Epigenetic therapy of hematological malignancies: where are we now?

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Abstract: A growing amount of evidence points towards alterations in epigenetic machinery as a leading cause in disease initiation and progression. Like genetic alterations, misregulation of the epigenetic regulators can lead to abnormal gene expression. However, unlike genetic events, the epigenetic machinery may be targeted pharmacologically, potentially resulting in the reversal of a particular epigenetic state. The success of DNA methyltransferase and histone deacetylase inhibitors represents a proof of concept for the use of therapies intended to target the epigenome in the treatment of hematological malignancies. Nevertheless, the molecular mechanisms underlying the efficacy of these agents have not been completely elucidated. Recently, a large number of studies sequencing cancer cell genomes identified recurring mutations of epigenetic regulators, providing new insights into the molecular underpinnings of cancer. Consequently, the efforts to identify specific epigenetic inhibitors have been expanded in order to target particular subsets of patients. This review will summarize the progress made using the currently available epigenetic therapies and discuss some of the more recently identified targets whose inhibition may present potential avenues for the treatment of hematologic malignancies.

Keywords: epigenetics, methylation, MLL, EZH2

Introduction

The fate of any individual cell depends on the expression of genes that regulate its development, from the most primitive stages through terminal differentiation. While all cells contain an identical set of genetic information, various mechanisms exist to ensure that only a subset of genes, required for a particular cell's function, are being expressed at any given time. The sequence-encoded regulatory elements and sequence-specific transcription factors can only partially explain how this is achieved. In addition, chromatin architecture plays an important role in regulating gene expression. DNA and histone proteins are covalently modified, signaling for a particular transcriptional state, all while leaving the underlying DNA sequence intact. Collectively, these epigenetic mechanisms affect all known biological processes and play a critical role in normal development and the disease state.

Epigenetic regulation is exerted through covalent modifications of DNA and histones by enzymes that can add ('writers') or remove ('erasers') these

modifications to or from specific residues. In addition, other proteins ('readers') can interpret this epigenetic code through domains that specifically recognize certain modifications. Together, this leads to the recruitment of activator or repressor complexes that are responsible for alteration of chromatin structure and the induction or repression of transcription.

Methylation of DNA at the cytosine of the CpG dinucleotide is associated with gene silencing. While generally under represented genome wide, large clusters of CpG dinucleotides, or CpG islands, are commonly found in the 5' gene regulatory elements. Methylation of these islands leads to the recruitment of repressor complexes and transcriptional inactivation. DNA methylation is performed by a family of DNA methyltransferases (DNMTs), of which three main members have been identified in eukaryotic cells (throughout this review, human gene names and gene products are capitalized and italicized; mouse gene names and gene products have only the first letter capitalized and are also italicized;

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nomenclature that refers to protein products is the same as gene names but nonitalicized): DNMT1, DNMT3A, and DNMT3B [Bestor, 2000]. DNMT1 is considered a maintenance methyltransferase, whereas DNMT3A and DNMT3B catalyze *de novo* methylation of DNA. Human tumors are characterized by a redistribution of DNA methylation, with the hypomethylation and hypermethylation of certain genes defining particular tumor types. In general, human tumors are considered to be globally hypomethylated, which is thought to contribute to genomic instability and further mutations. However, locus-specific hypermethylation, often in CpG islands upstream of tumor suppressor genes, has been identified in many types of cancers, which results in gene silencing.

In chromatin, DNA is wrapped around histone proteins to form the nucleosome. The nucleosome is composed of four core histones, H2A, H2B, H3, and H4, all of which have a histone 'tail' that does not bind to the DNA. The histone tail can undergo a variety of post-translational modifications, including methylation, acetylation, phosphorylation, and ubiquitination, with methylation and acetylation being the most studied. Depending on the residues being modified, identical modifications can have opposing consequences. Trimethylation of lysine 4 on histone H3 (H3K4me3) in the promoter region is associated with actively transcribed genes, whereas trimethylation of lysine 27 on histone H3 (H3K27me3) in the same region is associated with gene repression. In addition, these two normally mutually exclusive modifications can coexist on the same loci under certain conditions, such as in stem cells [Bernstein *et al.* 2006]. These bivalent marks (H3K4me3/H3K27me3) allow stem cell genes to maintain a poised state, enabling the genes to be quickly activated or repressed upon lineage selection. Genes that are activated upon differentiation lose the H3K27me3 mark, and repressed genes lose the H3K4 trimethylation. This illustration of combinatorial regulation is an example of the so-called 'histone code', by which specific enzymes can alter chromatin structure through modification of histones, effectively shifting the transcription of a target gene.

Many recent studies have linked misregulation of the epigenetic pathways to oncogenesis. Genetic changes, including mutations, deletions, amplifications, and translocations of genes encoding for the epigenetic machinery, have epigenetic

consequences, including genome-wide changes in chromatin or DNA methylation content in the cancer cell. While the underlying genetic anomaly is not easily remedied, the aberrant activity of epigenetic enzymes affected by the genetic lesions is theoretically targetable by small molecules. These include molecules that can affect the writers and erasers of the epigenetic code, as well as molecules that can interfere with the ability of the readers to interact with particular epigenetic modules. Currently, the only clinically tested epigenetic therapies involve inhibition of histone deacetylases (HDACs) and DNMTs. While promising, these therapies are pleiotropic, and without a better understanding of the molecular mechanism of action of these drugs, it will be difficult to improve their efficacy. A new generation of directed epigenetic therapies involves targeting specific epigenetic regulators whose function may be altered in a particular disease subset. Here, we briefly summarize some of the existing clinical data involving epigenetic therapies in hematological malignancies and discuss the development of the new molecules that may present more precise strategies for the treatment of patients with cancer.

DNA methylation machinery in normal hematopoiesis and malignancy

The DNMT family of genes has proven to be important in hematopoiesis and cancer development. Loss of *Dnmt1* in adult hematopoietic stem cells (HSCs) leads to numerous defects of HSCs *in vivo*, including impaired self-renewal, bone marrow niche retention, and altered patterns of DNA methylation and gene expression [Trowbridge *et al.* 2009]. In addition, mice with a *Dnmt1* deficiency cannot suppress key myeloid regulators and thus cannot differentiate into lymphoid progeny [Broske *et al.* 2009]. Earlier work found that loss of either *Dnmt3a* or *Dnmt3b* alone did not have any effect on HSC function, but a combined deficiency of these molecules led to impaired self-renewal capacity in HSCs [Tadokoro *et al.* 2007]. However, a more recent study showed that conditional ablation of *Dnmt3a* in HSCs resulted in impaired differentiation *in vivo*, along with increased self-renewal of HSCs in the bone marrow [Challen *et al.* 2012]. *Dnmt3a*-deficient cells have both hypomethylation and hypermethylation of specific loci, many of which are associated with changes observed in human hematopoietic malignancies [Challen *et al.* 2012].

The advent of massively parallel DNA sequencing is one technique that has allowed for the discovery of previously undefined mutations in cancers, notably those involved in epigenetic regulation. In 2010, this technology revealed that *DNMT3A* harbored mutations in 20% of patients with acute myeloid leukemia (AML), the most common being the R882 mutation [Ley *et al.* 2010; Yamashita *et al.* 2010; Yan *et al.* 2011]. Patients with a *DNMT3A* mutation have a significantly poorer outcome than those without. The mutant protein showed reduced enzymatic activity in *in vitro* studies, and was associated with altered DNA methylation and expression profiles [Yan *et al.* 2011]. *DNMT3A* mutations are also found in myelodysplastic syndrome (MDS) and are thought to occur early during the disease, resulting in a more rapid progression to AML [Walter *et al.* 2011]. Prior work has shown that there are distinct patterns of DNA methylation that characterize AML subtypes, but how these patterns contribute to disease progression is still not fully defined [Figueroa *et al.* 2009, 2010b].

Another gene family that has come to light as important in epigenetic control is the *TET* (*Ten-Eleven-Translocation*) family. *TET1–3* all contain enzymatic activity allowing for the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) [Ko *et al.* 2010; Tahiliani *et al.* 2009]. 5-hmC is hypothesized to be an intermediate in the demethylation of DNA, and *TET* enzymatic activity is considered related to the base excision repair pathway of DNA [Guo *et al.* 2011]. However, the precise function of 5-hmC is still incompletely understood and requires further investigation. In 2002, *TET1* was identified as a fusion partner of *MLL* in patients with AML [Lorsbach *et al.* 2003; Ono *et al.* 2002], and mutations of *TET2* were identified in AML, MDS, myeloproliferative neoplasm, and chronic myelomonocytic leukemia several years later [Abdel-Wahab *et al.* 2009; Delhommeau *et al.* 2009; Langemeijer *et al.* 2009]. *TET2* mutations include deletions, truncations, missense and nonsense mutations, usually resulting in gene inactivation. Often, mutations of *TET2* are found in combination with other mutations seen in myeloid malignancies, such as *ASXL1* [Abdel-Wahab *et al.* 2011], suggesting that it may be a part of a synergistic effect in disease progression.

Mutations in the isocitrate dehydrogenases, *IDH1* and *IDH2*, are also implicated in cancer progression. Mutant *IDH1* and *IDH2* were initially

identified in glioma [Parsons *et al.* 2008; Yan *et al.* 2009], but recurrent mutations have also been characterized in MDS and AML [Green and Beer, 2010; Mardis *et al.* 2009]. Under normal conditions, *IDH1/2* are part of the citric acid cycle and act to catalyze the production of α -ketoglutarate (α KG) from isocitrate. However, when mutations occur in either enzyme, an aberrant metabolite known as 2-hydroxyglutarate is produced instead, inhibiting the activity of α KG-dependent enzymes, which include the *TET* proteins and the Jumonji (JmjC) family of histone demethylases [Xu *et al.* 2011]. Expression of mutant *IDH1/2* or a deficiency of *TET2* in normal hematopoietic cells leads to an expansion of stem cell function and a differentiation blockade consistent with a proleukemogenic effect [Moran-Crusio *et al.* 2011; Quivoron *et al.* 2011]. *IDH1/2*-mutant AMLs have a distinct DNA hypermethylation signature and are found to be mutually exclusive with *TET2* mutations, suggesting that they act within the same pathway [Figueroa *et al.* 2010a].

Current epigenetic therapies in cancer

DNA methylation is a reversible process, thus making it an attractive and viable target for cancer therapeutics. There are two main US Food and Drug Administration (FDA)-approved DNA hypomethylating agents, 5-azacitidine (5-aza) and decitabine (5-aza-deoxycytidine), which have proven efficacious in treating human tumors. 5-aza is primarily incorporated into RNA and at a lower frequency into DNA, whereas decitabine is incorporated into DNA. Both drugs covalently trap the DNMT enzymes to cytosine residues, preventing them from completing the enzymatic reaction. These compounds act generally at CpG dinucleotides, but do not show any specificity for particular DNA loci [Kihlslinger and Godley, 2007]. The precise mechanism of action of these drugs is still unclear, but one hypothesis is that this trapping of DNMTs leads to the degradation of these factors, resulting in overall reduced DNMT levels, and accordingly gene hypomethylation and re-expression of previously silenced loci.

Although 5-aza and decitabine have been used to treat numerous types of human tumors, they have been perhaps most successful against the hematologic malignancies, specifically MDS and AML. 5-aza was shown to have a significant response rate in patients with MDS, along with

reduced risk of leukemic transformation and increased survival, which helped lead to its FDA approval in 2004 [Silverman *et al.* 2002]. A large-scale clinical study known as AZA-001 helped establish 5-aza as the preferred treatment for patients with high-risk MDS [Fenaux *et al.* 2009]. Fewer data exist regarding the usage of these compounds in treating lower-risk MDS. Dosing of DNMT inhibitors has always been challenging, but it appears that a more prolonged administration of the drugs results in decreased cell cycle inhibition, allowing for greater incorporation of the drugs into the DNA and a reversal of methylation patterns [Gore, 2011]. Both of these drugs are normally administered via injection, but an oral form of 5-aza is also being investigated, and appears to be almost as efficient at demethylating particular DNA loci, as the majority of patients developed a clinical response [Garcia-Manero *et al.* 2011; Ziemba *et al.* 2011].

A number of other compounds that are reported to inhibit DNMTs also exist. Virtual screening efforts have led to the discovery of compounds like RG108 and NSC14778 [Brueckner *et al.* 2005; Kuck *et al.* 2010]. Molecular modeling and other computational approaches have also been used to develop specific inhibitors of the DNMTs through ligand-binding interactions. The crystal structure of DNMT1 was recently published [Song *et al.* 2011] and was used to identify non-nucleoside DNMT1 inhibitors, at least one of which appears to have promising activity for epigenetic therapy [Yoo *et al.* 2012]. In addition, some drugs that are approved for other applications have DNMT inhibitory activity, such as the nucleoside analog and antiarrhythmic drug procainamide [Halby *et al.* 2012].

HDAC inhibitors (HDACi) are another class of drugs that have become more prominent in the treatment of hematopoietic diseases. These inhibitors promote the retention of acetyl groups on the tails of histone proteins, which allows for a more active, open chromatin conformation. The first HDACi, vorinostat, received FDA approval in 2006 for the treatment of cutaneous T-cell lymphoma [Mann *et al.* 2007]. Other HDACi, such as sodium phenylbutyrate, have existed for a number of years, and have given rise to second-generation compounds. These include entinostat and panobinostat, and have moderate effects in treating MDS, AML and acute lymphocytic leukemia when used alone [Dimicoli *et al.* 2012; Giles *et al.* 2006].

Although single-agent therapy has proven efficacious, mostly for hypomethylating agents, combinatorial studies have been done to enhance the effectiveness of these drugs. In the case of HDACi, induced expression of highly methylated genes is generally not detected when these compounds are administered alone. However, administration of a hypomethylating agent in combination with an HDAC inhibitor in MDS and AML have been shown to promote re-expression of epigenetically silenced genes, along with major clinical responses [Cameron *et al.* 1999; Gore *et al.* 2006]. In contrast, another study showed that administration of 5-aza and entinostat did not show a correlation between clinical response and reversal of DNA methylation or gene expression of tumor suppressor genes [Fandy *et al.* 2009]. Interestingly, treatment with both drugs led to an increase in γ -H2AX in peripheral blood mononuclear cells, indicating that DNA damage was occurring. Thus, even though these therapies are supposedly targeting the epigenome, further studies are still required to pinpoint how these drugs interact, as it is still highly possible that other mechanisms, such as DNA damage, apoptosis, or immune response are accountable for the responses observed in patients.

Emerging epigenetic therapies

MLL-associated leukemias

Histone methyltransferases (HMTs) can be subdivided based on the nature of their substrate to lysine and arginine methyltransferases. Histone methylation enhances the combinatorial power of the histone code due to the fact that lysine residues can be mono-, di-, or trimethylated, while arginines can be monomethylated or symmetrically or asymmetrically dimethylated. Each one of these methylation states can have a specific biological effect. Except for DOT1L (KMT4), all lysine methyltransferases contain the conserved SET (Suppressor of variegation, Enhancer of Zeste, and Trithorax) domain that is the enzymatic engine of the protein.

MLL (Mixed Lineage Leukemia, KMT2A) is a histone methyltransferase that specifically methylates of lysine 4 on histone H3, a mark found in the promoters of the transcriptionally active genes [Nakamura *et al.* 2002]. Rearrangements of *MLL* occur in about 10% of all acute leukemias, and are found in more than 70% of cases of infant leukemia. *MLL* has been found fused to more

than 70 different partner proteins, and in each case, the N-terminus of MLL is maintained in the fusion protein [Popovic and Zeleznik-Le, 2005]. Given that the SET domain of MLL is located on the C-terminus, it would be expected that the lack of the enzymatic activity silences the MLL targets, such as *HOX* genes, leading to the leukemic phenotype. However, MLL translocations lead to enhanced expression of the MLL target genes, suggesting that the aberrant gene activation is not linked to histone methylation by the SET domain of MLL.

While there is not a single unifying property of the MLL fusion partners that can account for an increase in gene transcription, many of the partners are members of large super elongation complexes (SECs) [Lin *et al.* 2010]. The SECs include the positive transcription elongation factor b that can stimulate promoter-arrested RNA polymerase by phosphorylation, allowing for transcriptional elongation and promoter clearance [Yang *et al.* 2005]. Other members of SECs include the BRD family of epigenetic readers that recognize acetylated histones through their tandem bromodomain module and play a role in the recruitment of the complexes to chromatin [Benedikt *et al.* 2011]. Two studies simultaneously showed that inhibition of the BRD–histone interaction using small molecule inhibitors, JQ1 and I-BET151, induce differentiation and apoptosis of leukemic cells and extend survival of mice transplanted with cells expressing MLL fusion proteins [Dawson *et al.* 2011; Zuber *et al.* 2011]. In each case, BRD inhibition abrogated *c-MYC* transcriptional networks, through direct inhibition of *c-MYC* transcription. This finding prompted a broader use of BRD inhibitors in malignancies like multiple myeloma, in which activation of *c-MYC* had been shown to play an important role in disease development [Delmore *et al.* 2011]. Treatment of multiple myeloma cell lines with JQ1 leads to decreased cell growth, including cells resistant to other forms of antimyeloma therapeutic agents, such as melphalan and dexamethasone. These findings raise a possibility that BRD inhibition may have a wide therapeutic scope in hematopoietic and other malignancies.

Besides recruitment of SECs, other MLL fusion partners can recruit smaller protein complexes, which include DOT1L methyltransferase, specific for methylation of lysine 79 on histone H3 (H3K79), another mark associated with actively transcribed genes [Bitoun *et al.* 2007]. Consequently, *HOX*

genes and other MLL targets are enriched for H3K79 methylation in the presence of MLL fusion proteins. In the absence of *Dot1l*, the MLL fusions are unable to transform bone marrow progenitor cells, and depletion of *Dot1l* from MLL-AF9 transformed cells leads to decreased expression of MLL-AF9 target genes [Bernt *et al.* 2011; Chang *et al.* 2010]. Together, these data suggest that inhibition of DOT1L activity may be a promising way of targeting leukemia-associated genes turned on by MLL fusion proteins.

With that in mind, Daigle and colleagues identified EPZ004777 as a potent and selective inhibitor of DOT1L [Daigle *et al.* 2011]. The inhibitor specifically decreased H3K79 methylation of all the cell lines tested, but interestingly, it only inhibited growth and induced apoptosis of cell lines harboring MLL rearrangements. Proliferation of cell lines without MLL fusion proteins was not affected by EPZ004777, even though the levels of H3K79 methylation were dramatically reduced. The inhibitor was well tolerated in the *MLL-AF9* leukemia mouse model, without adverse effect on normal hematopoiesis. This is a critical finding given that, in mice, normal hematopoiesis is affected by *Dot1l* loss, leading to anemia and depletion of the HSC compartment [Jo *et al.* 2011]. Together, this suggests that DOT1L addiction is specific to cells expressing MLL fusion proteins, enhancing the therapeutic potential of DOT1L inhibition.

MLL fusion proteins can also be inhibited through impairment of their recruitment to their target genes. Normally, both the wild-type MLL and the MLL fusion proteins depend on the protein MENIN for proper recruitment to the *HOX* cluster and other targets [Yokoyama *et al.* 2005]. *MENIN* is a tumor suppressor gene that interacts with the N-terminal portion of MLL, which is maintained within the MLL fusions. The presence of *MENIN* is required for proper expression of *MLL* target genes and for leukemia induction by MLL fusion proteins. In a recent study, Grembecka and colleagues used a small molecule inhibitor, MI-2, to prevent MLL interaction with MENIN [Grembecka *et al.* 2012]. As a result, MI-2 inhibited growth and colony formation of cells transformed with MLL fusion proteins by preventing the fusion proteins from being properly recruited to their transcriptional targets. Because normal MLL function may also be impaired using MI-2 inhibitor, additional studies are needed to assess the possible effects

these types of molecules may have on normal hematopoiesis.

Collectively, in the preclinical setting, various epigenetic targeting approaches have shown promise for the treatment of MLL-associated leukemias. With improved pharmacokinetic properties and increased efficacy *in vivo*, it is likely that these agents will soon enter clinical trial.

EZH2 and H3K27 methylation

Enhancer of Zeste 2 (EZH2, KMT6) is the catalytic subunit of the Polycomb Repressive Complex 2 (PRC2), which catalyzes dimethylation and trimethylation of lysine 27 on histone H3 (H3K27). PRC2 consists of three additional core components, SUZ12, EED and RbAp46/48, which are required for the methylation function of the complex [Morey and Helin, 2010]. The methylation of lysine 27 elicits gene repression and alterations of this epigenetic mark are common in cancer and development. EZH2 is overexpressed in a wide range of cancers and its upregulation is associated with enhanced self-renewal, cell migration and an increase in genomic instability [Albert and Helin, 2010; Bracken *et al.* 2006; Kleer *et al.* 2003]. Consistent with an oncogenic role, recurrent gain-of-function somatic mutations have been described in approximately 30% of diffuse large B-cell lymphomas and 10% of follicular lymphomas [Morin *et al.* 2010]. Wild-type EZH2 converts unmethylated H3K27 to H3K27me1, and to a lesser extent me2 and me3. Mutations of tyrosine 641 (Y641) within the SET domain lead to altered substrate specificity and recognition of the H3K27me1, produced by the wild-type protein, and its conversion to H3K27me3 [Sneeringer *et al.* 2010]. Because the Y641X mutations are always heterozygous, it can be concluded that the effect of the mutant protein greatly depends on the enzymatic activity of the other wild-type allele. Consequently, the lymphoma cell lines carrying the Y641 mutations show a global increase in H3K27me3 mark [Sneeringer *et al.* 2010; Yap *et al.* 2011]. Normally, the germinal center reaction activates EZH2 expression in B cells, where its role is to suppress the DNA damage response pathways that would otherwise be stimulated due to somatic hypermutation during antibody production [Velichutina *et al.* 2010]. A global increase in H3K27me3 due to the Y641 mutations may alter these and other important pathways that enhance cell survival and induce transformation. It will be of interest to determine how EZH2

mutations may affect other epigenetic mechanisms such as DNA methylation, given that EZH2 can directly interact with DNMTs and control their activity [Vire *et al.* 2006].

The presence of the H3K27me mark depends not only on EZH2, but also on the demethylating enzymes, UTX/UTY, and JMJD3. All three of these proteins belong to the Jumonji family of demethylases, and their cellular roles are tissue specific and context dependent [Agger *et al.* 2007; Hubner and Spector, 2011]. Somatic mutations in *UTX* have been described in a number of cancers, including AML and up to 10% of multiple myelomas [van Haaften *et al.* 2009]. Interestingly, many of the *UTX* mutant tumors also have a loss of *UTY*, presumably leading to a major loss of H3K27 demethylation activity. Similar to the gain-of-function EZH2 mutations, alterations in *UTX/UTY* lead to an increase in H3K27 methylation. However, this increase seems to occur more focally, on specific *UTX* targets. The normal roles of *UTX*, *JMJD3* and other members of the *JmjC* family may also be affected in the presence of the previously mentioned *IDH1/2* mutations, commonly found in myeloid neoplasia due to the production of the aberrant metabolite 2-hydroxyglutarate [Ward *et al.* 2010]. Indeed, Xu and colleagues showed that *IDH1* mutations induce hypermethylation of a number of histone residues, including H3K27 [Xu *et al.* 2011].

Together, many hematopoietic tumors carry genetic alterations that lead to increased lysine 27 methylation and thus may be amenable to therapies with EZH2 inhibitors. The S-adenosylhomocysteine hydrolase inhibitor 3-deazaneplanocin A (DZNeP) is the only EZH2 inhibitor described to date [Tan *et al.* 2007]. DZNeP induces the degradation of EZH2 protein, leading to a global reduction in H3K27 methylation and a promotion of apoptosis. However, DZNeP seems to lack specificity for EZH2 and can affect methylation of various histone residues, hence more specific inhibitors are needed [Miranda *et al.* 2009]. The recently introduced compounds from GlaxoSmithKline and Epizyme present the first generation of specific inhibitors of EZH2 [McCabe *et al.* 2012; Knutson *et al.* 2012]. These compounds show high preference towards EZH2 and do not inhibit the action of other HMTs, including the functional homologue EZH1. Initial experimental data suggest high sensitivity of the Y641X-expressing lymphoma cell lines to these agents, supporting the possibility of moving these compounds towards the clinic for the

treatment of patients with various *EZH2* anomalies [McCabe *et al.* 2012; Knutson *et al.* 2012].

While the role of *EZH2* as an oncogene has been studied and documented extensively, in myeloid neoplasia *EZH2* is also commonly altered by deletions and mutations that lead to the loss of *EZH2* function, suggesting that this factor can have a possible role as a tumor suppressor [Ernst *et al.* 2010; Nikoloski *et al.* 2010]. The *EZH2* gene is located on chromosome 7q, a region of frequent chromosomal deletions. These deletions can occur concomitantly with the disrupting mutations of the alternative *EZH2* allele, indicating absence of any functional *EZH2* protein. As expected, in the absence of *EZH2* cells show a global decrease in H3K27 methylation. *EZH2* mutations are found throughout the gene and their presence is associated with an adverse prognosis. Other members of the PRC2 complex, *SUZ12* and *EED*, are also mutated in myeloid malignancies, albeit at a lower frequency [Kroeze *et al.* 2012]. More recently, mutations in PRC2 members and other chromatin modifiers have also been demonstrated in T-cell acute lymphoblastic leukemia [Zhang *et al.* 2012]. Interestingly, the t(4;14) translocation present in about 15% of multiple myelomas leads to overexpression of the *MMSET/NSD2/WHSC1* protein, a histone methyltransferase specific for dimethylation of lysine 36 on histone H3 (H3K36me2) [Li *et al.* 2009; Martinez-Garcia *et al.* 2011]. Overexpression of *MMSET* leads to a global increase in K36me2 and a concomitant decrease in H3K27me2/3 across the genome [Martinez-Garcia *et al.* 2011]. *MMSET* levels are also frequently elevated in advanced solid tumors [Hudlebusch *et al.* 2011]. In effect, *MMSET* overexpression may phenocopy loss of *EZH2* function. t(4;14)+ myelomas and other *EZH2* loss-of-function cancers may be good candidates for therapy using H3K27 demethylase inhibitors that are yet to be developed.

The presence of both activating and inactivating alterations of *EZH2* in different cancers suggest that the biological outcome of H3K27 alterations may be tissue specific. It remains to be determined whether all mutations affect a common subset of genes and regulatory pathways, or whether the role of *EZH2* changes between different malignancies. Nevertheless, the frequent occurrence of genetic lesions disturbing the H3K27 balance suggests that this epigenetic mark is tightly controlled, with either loss or gain of *EZH2* function potentially being oncogenic. This

critical balance may also provide additional challenges for discovery of safe and effective inhibitors of *EZH2*.

Conclusions

The recent discoveries of mutations in genes that regulate DNA and histone lysine methylation have helped provide insight into disease progression in a number of hematologic malignancies. The reversible nature of epigenetic marks made by different regulatory elements make them highly attractive targets for cancer therapies. Hypomethylating agents and HDAC inhibitors in clinical use represent the initial agents that exploit epigenetic alterations to benefit patient outcome. Inhibitors that target particular epigenetic regulators, such as the BRD family and inhibitors of *EZH2*, may prove to be potent compounds in the treatment of many human tumors. Further work is needed to determine whether such agents elicit therapeutic response through specific changes in gene expression, resulting in the re-expression of tumor suppressor genes, silencing of oncogenes, or the reactivation of downstream targets that prevent cancers from growing unchecked. Regardless, it is hoped that these molecules will be effective for treatment of not only hematological malignancies but also of other diseases with epigenetic lesions.

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