REVIEW ARTICLE

MECHANISMS OF DISEASE

Targeting Epigenetic Readers in Cancer

Mark A. Dawson, M.D., Ph.D., Tony Kouzarides, Ph.D., and Brian J.P. Huntly, M.D., Ph.D.

THE PRINCIPAL TENET IN ONCOLOGY IS THAT CANCER IS A DISEASE THAT IS initiated and driven by somatic aberrations of our genome. The challenge is to decipher how these genomic alterations culminate in malignant transformation and how they can be targeted for therapeutic gain. Molecular insights provided by the study of hematopoietic cancers have elucidated many fundamental principles in cancer biology.

Recurrent chromosomal translocations involving transcriptional regulators in several hematopoietic cancers illustrate the importance of transcriptional dysregulation in cancer.[1] The earliest detected translocations provided examples of abnormalities in both transcriptional activation and transcriptional repression.[1,2] The introduction of more refined techniques in molecular biology has led to the identification of further recurrent translocations and somatic mutations that result in pathognomonic transcriptional alterations.[3] These gene-expression signatures have diagnostic utility as well as prognostic significance, but as yet few new treatments have emerged. The task of cataloguing all the genomic aberrations in cancer has now begun in earnest.[4] Using next-generation sequencing platforms, the International Cancer Genome Consortium has already provided an unparalleled annotation of recurrent somatic mutations in protein-coding genes for a large variety of cancers.[5] These efforts have brought into focus a new central theme: recurrent mutations in epigenetic regulators, which are especially prevalent in the hematopoietic cancers (Table 1).

From the Department of Haematology, Cambridge Institute for Medical Research (M.A.D., B.J.P.H.), Cambridge University Hospitals National Health Service Foundation Trust (M.A.D., B.J.P.H.), the Gurdon Institute and Department of Pathology (M.A.D., T.K.), and the Wellcome Trust and Medical Research Council Cambridge Stem Cell Institute (B.J.P.H.), University of Cambridge — all in Cambridge, United Kingdom. Address reprint requests to Dr. Huntly at the Department of Haematology, Cambridge Institute for Medical Research, University of Cambridge, Cambridge CB2 0XY, United Kingdom, or at bjph2@cam.ac.uk.

N Engl J Med 2012;367:647-57.
DOI: 10.1056/NEJMra1112635

EPIGENETICS AND CHROMATIN BIOLOGY

The term "epigenetics" (see the Glossary) remains the object of contention and ambiguity.[6,7] It was originally coined by Waddington to describe heritable changes in gene expression and cellular phenotype that were independent of alterations in the DNA sequence.[7] Epigenetics is most frequently used to describe the study of chromatin biology, and this will be the definition used in this review. Chromatin is the macromolecular complex of DNA and histone proteins. It provides the scaffold for the packaging of our entire genome and contains the heritable material of eukaryotic cells.

The basic functional unit of chromatin is the nucleosome, which consists of an octamer containing two each of the histones H2A, H2B, H3, and H4, around which 147 bp of DNA are wrapped[6] (Fig. 1A). Nucleosomes compact and package DNA in a dynamic and highly controlled fashion that caters to the multitude of DNA-based processes. Consecutive nucleosomes are separated by unwrapped linker DNA, typically between 20 and 50 bp in length.[8] Wrapped nucleosomal DNA is inherently less accessible than linker DNA, thus the genomic positioning and compaction of nucleosomes strongly influences the ability of proteins to bind target sequences within DNA and to carry out their function. Broadly speaking, chromatin can be divided into two disparate states: heterochromatin, which is tightly packaged and contains primarily inactive genes, and euchromatin, which has a more relaxed con-

Table 1. Epigenetic Regulators with Reader Domains Recurrently Mutated in Cancer.*

Epigenetic Regulator	Epigenetic Site Modified	Epigenetic Reader Domain	Tumor Types
Catalytically active epigenetic readers			
DNA methyltransferase			
DNMT3A	5mC	PWWP	Acute myeloid leukemia, myelodysplastic syndrome, myeloproliferative neoplasms
Histone acetyltransferases			
KAT3A (CBP)	H2AK5 H2BK12–K15 H3K14–K18 H4K5–K8	Bromodomain	Acute myeloid leukemia, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, non-Hodgkin's lymphoma, and transitional-cell bladder cancer
KAT3B (p300)	H2AK5 H2BK12–K15 H3K14–K18 H4K5–K8	Bromodomain	Colorectal, breast, pancreatic, acute myeloid leukemia, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, transitional-cell bladder cancer
KAT6A (MOZ)	H3K14	PHD finger	Acute myeloid leukemia, myelodysplastic syndrome
KAT6B (MORF)	H3K14	PHD finger	Acute myeloid leukemia, uterine leiomyoma
Histone methyltransferases			
MT2A (MLL1)	H3K4	Bromodomain PHD finger	Acute myeloid leukemia, acute lymphoblastic leukemia, transitional-cell bladder cancer
KMT2B (MLL2)	H3K4	PHD finger	Medulloblastoma, renal, diffuse large B-cell lymphoma, follicular lymphoma
KMT2C (MLL3)	H3K4	PHD finger	Medulloblastoma, transitional-cell bladder cancer
KMT3B (NSD1)	H3K36	PWWP PHD finger	Acute myeloid leukemia
MMSET	H3K36 H4K20	PWWP PHD finger	Multiple myeloma
NSD3	H3K36	PWWP PHD finger	Acute myeloid leukemia
Histone demethylases			
KDM5A (JARID1A)	H3K4	PHD finger	Acute myeloid leukemia
KDM5C (JARID1C)	H3K4	PHD finger	Renal
Chromatin-remodeling enzymes			
SMARCA4 (BRG1)		Bromodomain	Lung, rhabdoid, medulloblastoma, breast, prostate, pancreas
SMARCA2 (BRM)		Bromodomain	Squamous-cell carcinomas of the head and neck
Noncatalytic epigenetic readers			
BRD1		Bromodomain PHD finger	Acute lymphoblastic leukemia
BRD3		Bromodomain	NUT midline carcinoma
BRD4		Bromodomain	NUT midline carcinoma
TRIM33		Bromodomain PHD finger	Papillary thyroid
PBRM1		Bromodomain	Renal, breast
ING1		PHD finger	Melanoma, breast
ING4		PHD finger	Head and neck
MSH6		PWWP	Colorectal

* The epigenetic regulators listed contain chromatin reader domains and have been described as mutated in the cancer genomes sequenced to date. Only genes that have been reproducibly detected on multiple occasions are listed. The names in parentheses are those in common use. MLL denotes mixed-lineage leukemia, NUT nuclear protein in testis, PHD plant homeodomain, and PWWP the domain characterized by the proline–tryptophan–tryptophan–proline motif. Information courtesy of Dr. Peter Campbell, Cancer Genome Project, Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

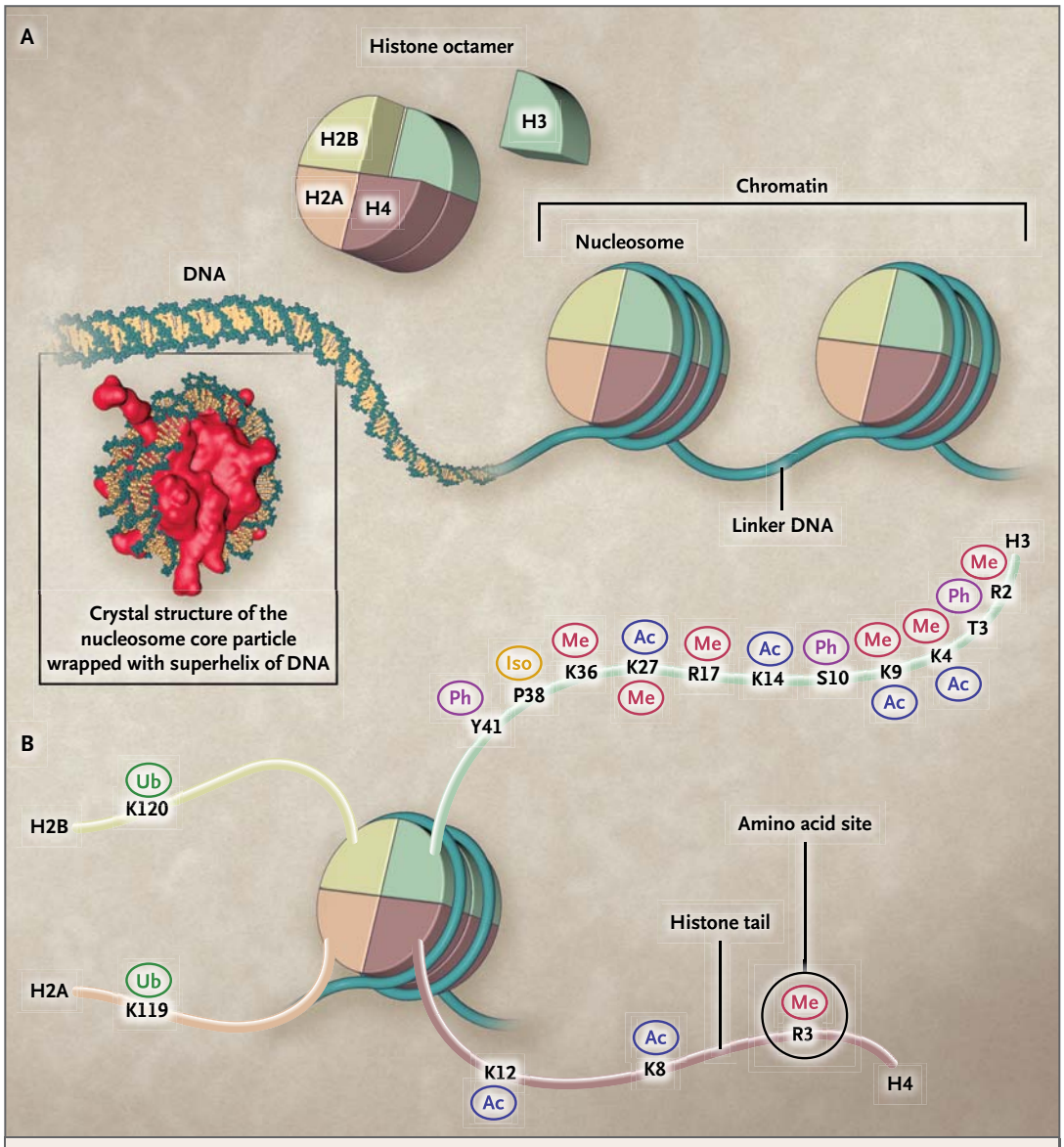


Figure 1. The Nucleosome.

The basic functional unit of chromatin is the nucleosome (Panel A), which is composed of a histone octamer around which DNA is wrapped. Octamers are separated by linker DNA. The histone octamer is assembled from a histone H3:H4 tetramer and two H2A:H2B dimers. The histone tails of all four core histones are subject to a variety of post-translational modifications (Panel B). These include methylation (Me), acetylation (Ac), phosphorylation (Ph), ubiquitylation (Ub), and proline isomerization (Iso), all of which occur at the site of a specific amino acid, such as K4 and K9 on the histone H3 tail. The same histone amino acid may be subject to different post-translational modifications, which may facilitate different biologic outcomes.

formation that provides a more permissive environment for active transcription. Several factors influence both local and global chromatin architecture, but perhaps the most influential elements that coordinate this process are the covalent modifications of either DNA or histones (Fig. 1B).[8] These chromatin modifications play an instructive role in regulating DNA-templated processes, including transcription, repair, and replication.[6,9]

Chromatin modifications serve two main functions: first, they physically enhance or weaken the

noncovalent interactions between histones or between histones and DNA, determining accessibility to specific DNA loci, and second, they provide an informative platform for the recruitment of epigenetic regulators. At least 4 different DNA modifications[10,11] and at least 16 distinct classes of histone modification have been described (Fig. 1B).[9,12] All of these chemical modifications are dynamic; they are laid down by "chromatin writers" and removed by "chromatin erasers" in a highly regulated manner (Fig. 2A). Chromatin immunoprecipitation coupled with next-generation sequencing has made it possible to annotate comprehensive global maps in specific cellular contexts for many of these modifications.[13,14] The vast array of modifications are not random and often adopt a predictable genomic distribution that can be used to define specific cellular processes, such as active transcription.[14] A fundamental advance in our understanding of chromatin regulation was the realization that many chromatin regulators possess specialized domains that allow these proteins to survey the epigenetic landscape and dock at specific regions within the genome. The bind-

Glossary

Acetylation: A reaction that results in the addition of a functional acetyl group to an organic compound. Deacetylation is the removal of the acetyl group. Acetylation is a post-translational chemical modification of both histones and nonhistone proteins.

Bromodomain and extraterminal (BET) proteins: A family of proteins (BRD2, BRD3, BRD4, and BRDT) characterized by tandem bromodomains that interact with acetylated histones and influence gene expression, cell-cycle regulation, and development.

Bromodomains: Regions within proteins capable of recognizing acetylated histones. Proteins containing bromodomains are involved in transcription, DNA repair, replication, and chromosome condensation.

Chromatin: The complex of DNA and protein that composes chromosomes. Chromatin allows the nearly 2 m of DNA material in each cell of the body to fit in a nucleus measuring just 4 to 8 μm in diameter. Chromatin provides the context for mitosis, meiosis, and gene expression. Changes in chromatin structure are affected by modifications in DNA and histones.

Chromatin immunoprecipitation (ChIP) assay: An experimental procedure designed to determine whether a given protein binds to a specific DNA sequence in the cell. DNA-bound protein is cross-linked to DNA, chromatin is then isolated, and the DNA–protein complexes are sheared into small fragments. Antibodies to the proteins are used to pull down fragments of DNA bound to specific proteins. The presence and quantification of specific or global DNA sequences can then be detected by polymerase-chain-reaction assay or massively parallel sequencing (ChIP-Seq) assay.

Chromodomains: Regions in proteins that are capable of detecting and binding methylated histones. Examples include the chromodomain of heterochromatin protein 1 (HP1), which recognizes the trimethylation of lysine 9 on histone H3 (H3K9me3).

Epigenetic erasers: Proteins capable of removing the chemical modifications of DNA or histones.

Epigenetic or chromatin readers: Protein complexes capable of detecting the precise temporal and spatial patterns of chromatin modifications and subsequently initiating regulatory processes based on these patterns. Readers are capable of initiating or silencing transcription, DNA repair, and other vital processes. Some readers are the products of tumor-suppressor genes.

Epigenetic writers: Proteins capable of adding chemical modifications to DNA or histones.

Epigenetics: The study of heritable changes to DNA structure that do not alter the underlying sequence; DNA methylation and histone modification are well-known examples.

Interactome: The complete set of physical molecular interactions among proteins that take place within a cell.

Linker DNA: The double-stranded DNA between two nucleosome cores.

Mediator complex: A multiprotein complex (comprising up to 26 subunits in humans) required for transcription. The complex binds to the C-terminal domain of RNA polymerase II and bridges the enzyme to transcription factors.

Methylation: The chemical addition of a methyl group (CH_3) to a substrate. Relevant targets for methylation include DNA, where it is generally focused on cytosine–phosphate–guanine (CpG) sites and results in the generation of 5-methylcytosine from cytosine. Protein methylation generally occurs on either arginine or lysine residues. Arginine may be monomethylated or dimethylated; lysine may be monomethylated, dimethylated, or trimethylated. Each of these states of methylation may convey different information.

Next-generation sequencing: A variety of new techniques that can generate a DNA sequence from a single molecule of DNA rather than from pools of DNA templates, allowing hundreds of millions of DNA fragments to be sequenced at the same time on a single platform (also known as massively parallel sequencing).

Nucleosome: The fundamental building block of chromatin; it is composed of 147 base pairs of DNA wrapped around two copies each of four histone proteins, H2A, H2B, H3, and H4.

NUT-midline carcinoma: A poorly differentiated midline epithelial neoplasm of the aerodigestive tract, characterized by a t(15;19) translocation; this genetic alteration creates a fusion protein between the *NUT* (nuclear protein in testis) gene on chromosome 15 and the *BRD4* (bromodomain 4) gene on chromosome 19.

Plant homeodomain (PHD) finger: A region within a protein capable of detecting methylated histones. Examples include the PHD finger of RAG2, which recognizes the trimethylation of lysine 4 on histone H3 (H3K4me3).

Glossary (Continued)

Polymerase-associated factor complex (PAFc): A protein complex that facilitates transcriptional initiation and elongation, in part through the recruitment of the MLL compass complex and the RNF20–RAD6 complex, which leads to H3K4 trimethylation and H2AK119 monoubiquitylation.

Positive transcription elongation factor b (P-TEFb) complex: A complex comprising CDK9 and cyclin T1 or T2 that phosphorylates the C-terminal domain of RNA polymerase II and the NELF–DSIF (negative elongation factor–DRB sensitivity-inducing factor) inhibitory complex, thereby facilitating the release of RNA polymerase II from a paused state into productive transcriptional elongation.

Proline–tryptophan–tryptophan–proline motif: A sequence of amino acids that make up the PWWP domain, which characterizes proteins involved in DNA methylation, DNA repair, and transcription regulation. Proteins with this motif are capable of detecting and binding methylated histones. Examples include the PWWP domain of the protein BRPF1, which recognizes the trimethylation of lysine 36 on histone H3 (H3K36me3).

RNA interference (RNAi) screening: The inhibition of gene expression by small noncoding RNA molecules. These molecules can hybridize with messenger RNA (mRNA) in the cell and direct the destruction of the message by Dicer, an endonuclease. Experimental screening is performed by exposing cells to many different RNAi sequences and assessing the effect on cell function or specific gene expression.

Sumoylation: A post-translational modification that plays a role in various cellular processes. Small ubiquitin-like modifier, or SUMO, proteins are a family of covalently linked small proteins (consisting of approximately 100 amino acids, or 12 kD) that are enzymatically attached to specific proteins (in a process analogous to ubiquitylation). Sumoylation influences the function of proteins by inhibiting the usual interaction between the target of sumoylation and another protein. The interaction involves the provision of a new binding surface or the initiation of conformational changes in the target protein. Sumoylated proteins are not destined for destruction. Chemically, sumoylation involves the formation of a peptide bond between a lysine on the target protein and a C-terminal glycine on the SUMO protein that is generated by the cleavage of the last four amino acids in the SUMO protein.

Superelongation complex (SEC): An assembly of transcription elongation factors that aids in the reinitiation of transcription by the proximal-promoter–paused RNA polymerase II.

Tudor domains: Regions within proteins that are capable of detecting and binding methylated histones. An example is the tudor domain of 53BP1, which recognizes the dimethylation of lysine 20 on histone H4 (H4K20me2).

Ubiquitylation: The ubiquitins are a family of small proteins that control the activity of larger proteins by reversibly marking them — either for destruction by the proteasome or for transport to different cellular compartments. This marking process involves a cascade of three types of enzymes known as E1, E2, and E3. The E1 enzyme activates ubiquitin and transfers it to an E2 enzyme, which interacts with a specific E3 partner to mediate the ligation of the ubiquitin moiety to the target protein. There are hundreds of E3 ubiquitin ligases, each of which (with help from various E2 conjugating enzymes) selects the specific protein that will receive the ubiquitin tag, thus determining the destination — such as the proteasome or the nucleus — of the protein. A major class of E3 ubiquitin ligases is the RING (named for "really interesting new gene") group of proteins, which act as scaffolds that juxtapose the target protein and the catalytic ubiquitin E2 enzyme.

ing modules within these "chromatin readers" recognize different covalent modifications of the nucleosome and assemble functional complexes onto specific loci to facilitate DNA-templated processes (Fig. 2B).[15,16]

In contrast with the static somatic alterations in DNA, the dynamic plasticity of the epigenome lends itself well to therapeutic manipulation. Epigenetic therapies targeting the catalytic activity of chromatin regulators have been developed and approved by the Food and Drug Administration for use in a small number of hematopoietic cancers.[17-19] These therapies include small molecules that target epigenetic writers (DNA methyltransferase inhibitors)[17,18] and epigenetic erasers (histone deacetylase inhibitors).[19] Despite these clinical successes, the pleiotropic effects of these compounds have made it difficult to decipher their exact molecular mechanism of action and have hampered their broader application in oncology. More recently, it has been possible to develop and deploy small molecules that specifically target the protein–protein interaction modules of certain epigenetic readers. This new therapeutic approach will be the primary subject of this review.

EPIGENETIC READERS

Many chromatin regulators, including several catalytic enzymes, act as "chromatin readers," possessing specialized domains that bind to distinct covalent modifications of the nucleosome and respond to information conveyed by upstream signaling cascades.[15] Critical residues within the binding pocket of the reader domain confer a preference for specific modification states, whereas residues outside the binding pocket contribute to determining the histone-sequence specificity. This modular combination allows proteins with similar binding domains to dock at different modified residues, or at the same amino acid displaying a different modification state.

For example, lysine residues have been shown to contain at least eight different covalent modifications, including acetylation, methylation, ubiquitylation, and sumoylation. Further complexity

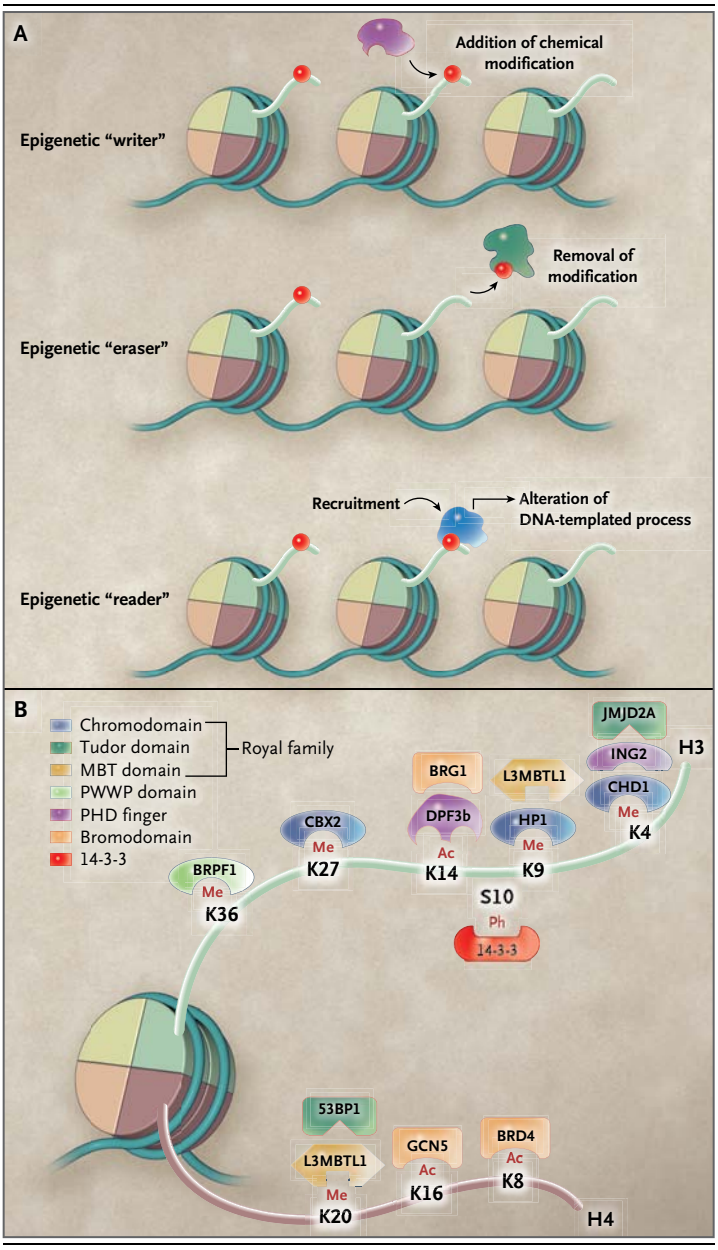


Figure 2. Epigenetic Regulation.

The main epigenetic regulators (Panel A) can be broadly categorized as epigenetic writers, which include the chromatin enzymes responsible for the deposition of covalent modifications on histones and DNA, the epigenetic erasers, enzymes that catalyze the removal of the covalent modifications of histones and DNA, and the epigenetic readers, proteins with specialized binding domains that recognize and bind to covalent modifications of histones and DNA. Epigenetic reader domains (Panel B) consist of specialized protein–protein interaction motifs that recognize and discriminate between various post-translational modifications. Domains within a class or family can have subtle variations that alter their preferred binding substrate. For instance, some plant homeodomain (PHD) fingers prefer binding to either unmodified or monomethylated lysines, whereas others show a binding preference for trimethylated lysines. The primary epigenetic reader domains shown represent those described in the literature to date. Panel B is adapted from Bannister and Kouzarides.[14]

exists by virtue of the fact that each lysine residue may be unmethylated, monomethylated, dimethylated, or trimethylated. For instance, the plant homeodomain (PHD) finger is a region within a protein that is capable of detecting methylated histones. The PHD fingers of the proteins BHC80 and DNMT3L preferentially bind unmethylated lysine residues,[20,21] whereas the PHD finger of ING2 binds most avidly to dimethylated and trimethylated lysines.[22,23] Other, similar methyl–lysine-recognition motifs exist, including chromodomains, tudor domains, and the PWWP domain, named for its characteristic proline–tryptophan–tryptophan–proline motif (Fig. 2B).[24-26] Notably, when the same lysine residue undergoes another modification, such as acetylation, it may then provide docking sites for other proteins containing acetyl–lysine binding domains, such as bromodomains.[27,28] Finally, to add to the complexity, many chromatin regulators have more than one type of reader domain, and their chromatin binding can be further influenced by neighboring histone modifications, so-called multivalent engagement-of-histone modifications.[29] These examples highlight the multifaceted mechanisms that chromatin readers use to decipher the intricate epigenetic landscape. (The best-characterized protein-binding pockets contained within chromatin-associated proteins are summarized in Fig. 2B.)

The importance of chromatin reader domains in maintaining homeostasis was initially revealed in the seminal observation that mutations in the PHD finger of *RAG2* abrogate the protein's ability to bind trimethylated-H3K4, reduce V(D)J recombination (in which variable, diverse, and joining gene segments are randomly combined), and result in immunodeficiency syndromes.[30] Mutations that abrogate the chromatin-reading capacity of many epigenetic regulators play an influential role in a variety of diseases, including cancer.[31] Using these chromatin reader modules as therapeutic targets may offer a unique opportunity to tailor therapies to specific diseases. An exemplar of this process has recently been provided with small molecules that specifically and avidly inhibit the tandem bromodomains of the

bromodomain and extraterminal (BET) family of proteins.[32-34]

BET BROMODOMAIN INHIBITORS

Bromodomains are highly conserved motifs present in a number of proteins throughout phylogeny.[27] More than 40 different human proteins contain a bromodomain, and some have multiple bromodomains. They can be clustered into nine major families according to sequence identity. Although the acetyl–lysine binding pocket for all bromodomains is hydrophobic, there can be considerable variation in the electrostatic interactions at the opening of the pocket among bromodomain families. This variation determines the specificity of individual bromodomains and provides the opportunity to develop specific small molecules that are targeted against certain families of bromodomains, including the BET proteins.[35,36]

The BET family has four members, including bromodomain-containing proteins 2, 3, and 4 (BRD2, BRD3, and BRD4), whose expression is ubiquitous, and BRDT, whose expression is confined to the germ cells. BET proteins share a common structural design, featuring a tandem bromodomain at the N-terminal end of the protein, and play an integral role in transcription and cell growth.[37] Early studies characterizing the BET proteins showed that BRD4 is associated with the mediator complex,[38] an important multicomponent protein complex facilitating the initiation of transcription.[39] BRD4 also associates with the active form of positive transcription elongation factor b (P-TEFb), a complex that regulates RNA polymerase II activity at the onset of transcriptional elongation.[40,41] BET proteins also maintain an association with chromatin throughout mitosis, and this feature facilitates "gene bookmarking," a process of rapid transcriptional reactivation of critical genes after mitosis.[42] Underscoring the physiological importance of the BET proteins in cellular homeostasis is the observation that the experimental knockout of either *Brd2*[43] or *Brd4*[44] in mice results in early embryonic lethality.

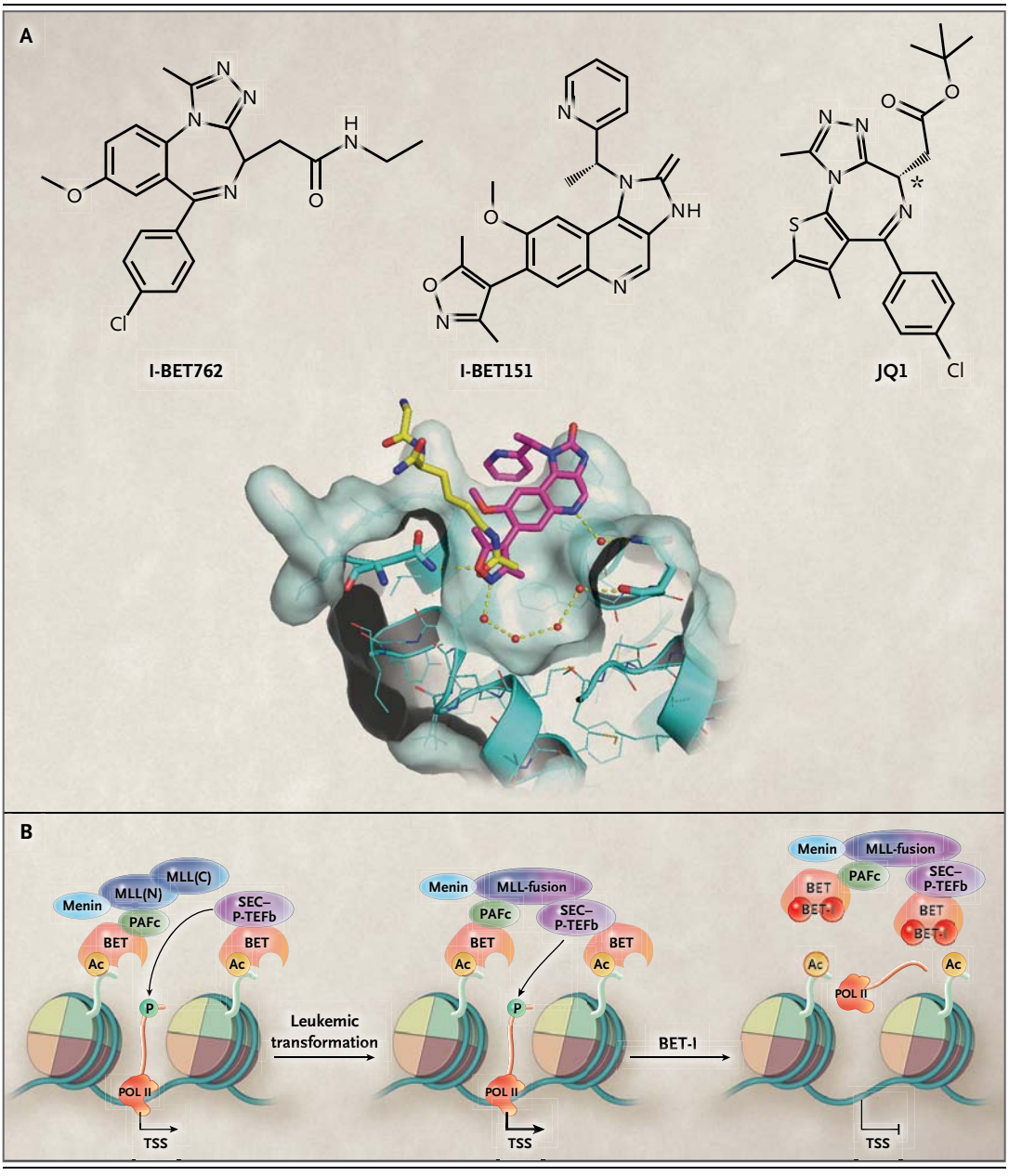
The essential nature of the BET proteins and their fundamental importance in the regulation of transcription has increased interest in elucidating the molecular mechanisms of their action. These efforts will be aided considerably by the recent identification of the complete nuclear BET-protein interactome.[32] In this study, three complementary global proteomic strategies were used to isolate the nuclear complexes containing the BET proteins. These data show that BET proteins are integral components of a large number of nuclear protein complexes that play a role in DNA replication, chromatin remodeling, DNA damage, and transcriptional regulation. Recurrent translocations of both *BRD3* and *BRD4* underpin the pathogenesis of NUT (or nuclear protein in testis) midline carcinoma,[33] and more recently, RNA interference (RNAi) screening strategies have been used to identify a central role for BRD4 in acute myeloid leukemia.[45] These findings have provided the impetus for investigating the potential of BET bromodomain inhibitors as novel anticancer agents.

BET INHIBITORS IN CANCER

Three different BET inhibitors (I-BET762,[34] JQ1,[33] and I-BET151[32]) show highly specific binding to both of the tandem domains of BRD2, BRD3, and BRD4 and inhibit their ability to engage with acetyl–lysine residues (Fig. 3A). These agents inhibit growth in a range of hematopoietic malignant cell lines[32,45-47] and in human NUT midline carcinoma cell lines.[33] Although the therapeutic efficacy of BET inhibition has not yet been tested in patients, its efficacy has been shown in vivo in murine models.

NUT MIDLINE CARCINOMA

NUT midline carcinoma is a rare but aggressive epithelial malignant disease that is invariably fatal, with a mean survival of less than 1 year.[48] It is most often manifested in the midline of the upper aerodigestive tract and mediastinum. Histologic diagnosis of NUT midline carcinoma is difficult, and confirmation relies primarily on the demonstration of rearrangement of the *NUT* gene with the use of fluorescence in situ hybridization.[48] *BRD4* and *BRD3* represent the *NUT* translocation partners in the majority of cases, and these fusions result in the aberrant localization of NUT to chromatin. Knockdown of the NUT fusions resulted in dramatic differentiation and growth arrest of the malignant cells, providing a therapeutic rationale for the targeting of BET proteins. Remarkably, the BET bromodomain inhibitor JQ1 was able to displace the BRD4–NUT fusion protein from chromatin and induce a rapid differentiation and arrest of proliferation in NUT midline carcinoma cell lines.[33] In addition, JQ1 showed excellent efficacy



in murine xenograft models of NUT midline carcinoma, resulting in tumor differentiation and regression and increased survival.[33]

ACUTE MYELOID LEUKEMIA WITH *MLL* TRANSLOCATIONS

Recurrent translocations of *MLL*, the mixed-lineage leukemia gene, result in aggressive leukemias with a poor prognosis that are often refractory to conventional therapies.[49] More than 70 different *MLL*-translocation partners have been identified in leukemia.[49] Despite this variation, a central abnormality in transcriptional elongation appears to underpin the molecular pathogenesis of this disease.[50] Many MLL fusion partners are members of the superelongation complex (SEC), an important regulator of transcriptional elongation.[51-53] Moreover, the N-terminal portion of MLL, a region conserved in all MLL fusion proteins, associates with another critical transcriptional complex called the polymerase-associated factor complex (PAFc).[54,55] The functional integrity of these complexes is critical for malignant transformation by MLL fusion proteins.[52-55] Two recent studies using

Figure 3 (facing page). The BET Inhibitors.

In Panel A, the chemical structures of the bromodomain and extraterminal (BET) inhibitors I-BET762, I-BET151, and JQ1 are shown, as is a surface diagram of the binding of I-BET151 (magenta). I-BET151 binds in the first bromodomain of BRD4, displacing the acetylate H3K14 peptide (yellow). In Panel B (left), the histone methyltransferase mixed-lineage leukemic (MLL) complex is enzymatically cleaved to generate two subunits. The amino (N) terminal subunit interacts with several cofactors, including menin and the polymerase-associated factor complex (PAFc). The carboxy (C) terminal subunit contains the methyltransferase activity and is lost in the various chromosomal translocations observed in acute myeloid and lymphoblastic leukemia. Many of the common translocation partners of MLL (including AF9, ENL, AF4, and ELL) are members of a critical transcription elongation complex called the superelongation complex (SEC). This complex also contains the positive transcription elongation factor b (P-TEFb) complex, which is composed of CDK9 and cyclin T1 or T2. The SEC–P-TEFb complex phosphorylates (P) RNA polymerase II (POL II), facilitating transcriptional elongation. MLL fusions therefore abnormally co-opt the activity of these complexes, which leads to aberrant transcriptional programs that culminate in leukemia (middle). Recent studies[32] showing that BET proteins are integral components of both the PAFc and SEC–P-TEFb complexes provide the rationale and molecular mechanism for the preclinical results observed when MLL-fusion leukemias are treated with the BET bromodomain inhibitors (BET-I). A proposed model (right) for the mechanism of action of BET inhibitors in MLL-translocated leukemia is that they prevent the binding of the BET-associated complexes (SEC and PAFc) to acetylated histones on chromatin. As a result, there is a decrease in the transcriptional activity exerted by the leukemic MLL fusions. TSS denotes the transcriptional start site of a gene critical to the maintenance of leukemia.

complementary approaches have identified an essential role for the BET proteins in a broad range of acute myeloid leukemias, including MLL-translocated leukemias.[32,45]

Dawson and colleagues used a global proteomic approach that identified BRD3 and BRD4 as key components of both PAFc and SEC.[32] In contrast, Zuber and colleagues used an RNAi screen to show that the depletion of BRD4 dramatically reduced the viability of MLL-AF9 leukemia in vitro and in vivo.[45] Both JQ1 and the novel BET inhibitor I-BET151 showed remarkable efficacy in vitro and in vivo against MLL fusion leukemia, resulting in the rapid induction of cell-cycle arrest and apoptosis. Both studies highlighted a reduction in expression of critical regulators of transformation, including MYC, BCL2, and CDK6 (a cyclin-dependent protein kinase), after treatment with a BET inhibitor. Dawson and colleagues also found that the therapeutic efficacy of I-BET151 could be attributed at least in part to the inhibition of BRD3–BRD4-mediated recruitment of PAFc and SEC to chromatin (Fig. 3). These molecular events resulted in reduced recruitment of RNA polymerase II to the promoters of crucial oncogenes such as *MYC*, *BCL2*, and *CDK6*.[32] BET inhibition was also effective against primary human acute myeloid leukemia cells in vitro.[32,45] Notably, in primary human MLL-translocated leukemia cells, BET inhibition dramatically reduced the clonogenic capacity of the leukemia stem-cell compartment, suggesting that disease eradication may be possible.

Although Zuber and colleagues focused primarily on MLL-translocated leukemias, they also showed the efficacy of BET inhibition in a range of acute myeloid leukemia subtypes in which MLL rearrangement was absent. These findings raise the intriguing possibility that BET inhibition may be a more broadly applicable therapy in this genetically heterogeneous disease.

MULTIPLE MYELOMA AND BURKITT'S LYMPHOMA

Multiple myeloma is an incurable plasma-cell dyscrasia. Although the disease is genetically heterogeneous,[56] disordered expression of the myelocytomatosis viral oncogene homolog MYC is a prominent feature, providing the rationale to test for BET inhibition.[57] Similarly, Burkitt's lymphoma, an aggressive lymphoproliferative disorder, is characterized by recurrent translocations of *MYC*, most frequently involving the *IGH* locus that results in constitutive high-level expression of *MYC*. Several multiple myeloma cell lines show a dramatic response to BET inhibition with JQ1.[46,47] Treatment with JQ1 leads to a profound cell-cycle arrest and apoptosis of the cell lines; this cellular phenotype is associated with a reduction in *MYC* transcription and protein expression. This transcriptional repression of *MYC* is linked to a reduction in the binding of chromatin by BRD4, upstream of the *MYC* promoter. Delmore and colleagues further translated these findings into work with primary human multiple myeloma cells and murine models of multiple myeloma, further indicating the therapeutic potential of BET inhibition in this disease.[46] In addition, Mertz and colleagues extended their findings to reveal a survival benefit in murine xenograft models of Burkitt's lymphoma and acute myeloid leukemia.[47]

Taken together, the studies involving BET bromodomain inhibitors further validate the use of epigenetic targets in cancer therapy. They show that it is possible to target epigenetic readers as well as catalytic writers and erasers. Moreover, the studies establish that the specific targeting of protein–protein interactions can have antitumor effects in vitro and in animal models. Several questions warrant further investigation. It is unclear why these drugs have effects in hematopoietic cancers but not in the majority of common solid tumors, including malignant tumors of the breast and cervix.[47] It is also unclear why a compound that inhibits the localization of proteins germane to transcription in general alters the expression of only a few hundred specific and reproducible genes. Although we have some insights into the molecular mechanism determining efficacy in the MLL fusion leukemias[32] and in NUT midline carcinoma,[33] it is largely unclear what molecular events dictate efficacy in the other sensitive cancers. A central theme in all the studies cited is the down-regulation of MYC. However, even though MYC has a prominent role in cancer,[58] it is unlikely that the profound effects observed with the introduction of BET inhibition are mediated solely by MYC inhibition. There are many malignant cell lines that overexpress MYC yet fail to respond to BET inhibition[47]; MYC expression is not always affected by BET inhibition,[47] and MYC down-regulation is not predictive of a response to BET inhibition.[32,45] Furthermore, MYC overexpression fails to prevent the apoptosis induced by BET inhibition.[45] Identification of the complete BET protein interactome has shown that the BET proteins are integral components of a large number of nuclear protein complexes.[32] This finding suggests that BET proteins are involved with a variety of molecular mechanisms.

CONCLUSIONS

Cancer epigenetics is an area of ongoing research that continues to inform our understanding of the molecular pathogenesis of cancer and to identify novel therapeutic targets. Advances in medicinal chemistry have now made it possible to specifically target not just the catalytic activity of epigenetic regulators but also the protein–protein interaction modules that localize many of these proteins to chromatin. However, as is the case for the majority of tumors sensitive to BET inhibition, epigenetic therapeutic targets are not necessarily mutated in sensitive tumor types. Therefore, simple mutational screening may not provide a predictor of response. Potential means of identifying sensitive tumor types include combining drug sensitivity with mutational screening, as has recently been reported in two large screening studies,[59,60] and using transcriptional or epigenetic biomarkers to predict response. The successful use of the bromodomain inhibitors in cancer therapy is likely to further energize basic scientists, clinicians, and the pharmaceutical industry to search for compounds that may similarly target and disrupt other chromatin reader motifs, including chromodomains and PHD fingers, which are already known to play key roles in certain cancers.[31]

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Andy Bannister for reviewing an earlier version of this article and for helpful comments; and Rab Prinjha and Chun Wa Chung for providing the chemical structures and surface structure used in Figure 3A.

REFERENCES

1. Rowley JD. Chromosomal translocations: revisited yet again. Blood 2008; 112:2183-9.
2. Chen J, Odenike O, Rowley JD. Leukaemogenesis: more than mutant genes. Nat Rev Cancer 2010;10:23-36.
3. Wouters BJ, Löwenberg B, Delwel R. A decade of genome-wide gene expression profiling in acute myeloid leukemia: flashback and prospects. Blood 2009;113: 291-8.
4. Stratton MR, Campbell PJ, Futreal PA. The cancer genome. Nature 2009;458:719-24.
5. Forbes SA, Bindal N, Bamford S, et al. COSMIC: mining complete cancer genomes in the Catalogue of Somatic Mutations in Cancer. Nucleic Acids Res 2011; 39:D945-D950.
6. Allis CD, Jenuwein T, Reinberg D. Epigenetics. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 2007.
7. Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. An operational definition of epigenetics. Genes Dev 2009;23: 781-3.
8. Segal E, Widom J. What controls nucleosome positions? Trends Genet 2009; 25:335-43.
9. Kouzarides T. Chromatin modifications and their function. Cell 2007;128: 693-705.
10. Baylin SB, Jones PA. A decade of exploring the cancer epigenome — biological and translational implications. Nat Rev Cancer 2011;11:726-34.
11. Wu H, Zhang Y. Mechanisms and functions of Tet protein-mediated 5-methylcytosine oxidation. Genes Dev 2011;25: 2436-52.
12. Tan M, Luo H, Lee S, et al. Identification of 67 histone marks and histone lysine crotonylation as a new type of histone modification. Cell 2011;146:1016-28.
13. Barski A, Cuddapah S, Cui K, et al. High-resolution profiling of histone methylations in the human genome. Cell 2007; 129:823-37.
14. Rando OJ, Chang HY. Genome-wide views of chromatin structure. Annu Rev Biochem 2009;78:245-71.

15. Taverna SD, Li H, Ruthenburg AJ, Allis CD, Patel DJ. How chromatin-binding modules interpret histone modifications: lessons from professional pocket pickers. Nat Struct Mol Biol 2007;14:1025-40.
16. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res 2011;21:381-95.
17. Tefferi A, Vardiman JW. Myelodysplastic syndromes. N Engl J Med 2009; 361:1872-85.
18. Garcia-Manero G, Fenaux P. Hypomethylating agents and other novel strategies in myelodysplastic syndromes. J Clin Oncol 2011;29:516-23.
19. Foss FM, Zinzani PL, Vose JM, Gascoyne RD, Rosen ST, Tobinai K. Peripheral T-cell lymphoma. Blood 2011;117: 6756-67.
20. Lan F, Collins RE, De Cegli R, et al. Recognition of unmethylated histone H3 lysine 4 links BHC80 to LSD1-mediated gene repression. Nature 2007;448:718-22.
21. Ooi SK, Qiu C, Bernstein E, et al. DNMT3L connects unmethylated lysine 4 of histone H3 to de novo methylation of DNA. Nature 2007;448:714-7.
22. Peña PV, Davrazou F, Shi X, et al. Molecular mechanism of histone H3K4me3 recognition by plant homeodomain of ING2. Nature 2006;442:100-3.
23. Shi X, Hong T, Walter KL, et al. ING2 PHD domain links histone H3 lysine 4 methylation to active gene repression. Nature 2006;442:96-9.
24. Bannister AJ, Zegerman P, Partridge JF, et al. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. Nature 2001;410:120-4.
25. Lachner M, O'Carroll D, Rea S, Mechtler K, Jenuwein T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. Nature 2001;410:116-20.
26. Fischle W, Wang Y, Jacobs SA, Kim Y, Allis CD, Khorasanizadeh S. Molecular basis for the discrimination of repressive methyl-lysine marks in histone H3 by Polycomb and HP1 chromodomains. Genes Dev 2003;17:1870-81.
27. Dhalluin C, Carlson JE, Zeng L, He C, Aggarwal AK, Zhou MM. Structure and ligand of a histone acetyltransferase bromodomain. Nature 1999;399:491-6.
28. Jacobson RH, Ladurner AG, King DS, Tjian R. Structure and function of a human TAFII250 double bromodomain module. Science 2000;288:1422-5.
29. Ruthenburg AJ, Li H, Patel DJ, Allis CD. Multivalent engagement of chromatin modifications by linked binding modules. Nat Rev Mol Cell Biol 2007;8:983-94.
30. Matthews AG, Kuo AJ, Ramón-Maiques S, et al. RAG2 PHD finger couples histone H3 lysine 4 trimethylation with V(D)J recombination. Nature 2007;450: 1106-10.
31. Chi P, Allis CD, Wang GG. Covalent histone modifications — miswritten, misinterpreted and mis-erased in human cancers. Nat Rev Cancer 2010;10:457-69.
32. Dawson MA, Prinjha RK, Dittmann A, et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 2011;478: 529-33.
33. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. Nature 2010;468:1067-73.
34. Nicodeme E, Jeffrey KL, Schaefer U, et al. Suppression of inflammation by a synthetic histone mimic. Nature 2010;468: 1119-23.
35. Chung CW, Dean AW, Woolven JM, Bamborough P. Fragment-based discovery of bromodomain inhibitors part 1: inhibitor binding modes and implications for lead discovery. J Med Chem 2012;55:576-86.
36. Bamborough P, Diallo H, Goodacre JD, et al. Fragment-based discovery of bromodomain inhibitors part 2: optimization of phenylisoxazole sulfonamides. J Med Chem 2012;55:587-96.
37. Chiang CM. Brd4 engagement from chromatin targeting to transcriptional regulation: selective contact with acetylated histone H3 and H4. F1000 Biol Rep 2009;1:98.
38. Jiang YW, Veschambre P, Erdjument-Bromage H, et al. Mammalian mediator of transcriptional regulation and its possible role as an end-point of signal transduction pathways. Proc Natl Acad Sci U S A 1998;95:8538-43.
39. Malik S, Roeder RG. The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. Nat Rev Genet 2010;11:761-72.
40. Yang Z, Yik JH, Chen R, et al. Recruitment of P-TEFb for stimulation of transcriptional elongation by the bromodomain protein Brd4. Mol Cell 2005;19: 535-45.
41. Jang MK, Mochizuki K, Zhou M, Jeong HS, Brady JN, Ozato K. The bromodomain protein Brd4 is a positive regulatory component of P-TEFb and stimulates RNA polymerase II-dependent transcription. Mol Cell 2005;19:523-34.
42. Zhao R, Nakamura T, Fu Y, Lazar Z, Spector DL. Gene bookmarking accelerates the kinetics of post-mitotic transcriptional re-activation. Nat Cell Biol 2011;13: 1295-304.
43. Shang E, Wang X, Wen D, Greenberg DA, Wolgemuth DJ. Double bromodomain-containing gene Brd2 is essential for embryonic development in mouse. Dev Dyn 2009;238:908-17.
44. Houzelstein D, Bullock SL, Lynch DE, Grigorieva EF, Wilson VA, Beddington RS. Growth and early postimplantation defects in mice deficient for the bromodomain-containing protein Brd4. Mol Cell Biol 2002;22:3794-802.
45. Zuber J, Shi J, Wang E, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature 2011;478:524-8.
46. Delmore JE, Issa GC, Lemieux ME, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 2011;146:904-17.
47. Mertz JA, Conery AR, Bryant BM, et al. Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc Natl Acad Sci U S A 2011;108:16669-74.
48. French CA. Demystified molecular pathology of NUT midline carcinomas. J Clin Pathol 2010;63:492-6.
49. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. Nat Rev Cancer 2007;7:823-33.
50. Mohan M, Lin C, Guest E, Shilatifard A. Licensed to elongate: a molecular mechanism for MLL-based leukaemogenesis. Nat Rev Cancer 2010;10:721-8.
51. Mueller D, García-Cuellar MP, Bach C, Buhl S, Maethner E, Slany RK. Misguided transcriptional elongation causes mixed lineage leukemia. PLoS Biol 2009;7(11): e1000249.
52. Lin C, Smith ER, Takahashi H, 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; 37:429-37.
53. Yokoyama A, Lin M, Naresh A, Kitabayashi I, Cleary ML. A higher-order complex containing AF4 and ENL family proteins with P-TEFb facilitates oncogenic and physiologic MLL-dependent transcription. Cancer Cell 2010;17:198-212.
54. Milne TA, Kim J, Wang GG, et al. Multiple interactions recruit MLL1 and MLL1 fusion proteins to the HOXA9 locus in leukemogenesis. Mol Cell 2010;38:853-63.
55. Muntean AG, Tan J, Sitwala K, et al. The PAF complex synergizes with MLL fusion proteins at HOX loci to promote leukemogenesis. Cancer Cell 2010;17:609-21.
56. Chapman MA, Lawrence MS, Keats JJ, et al. Initial genome sequencing and analysis of multiple myeloma. Nature 2011; 471:467-72.
57. Shou Y, Martelli ML, Gabrea A, et al. Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation and tumor progression in multiple myeloma. Proc Natl Acad Sci U S A 2000;97:228-33.
58. Meyer N, Penn LZ. Reflecting on 25 years with MYC. Nat Rev Cancer 2008;8:976-90.
59. Garnett MJ, Edelman EJ, Heidorn SJ, et al. Systematic identification of genomic markers of drug sensitivity in cancer cells. Nature 2012;483:570-5.
60. Barretina J, Caponigro G, Stransky N, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature 2012;483:603-7.