PROSPECT
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Key Roles of Histone Methyltransferase and Demethylase in Leukemogenesis

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ABSTRACT

A growing body of evidence has underlined the involvement of histone methyltransferases and demethylases in leukemia development. These findings can be roughly classified into two categories according to their association with leukemia. On the one hand, these histone modifiers are recruited to DNA by specific affinities of aberrantly expressed transcription factors or fusion proteins, and induce chromatin modifications to regulate target gene expression. Epigenetic regulators may function as oncogenes in this context. On the other hand, recent studies have identified inactivating mutations of some key histone modulators in myeloid malignancies and these results suggest that they act as tumor suppressors. Profound understanding of these findings in the two categories will help us consider clinical applications of epigenetic drugs. In this prospect we will review the leukemogenic mechanisms clarified by the epigenetic approach and the current findings on genetic aberrations in each methyltransferase or demethylase, and discuss the potential of medical intervention in leukemia or leukemia stem cells targeting histone modifiers. J. Cell. Biochem. 112: 415–424, 2011.

KEY WORDS: LEUKEMIA; HISTONE METHYLTRANSFERASE; DEMETHYLASE; TRANSCRIPTION FACTOR; MUTATION

Despite continuous efforts to elucidate the molecular mechanisms that underlie the development of leukemia and to design novel agents to eradicate leukemic cells, most of the current therapies for leukemias are still based on intensive chemotherapy and/or hematopoietic stem cell transplantation, although some exceptions exist such as imatinib for chronic myelogenous leukemia (CML) and all-*trans* retinoid acid (ATRA) for acute promyelocytic leukemia (APL), both of which have markedly improved the prognosis by inhibiting the activities of key molecules critical for leukemogenesis. The refractoriness of leukemia to conventional therapy is partly explained by the existence of persistent leukemia stem cells (LSCs). A large number of studies have focused on the properties of LSC, which are characterized by limitless self-renewal and possibly confer resistance to conventional chemotherapy as they reside in a largely quiescent state with regard to cell-cycle activity. Several key genes or signaling pathways have been reported to play a role in the regulation of LSC self-renewal, such as the BMI1, HOX genes, WNT/β-catenin signaling, and Hedgehog signaling [Ferrando et al., 2003; Lessard and Sauvageau, 2003; Zhao et al., 2007; Dierks et al., 2008; Wang et al., 2010]; therefore, inhibition of multiple molecular targets may be required for eradicating LSC. On the other hand, histone methyltransferases and demethylases have been implicated as key regulators for the self-renewal and maintenance of hematopoietic stem cells (HSCs). The roles of histone modifications in stem cell biology suggest their oncogenic potential for chromatin remodeling in cancer stem cells or LSCs. For example, polycomb group (PcG) proteins have been reported to regulate multiple target genes and signaling pathways, including HOX genes, WNT/β-catenin, and Hedgehog [Bracken et al., 2006; Lee et al., 2006]. Thus, intervention in the functions of histone methyltransferases and demethylases can be a good candidate for leukemia or LSC treatment. As the first step in this direction, understanding the roles of these histone modifiers in normal and malignant cells is fundamental. We will review the relevant literature demonstrating critical findings of several histone methyltransferases and demethylases in the development of leukemia (Table I) and argue about the potential of epigenetic intervention for treatment of leukemia by modulating the activities of histone modifiers.

THE ROLE OF POLYCOMB GROUP PROTEINS IN LEUKEMIAS

PcG proteins regulate a variety of biological processes such as the cell cycle, apoptosis, stem cell differentiation, senescence, and cancer development by repressing a wide variety of target genes. PcG proteins form two distinct macromolecular complexes called

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TABLE I. Pathogenetic Roles of Histone Modifiers in Leukemia

Group/specificity/ transcriptional activity	Histone modifiers	Disorders	Molecular mechanisms	Refs.
Polycomb group proteins, H3K27me3/H2AK119ub repression	PRC2	APL	Interact with PML/RARα	Villa et al. [2007]
	PRC2 and PRC1	APL	Interact with PLZF/RARα	Boukarabila et al. [2009]
	CBX8	MLL leukemia	Interact with ENL and AF9	Garcia-Cuellar et al. [2001], Hemenway et al. [2001]
	RING1	MLL leukemia	Interact with ENL	Mueller et al. [2007]
	CXB4, BMI1	MLL leukemia	Interact with MLL	Xia et al. [2003]
	L3MBTL1	ETV6-related	Interact with ETV6	Boccuni et al. [2003]
	BMI1	AML	Overexpression	Chowdhury et al. [2007], Mohty et al. [2007]
	BMI1	MDS	Overexpression	Mihara et al. [2006]
	BMI1	CML	Overexpression	Bhattacharyya et al. [2009]
	SUZ12	CML	Overexpression	Pizzatti et al. [2010]
	EZH2 and MEL18	AML	Overexpression	Grubach et al.
	EZH2	Myeloid disorders	Inactivating mutation	Ernst et al. [2010], Nikoloski et al. [2010]
	Rae28	B-ALL	Deletion	Tokimasa et al. [2001]
	EPC1	ATLL	EPC1/ASXL2, mutation	Nakahata et al. [2009]
	ASXL1	Myeloid disorders	Mutation	Gelsi-Boyer et al. [2009]
SUV39H1 and G9a H3K9me2/me3 repression	SUV39H1 and G9a	Evi1 Leukemia	Interact with Evi1	Cattaneo and Nucifora [2008], Goyama et al. [2008, 2009], Spensberger and Delwel [2008]
	SUV39H1	APL	Interact with PML/RARα	Carbone et al. [2006]
	SUV39H1	AML1-related*6	Interact with AML1	Chakraborty et al. [2003], Reed-Inderbitzin et al. [2006]
DOT1L H3K79methylation activation	DOT1L	AML, ALL, acute undifferentiated leukemia	Interact with MLL fusions, SET/NUP214, CALM/AF10	Okada et al. [2005], Caudell, Vlierberghe et al.
PRMT, H3R8/H4R3 methylation activation	PRMT1	AML	Interact with MLL/EEN via Sam68	Cheung et al.
	PRMT5	Lymphoid malignancies	Overexpression	Wang et al. [1998, 2001, 2007, 2009, 2010]
Other methylases	NSD1	ALL	Form NUP98/NSD1	Cerveira et al. [2003]
	MLL5	B-ALL	Missense mutation	Emerling et al.
Demethylases	UTX	AML, CML, T-ALL	Inactivating mutation	Mullighan et al. [2007]
	JARID1A	AML	Form NUP98/JARID1A	van Zutven et al. [2006]
	JMJD1B	AML, MDS	Deletion, point mutation	Hu et al.
	LSD1	CML, T-ALL	Interact with TAL1	Hu et al. [2001]

polycomb repressive complex (PRC) 1 and 2. Major subunits of PRC2 are EZH2, SUZ12 and EED, whereas PRC1 is composed of more diverse components, such as BMI1, RING, HPH, and CBX family. PRC2 and PRC1, respectively, possess histone 3 lysine 27 (H3K27) trimethyltransferase and histone 2A lysine 119 (H2AK119) E3 ubiquitin ligase activities, and these histone modifications are essential for gene silencing mechanisms. Recent reports have revealed the implications of PcG proteins in several subtypes of leukemia.

ACUTE PROMYELOCYTIC LEUKEMIA (APL)

Recent findings have demonstrated that some fusion proteins or key transcription factors play an important role in leukemogenesis through physical interaction with PcG complexes. APL is characterized by a differentiation block resulting in an over-growth of promyelocytes. All-*trans* retinoid acid (ATRA) treatment has greatly improved the outcome of patients with APL by relieving the differentiation block of APL cells. PML/RARα fusion protein is a product of balanced reciprocal chromosomal translocation t(15;17) and is responsible for 99% of cases of APL. Villa et al. [2007] demonstrated that this chimeric protein recruits PRC2 to RARβ2 promoter, one of the established targets of PML/RARα. RARβ2 mRNA expression and cell differentiation reverted by RNAi-mediated inactivation of PRC2 in an APL cell line, NB4. Importantly, repressive histone marks induced by PRC2 recruitment also reverted by in vitro ATRA treatment, resulting in increased cell differentiation in human APL cells (Fig. 1A).

On the other hand, APL patients with PLZF/RARα translocation showed resistance to ATRA, leading to a poor prognosis. Boukarabila et al. [2009] investigated the molecular mechanisms of this phenomenon in association with PcG proteins. They showed that both PLZF and PLZF/RARα bind to PRC1, whereas PML/RARα does not. Interestingly, PLZF/RARα-mediated PRC2 recruitment to RARβ2 promoter was canceled by ATRA treatment, whereas PRC1 was still enriched at the RARβ2 promoter after ATRA treatment and seemed to play a role in persistent repression of RARβ2 (Fig. 1B). As PML/RARα and PLZF/RARα are both degraded upon ATRA treatment, they argued that some of the chromatin-bound fraction of PLZF/RARα remains protected through the incorporation of PLZF/RARα into the PRC1 complex or the highly compact nature of the chromatin once targeted by PRC1. These observations implied the important notion that targeting PRC2 may not be enough to eradicate PLZF/RARα-associated APL or possibly, other subtypes of leukemia in which PRC1 mediates the leukemogenic potential; therefore, PRC1 should be another target for therapeutic intervention.

LEUKEMIA WITH MLL REARRANGEMENTS

The mixed lineage leukemia (MLL) gene is a human homologue of genetically defined trithorax group (trxG) proteins involved in

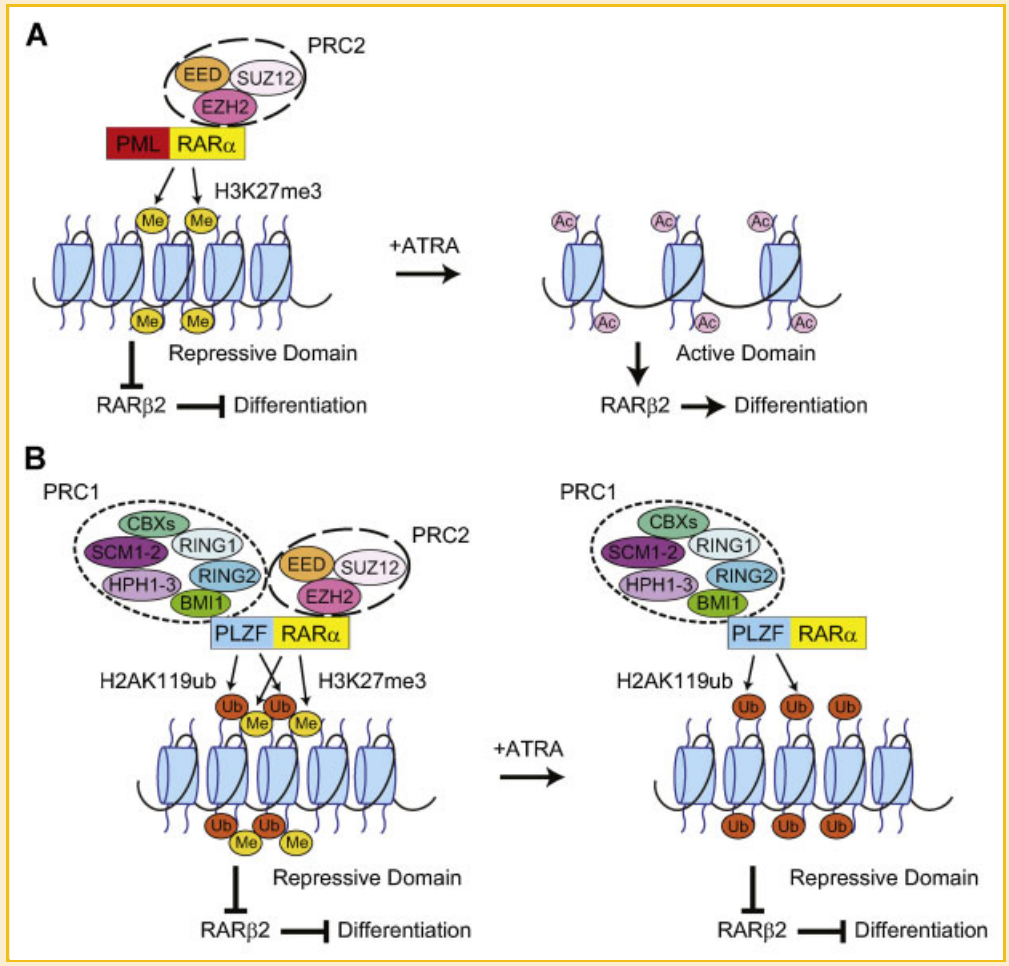

Fig. 1. Schematic representations of the polycomb repressive complexes and APL-related fusion proteins. The PML/RARα-PRC2 complex is degraded upon ATRA treatment which leads to re-expression of PML/RARα target genes (A), whereas the PLZF/RARα-PRC2-PRC1 complex is only partly degraded by ATRA, resulting in persistant repression of target genes (B), although it is unknown why PLZF/RARα shows resistance to ATRA-induced degradation.

transcriptional activation via changes of the chromatin architecture. Clinically, leukemia patients with MLL gene rearrangements show unique properties. MLL rearrangements are observed in >70% of cases of infant acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL), while they are found in up to 10% of adult AML. MLL rearrangements are also detected in patients with therapy-related leukemia who were previously treated with topoisomerase II inhibitors. MLL is a frequent target of recurrent chromosomal translocations found in human acute leukemias. AF4, AF9, and ENL are the three most common fusion partners of MLL, which are derived from t(4;11)(q21;q23), t(9;11)(p22;q23), and t(11;19)(q23;p13.3), respectively. Overcoming leukemia with MLL rearrangements is one of the most challenging issues in hematological malignancies as it is associated with multiple phenotypes, a young age at diagnosis, and poor prognosis in general.

Some reports have been published in relation to PcG proteins, particularly regarding the physical interaction between PRC1 and MLL or its fusion partners. Specifically, CBX8 (also known as PC3 and RC1) binds to ENL and AF9 [Garcia-Cuellar et al., 2001; Hemenway et al., 2001] and RING1 (also known as RING1A or RNF1) has also been reported to be an interaction partner with ENL [Mueller et al., 2007]. MLL itself interacts with CBX4 (also known as NBP16, PC2, or hPC2) and BMI1 through the cysteine-rich CXXC region of the MLL repression domain, which is retained in chromosomal translocations [Xia et al., 2003]. Furthermore, Xia et al. showed that the MLL repression domain can decrease reporter activity in concert with BMI1. In addition, MLL or its fusion partners are associated with corepressor CtBP and histone deacetylases (HDACs) that act as repressive epigenetic regulators [Xia et al., 2003]. Although much attention has focused on the MLL or MLL fusion protein-mediated positive regulation of well-established targets such as HOX genes and Meis1, these results suggest that MLL fusion proteins play a role in the repression of putative MLL target genes through the recruitment of an elaborate complex consisting of these epigenetic regulators or others molecules (Fig. 2). In fact, our preliminary experiments indicated that bone marrow cells retrovirally transduced with MLL/ENL or leukemia cell lines with MLL rearrangements were highly sensitive to DZNep, a PRC2 inhibitor, which raised the possibility that some repressive targets are critical for MLL

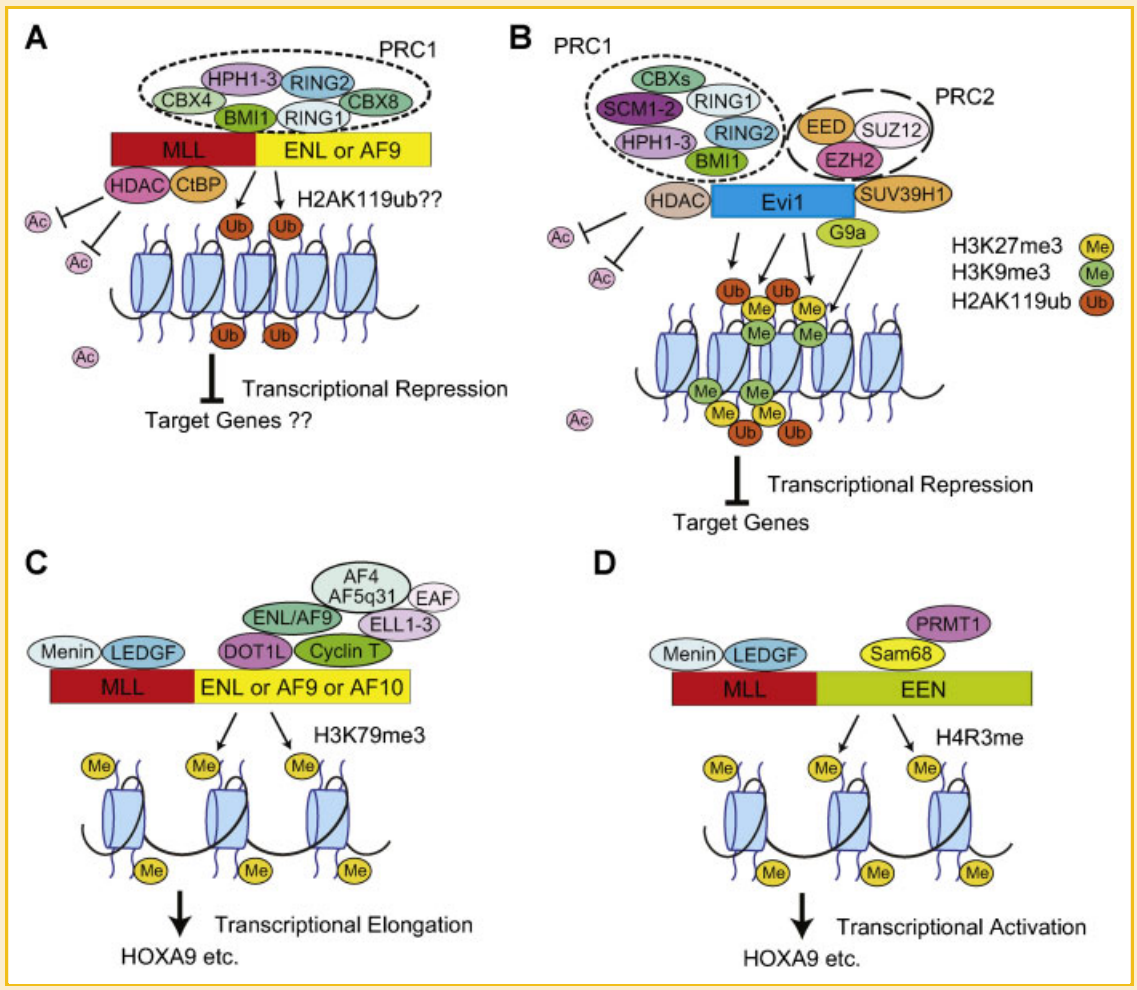

Fig. 2. A,C,D: Schematic representations of transcriptional regulation by MLL fusion proteins and their interaction proteins, including a proposal model of PRC1-mediated suppression of putative MLL fusion target genes (A). B: Leukemia oncogene Evi1 represses its targets through recruitment of polycomb group proteins, SUV39H1, G9a, and histone deacetylases.

rearrangement-induced transformation, although further investigation is required.

CHRONIC MYELOGENOUS LEUKEMIA (CML)

CML is characterized by the presence of the Philadelphia (Ph) chromosome that results from a (9;22)(q34;q11) reciprocal translocation that juxtaposes the c-abl oncogene 1 (ABL1) gene on chromosome 9 with the breakpoint cluster region (BCR) gene on chromosome 22, leading to the generation of the BCR/ABL fusion gene with enhanced ABL1 kinase activity. Some PcG proteins are known to be highly expressed in CML, particularly CML in advanced phases. The most intensely investigated gene is BMI1, which is implicated in normal and LSC proliferation [Lessard and Sauvageau, 2003]. Two groups have reported that BMI1 expression is significantly higher in the accelerated phase and blastic crisis than in the chronic phase [Mohty et al., 2007; Bhattacharyya et al., 2009]. Of clinical importance is that BMI1 can be utilized as a molecular marker to predict prognosis in myeloid disorders [Mihara et al., 2006; Chowdhury et al., 2007; Mohty et al., 2007]. In fact, a recent study revealed that co-expression of BMI1 and BCR/ABL in human $CD34^+$ cells induced leukemia in a transplantation model using NOD-SCID mice, but single overexpression of BMI1 or BCR/ABL did not [Rizo et al., 2010]. Overexpression of BMI1 in $CD34^+$ cells derived from patients with CML in the chronic phase activated their proliferation and self-renewal capacity, which is consistent with the expression profiles described above. These results suggested that BMI1 collaborates with BCR/ABL to induce disease progression. Although the mechanisms of BMI1 up-regulation have not been fully elucidated, BCR/ABL has been shown to positively regulate BMI1 mRNA expression, and BMI1 is also susceptible to posttranscriptional regulation in CML cells [Bhattacharyya et al., 2009]. Mohty et al. demonstrated that samples from CML patients displayed significantly higher levels of E2F1 than healthy controls and they speculated that E2F1 is responsible for BMI1 activation in CML, as BMI1 was reported to be a direct target of E2F1 [Nowak et al., 2006; Mohty et al., 2007]. Regarding PRC2 and CML, Pizzatti et al. reported that SUZ12 is overexpressed in bone marrow samples of patients in blastic crisis [Pizzatti et al., 2010]. They have also

demonstrated that SUZ12 is a target gene of non-canonical and canonical WNT pathway, as WNT1, WNT5, and WNT11 could directly enhance SUZ12 transcription, respectively, although there were some difference in the affinities between these WNT pathway members and the SUZ12 promoter. By genetically targeting β-catenin, Wnt signaling was demonstrated to regulate long-term reconstitution of the hematopoietic system [Zhao et al., 2007]. In addition, they revealed that Wnt signaling is essential for the self-renewal of LSCs in the murine CML model. Taken together, the BCR/ABL-Wnt-PcG axis may have pathophysiological implications in the development of CML, as aberrant expression of PcG proteins has the potential to contribute to the reversion of cells to a more stem cell-like phenotype.

ETV6-RELATED LEUKEMIAS

Another example is the interaction between ETV6 (also known as TEL) and L3MBTL1 (H-L(3)MBT or L3MBTL) [Boccuni et al., 2003], a member of the PcG proteins that functions as a HDAC-independent transcriptional suppressor and is possible candidate for a 20q deletion-associated myeloproliferative neoplasm [Li et al., 2004; Perna et al., 2010]. ETV6 is a transcription factor crucial for definitive hematopoiesis [Wang et al., 1998] and more than 40 translocations involving ETV6 have been documented so far in a wide variety of hematological malignancies. Known partner genes can be broadly classified into two groups; tyrosine kinases (such as PDGFβR, ABL1, JAK2, and SYK) and transcription factors (AML1, Evi1, CDX2, and PAX5, for example). Additional studies aimed at understanding the role of L3MBTL1 in ETV6-associated neoplasms are warranted.

MUTATIONS OF POLYCOMB GROUP PROTEINS IN MYELOID MALIGNANCIES

One of the most important findings in PcG is the identification of mutations of EZH2 in myeloid malignancies [Ernst et al., 2010; Nikoloski et al., 2010]. Human EZH2 is located on chromosome 7q36, and 7- or del(7q) is frequently detected in myelodysplastic syndrome (MDS) and AML, and is associated with poor prognosis [Haase et al., 2007]. The two groups identified recurrent somatic mutation of EZH2 in MDS, myeloproliferative neoplasms (MPNs), and MDS/MPNs using high-resolution SNP arrays. Most of the missense mutations were found in the evolutionarily conserved CXC-SET domain and domain II, which are critical for histone methyltransferase potential and interaction with SUZ12, respectively; therefore, these mutations were predicted to be loss-of-function mutations, suggesting that EZH2 functions as a tumor suppressor in myeloid malignancies. Of particular note is that EZH2 mutations were not detected in 54 AML patients with 7-/del(7q) [Ernst et al., 2010], indicating the need for further investigation to identify possible tumor suppressor genes on chromosome 7. Interestingly, leukemia with 3q26 rearrangements is closely related to both Evi1 overexpression, hereinafter described in detail, and chromosomal abnormalities involving chromosome 7. De Weer et al. [2010] addressed this issue using high-resolution array comparative genomic hybridization analysis and presented some candidate genes for tumor suppressors on 7q in AML patients or AML cell lines with 3q26 rearrangements.

The RAE28 (also known as HPH1, EDR1, and PHC1) gene is a member of PRC1 and is located on chromosome 12p13. Tokimasa et al. [2001] reported that RAE28 expression was not detected in four of 43 children with B-ALL. Surface marker profiles showed that all four patients had the B-cell precursor phenotype, which is in accord with B-cell maturation arrest in rae28$^{-/-}$ mice. 12p is involved in approximately 30% of patients with ALL and RAE28 is thus a good candidate as the target of 12p abnormalities. This point should be pursued on a larger scale.

Another example of genetic alteration in PcG proteins is enhancer of polycomb1 (EPC1) in adult T-cell leukemia (ATL) [Nakahata et al., 2009]. EPC1 was mapped on chromosome 10p11.2 and 10p11 is a frequent chromosomal breakpoint in acute-type ATL. Investigation of a small number of ATL patient samples and ATL cell lines revealed the existence of the EPC1/ASXL2 fusion gene and truncated EPC1 gene. Pathogenetic mechanisms of these mutations should be clarified in the future.

SUV39H1/G9a

LEUKEMIA WITH HIGH EVI1 EXPRESSION

The ecotropic viral integration-site 1 (Evi1) is a unique gene in that it was the only gene whose overexpression was found to define a cluster in a large-scale gene expression profiling of AML, which accounts for approximately 10% of cases of AML [Valk et al., 2004]. The patients classified into this cluster show a poor outcome [Valk et al., 2004]. Aberrant expression of Evi1 is also implicated in the development of MDS and chronic myelogenous leukemia (CML). The human Evi1 gene is located on chromosome 3q26 and rearrangements involving this region, including t(3;21), t(3;12), t(3;3), and inv(3), often activate Evi1 expression in myeloid malignancies. Overexpression of Evi1 can occur in patients without 3q26 rearrangements by an unknown mechanism. In the normal murine hematopoietic system, Evi1 is predominantly expressed in the HSC fraction, and gene targeting strategies have revealed that Evi1 is critical for the proliferation and maintenance of hematopoietic stem cells [Yuasa et al., 2005; Goyama et al., 2008]. Evi1 functions as a transcription factor and recent studies have identified Gata2 and Pbx1 as target genes of Evi1 [Yuasa et al., 2005; Shimabe et al., 2009]. In addition to its DNA-binding potential, Evi1 binds to SMAD3 and CtBP proteins, both of which are necessary for efficient inhibition of growth-suppressive signaling of TGF-β [Kurokawa et al., 1998; Izutsu et al., 2001].

Recently, our group and others reported that Evi1 interacts with SUV39H1 and G9a [Cattaneo and Nucifora, 2008; Spensberger and Delwel, 2008; Goyama et al., 2009], both of which are H3K9 specific methyltransferases associated with gene silencing [Rea et al., 2000; Tachibana et al., 2001]. In reporter assays, SUV39H1 was shown to enhance transcriptional repression by Evi1, whereas the significance of G9a in transcriptional regulation in connection with Evi1 has not been clarified. In functional assays, however, our group demonstrated that RNAi-based knockdown of SUV39H1 or G9a specifically reduced their colony-forming activity of Evi1-transduced bone marrow progenitors [Goyama et al., 2009], suggesting that Evi1 forms a complex with H3K9 methyltransferases and epigenetically represses putative Evi1 target genes whose downregulation is

essential for Evi1-induced transformation. Furthermore, our recent study revealed that Evi1 also interacts with PcG proteins. We identified a repressive target gene of Evi1 in bone marrow cells, which is epigenetically regulated by Evi1-dependent recruitment of PRC2 and PRC1, but not by SUV39H1 or G9a [Yoshimi et al., in submission] (Fig. 2B). These findings and future investigations will provide novel therapeutic targets for leukemia with activated Evi1, and the epigenetic approach may be one of the clues to elucidate the molecular mechanisms of Evi1-mediated leukemogenesis.

SUV39H1 AND OTHER TRANSCRIPTIONAL REGULATORS

Carbone et al. [2006] showed that PML/RARα fusion protein physically interacts with SUV39H1 and represses its target genes, such as RARβ2, NFE2, PSCD4, and MYO1F, in a synergistic manner. The authors proposed a model that PML/RARα forms higher order complexes composed of SUV39H1, HDACs, and DNA methyltransferases (DNMTs) at its target gene promoters and reduces their expression through H3K9 methylation, histone deacetylation, and DNA methylation.

AML1 (also known as RUNX1, PEBP2aB, and CBFA2) was originally identified as a gene located at the breakpoint of t(8;21) translocation. AML1 functions as a transcription factor that binds to target gene promoters by recognizing specific DNA sequences to regulate hematopoietic gene expression. In addition, t(12;21) and t(3;21) encode ETV6/AML1 and AML1/Evi1, respectively, and these fusion proteins have potential as transcriptional repressors by interacting with epigenetic silencers, as described above. Two groups have reported the interaction between AML1 and SUV39H1 [Chakraborty et al., 2003; Reed-Inderbitzin et al., 2006]. Chakraborty et al. also showed using luciferase reporter assays that SUV39H1 cancels M-CSFR transcriptional activation by AML1, possibly via SUV39H1's function to abrogate AML DNA binding. In addition, Reed-Inderbitzin et al. demonstrated the binding of AML1 with HDAC1 and HDAC3 as well as SUV39H1. Thus, AML1 may recruit these epigenetic regulators when it represses the expression of target genes. Given that inv(16) fuses the AML1 associating factor CBFβ (core binding factor β) to the MYH11 (smooth muscle myosin heavy-chain gene), it is possible that CBFβ/MYH11 interacts with SUV39H1 or other histone methyltransferases/deacetylases, such as PcG proteins, because CBX4, a component of PRC1, was found in a complex with SUV39H1 [Sewalt et al., 2002].

Taken together, several transcription factors or oncogenic fusion proteins which are critical for normal hematopoiesis or leukemogenesis may exert transcriptionally repressive potential by recruiting higher order chromatin remodeling complexes, presumably like PcG-SUV39H1-HDACs-DNMTs.

DOT1L

DOT1L, a histone methyltransferase that methylates lysine 79 residues in histone H3 (H3K79), has been intensely investigated in relation to leukemia with MLL rearrangements. MLL fusion proteins may activate leukemogenic gene expression, such as HOX genes and Meis1, whereas most MLL translocations lose the SET domain that mediates H3K4 methylation. Identification of the interaction between MLL fusion partners (AF10, AF4, AF9, ENL, etc.) and DOT1L shed light on the mechanisms of transcriptional activation. Originally, AF10 was found to interact with DOT1L in a yeast two-hybrid screen [Okada et al., 2005]. RNAi-mediated knockdown of DOT1L showed that DOT1L is essential for the bone marrow transformation induced by MLL/AF10. MLL/AF10 was demonstrated to recruit DOT1L to MLL target genes such as HOXA7 and HOXA9, induce H3K79me2 enrichment, and up-regulate the expression of these genes in vitro (Fig. 2C). Krivtsov et al. [2008] established an excellent MLL/AF4 disease model using a conditional expression and showed that the DOT1L-mediated H3K79 methylation pattern and gene expression signature are shared by murine MLL/AF4-induced ALL and human ALL with MLL rearrangements. They also exhibited that H3K79me2 profiles can distinguish human MLL-rearranged ALL from other subtypes of ALL. There remains a difficulty in phenotypically recapturing human leukemia with MLL/AF4, as it almost entirely shows early pro-B or biphenotypic leukemia while more than half of diseased mice presented with myeloid phenotypes. These data suggested, however, that DOT1L/H3K79me2 may be a therapeutic target of MLL-rearranged leukemia.

PRMT1

As for the association between histone modifications and leukemogenic mechanisms in leukemia with MLL rearrangements, PRMT1 seems to link to leukemia development independently of DOT1L. PRMT1 is a histone H4-specific methyltransferase that is involved in diverse processes, such as transcriptional activation, protein localization, and signal transduction [Strahl et al., 2001; Wang et al., 2001]. Cheung et al. focused on the transforming potentials of MLL/EEN and found that the SH3 domain, located in the carboxy-terminal end of EEN, is the minimal transformation domain that is necessary and sufficient for MLL/EEN-mediated transformation, and mass spectrometry identified Sam68 as a protein interacting with the SH3 domain. Sam68 has been known to recruit PRMT1 and histone acetyltransferase CBP, and these proteins were found in a complex with MLL/EEN. ChIP assays suggested that MLL/EEN recruits PRMT1-Sam68-CBP complex to the HOXA9 locus and activates the gene expression of HOXA9 via H4R3 methylation and H4 acetylation (Fig. 2D). Although the clinical relevance of these findings has not been addressed, these data showed the further potential of therapeutic intervention for MLL leukemia.

On the other hand, PRMT1 plays an important role in the regulation of the glucocorticoid (GC) responsiveness of ALL leukemic cells, which is critical for the treatment of ALL [van Galen et al., 2010]. The authors showed that B-cell translocation 1 (BTG1) recruits PRMT1 to GC receptor gene promoter and up-regulates GC receptor expression, which in turn positively regulates GCs responsiveness. As BTG1 is frequently deleted in ALL [Mullighan et al., 2007], they argued that inactivation of BTG1 by genomic alterations is responsible for the resistance to GCs.

NSD1

Nuclear receptor-binding SET domain protein 1 (NSD1) has methyltransferase activity specific for H3K36 [Rayasam et al., 2003], which is associated with transcriptional elongation, and mutations of NSD1 were responsible for Sotos syndrome, which is characterized by childhood overgrowth, facial dysmorphism, advanced bone age, seizures, and mental retardation [Kurotaki et al., 2002]. In a small case study, NUP98/NSD1 fusion product was detected in one out of 20 childhood AML patients (5%) [Cerveira et al., 2003], and Wang et al. investigated the molecular functions of NUP98/NSD1 for leukemogenesis in relation to H3K36 methylation. The authors demonstrated that NUP98/NSD1 enforces expression of the HOXA genes as well as Meis1, and induces AML in transplantation assays [Wang et al., 2007]. They proposed a model in which HOXA9 gene expression is up-regulated via NSD1-mediated H3H36 methylation and NUP98-mediated histone acetylation, both of which prevent PcG-induced silencing of HOXA genes during normal myeloid differentiation.

HISTONE DEMETHYLASES

Only limited amounts of data are currently available regarding the involvement of histone demethylases in leukemogenesis. UTX (also known as KDM6A) is a member of the Jumonji C family and has been proved to catalyze the demethylation of H3K27me2/me3 [Agger et al., 2007]. Recent large-scale systematic mutational screens of the coding genome revealed inactivating somatic mutations of UTX in multiple cancer samples, including cell lines established from AML, CML in blastic crisis, and T-ALL [Mullighan et al., 2007]. Reinduction of UTX in UTX null carcinoma cell lines KYSE-180 and KYSE-450 significantly decreased the proliferation activity of these cells with transcriptional changes in the gene sets associated with polycomb targets, indicating that UTX acts as a tumor suppressor in these cells; however, how the inactivating mutations work or how enhanced H3K27me3 marks exert oncogenic properties in leukemia cells remains unknown. Given that inactivating mutations of EZH2 are also detected in myeloid malignancies, balanced regulation of histone marks and consequent gene expression may be critical for protecting cells from developing malignancies. It is also likely that unknown mechanisms contribute to epigenetic changes. Comprehensive studies using murine models that harbor mutations in EZH2 or UTX will be helpful for understanding the leukemogenic potentials of these mutations and for elucidation of the roles of histone modifications in tumorigenesis.

Another example of genetic alterations in histone demethylases was found in a patient with AML M7 who had a cryptic t(11;21;12)(p15;p13;p13) complex variant, which produced NUP98/JARID1A fusion protein [van Zutven et al., 2006]. This fusion protein includes the first 13 exons of NUP98 and exons 28–31 of JARID1A. JARID1A (also known as KDM5A, RBBP2, and RBP2) is a H3K4 demethylase that regulates target genes of PcG in a coordinated manner by interacting with PRC2 [Pasini et al., 2008]. In NUP98/JARID1A fusion protein, the JARID1A portion retains its PHD finger, which recognizes H3K4me3. Wang et al. [2009] demonstrated that this fusion protein induces leukemia in mouse models and that the interaction between the PHD finger of the fusion protein and H3K4me3 is essential for leukemic transformation. Detailed ChIP assays on HOXA gene loci using NUP98/JARID1A-transduced bone marrow cells revealed an unique feature of this fusion protein that NUP98/JARID1A forms a boundary factor that prevents H3K4me3 marks from the JARID1A/PRC2 complex-mediated demethylation program and provides a discriminative gene expression signature of leukemic stem cells, possibly by collaboration with components of H3K4 methyltransferase complex/histone acetyltransferase complex, such as RBBP5, MLLs, and WDR5.

JMJD1B (also known as KDM3B, 5qNCA, JHDM2B, and NET22) is a H3K9me2/me1 demethylate [Klose et al., 2006] and its high expression was observed in hematopoietic cells, including $CD34^{+}$ cells and AML cell lines. JMJD1B is located on chromosome 5q31 and this region is frequently deleted in AML and MDS [Pedersen-Bjergaard et al., 1990]. Hu et al. [2001] demonstrated a possible point mutation in a highly conserved region of JMJD1B in the KG-1 AML cell line. The authors also showed that overexpression of JMJD1B suppressed clonogenic growth of the MUTZ-1 cell line derived from AML patients with chromosome 5 deletion. These data indicated that JMJD1B may have a role in leukemic cell proliferation and that it is a candidate for del(5q) targets.

FINAL CONCLUSIONS AND FUTURE DIRECTIONS

Recent extensive studies resulted in the identification of somatic mutations in several epigenetic regulators with a high frequency in myeloid malignancies, as we described above. In addition, TET2 and ASXL1, which are putative regulators of DNA methylation and PcG/TrxG functions, respectively, are also found to be frequently mutated in myeloid malignancies [Delhommeau et al., 2009; Gelsi-Boyer et al., 2009]. These findings suggest that unbalanced epigenetic regulation frequently occurs in hematological disorders and is crucial for malignant transformation of hematopoietic cells. The notion that unbalanced regulation induces cancer is partly supported by the controversial results that both inactivating mutations of EZH2 and UTX are found in hematological malignancies, although it remains to be elucidated whether these mutations act in opposite directions. Analyses of UTX mutations in large-scale are also required using patient samples.

The attempt to inhibit histone methyltransferases and demethylases has just begun. For example, *S*-adenosylhomocysteine hydrolase inhibitor 3-deazaneplanocin A (DZNep), an inhibitor of PRC2, was effective against a few AML cell lines and primary AML cells in vivo and in vitro [Fiskus et al., 2009]. In our preliminary experiments, DZNep possibly impaired normal HSC, leading to severe pancytopenia at the same dose with prolonged administration. In this regard, it will be helpful to determine the level of PRC2 required for the survival of cancerous versus normal cells in vivo and what differential effects of PRC2 loss are in these cells. Furthermore, recurrent inactivating mutations of EZH2 in myeloid disorders raised the possibility that administration of DZNep induces

other myeloid malignancies, as the authors previously mentioned [Ernst et al., 2010]. Pathogenetic fusion products and aberrantly expressed transcription factors interact or possibly interact with PcG proteins in approximately 40% of cases of AML according to the clustering based on gene expression signature, as shown above; therefore, these interactions are very attractive targets, at least for AML treatment. Comprehensive analyses of animal models of EZH2 mutations, UTX mutations, and DZNep administration using genome-wide mapping of chromatin and the DNA methylation state and gene expression profiling will provide profound insight into the role of histone methylation in leukemogenesis, with suggestions for the clinical application of epigenetic drugs like DZNep.

Further potential of therapeutic intervention targeting epigenetic regulators may be small molecules that directly inhibit histone methyltransferases or demethylases [Kubicek et al., 2007]; however, the same problem as described above may apply to these inhibitors if small molecules globally suppress targeted enzymes. Given that histone methyltransferases seem to have oncogenic functions in the presence of chromosomal translocations or aberrantly expressed transcription factors, the discovery of specific inhibitors that disrupt interaction between methyltransferases and transcription factors may improve the specificity and safety of the treatment.

Although marked clinical heterogeneity makes it difficult to develop universal inhibitors of leukemia, investigations of the pattern of epigenetic changes or molecular interaction between methyltransferases and key regulators of hematopoiesis are in process to obtain critical insights into the mechanisms of leukemogenesis in several subtypes of leukemia defined by morphology, karyotype, acquired gene mutations, or gene expression signatures. Continuously evolving molecular tools make it possible, to some extent, to screen the molecular properties of HSC and LSC, rare populations of cells. These studies may provide opportunities to overcome the difficulty of treating heterogeneous diseases by presumably targeting multiple signaling pathways that are essential for the maintenance of LSC. Epigenetic investigations comparing HSC with LSC or LSC with non-LSC leukemic cells should be performed more extensively in the future to develop novel stem cell-directed therapies.

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