1991;88(8):3470-4.
27. Robertson KD, Uzvolgyi E, Liang G, Talmadge C, Sumegi J, Gonzales FA, et al. The human DNA methyltransferases (DNMTs) 1, 3a and 3b: coordinate mRNA expression in normal tissues and overexpression in tumors. Nucleic Acids Res. 1999;27(11):2291-8.
28. Yan XJ, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, et al. Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocytic leukemia. Nat Genet. 2011;43(4):309-15.
29. Yamashita Y, Yuan J, Suetake I, Suzuki H, Ishikawa Y, Choi YL, et al. Array-based genomic resequencing of human leukemia. Oncogene. 2010;29(25):3723-31.
30. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, et al. DNMT3AMutations in Acute Myeloid Leukemia. N Engl J Med. 2010;363(25): 2424-33.
31. Hou HA, Kuo YY, Liu CY, Chou WC, Lee MC, Chen CY, et al. DNMT3A mutations in acute myeloid leukemia: stability during disease evolution and clinical implications. Blood. 2012;119(2):559-68.
32. Thol F, Damm F, Ludeking A, Winschel C, Wagner K, Morgan M, et al. Incidence and prognostic influence of DNMT3A mutations in acute myeloid leukemia. J Clin Oncol. 2011;29(21):2889-96.
33. Metzeler KH, Walker A, Geyer S, Garzon R, Klisovic RB, Bloomfield CD, et al. DNMT3A mutations and response to the hypomethylating agent decitabine in acute myeloid leukemia. Leukemia. 2012;26(5):1106-7.
34. Dinardo CD, Patel KP, Garcia-Manero G, Luthra R, Pierce S, Borthakur G, et al. Lack of association of IDH1, IDH2 and DNMT3A mutations with outcome in older patients with acute myeloid leukemia treated with hypomethylating agents. Leuk Lymphoma. 2014.
35. Kim SJ, Zhao H, Hardikar S, Singh AK, Goodell MA, Chen T. A DNMT3A mutation common in AML exhibits dominant-negative effects in murine ES cells. Blood. 2013;122(25):4086-9.
36. Russler-Germain DA, Spencer DH, Young MA, Lamprecht TL, Miller CA, Fulton R, et al. The R882H DNMT3A mutation associated with AML dominantly inhibits wild-type DNMT3A by blocking its ability to form active tetramers. Cancer Cell. 2014;25(4): 442-54.
37. Xu J, Wang YY, Dai YJ, Zhang W, Zhang WN, Xiong SM, et al. DNMT3A Arg882 mutation drives chronic myelomonocytic leukemia through disturbing gene expression/DNA methylation in hematopoietic cells. Proc Natl Acad Sci USA. 2014;111(7): 2620-5.
38. Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M, et al. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. Leukemia. 2011;25(7):1153-8.
39. Stegelmann F, Bullinger L, Schlenk RF, Paschka P, Griesshammer M, Blersch C, et al. DNMT3A mutations in myeloproliferative neoplasms. Leukemia. 2011;25(7):1217-9.
40. Fried I, Bodner C, Pichler MM, Lind K, Beham-Schmid C, Quehenberger F, et al. Frequency, onset and clinical impact of somatic DNMT3A mutations in therapy-related and secondary acute myeloid leukemia. Haematologica. 2012;97(2):246-50.
41. Challen GA, Sun D, Jeong M, Luo M, Jelinek J, Berg JS, et al. Dnmt3a is essential for hematopoietic stem cell differentiation. Nat Genet. 2012;44(1):23-31.
42. Shlush LI, Zandi S, Mitchell A, Chen WC, Brandwein JM, Gupta V, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506(7488): 328-33.
43. Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig

R, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol. 2002;20 (10):2429-40.
44. Kantarjian H, Issa JP, Rosenfeld CS, Bennett JM, Albitar M, DiPersio J, et al. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer. 2006;106(8):1794-803.
45. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Finelli C, Giagounidis A, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. Lancet Oncol. 2009;10(3):223-32.
46. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, Santini V, Gattermann N, Germing U, et al. Azacitidine prolongs overall survival compared with conventional care regimens in elderly patients with low bone marrow blast count acute myeloid leukemia. J Clin Oncol. 2010;28(4):562-9.
47. Grövdal M, Karimi M, Khan R, Aggerholm A, Antunovic P, Astermark J, et al. Maintenance treatment with azacytidine for patients with high-risk myelodysplastic syndromes (MDS) or acute myeloid leukaemia following MDS in complete remission after induction chemotherapy. Br J Haematol. 2010;150(3):293-302.
48. Sauntharajah Y. Key clinical observations after 5-azacytidine and decitabine treatment of myelodysplastic syndromes suggest practical solutions for better outcomes. Hematology Am Soc Hematol Educ Program. 2013;2013:511-21.
49. Platzbecker U, Germing U. Combination of azacitidine and lenalidomide in myelodysplastic syndromes or acute myeloid leukemia-a wise liaison? Leukemia. 2013;27 (9):1813-9.
50. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, et al. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. Science. 2009;324(5929):930-5.
51. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. Nature. 2010;466(7310):1129-33.
52. He YF, Li BZ, Li Z, Liu P, Wang Y, Tang Q, et al. Tet-mediated formation of 5-carboxylcytosine and its excision by TDG in mammalian DNA. Science. 2011;333 (6047):1303-7.
53. Ito S, Shen L, Dai Q, Wu SC, Collins LB, Swenberg JA, et al. Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. Science. 2011;333 (6047):1300-3.
54. Branco MR, Ficz G, Reik W. Uncovering the role of 5-hydroxymethylcytosine in the epigenome. Nat Rev Genet. 2012;13(1):7-13.
55. Wu H, Zhang Y. Tet1 and 5-hydroxymethylation: a genome-wide view in mouse embryonic stem cells. Cell Cycle. 2011; 10(15):2428-36.
56. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Masse A, et al. Mutation in TET2 in myeloid cancers. N Engl J Med. 2009;360(22):2289-301.
57. Langemeijer SM, Kuiper RP, Berends M, Knops R, Aslanyan MG, Massop M, et al. Acquired mutations in TET2 are common in myelodysplastic syndromes. Nat Genet. 2009;41(7):838-42.
58. Solary E, Bernard OA, Tefferi A, Fuks F, Vainchenker W. The Ten-Eleven Translocation-2 (TET2) gene in hematopoiesis and hematopoietic diseases. Leukemia. 2014;28(3):485-96.
59. Abdel-Wahab O, Mullally A, Hedvat C, Garcia-Manero G, Patel J, Wadleigh M, et al. Genetic characterization of TET1, TET2, and TET3 alterations in myeloid malignancies. Blood. 2009;114(1):144-7.
60. Kosmider O, Gelsi-Boyer V, Ciudad M, Racoeur C, Jooste V, Vey N, et al. TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. Haematologica. 2009;94(12):1676-81.
61. Tefferi A, Pardanani A, Lim KH, Abdel-Wahab O, Lasho TL, Patel J, et al. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. Leukemia. 2009;23(5):905-11.
62. Itzykson R, Kosmider O, Cluzeau T, Mansat-De Mas V, Dreyfus F, Beyne-Rauzy O, et al. Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias. Leukemia. 2011;25(7): 1147-52.
63. Cancer Genome Atlas Research N. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059-74.
64. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, et al. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell. 2010;18(6):553-67.
65. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, et al. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature. 2009;462(7274): 739-44.
66. Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Coller HA, et al. The common feature of leukemia-associated IDH1 and IDH2 mutations is a neomorphic enzyme activity converting alpha-ketoglutarate to 2-hydroxyglutarate. Cancer Cell. 2010;17(3): 225-34.
67. Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH, et al. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer cell. 2011;19(1):17-30.
68. Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O, et al. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature. 2012;483(7390):474-8.
69. Chen C, Liu Y, Lu C, Cross JR, Morris JPt, Shroff AS, et al. Cancer-associated IDH2 mutants drive an acute myeloid leukemia that is susceptible to Brd4 inhibition. Genes Dev. 2013;27(18):1974-85.
70. Kats LM, Reschke M, Taulli R, Pozdnyakova O, Burgess K, Bhargava P, et al. Proto-oncogenic role of mutant IDH2 in leukemia initiation and maintenance. Cell Stem Cell. 2014;14(3):329-41.
71. Schnittger S, Haferlach C, Ulke M, Alpermann T, Kern W, Haferlach T. IDH1 mutations are detected in 6.6% of 1414 AML patients and are associated with intermediate risk karyotype and unfavorable prognosis in adults younger than 60 years and unmutated NPM1 status. Blood. 2010;116(25):5486-96.
72. Abbas S, Lugthart S, Kavelaars FG, Schelen A, Koenders JE, Zeilemaker A, et al. Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value. Blood. 2010;116(12): 2122-6.
73. Green CL, Evans CM, Hills RK, Burnett AK, Linch DC, Gale RE. The prognostic significance of IDH1 mutations in younger adult patients with acute myeloid leukemia is dependent on FLT3/ITD status. Blood. 2010;116(15):2779-82.
74. Green CL, Evans CM, Zhao L, Hills RK, Burnett AK, Linch DC, et al. The prognostic significance of IDH2 mutations in AML depends on the location of the mutation. Blood. 2011;118(2):409-12.
75. Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Kronke J, Bullinger L, et al. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. J Clin Oncol. 2010;28(22):3636-43.
76. Wang F, Travins J, DeLaBarre B, Penard-Lacronique V, Schalm S, Hansen E, et al. Targeted inhibition of mutant IDH2 in leukemia cells induces cellular differentiation. Science. 2013;340(6132):622-6.
77. Chaturvedi A, Araujo Cruz MM, Jyotsana N, Sharma A, Yun H, Gorlich K, et al. Mutant IDH1 promotes leukemogenesis in vivo and can be specifically targeted in human AML. Blood. 2013;122(16):2877-87.
78. Kouzarides T. Chromatin modifications and their function. Cell. 2007;128(4):693-705.
79. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res. 2011;21(3):381-95.
80. Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, Chan WI, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature. 2011;478(7370): 529-33.
81. Shahbazian MD, Grunstein M. Functions of site-specific histone acetylation and deacetylation. Ann Rev Biochem. 2007;76:75-100.
82. Kleff S, Andrulis ED, Anderson CW, Sternglanz R. Identification of a gene encoding a yeast histone H4 acetyltransferase. J Biol Chem. 1995;270(42):24674-7.
83. Pasqualucci L, Dominguez-Sola D, Chiarenza A, Fabbri G, Grunn A, Trifonov V, et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma. Nature. 2011;471(7337):189-95.
84. Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, et al. CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature. 2011;471 (7337):235-9.
85. Sobulo OM, Borrow J, Tomek R, Reshmi S, Harden A, Schlegelberger B, et al. MLL is fused to CBP, a histone acetyltransferase, in therapy-related acute myeloid leukemia with a t(11;16)(q23;p13.3). Proc Nl Acad Sci USA. 1997;94(16):8732-7.
86. Taki T, Sako M, Tsuchida M, Hayashi Y. The t(11;16)(q23;p13) translocation in myelodysplastic syndrome fuses the MLL gene to the CBP gene. Blood. 1997;89(11):3945-50.
87. Carapeti M, Aguiar RC, Goldman JM, Cross NC. A novel fusion between MOZ and the nuclear receptor coactivator TIF2 in acute myeloid leukemia. Blood. 1998; 91(9):3127-33.
88. Lavau C, Du C, Thirman M, Zeleznik-Le N. Chromatin-related properties of CBP fused to MLL generate a myelodysplastic-like syndrome that evolves into myeloid leukemia. EMBO J. 2000;19(17):4655-64.
89. Huntly BJ, Shigematsu H, Deguchi K, Lee BH, Mizuno S, Duclos N, et al. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. Cancer Cell. 2004;6(6):587-96.
90. Deguchi K, Ayton PM, Carapeti M, Kutok JL, Snyder CS, Williams IR, et al. MOZ-TIF2-induced acute myeloid leukemia requires the MOZ nucleosome binding motif and TIF2-mediated recruitment of

CBP. Cancer Cell. 2003;3(3):259-71.
91. Wang L, Gural A, Sun XJ, Zhao X, Perna F, Huang G, et al. The leukemogenicity of AML1-ETO is dependent on site-specific lysine acetylation. Science. 2011;333(6043):765-9.
92. Bowers EM, Yan G, Mukherjee C, Orry A, Wang L, Holbert MA, et al. Virtual ligand screening of the p300/CBP histone acetyltransferase: identification of a selective small molecule inhibitor. Chem Biol. 2010;17(5):471-82.
93. Gao XN, Lin J, Ning QY, Gao L, Yao YS, Zhou JH, et al. A histone acetyltransferase p300 inhibitor C646 induces cell cycle arrest and apoptosis selectively in AML1-ETO-positive AML cells. PloS One. 2013;8(2):e55481.
94. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov. 2006;5(9):769-84.
95. Grignani F, De Matteis S, Nervi C, Tomassoni L, Gelmetti V, Cioce M, et al. Fusion proteins of the retinoic acid receptor-alpha recruit histone deacetylase in promyelocytic leukaemia. Nature. 1998;391(6669):815-8.
96. He LZ, Guidez F, Tribioli C, Peruzzi D, Ruthardt M, Zelent A, et al. Distinct interactions of PML-RARalpha and PLZF-RARalpha with co-repressors determine differential responses to RA in APL. Nat Genet. 1998;18(2):126-35.
97. Lin RJ, Nagy L, Inoue S, Shao W, Miller WH, Jr., Evans RM. Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature. 1998;391(6669):811-4.
98. Fazi F, Zardo G, Gelmetti V, Travaglini L, Ciolfi A, Di Croce L, et al. Heterochromatic gene repression of the retinoic acid pathway in acute myeloid leukemia. Blood. 2007;109(10):4432-40.
99. He LZ, Tolentino T, Grayson P, Zhong S, Warrell RP, Jr., Rifkind RA, et al. Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. J Clin Invest. 2001;108(9):1321-30.
100. Bojang P, Jr., Ramos KS. The promise and failures of epigenetic therapies for cancer treatment. Cancer Treat Rev. 2014;40(1):153-69.
101. Dickinson M, Johnstone RW, Prince HM. Histone deacetylase inhibitors: potential targets responsible for their anti-cancer effect. Invest New Drugs. 2010;28 (Suppl 1):S3-20.
102. Bots M, Verbrugge I, Martin BP, Salmon JM, Ghisi M, Baker A, et al. Differentiation therapy for the treatment of t(8;21) acute myeloid leukemia using histone deacetylase inhibitors. Blood. 2014;123(9):1341-52.
103. Huang B, Yang XD, Zhou MM, Ozato K, Chen LF. Brd4 coactivates transcriptional activation of NF-kappaB via specific binding to acetylated RelA. Mol Cell Biol. 2009;29(5):1375-87.
104. Lamonica JM, Deng W, Kadauke S, Campbell AE, Gamsjaeger R, Wang H, et al. Bromodomain protein Brd3 associates with acetylated GATA1 to promote its chromatin occupancy at erythroid target genes. Proc Natl Acad Sci USA. 2011;108(22):E159-68.
105. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, et al. Suppression of inflammation by a synthetic histone mimic. Nature. 2010;468(7327):1119-23.
106. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, et al. Selective inhibition of BET bromodomains. Nature. 2010;468(7327):1067-73.
107. Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature. 2011;478(7370):524-8.
108. Dawson MA, Gudgin EJ, Horton SJ, Giotopoulos G, Meduri E, Robson S, et al. Recurrent mutations, including NPM1c, activate a BRD4-dependent core transcriptional program in acute myeloid leukemia. Leukemia. 2014;28(2):311-20.
109. Loven J, Hoke HA, Lin CY, Lau A, Orlando DA, Vakoc CR, et al. Selective inhibition of tumor oncogenes by disruption of super-enhancers. Cell. 2013;153(2):320-34.
110. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011;146(6):904-17.
111. Mertz JA, Conery AR, Bryant BM, Sandy P, Balasubramanian S, Mele DA, et al. Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc Natl Acad Sci U S A. 2011;108(40):16669-74.
112. Ott CJ, Kopp N, Bird L, Paranal RM, Qi J, Bowman T, et al. BET bromodomain inhibition targets both c-Myc and IL7R in high-risk acute lymphoblastic leukemia. Blood. 2012;120(14):2843-52.
113. Allis CD, Berger SL, Cote J, Dent S, Jenuwien T, Kouzarides T, et al. New nomenclature for chromatin-modifying enzymes. Cell. 2007;131(4):633-6.
114. Collins RE, Tachibana M, Tamaru H, Smith KM, Jia D, Zhang X, et al. In vitro and in vivo analyses of a Phe/Tyr switch controlling product specificity of histone lysine methyltransferases. J Biol Chem. 2005;280(7):5563-70.
115. Wilson JR, Jing C, Walker PA, Martin SR, Howell SA, Blackburn GM, et al. Crystal structure and functional analysis of the histone methyltransferase SET7/9. Cell. 2002;111(1):105-15.
116. Barski A, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, et al. High-resolution profiling of histone methylations in the human genome. Cell. 2007;129(4):823-37.
117. Rea S, Eisenhaber F, O'Carroll D, Strahl BD, Sun ZW, Schmid M, et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. Nature. 2000;406(6796):593-9.
118. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet. 2007;39(3):311-8.
119. Cecere G, Hoersch S, Jensen MB, Dixit S, Grishok A. The ZFP-1(AF10)/DOT-1 complex opposes H2B ubiquitination to reduce Pol II transcription. Mol Cell. 2013;50(6):894-907.
120. Bernt KM, Armstrong SA. Targeting epigenetic programs in MLL-rearranged leukemias. Hematology Am Soc Hematol Educ Program. 2011;2011:354-60.
121. Slany RK. When epigenetics kills: MLL fusion proteins in leukemia. Hematol Oncol. 2005;23(1):1-9.
122. De Braekeleer M, Morel F, Le Bris MJ, Herry A, Douet-Guilbert N. The MLL gene and translocations involving chromosomal band 11q23 in acute leukemia. Anticancer Res. 2005;25(3B):1931-44.
123. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. Nat Rev Cancer. 2007;7(11):823-33.
124. Meyer C, Hofmann J, Burmeister T, Groger D, Park TS, Emerenciano M, et al. The MLL recombinome of acute leukemias in 2013. Leukemia. 2013.
125. Luo Z, Lin C, Shilatifard A. The super elongation complex (SEC) family in transcriptional control. Nature reviews Molecular cell biology. 2012;13(9):543-7.
126. Nguyen AT, Zhang Y. The diverse functions of Dot1 and H3K79 methylation. Genes Dev. 2011;25(13):1345-58.
127. Dawson MA, Kouzarides T. Cancer epigenetics: from mechanism to therapy. Cell. 2012;150(1):12-27.
128. Dawson MA, Kouzarides T, Huntly BJ. Targeting epigenetic readers in cancer. N Engl J Med. 2012;367(7):647-57.
129. Lin C, Smith ER, Takahashi H, Lai KC, Martin-Brown S, Florens L, et al. AFF4, a component of the ELL/P-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. Mol Cell. 2010;37(3):429-37.
130. Bernt KM, Zhu N, Sinha AU, Vempati S, Faber J, Krivtsov AV, et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. Cancer Cell. 2011;20(1):66-78.
131. Mueller D, Bach C, Zeisig D, Garcia-Cuellar MP, Monroe S, Sreekumar A, et al. A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. Blood. 2007;110(13):4445-54.
132. Milne TA, Martin ME, Brock HW, Slany RK, Hess JL. Leukemogenic MLL fusion proteins bind across a broad region of the Hox a9 locus, promoting transcription and multiple histone modifications. Cancer Res. 2005;65(24):11367-74.
133. Okada Y, Feng Q, Lin Y, Jiang Q, Li Y, Coffield VM, et al. hDOT1L links histone methylation to leukemogenesis. Cell. 2005;121(2):167-78.
134. Jo SY, Granowicz EM, Maillard I, Thomas D, Hess JL. Requirement for Dot1l in murine postnatal hematopoiesis and leukemogenesis by MLL translocation. Blood. 2011;117(18):4759-68.
135. Nguyen AT, He J, Taranova O, Zhang Y. Essential role of DOT1L in maintaining normal adult hematopoiesis. Cell Res. 2011;21(9):1370-3.
136. Feng Y, Yang Y, Ortega MM, Copeland JN, Zhang M, Jacob JB, et al. Early mammalian erythropoiesis requires the Dot1L methyltransferase. Blood. 2010;116(22):4483-91.
137. Guenther MG, Lawton LN, Rozovskaia T, Frampton GM, Levine SS, Volkert TL, et al. Aberrant chromatin at genes encoding stem cell regulators in human mixed-lineage leukemia. Genes Dev. 2008;22(24):3403-8.
138. Krivtsov AV, Feng Z, Lemieux ME, Faber J, Vempati S, Sinha AU, et al. H3K79 methylation profiles define murine and human MLL-AF4 leukemias. Cancer Cell. 2008;14(5):355-68.
139. Daigle SR, Olhava EJ, Therkelsen CA, Majer CR, Sneeringer CJ, Song J, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. Cancer Cell. 2011;20(1):53-65.
140. Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, Boriack-Sjodin PA, et al. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood. 2013;122(6):1017-25.
141. Grembecka J, He S, Shi A, Purohit T, Muntean AG, Sorenson RJ, et al. Menin-MLL inhibitors reverse oncogenic activity of MLL fusion proteins in leukemia. Nat Chem Biol. 2012;8(3):277-84.
142. Yokoyama A, Somervaille TC, Smith KS, Rozenblatt-Rosen O, Meyerson M, Cleary ML. The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. Cell. 2005;123(2):207-18.
143. Caslini C, Yang Z, El-Osta M, Milne TA, Slany RK, Hess JL. Interaction of MLL amino

terminal sequences with menin is required for transformation. Cancer Res. 2007;67(15):7275-83.
144. Grembecka J, He S, Senter TJ, Pollock J, Han C, Upadhyay SK, et al. High-affinity small molecule inhibitors of the menin-Mixed Lineage Leukemia (MLL) interaction closely mimic a natural protein-protein interaction. J Med Chem. 2014.
145. Cao F, Townsend EC, Karatas H, Xu J, Li L, Lee S, et al. Targeting MLL1 H3K4 methyltransferase activity in mixed-lineage leukemia. Mol Cell. 2014;53(2):247-61.
146. Mishra BP, Zaffuto KM, Artinger EL, Org T, Mikkola HK, Cheng C, et al. The histone methyltransferase activity of MLL1 is dispensable for hematopoiesis and leukemogenesis. Cell Rep. 2014;7(4):1239-47.
147. Klauke K, Radulovic V, Broekhuis M, Weersing E, Zwart E, Olthof S, et al. Polycomb Cbx family members mediate the balance between haematopoietic stem cell self-renewal and differentiation. Nat Cell Biol. 2013;15(4):353-62.
148. Cao R, Tsukada Y, Zhang Y. Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing. Mol Cell. 2005;20(6):845-54.
149. Eskeland R, Leeb M, Grimes GR, Kress C, Boyle S, Sproul D, et al. Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. Mol Cell. 2010;38(3):452-64.
150. Kondo Y, Shen L, Cheng AS, Ahmed S, Boumber Y, Charo C, et al. Gene silencing in cancer by histone H3 lysine 27 trimethylation independent of promoter DNA methylation. Nat Genet. 2008;40(6):741-50.
151. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010;42(2):181-5.
152. Sneeringer CJ, Scott MP, Kuntz KW, Knutson SK, Pollock RM, Richon VM, et al. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci U S A. 2010;107(49):20980-5.
153. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature. 2012;492(7427):108-12.
154. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. Nat Genet. 2010;42(8):722-6.
155. Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tonnissen ER, et al. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. Nat Genet. 2010;42(8):665-7.
156. Abdel-Wahab O, Pardanani A, Patel J, Wadleigh M, Lasho T, Heguy A, et al. Concomitant analysis of EZH2 and ASXL1 mutations in myelofibrosis, chronic myelomonocytic leukemia and blast-phase myeloproliferative neoplasms. Leukemia. 2011;25(7):1200-2.
157. Grossmann V, Kohlmann A, Eder C, Haferlach C, Kern W, Cross NC, et al. Molecular profiling of chronic myelomonocytic leukemia reveals diverse mutations in >80% of patients with TET2 and EZH2 being of high prognostic relevance. Leukemia. 2011;25(5):877-9.
158. Guglielmelli P, Biamonte F, Score J, Hidalgo-Curtis C, Cervantes F, Maffioli M, et al. EZH2 mutational status predicts poor survival in myelofibrosis. Blood. 2011;118(19):5227-34.
159. Score J, Hidalgo-Curtis C, Jones AV, Winkelmann N, Skinner A, Ward D, et al. Inactivation of polycomb repressive complex 2 components in myeloproliferative and myelodysplastic/myeloproliferative neoplasms. Blood. 2012;119(5):1208-13.
160. Kroeze LI, Nikoloski G, da Silva-Coelho P, van Hoogen P, Stevens-Linders E, Kuiper RP, et al. Genetic defects in PRC2 components other than EZH2 are not common in myeloid malignancies. Blood. 2012;119(5):1318-9.
161. Abdel-Wahab O, Adli M, LaFave LM, Gao J, Hricik T, Shih AH, et al. ASXL1 mutations promote myeloid transformation through loss of PRC2-mediated gene repression. Cancer Cell. 2012;22(2):180-93.
162. Gelsi-Boyer V, Trouplin V, Roquain J, Adelaide J, Carbuccia N, Esterni B, et al. ASXL1 mutation is associated with poor prognosis and acute transformation in chronic myelomonocytic leukaemia. Br J Haematol. 2010;151(4):365-75.
163. Scheuermann JC, de Ayala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Fraterman S, et al. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. Nature. 2010;465(7295):243-7.
164. Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature. 2003;423(6937):255-60.
165. Park IK, Qian D, Kiel M, Becker MW, Pihalja M, Weissman IL, et al. Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. Nature. 2003;423(6937):302-5.
166. Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS, et al. Role of histone H2A ubiquitination in Polycomb silencing. Nature. 2004;431(7010):873-8.
167. Buchwald G, van der Stoop P, Weichenrieder O, Perrakis A, van Lohuizen M, Sixma TK. Structure and E3-ligase activity of the Ring-Ring complex of polycomb proteins Bmi1 and Ring1b. EMBO J. 2006;25(11):2465-74.
168. Mohty M, Yong AS, Szydlo RM, Apperley JF, Melo JV. The polycomb group BMI1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia. Blood. 2007;110(1):380-3.
169. Mihara K, Chowdhury M, Nakaju N, Hidani S, Ihara A, Hyodo H, et al. Bmi-1 is useful as a novel molecular marker for predicting progression of myelodysplastic syndrome and patient prognosis. Blood. 2006;107(1):305-8.
170. Chowdhury M, Mihara K, Yasunaga S, Ohtaki M, Takihara Y, Kimura A. Expression of Polycomb-group (PcG) protein BMI-1 predicts prognosis in patients with acute myeloid leukemia. Leukemia. 2007;21(5):1116-22.
171. Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell. 2004;119(7):941-53.
172. van Haaften G, Dalgliesh GL, Davies H, Chen L, Bignell G, Greenman C, et al. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nat Genet. 2009;41(5):521-3.
173. Mar BG, Bullinger L, Basu E, Schlis K, Silverman LB, Dohner K, et al. Sequencing histone-modifying enzymes identifies UTX mutations in acute lymphoblastic leukemia. Leukemia. 2012;26(8):1881-3.
174. Kruidenier L, Chung CW, Cheng Z, Liddle J, Che K, Joberty G, et al. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. Nature. 2012;488(7411):404-8.
175. Harris WJ, Huang X, Lynch JT, Spencer GJ, Hitchin JR, Li Y, et al. The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells. Cancer Cell. 2012;21(4):473-87.
176. Schenk T, Chen WC, Gollner S, Howell L, Jin L, Hebestreit K, et al. Inhibition of the LSD1 (KDM1A) demethylase reactivates the all-trans-retinoic acid differentiation pathway in acute myeloid leukemia. Nat Med. 2012;18(4):605-11.
177. Berglund L, Bjorling E, Oksvold P, Fagerberg L, Asplund A, Szigyarto CA, et al. A gene-centric Human Protein Atlas for expression profiles based on antibodies. Mol Cell Proteomics. 2008;7(10):2019-27.
178. Lokken AA, Zeleznik-Le NJ. Breaking the LSD1/KDM1A addiction: therapeutic targeting of the epigenetic modifier in AML. Cancer Cell. 2012;21(4):451-3.
179. Wang GG, Song J, Wang Z, Dormann HL, Casadio F, Li H, et al. Haematopoietic malignancies caused by dysregulation of a chromatin-binding PHD finger. Nature. 2009;459(7248):847-51.
180. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.
181. Baek SH. When signaling kinases meet histones and histone modifiers in the nucleus. Mol Cell. 2011;42(3):274-84.
182. Dawson MA, Bannister AJ, Gottgens B, Foster SD, Bartke T, Green AR, et al. JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. Nature. 2009;461(7265):819-22.
183. Dawson MA, Foster SD, Bannister AJ, Robson SC, Hannah R, Wang X, et al. Three distinct patterns of histone H3Y41 phosphorylation mark active genes. Cell Rep. 2012;2(3):470-7.
184. Liu F, Zhao X, Perna F, Wang L, Koppikar P, Abdel-Wahab O, et al. JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation. Cancer Cell. 2011;19(2):283-94.
185. Marcucci G, Maharry K, Wu YZ, Radmacher MD, Mrozek K, Margeson D, et al. IDH1 and IDH2 gene mutations identify novel molecular subsets within de novo cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. J Clin Oncol. 2010;28(14):2348-55.
186. Metzeler KH, Becker H, Maharry K, Radmacher MD, Kohlschmidt J, Mrozek K, et al. ASXL1 mutations identify a high-risk subgroup of older patients with primary cytogenetically normal AML within the ELN Favorable genetic category. Blood. 2011;118(26):6920-9.