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EPIGENETICS IN HEMATOLOGY

Recent progress toward epigenetic therapies: the example of mixed lineage leukemia

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The importance of epigenetic gene regulatory mechanisms in normal and cancer development is increasingly evident. Genome-wide analyses have revealed the mutation, deletion, and dysregulated expression of chromatin-modifying enzymes in a number of cancers, including hematologic malignancies. Genome-wide studies of DNA methylation and histone modifications are beginning to reveal the landscape of cancer-specific chromatin patterns. In parallel, recent genetic loss-of-function studies in murine models are demonstrating functional involvement of chromatin-modifying enzymes in malignant cell proliferation and self-renewal. Paradoxically, the same chromatin modifiers can, depending on cancer type, be either hyperactive or inactivated. Increasingly, cross talk between epigenetic pathways is being identified. Leukemias carrying *MLL* rearrangements are quintessential cancers driven by dysregulated epigenetic mechanisms in which fusion proteins containing N-terminal sequences of MLL require few or perhaps no additional mutations to cause human leukemia. Here, we review how recent progress in the field of epigenetics opens potential mechanism-based therapeutic avenues. (*Blood*. 2013; 121(24):4847-4853)

Introduction

The completion of the human genome project and the development of next-generation sequencing technologies have profoundly changed the study of cancer. Recent years have witnessed the accumulation of unprecedented amounts of genomic data, and the catalog of both genetic and epigenetic alterations in cancer has grown significantly. In parallel, the importance of chromatin modifications (or epigenetic mechanisms) in the regulation of tumor-associated gene expression is becoming better understood, prompting the development of therapeutic approaches that target these mechanisms in cancer cells. Epigenetic gene regulatory mechanisms can be broadly classified into chromatin remodeling, DNA cytosine methylation, and covalent histone modifications. Additional mechanisms such as non-coding RNA are also increasingly recognized. Large-scale sequencing projects of human cancer genomes have provided evidence that many epigenetic regulators are deleted and/or mutated in cancer,[1,2] suggesting an interplay between genetics and epigenetics in cancer development. Interestingly, mutations in a given epigenetic regulator can be activating or inactivating,[3-5] suggesting tumor promoting or tumor suppressor functions for the same gene product depending on cellular context. How mutations in epigenetic modifiers contribute to cellular transformation on a mechanistic level remains incompletely understood in most cancers. However, it is likely that mutations in epigenetic modifiers result in molecular vulnerabilities that can, pending more detailed mechanistic understanding, be exploited for future targeted therapies. Importantly, epigenetic modifications are potentially reversible. Many of the players involved are enzymes, which are generally considered more targetable by small-molecule drugs than other classes of molecules, such as transcription factors. Indeed, there is already precedence for efficacious clinical application of therapies directed toward epigenetic mechanisms. Hypomethylating agents such as 5-aza-2′deoxycytidine (decitabine) and 5-azacytidine (Vidaza) are approved for the treatment of myelodysplastic syndromes, and the histone deacetylase inhibitors vorinostat and romidepsin are approved for the treatment of cutaneous T-cell lymphoma. There is great interest in expanding the indications for these agents, and multiple clinical studies are ongoing. This success has spurred interest in the development of additional drugs that target epigenetic mechanisms. An emerging theme in epigenetics research is the concepts of "writers,"[6] "readers"[7] (or "binders"), and "erasers."[8] All three may open exciting avenues for targeted therapies as exemplified by recent studies that investigate writers such as DOT1L,[9-11] readers such as the acetyl-binding BET-domain protein BRD4,[12-14] or erasers such as the histone demethylase LSD1.[15]

Acute leukemia carrying a rearrangement of the mixed lineage leukemia (*MLL*) gene on chromosome band 11q23 is a prototypical cancer driven by epigenetic mechanisms. Typically, the 5′ end of the *MLL* gene is fused in frame to the 3′ portion of one of more than 70 known translocation partners. An alternative mechanism of transformation involves a partial tandem duplication of exons near the 5′ end of the *MLL* gene that may function via somewhat different mechanisms and will not be discussed here further. *MLL* rearrangements are observed in approximately 5% to 10% of acute myeloid leukemia (AML) and in >70% of infant acute lymphoblastic leukemia (ALL). They occur de novo or after chemotherapy exposure (typically topoisomerase 2 inhibitors such as etoposide or, less commonly, anthracyclines). The most common translocation partners are *AF9* in AML and *AF4* in ALL. *MLL* rearrangements are associated with standard risk in AML and poor prognosis in ALL. Event-free survival is approximately 50% for both *MLL*-rearranged de novo AML and ALL (which translates into an average-risk prognostic

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category for AML and a poor-risk category for pediatric ALL). These outcome statistics are unsatisfactory and illustrate the need for better therapies for these leukemias. Secondary disease arising from myelodysplastic syndrome or after exposure to topoisomerase 2 inhibitors carries a particularly dismal prognosis, even after allogeneic stem cell transplantation. Careful analysis has begun to delineate an impact of the fusion partner on outcome.[16] Other risk factors such as overexpression of *EVI1* have also been described.[17,18]

The past few years have seen a greatly increased understanding of the pathophysiology of *MLL*-rearranged leukemia. Here, we summarize some of the most recent findings and focus on mechanisms with therapeutic implications; we specifically discuss two aspects of *MLL*-rearranged leukemias: the direct effects of the leukemogenic fusion and the interaction of the MLL fusion with other epigenetic regulatory systems.

MLL proteins in gene expression

Wild-type (WT) MLL is a histone methyltransferase with specificity for lysine 4 on histone 3 (H3K4). MLL contains a catalytic SET domain also found in the position effect variegation modifier (Su(var)3-9), the Polycomb-group protein E(z), and the trithorax-group protein Trx). In humans, there are 3 types of complexes with H3K4 methylating activity: SETD1A/B-containing complexes (related to yeast COMPASS) are responsible for most cellular H3K4 trimethylation (H3K4me3).[19] MLL (MLL1/KMT2A) or MLL4 (KMT2B, also referred to as MLL2)-containing complexes, which are related to *Drosophila* Trx-containing complexes, play an important role in *HOX* gene regulation. *MLL3 (KMT2C)* or *MLL2 (KMT2D;* also referred to as *MLL4*) are paralogues with homology to *Drosophila* Trx-related (*trr*). They have been implicated in nuclear receptor–mediated gene activation via locus-specific catalysis of H3K4me3 and more recently, enhancer regulation (see "Other members of the MLL family in cancer and leukemia"). A fifth member of the *MLL* family, *MLL5*, is more closely related to *SETD5* than to other *MLL* genes and will not be discussed further here. The phylogeny and function of the different complexes have recently been reviewed.[20] WT MLL proteins are large and contain multiple functional domains. In mice, knock-in of sequences encoding a truncated Mll protein without histone methyltransferase activity results in a milder phenotype than complete disruption of *Mll* function,[21] and knockdown of *Mll* in mouse embryonic fibroblasts leads to decrease of H3K4me3 in only a minor proportion of genes.[19] The Set-, Trx-, and Trr-containing complexes all contain multiple proteins (including multiple epigenetic modifiers), as do their mammalian counterparts. Studies in knockout mice have demonstrated that *Mll* is important for embryonic development, body patterning, and proper *Hox* gene expression in mice. A conditional knockout mouse has revealed that *Mll* is strictly required for normal adult hematopoietic stem cells.[22] *MLL2 (KMT2D)* has recently received attention because it is mutated in several different cancers, with a particularly high incidence in lymphoma, and the related *MLL3* is frequently mutated in a number of solid tumors (eg, medulloblastoma[23]).

Mechanisms of MLL fusion–mediated transformation

The available evidence suggests that MLL fusion proteins generated by *MLL* translocations function by transcriptionally upregulating approximately 100 target genes. The best characterized direct binding targets of MLL fusions are distal *HoxA* cluster genes and *Meis1*. Overexpression of *HoxA9* and *Meis1* is sufficient for leukemic transformation of mouse bone marrow, which suggests a critical role for *HoxA* cluster genes and *Meis1* in MLL fusion–mediated transformation. The specific role of the other MLL fusion binding targets is incompletely understood. Forced expression of *MLL-AF9* or combined forced expression of *HoxA9* and *Meis1* leads to in vivo leukemia arising from transduced hematopoietic stem cells. However, only *MLL-AF9*, but not the combination of *HoxA9-Meis1* efficiently transforms committed granulocyte-macrophage progenitors, and this re-establishing of self-renewal on a non–self-renewing population (granulocyte-macrophage progenitors)[24] and the development of drug resistance[25] have been linked to activation of the β-catenin pathway by *MLL-AF9*, independent of *HoxA9* and *Meis1* function. How β-catenin signaling is activated by *MLL-AF9* remains to be determined, although it appears that cell intrinsic, rather than niche-derived Wnt signals, plays a predominant role.[26] These data suggest that modulation of β-catenin signaling may be of therapeutic value in *MLL*-rearranged leukemia. The individual contribution to cellular transformation by individual direct MLL-AF9 binding targets other than *HoxA9* and *Meis1* is less well characterized. Interestingly, some of the direct binding targets of MLL-AF9 are in fact tumor suppressors (eg, *Cdkn1b*).

Recruitment of MLL fusion proteins

WT MLL is recruited to chromatin via several protein-protein interactions that involve menin/LEDGF,[27,28] PAF,[29,30] and PHD[29,31] fingers. A candidate therapeutic strategy is to interfere with recruitment of MLL fusions to chromatin. Fusion proteins invariably lose the PHD fingers, and artificial fusions, including the WT MLL PHD fingers, lose their transforming activity.[32] Importantly, structural data suggest a role of the PHD finger in shutting off the WT MLL program during normal hematopoietic differentiation.[33] Pharmacologic interference with the menin-MLL fusion interaction has shown promise as a therapeutic strategy for *MLL*-rearranged leukemia.[34] Interfering with the CXXC-PAF interaction may also be a viable strategy,[30] although a chemical inhibitor of this interaction has not been reported to the best of our knowledge.

Transcriptional upregulation mediated by MLL fusion proteins

The precise mechanism MLL fusions use to upregulate target genes is incompletely understood, but significant progress has been made. In leukemogenic fusions, the catalytic SET domain, which is located at the C-terminus, is invariably lost; however, MLL dimerizes, and WT MLL is required for appropriate target locus histone modifications, including H3K4me3, H3K79me2, and the transforming activity of MLL-AF9 in a murine model.[35] Interestingly, the Polycomb-group protein Cbx8, traditionally implicated in transcriptional silencing via Polycomb repressive complex (PRC)1, has been shown to be required for full induction of MLL fusion target gene expression.[36] This function is independent of PRC1 and instead involves the histone acetyl transferase Tip60, which is a modulator of an Myc transcriptional program found to be important in embryonic stem cells[37] and *MLL*-rearranged leukemias.[15,38,39] It is also noteworthy that MLL binds a number of protein complexes implicated in transcriptional elongation, including EAP,[40] AEP,[41] and SEC.[42] P-TEFb (CDK9/cyclinT1) is found in these partially overlapping elongation-associated protein complexes that are important for MLL fusion–induced leukemia in mouse models.[41,42] Small-molecule inhibitors of CDK9, such as the flavonoids, are in various stages of preclinical

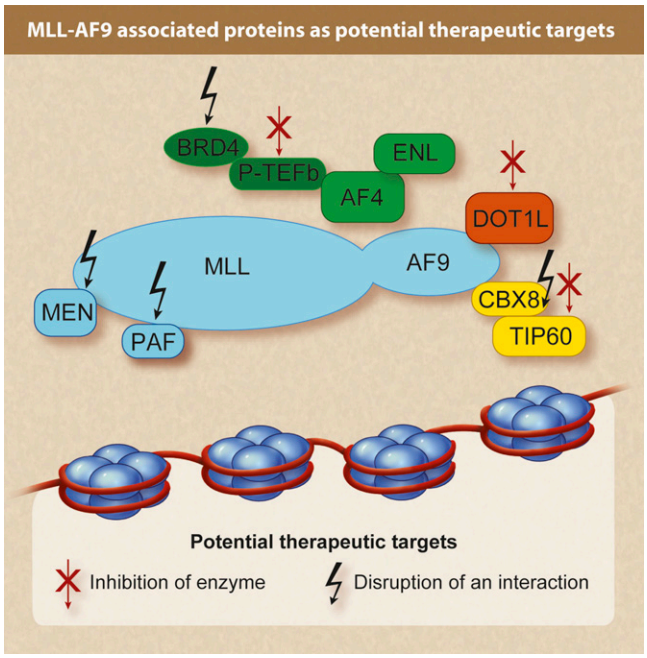

Figure 1. Schematic of the MLL-AF9 fusion and select binding partners. Arrows indicate potential molecular therapeutic targets. Red arrows indicate targets amenable to enzymatic inhibition. Black arrows indicate targets amenable to inhibition of protein-protein interaction. (Professional illustration by Debra T. Dartez.)

and clinical development for other malignancies and may represent a therapeutic opportunity for *MLL*-rearranged leukemias.

The histone methyltransferase DOT1L has been reported to be part of transcriptional elongation–associated multiprotein complexes EAP (but not AEP/SEC), and there are conflicting data regarding the simultaneous versus sequential recruitment of P-TEFb and DOT1L to chromatin. DOT1L is the only known methyltransferase for lysine 79 in histone 3 (H3K79me1/2/3) and was originally discovered as a disruptor of telomeric silencing in yeast.[43,44] DOT1L was originally implicated in leukemia when it was shown to interact with the MLL fusion partner AF10 in a yeast two-hybrid screen.[45] Abnormally high K79me2 signal was then found to be associated with genes directly bound by the more common MLL fusion protein MLL-AF4 in murine and human leukemias, suggesting that DOT1L may have a broader role in *MLL*- rearranged leukemias independent of the fusion partner.[46,47] Importantly, in genetic loss-of-function mouse models, *Dot1l* is required for leukemia initiation and maintenance by MLL-AF9[9-11,48] and by 3 additional particularly aggressive fusions: MLL-AF10, CALM-AF10,[49] and MLL-AF6.[50] Interestingly, AF6 is a cytosolic protein that does not appear to bind any of the nuclear DOT1L-containing protein complexes but mediates dimerization of the fusion,[51] a feature found to be sufficient for MLL-mediated transformation in some model systems. Genetic or pharmacologic inactivation of *Dot1l* leads to the collapse of an MLL fusion–driven transcriptional program that is upregulated via MLL fusion binding.[9] These genetic studies coincided with the development of small-molecule DOT1L inhibitors that have been shown to selectively target *MLL*-rearranged leukemia cell lines.[52,53] Consequently, the clinical evaluation of DOT1L inhibitors has been initiated in a phase 1 study (NCT01684150). Several important questions arise from these studies: (1) Are there resistance mechanisms to DOT1L inhibition? (2) How will cooperating mutations in genes such as *N-RAS* and *FLT3* influence response to DOT1L inhibitors in vivo? and (3) Is there preferential activity on human leukemic stem cells over normal hematopoietic stem cells? Another important question for future preclinical studies is whether there are other malignancies sensitive to DOT1L inhibition. Improved molecular understanding of the direct action of MLL fusions, while still incomplete, has led to the discovery of several potential targeted therapies in varying stages of preclinical and clinical development (Figure 1). Given the toxicities of conventional therapeutic regimens for AML and *MLL*-rearranged ALL, the advent of these new areas of investigation is most welcome.

Interaction of MLL fusions with other epigenetic systems

There is a growing appreciation of the interplay between different epigenetic systems. Epigenetic readers, writers, and erasers are mutated, amplified, deleted, or transcriptionally dysregulated in many cancers. We limit ourselves here to a brief discussion of DNA methylation and PRC2 in the context of MLL-fusion leukemias.

DNA cytosine methylation

DNA methylation in mammals typically occurs in the carbon 5 position of the cytosine ring, in the context of CpG dinucleotides, leading to the formation of 5-methylcytosine (5mC). There is ample evidence for dysregulation of DNA methylation in cancer, including hematologic malignancies, with important therapeutic implications.[54] Here, we highlight a few points pertinent to MLL leukemia. Genome-wide analysis of DNA methylation patterns has revealed additional insights not provided by the study of the methylation status of individual gene loci (such as *CDKN2A*).[55,56] In AML, DNA methylation programs mostly segregate with known cytogenetic groups. However, novel subgroups of AML not previously suspected on the basis of their cytogenetics have been identified on the basis of the clustering of DNA methylation patterns. There is also a set of genes aberrantly methylated in all AML. Interestingly, AML can be globally either hypo- or hypermethylated,[55] demonstrating that too much or too little activity of an epigenetic pathway may contribute to leukemogenesis; this theme is also evident in the study of Polycomb proteins. Finally and importantly, DNA methylation patterns are linked to patient survival, suggesting clinical relevance to the detailed study of aberrant epigenetic changes.[56] Direct binding of DNA methyltransferases (DNMTs) by prognostically relevant transcription factors such as EVI1[17] has been reported, and hypermethylation associated with refractory disease has been linked to overexpression of *EVI1* in patients[17,18] and in mouse models.[57]

Recent data further underscore the functional importance of aberrant DNA methylation in murine models of MLL-AF9–mediated AML: clinical samples of *MLL*-rearranged leukemias show pronounced global hypomethylation.[55] This is mirrored by hypomethylation of murine MLL-AF9–mediated AML, with more chemosensitive progenitor-derived MLL-AF9 AML being hypomethylated compared with more resistant stem cell–derived disease.[57] It is unknown whether the changes in methylation simply reflect the leukemic cell of origin or are causally related to the observed differences in latency and chemosensitivity. A more detailed functional understanding awaits whole genome bisulfite sequencing coupled with genome-wide analysis of hydroxymethylcytosine (which bisulfite sequencing cannot distinguish from methylcytosine). Of clinical interest, genetically engineered

haploinsufficiency for the maintenance DNMT Dnmt1 in murine MLL-AF9 AML leads to a striking loss of self-renewal.[58]

These data imply that there is an optimal level of methylation for MLL-AF9 leukemia and suggest benefit to the clinical use of hypomethylating agents in *MLL*-rearranged AML. Another interesting finding in this study was the enrichment of derepressed genes in bivalent PRC2 targets, marked by both activating H3K4 trimethylation (placed by one of the MLL- or SET-containing complexes) and repressive H3K27 trimethylation (placed by PRC2, containing either enhancer of Zeste 2 [EZH2] or EZH1 as the active methyltransferase).[58]

A link between another important mechanism, the H3K9me2/3 axis, and DNA cytosine methylation has also been suggested.[59,60] A recent elegant study demonstrated that forced occupation of HP1a on a transgene via a chemical inducer of dimerization can lead to H3K9 methylation. Prolonged but not short-term HP1 occupation also leads to silencing by DNA methylation, and pharmacologic inhibition of DNMTs leads to an expected reversal of DNA methylation, and also, less expectedly, to reversal of H3K9 methylation.[61] More work is needed to analyze the genome-wide interplay of different epigenetic silencing mechanisms because this area clearly has therapeutic implications.

Polycomb genes

Polycomb-group genes are important developmental regulators involved in body patterning and *HOX* gene regulation. They are of considerable interest in cancer epigenetics. Several different Polycomb complexes have been described, and we limit ourselves here to highlighting how PRC2 intersects with MLL.

PRC2 consists of the core components embryonic ectoderm development (EED), suppressor of Zeste 12 (SUZ12), and a catalytic component, which can be an EZH2 or its less well characterized cousin, EZH1,[62,63] which can partially compensate *Ezh2* inactivation by preventing loss of H3K27me3 at some but not all PRC2 target gene loci.[39,63] Another protein, JARID2, is involved in recruitment of the complex to target loci[64-66] and is inactivated in human leukemia.[67] Other factors also associate with the PRC2 complex.[66] The canonical function of PRC2 is transcriptional repression of bound genes via trimethylation of histone 3 on lysine residue 27 (H3K27me3). A chromatin compacting function has been ascribed to EZH1.[62] However, it should be noted that both EZH1 binding[68,69] and EZH2 binding[70] have recently been associated with transcriptional activation. Furthermore, at least some histone methyltransferases can methylate nonhistone substrates. This phenomenon is best characterized for SETD7.[71] A function for Ezh2 in cardiac development through methylation-mediated direct inactivation of the transcription factor Gata4 has also recently been described for Ezh2.[72] Thus, it is likely that EZH2 can control cell state programs and perhaps cancer development at multiple levels.

Oncogenic function of PRC2

Early studies assessing *EZH2* in cancer found it to be overexpressed in cultured mantle cell lymphoma cell lines.[73] *EZH2* overexpression is associated with disease progression in prostate cancer,[74] a function that was recently reported to be independent of PRC2/H3K27me3.[75] Overexpression of *EZH2* was also found in other solid tumors in follow-up studies, and forced expression of *EZH2* was shown to mediate enhanced self-renewal[76] and proliferation.[77] A role for *EZH2* in cell cycle progression of cultured cells, downstream of *E2F*, has also been characterized.[78] In cancers of the hematopoietic system, both hyperactivity and inactivation of *EZH2* have been described. There is great interest in the development of PRC2 inhibitors for use in cancer therapy, and DZNep, reportedly a PRC2 inhibitor, was recently shown to have activity in cancer models[79] and specifically in leukemia.[80] However, the specificity of this molecule has been called into question.[81] Subsequent studies using more potent and specific EZH2 inhibitors have shown significant inhibition of cellular proliferation in lymphoma cell lines that harbor activating *EZH2* mutations.[82-84]

We and others recently investigated the role of PRC2 in MLL-AF9–dependent AML, and found that *Eed*, but not *Ezh2*, is strictly required for MLL-AF9 AML.[39,85] The inactivation of PRC2 components Ezh2[39,85] and Eed[39,86] leads to reduction and complete loss of self-renewal, respectively, in murine MLL-AF9 leukemia. The precise mechanistic underpinnings of the role of Ezh2/PRC2 in MLL-AF9 leukemia (gene repression vs gene activation and critically important target genes) are currently unknown. Given the apparent inverse correlation between leukemogenic potency of MLL-AF9 cells and degree of *Cdkn2a* derepression,[39] *Cdkn2a*, a known PRC2-repressed locus, is an important candidate locus. Double knockout of *Ezh2/Eed* and *Cdkn2a* is likely to shed more light on this issue. Normal adult hematopoietic stem cells are dependent on *Ezh1*,[87] but not *Ezh2*,[88] apparently via a *Cdkn2a*-dependent mechanism. Interestingly, a phenotype similar to the one observed after *Ezh2* inactivation in MLL-AF9 leukemia, including loss in transcription of Myc targets and upregulation of PRC2 targets, was recently described for short hairpin RNA (shRNA) –mediated and pharmacologic loss-of-function studies of the histone demethylase Lsd1.[15] Of note, the long noncoding RNA HOTAIR (previously implicated in PRC2 recruitment and cancer) has been described to physically link EZH2 and LSD1 proteins.[89] The functional significance of this is presently unknown but may merit further investigation. *MYC* is an important gene in many cancers and has been validated as a potential therapeutic target in genetic models. However, direct pharmacologic interference with *MYC* has proven difficult. Recently, indirect modulation of *MYC* levels by genetic and pharmacologic interference with the epigenetic reader of acetylated histones BRD4 has been demonstrated.[12-14] It appears that inhibition of epigenetic readers such as BRD4 and possibly CBX8, writers such as PRC2, and erasers such as LSD1 may open additional opportunities for targeting MYC, a long elusive therapeutic target. In summary, genetic models have demonstrated an important relationship between MLL and other epigenetic systems, pointing toward potential therapies.

Tumor suppressor function of Ezh2

Inactivating mutations in *EZH2* have been described in myelodysplastic syndrome, myeloproliferative disease, and T-cell ALL and are correlated with adverse prognosis. Inactivation of *Ezh2* in mice leads to T-cell ALL.[90] The precise mechanisms by which *EZH2* contributes to oncogenesis vs tumor suppression are unknown and deserve further study.

Other members of the MLL family in cancer and leukemia

Recurrent mutations in different epigenetic modifiers have been found in many cancers, including MLL family members. A query of the COSMIC database (performed in April 2013) reveals that there are relatively few mutations of *SETD1A* and *SETD1B* (68 and 12 unique samples, respectively, with simple mutations). There are also relatively few reported samples with simple mutations in *MLL*

(139) and *MLL4* (*KMT2B*; 89). However, there are substantially more frequent mutations of *trr*-homolog *MLL* family members *MLL2* (*KMT2D*; 321) and *MLL3* (370), suggesting a major tumor suppressive effect of these enzymes.[23,91] What is the oncogenic mechanism of *MLL2/MLL3* mutations? An only partially redundant role for *MLL3* and *MLL2* in the activation of p53 target genes has been reported.[92] In insects, Trr functions in nuclear receptor signaling,[93] and recent data from the analysis of genome-wide MLL2-binding and *MLL2*-inactivated cell lines support a role for *MLL2* in both p53 and nuclear receptor signaling.[94] Very recent data show a role for *trr* and *Utx* in enhancer function.[95] Given that multiple gene products with documented or suspected involvement in enhancer function/long-range enhancer-promotor interactions (*MLL2/4, CBP, UTX, CTCF*, cohesin complex members) have recently been shown to be mutated in cancer, it is tempting to speculate that there may be a unifying theme. Active enhancers appear to be associated with defined chromatin modifications (H3K4me1 and H3K27Ac),[96] at least in part mediated by Trr (in *Drosophila*)[95] and p300,[96] respectively. Prior to full activation, they pass through a "poised" stage (H4me1, K27me3) reminiscent of the earlier described poised promoter marked by H3K4me3 and H3K27me3. The removal of K27me3 on some enhancers is performed by Utx[97] and is required for the appropriate activation of developmental programs. It may be that a similar mechanism is responsible for differentiation block in hematopoietic and other cancers, a common and widely appreciated feature of malignancy. Future studies will undoubtedly shed more light on this issue.

Concluding remarks and outlook

Epigenetic mechanisms are clearly important in cancer. New data from the past few years illustrate 2 important concepts.

First, *MLL*-rearranged leukemias provide a paradigm for how epigenetic dysregulation can lead to cancer through inappropriate chromatin structure with subsequent activation of target genes with oncogenic activity. An improved molecular understanding of how MLL fusions upregulate binding targets has led to the identification of a number of potential mechanism-based therapeutic vulnerabilities for this poor-prognosis malignancy (Figure 1). We have focused here on therapeutic targets related to the recruitment and transcriptional effects of MLL fusions. Other strategies such as FLT3-kinase inhibition and interference with HOXA9/MEIS1 transcriptional activities[98,99] are also being explored, and their discussion here has been omitted due to space constraints. Both *MLL* translocations and *MLL* family member mutations are associated with, and probably mediate, a cellular differentiation block. We believe that future studies investigating the precise mechanisms involved are likely to uncover additional therapeutic opportunities.

Second, epigenetic pathways do not exist in a vacuum, and there is functional crosstalk. A better understanding of this crosstalk may aid in the development of more precisely targeted epigenetic therapies. For example, there may be synergies between the inhibition of PRC2 and DNA methylation. Given the frequent mutations in epigenetic modifiers, it seems likely that these mutations might sensitize the affected cancer cell to interventions that could be less toxic to cells with normal chromatin structure and regulation. Future studies will help further develop this important concept. Another understudied area concerns dynamic changes in the epigenetic makeup of cancer cells in response to external stimuli. An epigenetic drug persister phenotype in response to a targeted therapy (gefitinib) has been demonstrated.[100] Deconstructing the complicated interplay between different epigenetic regulatory mechanisms and other targeted therapeutic interventions in genetically defined murine models is likely to provide the required mechanistic insight for developing the next generation of therapeutics that target epigenetic mechanisms.

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Authorship

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