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Therapeutic Opportunities of Targeting Canonical and Noncanonical PcG/TrxG Functions in Acute Myeloid Leukemia

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Keywords

acute myeloid leukemia, Polycomb repressive complex, MLL, EZH2, synthetic lethality, antagonistic pleiotropy

Abstract

Transcriptional deregulation is a key driver of acute myeloid leukemia (AML), a heterogeneous blood cancer with poor survival rates. Polycomb group (PcG) and Trithorax group (TrxG) genes, originally identified in *Drosophila melanogaster* several decades ago as master regulators of cellular identity and epigenetic memory, not only are important in mammalian development but also play a key role in AML disease biology. In addition to their classical canonical antagonistic transcriptional functions, noncanonical synergistic and nontranscriptional functions of PcG and TrxG are emerging. Here, we review the biochemical properties of major mammalian PcG and TrxG complexes and their roles in AML disease biology, including disease maintenance as well as drug resistance. We summarize current efforts on targeting PcG and TrxG for treatment of AML and propose rational synthetic lethality and drug-induced antagonistic pleiotropy options involving PcG and TrxG as potential new therapeutic avenues for treatment of AML.

INTRODUCTION

Acute myeloid leukemia (AML) is a blood cancer characterized by the uncontrolled growth of myeloid blood cells in the bone marrow that interfere with the production and functions of normal blood cells. It is a highly heterogeneous disease with specific mutations targeting various components of transcriptional and epigenetic machineries, which have important prognostic value (91, 137) and feature in the recent update on the major AML subtypes by the World Health Organization (6). Despite AML's known molecular and cellular heterogeneity, almost all AML patients are currently treated with the same standard cytotoxic chemotherapeutics developed more than half a century ago. The average five-year overall survival rate for AML patients under age 60 is below 40%, and the prognosis in patients over age 60 is even worse, with a five-year overall survival rate of less than 10% (12, 131, 137). The only exception, and the first example of targeted therapy, is the treatment of the AML subtype acute promyelocytic leukemia with all-*trans* retinoic acid (ATRA); this treatment specifically targets the oncogenic transcription factor promyelocytic leukemia/retinoic acid receptor alpha (PML-RARA), which drives this subtype (29).

Compared with other cancers, AML has a remarkably low mutational burden (82), and many of the mutations affect transcription (91). A key level of transcriptional regulation involves nucleosomes, the fundamental unit of chromatin: DNA wrapped around an octamer of histones H2A, H2B, H3, and H4. Modifications to nucleosomes, including DNA methylation, covalent histone modifications (e.g., methylation, phosphorylation, acetylation, and ubiquitination), and ATP-dependent chromatin remodeling not only can affect the access of transcription factors, cofactors, and general transcriptional machinery to DNA but also provide a means to stably store and pass on information in the genome without changes to the DNA sequence (i.e., epigenetics). Mutations in AML affect transcriptional regulation at multiple levels, from transcription initiation, elongation, and RNA splicing and processing to the spatial three-dimensional organization of mammalian genomes into chromatin loops and topologically associating domains as well as epigenetic modifications that orchestrate the stable and heritable repression and/or activation of gene expression in generations of daughter cells. Aberrant DNA methylation in AML and its clinical implications have been recently reviewed (28, 135); here, we focus on two major classes of key epigenetic regulators, Polycomb group (PcG) and Trithorax group (TrxG) proteins, which have important functions in the covalent modification and remodeling of chromatin involved in AML biology.

POLYCOMB AND TRITHORAX

Origins and History

The PcG and TrxG genes were first identified in *Drosophila melanogaster* several decades ago as critical regulators in embryogenesis and differentiation processes. In general, these two groups of proteins antagonize each other in regulating the expression of key developmental genes, including the widely studied homeotic or HOX genes, and the gene expression patterns are passed on to subsequent generations as part of the epigenetic memory system (64). While PcG and TrxG are not required for the initiation of HOX gene expression, they are critical for the maintenance of the transcriptional state of HOX genes, which in turn, for example, define the identity of *Drosophila* body segments. Consistently, mutations in PcG genes lead to aberrant expression of HOX genes (73), whereas mutations in TrxG genes result in reduced expression of HOX genes (59). As a consequence, mutations of these genes cause homeotic transformations, phenocopying HOX-deficient phenotypes, in which the identity of an entire body segment is transformed into another.

PcG and TrxG proteins form and act in diverse multiprotein complexes, which possess specific chromatin-modifying enzymatic activities (64). *Drosophila* PcG proteins are organized into

Polycomb repressive complex 1 (PRC1, with H2A119 ubiquitination activity) and PRC2 (with H3K27 methylation activity); Pho repressive complex (PhoRC); Polycomb repressive deubiquitinase (PR-DUB, with H2A118 deubiquitination activity); and dRing-associated factors (dRAF, with both H2A118 ubiquitination and H3K36 demethylation activity), which shares subunits with PRC1. TrxG proteins arrange into *Drosophila* complexes of proteins associated with SET1 (dCOMPASS) and dCOMPASS-like complexes that share multiple subunits (both with H3K4 methylation activity); Trx acetylation complex 1 (TAC, with H3K4 methylation and H3K27 acetylation activity); and absent, small, or homeotic discs 1 (ASH1, with H3K36 dimethylation activity), as well as the Brahma-associated protein complex (BAP), polybromo-containing BAP complex (PBAP), and Kismet (KIS), which are ATP-dependent chromatin remodelers. In general, histone marks mediated by PcG, such as H2AK118ub, H3K27me3, and demethylation of H3K36, are strongly associated with gene repression, whereas TrxG-mediated H3K4 and H3K36 methylation, H3K27 demethylation and acetylation, and changes of nucleosome position are all associated with gene transcription. Functionally, in *Drosophila*, PcG and TrxG can tether to Polycomb response elements, which are specific DNA elements containing multiple transcription factor binding sites at target gene loci such as HOX loci to regulate their transcriptional states by mediating the appropriate chromatin modifications. The chromatin modification is then stably passed on to the next cell generation, which inherits the repressed or activated gene expression states. Several other functions have also been described for PcG and TrxG, such as roles in chromatin condensation and integrity (64), revealing that PcG and TrxG regulate their target genes at multiple levels, including local chromatin structure, higher-order chromatin organization, and global genome architecture.

Exceptions to the Canonical Antagonism in *Drosophila*

Although the classical view of antagonism holds true for many PcG and TrxG proteins, in which TrxG genes were originally defined as suppressors of PcG phenotypes in genetic screens (66), there are exceptions. One of the genetic screens utilizing a TrxG mutant phenotype to uncover novel TrxG genes identified several PcG genes [*Asx*, *E(PC)*, *E(z)*, *Psc*, *Su(z)*, and *Scm*] whose genetic loss failed to complement that TrxG phenotype and instead further enhanced the TrxG phenotype (47). Intriguingly, these PcG genes are distributed over all PcG complexes, raising the possibility that many PcG complexes may have the functionality not only to antagonize but also to synergize with TrxG, at least in cell-type- or context-specific manners. Similarly, one TrxG member, the GAGA factor encoded by the Trithorax-like (*Trl*) gene, interacts with PcG Pho and is required for PcG silencing (86). Although a new class for these proteins, enhancer of Polycomb and Trithorax (ETP), was proposed (47), it never gained sufficient traction in the field, and therefore the original antagonistic view about the functions of PcG and TrxG remains the most widely used. Additional functions of PcG and TrxG are being identified, including tissue-specific gene activation by PRC1 via chromatin looping (78) and alterations in RNA polymerase II (PolII) phosphorylation (95), as well as the critical roles of PcG and TrxG in cell cycle control, stem cell biology, and cancer. In this review, we describe the key mammalian PcG and TrxG complexes and their canonical/antagonistic and emerging noncanonical/synergistic functions, summarize their roles in AML disease biology, and discuss their potential as therapeutic targets for the treatment of AML.

PcG in Mammals

With the refinement of protein purification processes, an increasing number of PcG and TrxG subcomplexes with different activities and functions have been identified in mammalian cells. All mammalian PcG and TrxG complexes contain core components that often form the catalytic units and are shared among all subtypes, which then are further divided into specific subcomplexes

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according to their associated accessory proteins (**Supplemental Figures 1** and **2**). However, despite their increased complexity during evolution, canonical antagonistic functions of PcG and TrxG remain conserved on developmental genes such as HOX genes, and many mammalian PcG and TrxG proteins have homolog complexes and/or the same enzymatic activities as their *Drosophila* counterparts (**Figure 1*a***; **Supplemental Figures 1** and **2**).

PRC1

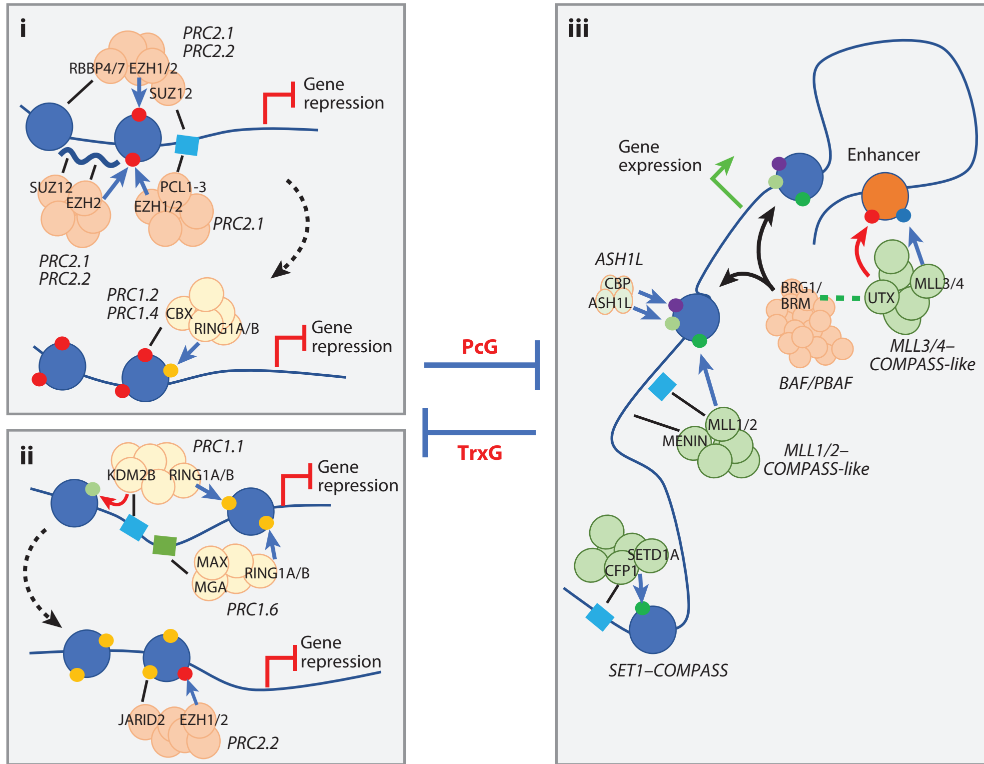
PRC1 is the most evolved and diverse PcG complex in mammals. It can be divided into canonical PRC1, whose subunit composition is homologous to that of *Drosophila* PRC1, and noncanonical PRC1 (ncPRC1), which constitutes all other PRC1 complexes (**Supplemental Figure 1*a***). The core components of all PRC1 complexes are the E3 ubiquitin ligases RING1A or RING1B and one of the six Polycomb group of ring finger (PCGF) proteins, which not only are sufficient to mediate basal levels of H2AK119ub in vitro (14, 74) but also act as a scaffold for PRC1 assembly. RING1 binds to one chromobox protein (CBX2, -4, -6, -7, or -8) in canonical PRC1 complexes or to RING1- and YY1-binding protein (RYBP) or its homolog YY1-associated factor (YAF2) in ncPRC1 complexes in a mutually exclusive manner (44) (**Supplemental Figure 1*a***). The incorporation of one out of six different PCGF proteins defines each PRC1 subcomplex associated with different accessory proteins, some of which mediate chromatin binding in the absence of Polycomb response elements in mammals.

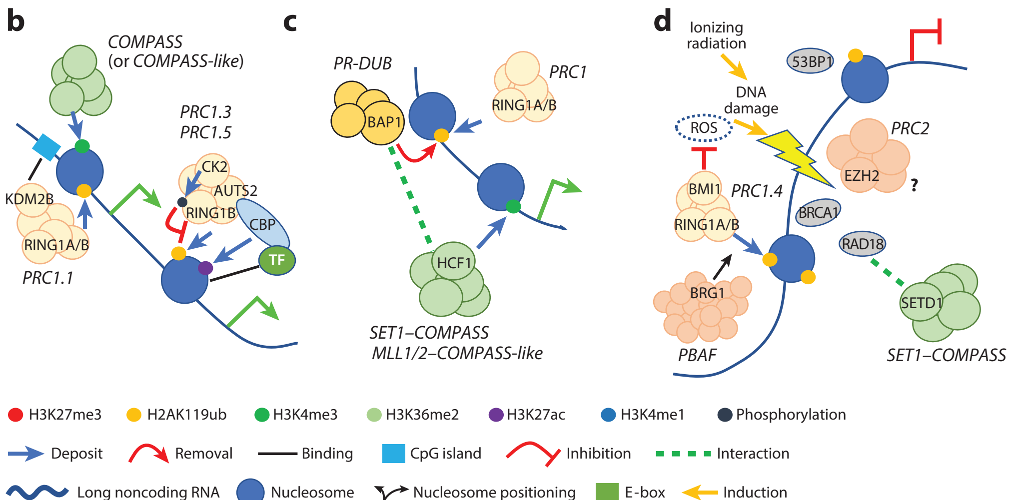
Two different modes of chromatin binding can be defined for PRC1: PRC2 dependent and PRC2 independent. The classical PRC2-dependent chromatin recruitment of PRC1 is mediated by CBX proteins, which recognize the H3K27me3 mark written by PRC2 (**Figure 1*a*, subpanel *i***). The more recently identified PRC2-independent chromatin binding of PRC1 is mediated, for example, by KDM2B, which binds to hypomethylated CpG islands, or by the MAX/MGA dimer, which binds to E-box DNA elements (**Figure 1*a*, subpanel *ii***). Thus, each PRC1 subcomplex possesses its own target locus specificity, which may partly overlap with that of other PRC1 complexes. While both canonical PRC1 complexes are strongly associated with gene repression (**Figure 1*a***), ncPRC1 can repress (PRC1.1, PRC1.6) (**Figure 1*a***) or activate (PRC1.1, PRC1.5, PRC1.3) (**Figure 1*b***) gene expression. Although not conserved as distinct complexes in mammals, members of the *Drosophila* PcG complexes dRAF and PhoRC are part of the mammalian PRC1.1 and PRC1.6 complexes, respectively (57, 121). Additional noncanonical functions for PRC1 in mammals have also been reported; a prominent example is the core component PCGF4 (BMI1), which is present in PRC1.4 and also implicated in the regulation of mitochondrial function and the DNA damage response (DDR) (77).

PRC2

RBBP4/7 and a trimeric enzymatic active core (EZH1/2, SUZ12, EED), which are sufficient to mediate H3K27 methylation in vitro (19, 93), form the core of mammalian PRC2 complexes. Strikingly, H3K27 methylation is mediated predominantly or even exclusively by PRC2. Mutually exclusive incorporation of accessory proteins further defines the PRC2.1 and PRC2.2 complexes. One of three Polycomb-like proteins (PCL1–3), Elongin BC, and either Polycomb repressive complex 2–associated protein (EPOP) or PRC2-associated LCOR isoform 1 or its paralog LCORL (PALI1/2) are present in PRC2.1, while PRC2.2 comprises Jumonji and AT-rich interaction domain 2 (JARID2) and adipocyte enhancer-binding protein 2 (AEBP2) (**Supplemental Figure 1*b***).

Similar to PRC1, the core components of PRC2 do not show any specific chromatin- or DNA-binding property. PRC2.1 and PRC2.2 are often targeted to chromatin or DNA by their specific





(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Model of PcG- and TrxG-mediated gene regulation. (*a*) The canonical antagonism of PcG-mediated gene repression and TrxG-mediated gene activation. (*i*,*ii*) PRC1 and PRC2 have different modes to target chromatin and can repress gene expression independently or in concert. The classical PRC2-dependent recruitment of PRC1 (**subpanel *i***) and the newer PRC1-dependent recruitment of PRC2 (**subpanel *ii***) are illustrated. As shown in **subpanel *i***, PRC2 binds to chromatin via direct interaction through RBBP4/7, through interaction with long noncoding RNA via EZH2/SUZ12, or to CpG islands via PCL1–3 and SUZ12, leading to trimethylation of H3K27. Then CBX protein containing PRC1.2/PRC1.4 complexes recognizes and binds the H3K27me3 mark and ubiquitinates H2AK119. As shown in **subpanel *ii***, PRC1.1 binds to CpG islands via KDM2B, or PRC1.6 binds E-boxes via MAX-MGA, leading to the monoubiquitination of H2AK119. Subsequently, JARID2 present in PRC2.2 recognizes and binds H2AK119ub, and PRC2.2 trimethylates H3K27. The deposition of H3K27me3 and/or H2AK119ub and the removal of H3K36me2 result in PcG-mediated gene repression. (*iii*) The antagonistic actions of TrxG are shown. SET1–COMPASS and MLL1/2–COMPASS-like complexes bind to chromatin at promoter regions by binding to CpG islands via CFP1 and MLL1/2, respectively. MLL3/4–COMPASS-like complexes are found at enhancer regions, and the H3K27me3 demethylase UTX also interacts with BRG1/BRM subunits of the BAF/PBAF chromatin remodeler, which affect the positioning of nucleosomes along DNA. The deposition of H3K4me3, H3K36me2, and H3K27ac; the removal of H3K27me3; and proper chromatin positioning positively regulate gene expression. (*b*–*d*) Noncanonical synergistic functions of PcG and TrxG proteins. As shown in panel *b*, PRC1.1 can be found at active genes, which are also enriched for H3K4me3, suggesting a potential synergism between PcG and TrxG in regulating these genes. PRC1.3 and PRC1.5 also have positive effects on gene expression. In panel *c*, deubiquitination of H2AK119ub by PR-DUB counteracts PcG repression and leads to gene activation. BAP1 also interacts with and stabilizes HCF1, which is a part of the SET1–COMPASS and MLL1/2–COMPASS-like complexes, revealing another potential synergism between PcG and TrxG complexes. As shown in panel *d*, both PcG and TrxG complexes play critical roles in the DNA damage response (DDR). PRC1 and PRC2 complexes are important for the recruitment of 53BP1 and BRCA1 to DNA damage sites. PRC1.4-mediated H2AK119ub is required for efficient DDR and the silencing of gene transcription near DNA damage sites, but the underlying mechanisms are poorly understood. Moreover, H2AK119ub mediated by PRC1.4 is facilitated by PBAF complexes, revealing a synergism between PcG and TrxG complexes in gene silencing at sites of DNA damage and recruitment of DDR proteins. On the other hand, the level of reactive oxygen species (ROS) is suppressed by BMI1, revealing a protective function of PcG in maintaining genomic integrity. Although EZH2 is required for efficient DDR, the H3K27 methylation at DNA damage sites is largely unchanged, and its underlying mechanisms remain poorly defined (as indicated by the question mark).

accessory proteins (**Figure 1*a***), all of which possess different affinities for specific histone modifications or DNA sequence, methylation, or topology (22). Also similar to PRC1, two different modes of recruitment of PRC2 to chromatin can be described: PRC1 independent and PRC1 dependent. While the latter mostly involves JARID2 recognizing the H2AK119ub mark mediated by PRC1 (**Figure 1*a*, subpanel *ii***), the former is much more diverse. PCL1–3 and SUZ12 bind to CpG islands, and RBBP4 and -7 possess binding affinity to chromatin, whereas EZH1/2 and SUZ12 can bind to long noncoding RNAs, all of which recruit PRC2 to chromatin (**Figure 1*a*, subpanel *i***). Together, the interdependent and independent modes of PRC1 and PRC2 recruitment to chromatin allow for fine-tuning of context-specific gene regulation by PRC1 and/or PRC2.

Intriguingly, cytoplasmic functions of EZH1 and EZH2 have also been described. In postmitotic muscle cells, the EZH1b isoform acts as an adaptive environmental sensor that controls the epigenome's structure by retaining Eed in the cytosol to prevent its assembly in the functional PRC2 complex (10), while cytosolic EZH2, SUZ12, and EED form a methyltransferase complex with a role in growth factor and T cell receptor–induced actin polymerization (112), in part through the methylation of TALIN, which links integrin molecules to the actin cytoskeleton (53).

PR-DUB

The ubiquitin C-terminal hydrolase BRCA1-associated protein 1 (BAP1) and one additional sex combs–like protein (ASXL1 or ASXL2) form the enzymatic active core of the mammalian H2A119ub-deubiquitinating PR-DUB complex (PR-DUB.1 with ASXL1 and PR-DUB.2 with ASXL2) (54). Both PR-DUB complexes share a similar set of accessory proteins (**Supplemental Figure 1*c***), including the chromatin-associated proteins MBD5 and MBD6, the transcription

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factors FOXK1 and FOXK2, the transcriptional coregulator host cell factor 1 (HCF1), the histone demethylase KDM1B, and the PcG protein OGT1 (32, 54), which is the only known *O*-GlcNAc transferase in mammals. Like those in PRC1 and PRC2 complexes, the accessory proteins of PR-DUB mediate binding of PR-DUB to chromatin or DNA targets.

TrxG in Mammals

The key TrxG complexes in mammals can be broadly divided into three groups: ATP-dependent chromatin remodelers, COMPASS and COMPASS-like complexes, and ASH1 chromatin modifiers (**Figure 1*a***; **Supplemental Figure 2**). Mammals possess several COMPASS and COMPASS-like complexes that mediate the bulk of H3K4 methylation in cells. WDR5, ASH2L, RBBP5, and DPY30 are core components shared in all COMPASS and COMPASS-like complexes (**Supplemental Figure 2*a***). In contrast to PRC1/2 core components, they lack any enzymatic activity and instead bind to DNA and chromatin. Different SET-domain-containing H3K4 methyltransferases with unique functionalities (96) are present in distinct COMPASS and COMPASS-like complexes. The H3K4 methyltransferase SETD1A/B forms SET1–COMPASS, which further possesses HCF1, WD repeat domain 82 (WDR82), and CXXC finger protein 1 (CPF1). While the MLL1/2–COMPASS-like complex is defined by MLL1/2 H3K4 methyltransferase, HCF1, and MENIN1 and is found predominantly at transcriptional start sites, the MLL3/4–COMPASS-like complex contains nuclear receptor coactivator 6 (NCOA6), PAXIP1-associated glutamate-rich protein 1 (PA1), PAX-interacting protein 1 (PTIP), and the H3K27 demethylase UTX and associates mainly with enhancer regions (**Figure 1*a***).

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In addition to H3K4 methylation, one TrxG-containing complex has been identified in mammals, comprising the H3K36 methyltransferase ASH1L, the H3K27 acetyltransferase CBP, RBB4/7, and MRG15/MRGX (105) (**Supplemental Figure 2*b***). TrxG proteins also define two ATP-dependent chromatin remodeling complexes, the mammalian SWI/SNF complexes, Brg/Brahma-associated factors (BAF), and polybromo- and Brg-associated factors (PBAF). The core proteins are BAF155, BAF70, BAF57, BAF47, BAF60A–C, BCL7A–C, BAF53A, and beta-actin. BAF further consists of the ATPase BRG1 or BRM as well as BAF250A/B, BRD9, BAF45B–D, and SS18, whereas PBAF includes exclusively BRG1 as well as BAF200, BRD7, BAF180, and BAF45A (**Supplemental Figure 2*c***). Together, the TrxG complexes mediate gene activation by covalently modifying the histones and positioning of nucleosomes that antagonize PcG-mediated repression (**Figure 1*a***).

Exceptions to the Canonical Antagonism in Mammals

As expected from the increased complexity of mammals, tissue-specific and noncanonical TrxG-agonistic functions have been described for PcG complexes. For instance, PRC1.5 was the first PRC1 complex where gene-activating functions were described and systematically dissected (43). The PRC1.5 complex contains casein kinase 2 and AUT2, which inhibit the H2A ubiquitination activity of PRC1.5 and recruit the histone acetyltransferase p300, respectively, leading to robust gene activation during neural development (**Figure 1*b***). In skin tissue, ncPRC1 promotes the expression of key skin regulatory genes, although the mechanisms remain poorly understood (26, 27). In addition to repressing gene expression, PRC1.1 is also found at actively transcribed genes that are also marked by H3K4me3 (121), suggesting a possible synergism between PcG and TrxG in regulating these genes (**Figure 1*b***).

BAP1, a core component of PR-DUB, promotes transcription (**Figure 1*c***), limiting PRC1 repressive activity by removing H2AK119ub (16) in HAP1 cells, which are a derivative of the near-haploid chronic myeloid leukemia cell line KBM-7. Moreover, HCF1 is present in PR-DUB,

SETD1–COMPASS, and MLL1/2–COMPASS-like complexes. BAP1 interacts with and stabilizes HCF1 (**Figure 1*c***), highlighting a potential role for PcG in maintaining the protein abundance of a TrxG complex member. On the other hand, a PRC2 subcomplex comprising EZH1 and SUZ12 was identified in developing erythroid cells that associates with actively transcribed genes and positive regulation of gene expression, although its role in the context of normal EZH2–PRC2 functions remains unclear (133).

EMERGING NONCANONICAL DNA DAMAGE RESPONSE FUNCTIONS OF PCG AND TRXG PROTEINS

Interestingly, an important role for PcG and TrxG in DDR is emerging and further expanding their functions beyond the classical antagonism of developmental gene expression (**Figure 1*d***). While an early study linked BMI1 (PCGF4) present in PRC1.4 to the regulation of mitochondrial function and the DDR pathway (77), likely through its transcriptional effects on key target genes implicated in reactive oxygen species and DDR, further studies showed that PRC1.4 is recruited to sites of DNA double-strand breaks and mediates H2AK119ub (48, 60). Although PRC1 recruitment and possibly H2AK119ub is important for DDR, including the recruitment of DDR proteins, the exact mechanisms remain poorly understood (120). Moreover, PRC2 is also recruited to sites of DNA damage, and downregulation of EZH2 decreases double-strand break repair, although H3K27me3 is not increased at damaged sites (17) (**Figure 1*d***). Notably, downregulation of EZH2 also induces cell cycle progression–dependent DNA damage (61), highlighting key functions of PRC2 in facilitating DDR.

Increasing evidence also suggests a role for TrxG in DDR. MLL is an effector protein in the mammalian S-phase checkpoint network that mediates H3K4 methylation at late replication origins and inhibits the loading of CDC45 onto the prereplication complex containing the MCM2–7 complex in order to delay DNA replication (76). During genotoxic stress, MLL is phosphorylated at serine 516 by ATR in the S phase, which disrupts its interaction and degradation by the SCF E3 ligase, leading to its accumulation and delayed replication. Conversely, MLL-deficient cells showed radioresistant DNA synthesis, genomic abnormalities, and S-phase checkpoint dysfunction, suggesting their ability to tolerate DNA damage and restart stalled replication forks, which could be rescued by the wild type but not by S516A or catalytic dead MLL mutants. Importantly, MLL fusions found in AML act as dominant negative mutants that abrogate the ATR-mediated stabilization of wild-type MLL, resulting in a compromised S-phase checkpoint and a severe radioresistant DNA synthesis phenotype. A study also recently showed that SETD1A, but not the shared COMPASS/COMPASS-like members RBB5 and ASH2L, interacts with the DDR protein RAD18 (**Figure 1*d***) and that knockdown of SETD1 abolishes ubiquitination of PCNA following induction of DNA damage, which is important for double-strand break repair (5). Moreover, Kakarougkas et al. (63) showed that PBAF promotes DNA double-strand break repair, reporting that PBAF is required not only for H2AK119ub at double-strand breaks but also, in concert with BMI1 and EZH2, for double-strand break–induced transcriptional silencing and efficient DNA repair, demonstrating an unexpected synergistic function between PcG and TrxG in DDR (**Figure 1*d***). The PBAF functions in DDR are controlled by ATM phosphorylation of BAF180. Lastly, a study has also reported that RAD21 plays a role in faithful DNA replication by preventing Myc-induced replicative stress (101).

ROLES OF PCG AND TRXG IN ACUTE MYELOID LEUKEMIA DISEASE BIOLOGY

Given the important functions of PcG and TrxG proteins in gene regulation and DDR, it is not surprising that they are frequently involved in disease settings. Deregulation and mutations of PcG

and TrxG are frequently found in cancer, including AML, in which they exhibit tumor suppressor or oncogenic functions. In this section, we review individual PcG and TrxG genes and their roles in AML disease development and/or maintenance.

PcG Proteins in Acute Myeloid Leukemia

The total loss of all PRC1 and ncPRC1 complexes mediated by the combined genetic knockout of the core components Ring1A and Ring1B abolished leukemic transformation in various murine AML models, including those with *MLL*, *PML-RARA*, and *MOZ-TIF2* fusion oncogenes (102, 109). Consistent with the function of PRC1 in repressing the *Ink4-Arf* locus (e.g., *p16Ink4a* and *p19Arf*) in normal and malignant development (92, 106), these studies also reported that derepression of the *Ink4a* locus and *Glis2* induces cell differentiation and leukemic suppression (109). Although PRC1 and ncPRC1 complexes are associated with different chromatin modifications and can have opposite effects on transcription, human AML cells show a surprising dependency on both complexes (121).

PCGF4/BMI1, which defines PRC1.4, is probably the best-studied PRC1 PcG member. Several studies have shown that BMI1 is required for leukemic transformation in a variety of AML models driven by various oncogenes, including *HOX9-MEIS1* (72), *AML1-ETO* and *PLZF-RARA* (111), and *CALM-AF10* (7). All of these studies used viral transduction of the oncogenes on hematopoietic stem and progenitor cells isolated from germline *Bmi1*$^{-/-}$ mice and showed the leukemic transformation–abolishing effects associated with derepression of the *Ink4a* locus. Consistent with its critical role in leukemic transformation, BMI1 not only is overexpressed in AML but also has prognostic value (103). Patients with higher BMI1 expression had significantly lower overall survival than those with lower BMI expression (104). However, BMI1 is present in only one canonical complex, PRC1.4, and how BMI1 exerts its oncogenic functions in the presence of other PRC1/ncPRC1 complexes remains poorly understood. The noncanonical BMI1 function outside of PRC1.4 described above may be equally important for its critical role in AML disease biology.

Several CBX proteins associated with canonical PRC1.2 and PRC1.4 complexes also display essential roles in leukemic transformation. All CBX family members share a conserved N-terminal chromodomain, binding to H3K27me3 and therefore linking canonical PRC1.4/PRC1.2 to PRC2, but display varying C-terminal sequences, accounting for their nonredundant functions (124). CBX8 directly interacts with two common MLL fusion proteins, AF9 and ENL, and its loss impairs leukemic transformation and, paradoxically, MLL fusion–mediated transcriptional activation (80, 113). MLL-AF9 uses CBX8 to recruit the histone acetyltransferase TIP60 to activate HOX gene expression (113), and binding of CBX8 by ENL also blocks Polycomb-mediated repression of HOX genes, which is important for efficient HOX gene expression by MLL-ENL (80). Loss of CBX2 (33) and CBX7 (62) also impairs AML cell proliferation and induces differentiation, whereas overexpression of CBX7 in human and murine hematopoietic stem and progenitor cells induces leukemia (62, 68). These oncogenic CBX functions appear to be tissue specific, as CBX7 showed tumor suppressor functions in other tissues, such as liver, lung, and brain (40, 45).

On the other hand, several PRC1.1 complex members are overexpressed or mutated in human AML, including BCOR and PCGF1. Consistent with an oncogenic role, knockdown of PCGF1 or KDM2B impaired AML cell proliferation (121). Studies using chromatin immunoprecipitation combined with sequencing (ChIP-seq) revealed that PRC1.1 also targets genes involved in metabolism, whereas canonical PRC1.2 and PRC1.4 mainly bind classical Polycomb target genes involved in developmental processes. However, BCOR shows tumor suppressor functions in AML. Mutated BCOR resulted in the expansion of myeloid progenitor cells, cooperated with

KRASG12D to induce AML, and regulated H2AK119ub and gene expression. Surprisingly, the upregulated genes that showed loss of H2AK119ub were developmental rather than metabolic genes, including HOX genes, which are classical and well-studied Polycomb targets and are essential for the leukemic phenotype in the mutated BCOR/KRASG12D model (65). These studies show that oncogenic and tumor suppressor roles are strictly context specific for PRC1 complex members—i.e., the function may change depending on the nature of the mutation(s), AML subtypes, and even cells of origin.

Studies have reported that BAP1, ASXL1, and ASXL2, the core components of PR-DUB, all have tumor suppressor functions in myeloid neoplasia, including AML. Although mutations in BAP1 are more frequent in solid tumors, where it constitutes a novel (familial) cancer syndrome (BAP1 tumor predisposition syndrome), they are rare in myeloid neoplasia. However, conditional loss of BAP1 in a mouse model led to myeloid transformation (32). Molecular characterization of the myeloid leukemia revealed no activation of HOX gene expression, but did show deregulation of several genes with immune system functions as a consequence of reduced protein levels of the epigenetic regulators and BAP1 substrates HCF1 and OGT. ASXL1 and ASXL2 mutations, on the other hand, are much more frequently found in AML (58, 97). While ASXL1 and ASXL2 mutations appear to be mutually exclusive, they tend to associate with different AML subgroups. ASXL1 mutations are found in many AML subtypes (91) and confer poor prognosis (46), whereas ASXL2 mutations are predominantly associated with the t(8;21) AML subtype (85), where they might be associated with better prognosis (134). Loss of ASXL1 in a mouse model also leads to myeloid transformation, although the mechanism is very different from loss of BAP1 (1). In *ASXL1* knockout cells, *HOXA* genes are upregulated and global levels of H3K27me3 are markedly reduced, which leads to the expression of genes poised for transcription. Loss of BAP1 or ASXL1 is insufficient to induce AML, but mutation or loss of ASXL1 has been shown experimentally to cooperate with NRASG12D (1) and CEBPA (30) to induce AML.

Oncogenic roles in supporting aberrant self-renewal have been identified for all three PRC2 core complex members—EED, EZH1/2, and SUZ12. Using a variety of AML models, including MLL fusion, AML1-ETO, and WT1 AML, several groups have shown that EZH2 is required for the maintenance of AML cells (8, 88, 110, 115). Although the mechanisms are poorly understood, current studies point to a derepression of known tumor suppressor and Polycomb target *CDKN2A* as well as genes involved in developmental and differentiation processes upon loss of EZH2. However, EZH2 tumor suppressor functions have also been reported in myeloid malignancies. EZH2 mutations are found in approximately 10–15% of cases in myelodysplastic syndromes (MDS) (100) and in secondary AML as well as in a few cases of de novo AML, suggesting opposite and context-dependent roles for EZH2 in disease initiation and maintenance, which were also recently experimentally illustrated (8). Interestingly, EZH2 mutations coexist with ASXL1 mutations in MDS patients and are associated with poor prognosis (94, 118), suggesting nonredundant functions of PRC2 and PR-DUB in protecting against myeloid transformation. While early evidence points toward an epigenetic component for the tumor suppressor role of EZH2 in disease initiation, future studies are needed to determine whether the dual role of EZH2 within PRC2 is mediated via epigenetic and/or other noncanonical functions, such as nonhistone protein methylation or DDR. For example, EZH1 is required for AML1-ETO AML because it mediates K43 methylation, which enables the full gene repressive activity of AML1-ETO (34) that is necessary for leukemic transformation. Similar to EZH2's role in AML maintenance, oncogenic roles for SUZ12 (107) and EED (31, 88, 107) were identified in MLL AML. Conversely, JARID2, which is part of PRC2.2, acts as a tumor suppressor in the progression from MDS or myeloproliferative neoplasms to secondary AML by recruiting PRC2 to epigenetically repress self-renewal genes in hematopoietic stem and progenitor cells (21). Together, these studies consistently highlight

the highly context-dependent roles of individual PRC complex members and their complexes in AML.

TrxG Proteins in Acute Myeloid Leukemia

SETD1A, the H3K4 methyltransferase of the SET1–COMPASS complex, was identified in a short hairpin RNA–based screen to be required for the growth of MLL-AF9 leukemia cells. Surprisingly, the internal FLOS region of SETD1A, which regulates DDR via direct interaction with cyclin K, is indispensable for AML cells, but the SET domain that mediates H3K4 methylation is not (56). Conversely, via its interaction with WDR82, NUP98 recruits the SET1–COMPASS complex to chromatin, which mediates the H3K4me3 that is necessary for gene transcription in AML cells, including those with NUP98 fusion proteins (41). Although oncogenic nonchromatin roles for SETD1A have been described, such as monomethylation of YAP-K342, which blocks nuclear export of YAP and promotes colorectal tumorigenesis (36), it is unclear whether SETD1A also plays such a role in AML.

While the other H3K4 methyltransferases of the COMPASS-like complexes also have roles in AML, they can be divided into oncogenic roles for MLL1/2–COMPASS-like complexes and tumor suppressor roles for MLL3/4–COMPASS-like complexes. Indeed, the first TrxG gene with clear oncogenic roles in AML was *MLL1*, which is structurally altered in 11q23 translocation (116) and accounts for approximately 5% of all AML cases (137). 11q23 translocations, the initiating mutational events in MLL AML, are characterized by the replacement of the MLL1 C terminus with one of more than 100 different fusion partners (84) that aberrantly recruit epigenetic modifying activities to MLL1 target loci such as HOX genes (138). Intriguingly, MLL2 is required for MLL AML, where it controls NF-κB, integrin B3, and IL-3 AML survival pathways (25), although the exact mechanism remains poorly understood. It is noteworthy that combined MLL1 and MLL2 loss exacerbated the single MLL2 effects. Consistently, loss of MENIN, part of the MLL1/2–COMPASS-like complexes, impaired leukemic growth of MLL AML concomitant with loss of HOX gene expression (136). More recently, MENIN's oncogenic role in the context of the MLL1/2–COMPASS-like complex was extended beyond MLL AML to NPM1-mutated AML, which is also characterized by aberrant HOX gene expression (70). Conversely, loss of MLL3 blocks hematopoietic stem and progenitor cell differentiation and enforces a self-renewal program by altering chromatin modifications, resulting in myeloid transformation (24). UTX, which is part of the MLL3/4–COMPASS-like complex, suppresses AML by repressing oncogenic ETS and the upregulation of tumor-suppressive GATA programs, although these functions seem to be independent of its H3K27 demethylation activity (51).

Interestingly, oncogenic roles for ASH1L have been reported for MLL AML, where the H3K36me2 written by ASH1L is preferentially bound by LEDGF, an MLL1-associated protein, which facilitates recruitment of MLL1/2–COMPASS-like complexes to chromatin at key leukemia target genes, including HOX genes (141). Consistently, point mutations in the seed sequence of miR-142-3p are found in AML, and loss of miR-142 leads to an increase in ASH1L protein and increased HOX gene expression, although loss of miR-142 was not sufficient to induce AML (117). Finally, genome-wide sequencing studies have identified mutations in genes encoding members of the cohesin complex (91). RAD21, the only known TrxG protein involved in cohesin functions, has tumor suppressor functions, as it negatively regulates hematopoietic self-renewal through repression of HOX genes (38).

Although many different SWI/SNF subunits are frequently mutated in solid tumors, the mutation incidence in AML is comparatively low (55). Despite this low incidence, potential tumor suppressor and oncogenic functions for SWI/SNF subunits in AML are emerging. Two studies have reported that BAF60b, in concert with CEBPE, plays a key role in myeloid differentiation

by regulating the expression of granule genes and chromatin accessibility (98, 130). While BAF47 loss-of-function mutations promote rhabdoid tumorigenesis (123), BAF47 is frequently downregulated in AML. BAF47 deficiency leads to increased expression of GEFs and was associated with Rac GTPase activation (23), which is important for AML survival (127). On the other hand, short hairpin RNA–based screens identified BRG1 as critical for AML cell proliferation and self-renewal (108, 142) by maintaining MYC transcription specifically in AML cells. Mechanistically, BRG1 binds to lineage-specific distal enhancer elements 1.7 Mb downstream of the MYC promoter, which are also occupied by a set of hematopoietic transcription factors, and circular chromosome conformation capture experiments showed preferential contact of the enhancer elements with the *MYC* gene (108). Moreover, different ATPases are used in BAF complexes expressed in normal hematopoiesis and AML. BAF complexes in long-term repopulating hematopoietic stem cells are assembled around BRM, whereas BAF complexes in AML predominantly incorporate BRG1. Surprisingly, while BRG1 is dispensable for long-term repopulating hematopoietic stem cell maintenance, it is required for the proliferative activity of long-term repopulating hematopoietic stem cells and downstream progenitors, highlighting the functional specialization of BAF complexes linked with the choice of alternative ATPases (15).

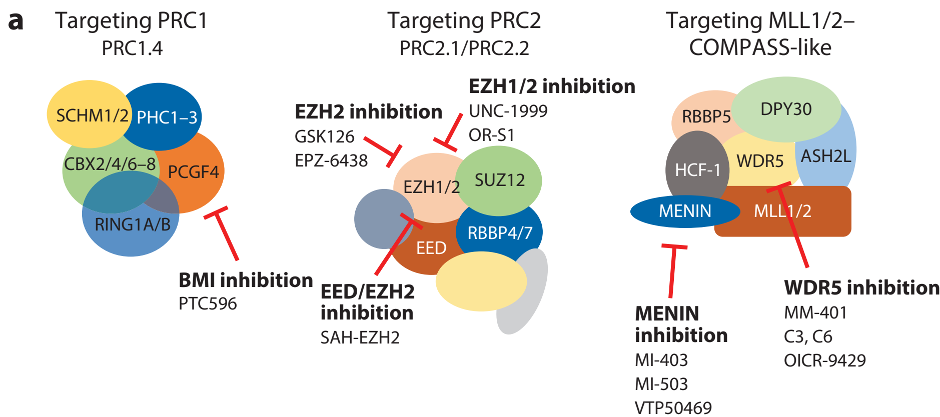
Crosstalk Between PcG and TrxG in Acute Myeloid Leukemia?

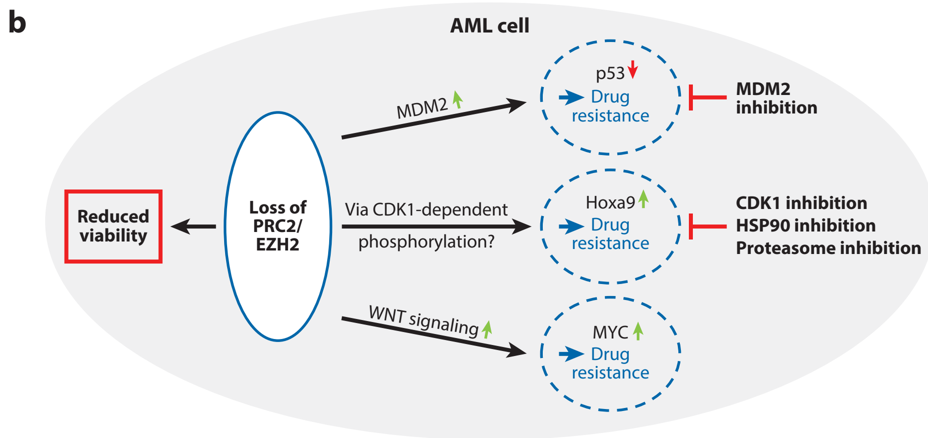
One of the examples of PcG and TrxG crosstalk/cooperation in AML disease biology is repression of HOX genes mediated by the RAD21/cohesin complex. This complex binds to CTCF sites (128), which helps to set up boundaries among individual HOX genes in the *HOXA* locus. RAD21 interacts with the PRC2 core component EED and recruits PRC2 to the CTCF sites within the HOX gene locus, where RAD21 is bound and PRC2 downregulates the expression of HOX genes via deposition of H3K27me3 (38). In addition, insulator functions of RAD21/cohesin may provide another means of TrxG/PcG crosstalk, in which RAD21/cohesin bound to CTCF sites organizes the domains where PRC1/2 repressive histone marks are deposited and prevents the spreading of these histone marks beyond the CTCF boundaries. Another example is the association of the ubiquitously expressed chromatin-associated transcriptional coregulator HCF1 with Polycomb PR-DUB and Trithorax SET1–COMPASS and MLL1/2–COMPASS-like complexes. While PR-DUB core member BAP1 interacts with HCF1 and OGT, deubiquitination by BAP1 protects these proteins from proteasomal degradation (32), and O-GlcNAcylation of HCF1 by OGT is necessary for HCF1 activation (20). Loss of BAP1 leads to reduced HCF1 levels, suggesting that BAP1 functions at least partly via HCF1. Conversely, although the exact role of HCF1 in SET1–COMPASS and MLL1/2–COMPASS-like complexes in AML is not known, HCF1 can tether the MLL2–COMPASS-like complex to E2F1 target genes for H3K4me3 to facilitate the transition from G1 to S phase in mammalian cells (140). These studies not only reveal functional roles for HCF1 in PcG and TrxG complexes but also provide an example of PcG-mediated stabilization of TrxG complex proteins. Finally, MLL AML is highly dependent on both PcG (canonical and noncanonical PRC1 and PRC2) and TrxG (MLL1/2–COMPASS-like), suggesting a degree of functional cooperation between PcG and TrxG in MLL AML disease biology, although the underlying mechanisms are incompletely understood.

TARGETING PCG AND TRXG AS A THERAPEUTIC APPROACH IN ACUTE MYELOID LEUKEMIA

Targeting of PcG and TrxG Complexes

Given their roles in AML disease biology and specific enzymatic activities, the targeting of PcG/TrxG represents a promising strategy in the treatment of AML (**Figure 2*a***). Indeed,





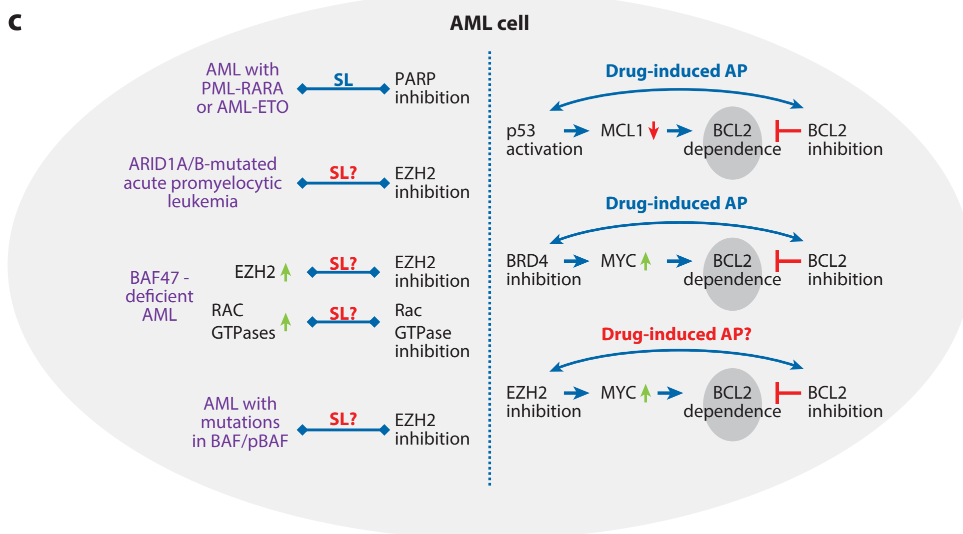


Figure 2

Targeting of PcG and TrxG in acute myeloid leukemia (AML). (*a*) Examples of small molecules that target components of different PcG and TrxG complexes and have shown efficacy in preclinical AML models. (*b*) Multifaceted effects of PRC2/EZH2 inhibition. While loss of PRC2/EZH2 is detrimental to AML cells of certain subtypes, it induces drug resistance through upregulation of HOXA9 or MYC or downregulation of p53. The indicated inhibitors have been shown to reverse the drug resistance phenotype induced by loss of PRC2/EZH2. (*c*) Experimentally verified drug-induced antagonistic pleiotropy (AP) and synthetic lethal (SL) combinations that have shown good efficacy in AML treatment (*blue*), along with examples of potential drug-induced AP and SL opportunities (*red*) whose therapeutic efficacy should be explored in AML models.

several compounds already show encouraging antileukemic activities in preclinical models through the disruption of complex assembly and/or downregulation of their enzymatic activities. PTC596, which targets PRC1.4 (**Figure 2*a***) and was identified in a high-throughput small-molecule library screen as a potent BMI1 inhibitor (89), has entered multiple phase 1 clinical trials in patients with solid tumors (ClinicalTrials.gov identifiers NCT02404480, NCT03206645, NCT03605550, and NCT03761095). Interestingly, PTC596 does not directly inhibit BMI1 enzymatic activity but leads to degradation of BMI1 protein. AML cells treated with PTC596 were arrested in G2/M phase and had reduced antiapoptotic MCL1 levels and increased apoptosis. PTC596 treatment induced apoptosis in primary AML samples in vitro and increased the survival of mice transplanted with MOLM-13 AML cells (89) or CALM-AF10 AML cells (7), demonstrating its potential in targeting AML cells.

Several inhibitors have been described that target PRC2 with promising antileukemia effects. Despite its dual role in disease initiation and maintenance, all the evidence so far depicts a functional requirement of EZH2 for full-blown AML, which makes it an attractive therapeutic target. 3-Deazaneplanocin A (DZNep) (**Figure 2*a***) indirectly inhibits EZH2 due to its SAH-hydrolase inhibitor activity, which in turn leads to an increased intracellular SAH concentration and degradation of the PRC2 complex (49). Although DZNep treatment depletes EZH2 levels, inhibits H3K27me3, leads to derepression of PRC2 target genes, and induces apoptosis in AML cells (39) but not normal cells (114), its in vivo efficacy in AML animal models as a single agent is less clear (39, 139). Other molecules, such as the selective EZH2 inhibitor GSK343 (122), also show antileukemia effects in vitro (8). EPZ-6438 (**Figure 2*a***), an EZH2 inhibitor currently in early-phase clinical trials (ClinicalTrials.gov identifier NCT01897571), increased the survival of mice transplanted with AML1-ETO AML cells (8). Moreover, the use of small molecules that inhibit both EZH1 and EZH2, such as UNC1999 (132) (**Figure 2*a***) or OR-S1 (42), in various AML mouse models resulted in significant survival differences compared with controls. Interestingly, efficient suppression of H3K27me3 and growth inhibition were not observed in MLL AML (132) with GSK126 (**Figure 2*a***), a potent and selective inhibitor of EZH2 catalytic activity with very good efficacy in lymphomas (83). However, GSK126 reduced H3K27me3 levels and inhibited growth in vitro in WT1 AML (110). Moreover, efforts to disrupt binding of core PRC2 components may represent alternative avenues, because a stabilized alpha-helix of EZH2 (SAH-EZH2) (**Figure 2*a***) peptide that selectively inhibits H3K27me3 by disrupting the EZH2/EED complex and reducing EZH2 protein levels also reduced the viability of MLL AML cells in vitro (67).

Early studies revealed that disruption of MLL1/2–COMPASS-like complexes with MM-401 (**Figure 2*a***), which inhibited MLL1 activity by blocking MLL1–WDR5 interaction and thus the complex assembly, induced growth arrest, apoptosis, and differentiation in MLL AML models in vitro (18). Other small-molecule antagonists of the MLL–WDR5 interaction, such as C3 and C6 or OICR-9429 (**Figure 2*a***), selectively inhibited proliferation and induced differentiation in MLL AML cells (3) or CEPBA p30-expressing human AML cells (52), respectively. Although certain AML subtypes, in particular MLL AML, appear to be quite sensitive to WDR5 inhibition, WDR5 assembles into multiple chromatin-modifying complexes, not just MLL1/2–COMPASS-like complexes, and the mechanism of WDR5 inhibition by targeting the WIN (WDR5 interaction) site is not fully understood (4). While MENIN exerts tumor suppressor functions by interacting with and inhibiting JUND activation in other tissues (2), inhibiting oncogenic MENIN function, such as the MENIN–MLL1 interaction, represents a promising avenue for targeting the MLL1/2–COMPASS-like complex in AML. The small-molecule inhibitors MI-403 and MI-503 (**Figure 2*a***) were the first to show in vivo efficacy in MLL AML mouse models (11). Moreover, VTP50469 (**Figure 2*a***), a novel and potent MENIN-MLL1 inhibitor with picomolar K_i (inhibitor constant), showed in vivo antileukemia effects not only in MLL AML (69) but also in

NPM1-mutated AML, another AML subtype characterized by aberrant HOX gene expression, where VTP50469 treatment was also suggested for AML disease prevention in individuals with clonal hematopoiesis of indeterminate potential or MDS with NPM1-mutant preleukemic clones (119).

Overcoming PRC2-Mediated Drug Resistance

Although targeting PRC2, including EZH2 catalytic activity, can be a promising strategy in the treatment of certain AML subtypes, recent reports have shown that loss of EZH2 also paradoxically emerges as a cause for therapy resistance (50, 81, 99) (**Figure 2*b***). Rathert et al. (99) reported that the suppression of the PRC2 complex promotes BET inhibitor resistance by facilitating the remodeling of regulatory pathways that restore the transcription of key targets, such as MYC. This process involves WNT signaling components that are recruited to a focal MYC enhancer in response to BET inhibition. On the other hand, Göllner et al. (50) showed that loss of endogenous EZH2 led to *HOXA7* and *HOXA9* gene activation, which caused resistance in AML cells. Loss of EZH2 resulted from enhanced CDK1-dependent phosphorylation of EZH2 at Thr487, which was then stabilized by HSP90 and followed by proteasomal degradation. Consistently, inhibition of HSP90, CDK1, and the proteasome all prevented EZH2 degradation and restored drug sensitivity in cell lines and primary patient samples. Finally, Maganti et al. (81) reported a critical function for MTF2 (PCL2) in targeting PRC2 to a gene regulator network that included MDM2. In MTF2-deficient AML cells, MDM2 is overexpressed and inhibits p53, leading to chemoresistance due to defects in cell cycle regulation and apoptosis. Overexpression of MTF2 or treatment with the MDM2 inhibitor Nutlin3A resensitized refractory AML cells to chemotherapy in vivo. Collectively, these studies establish a critical role for loss of PRC2 in mediating drug resistance but at the same time offer different avenues to overcome loss of PRC2-mediated resistance in refractory AML (**Figure 2*b***).

Targeting PcG and TrxG in Synthetic Lethal or Antagonistic Pleiotropy Settings

Another exciting area of research in targeting the PcG/TrxG complex for cancer and AML treatment is the potential use of specific synthetic lethality and antagonistic pleiotropy (AP) approaches that can potentially expand the scope and improve the efficacy of the treatments. Synthetic lethality and AP are particularly interesting to the cancer research community because they can reveal targets in cancer where the cancer-causing genes cannot be readily targeted. Synthetic lethality and AP are conceptually very similar and were pioneered in solid tumors as mechanistically driven anticancer treatment strategies. Synthetic lethality refers to the phenomenon in which perturbing two genes simultaneously results in the loss of viability but perturbing either of the genes individually does not; AP refers to the phenomenon in which a single pleiotropic gene exerts control over traits that are both beneficial and detrimental to fitness in a context-specific manner. For example, *BRCA1/2*-mutated cancer is defective in homologous DNA repair and very sensitive to the inhibition of PARP, which is critical for DDR in the absence of BRCA1/2 (13, 37). Thus, PARP inhibitors were identified as the first synthetic lethal drugs in the context of BRCA1/2-deficient cancer cells. Drug-induced AP occurs when the first cancer treatment positively affects the cancer cells' ability to respond to a second treatment, which would be ineffective without the first treatment taking place. As an example, BRAF V600E mutant melanomas are often treated with mitogen-activated protein kinase (MAPK) inhibitors as part of frontline treatment but frequently develop drug resistance via reactivation of the MAPK pathway, leading to higher levels of reactive oxygen species. Subsequent treatment with the HDAC inhibitor vorinostat suppresses SLC7A11,

increases the already elevated levels of reactive oxygen species, and causes selective apoptotic death only in drug-resistant cells (125), which otherwise has little effect in MAPK inhibitor–sensitive melanoma.

Examples of synthetic lethality in AML have been reported. Similar to the synthetic lethal PARP inhibitors in BRCA1/2-deficient cancer cells, the AML subtypes harboring AML1-ETO and PML-RARA fusions are very sensitive to PARP inhibition (**Figure 2*c***), in part because of their low expression of key homologous recombination–associated genes and impaired DDR. Conversely, DDR-proficient MLL AML expressing a high level of homologous recombination genes, including *HOXA9*, is resistant to PARP inhibition (35). Moreover, genome-wide CRISPR screens for essential genes across a panel of AML cell lines identified several synthetic lethal pairs involving oncogenic RAS, including *RAS* and the RAS effector genes *RAF1* and *SHOC2* as well as *RAS* and the *RCE1* and *ICMT* genes, which are involved in RAS maturation (126). Synthetic lethality was also observed by targeting EZH2 in *ARID1A*-mutated ovarian clear cell carcinoma (9). This is particularly interesting because it clearly shows that the antagonism between Polycomb PRC2(EZH2) and Trithorax SWI-SNF(ARID1A) also operates in cancer. While *ARID1A* is one of the most frequently mutated genes in cancer (71), it is only infrequently mutated in AML, except in acute promyelocytic leukemia, in which *ARID1A/B* mutations are second only to *WT1* mutations and impair the myeloid differentiation of NB4 cells upon ATRA treatment in vitro (79). Thus, it is tempting to speculate that EZH2 inhibition may also show synthetic lethality in *ARID1A/B*-mutated acute promyelocytic leukemia (**Figure 2*c***).

Perturbation of SWI/SNF activity may also be exploited in synthetic lethal targeting. Loss of the SWI/SNF component BAF47 increases SWI/SNF chromatin binding and leads to transcriptional upregulation of Rac GTPase GEFs (23), which play a critical role in MLL AML (87). To test the efficacy of this potential synthetic lethality, further studies are needed with Rac GTPase inhibitors in *BAF47*-mutated AML (**Figure 2*c***). Moreover, an additional synthetic lethality can be envisaged that utilizes loss of BAF47 and EZH2. Loss of BAF47 also leads to elevated levels of EZH2, which represses Polycomb targets. Importantly, this EZH2 activity is critical for loss of BAF47-mediated oncogenic transformation, and loss of EZH2 blocks tumor formation (129), which raises the possibility that *BAF47*-mutated AML may also respond to EZH2 inhibition (**Figure 2*c***). These targeting strategies could be potentially broadened to AML with mutations in SWI/SNF complexes, and future studies may shed light on this.

Drug-induced AP is gaining traction in the field to overcome drug resistance in AML. Although described as a synthetic lethality pair, p53 activation and BCL2 inhibition (90) can also be described as drug-induced AP (**Figure 2*c***). Mechanistically, p53 activation negatively regulates the Ras/Raf/MEK/ERK pathway and activates GSK3, which modulates degradation of MCL1, thus overcoming resistance to BCL2 inhibition in AML. Furthermore, BCL2 inhibition overcomes resistance to p53 activation by switching the cellular response from cell cycle arrest to apoptosis (90). Lin et al. (75) also recently reported another drug-induced AP example: Using a CRISPR screen in an AML cell line in combination with treatment using drugs currently approved or in clinical trial for AML, they identified drug-induced AP between the BRD4 inhibitor JQ1 and the BCL2 inhibitor ABT199 (**Figure 2*c***). They also identified the loss of PRC2 complex members EED and EZH2 as an ABT199 sensitizer, whereas loss of NSD2/3 and MYC scored in opposition to PRC2 members, revealing an AP axis featuring the reciprocal regulation of MYC by NSD2/3 and PRC2 core members EED and EZH2. The treatment order was important in obtaining the AP effect in vivo: AML cells that were treated first with JQ1 (a BRD4 inhibitor) and then ABT199 (a BCL2 inhibitor), but not cells treated in the reverse order, showed a significant reduction in leukemia burden (75). This raises the possibility that direct targeting of EZH2, leading to higher

MYC expression, could represent a more general approach to set up this particular AP trap in AML cells, making them vulnerable to BCL2 inhibition (**Figure 2*c***).

While a sound mechanistic understanding of individual AML mutations is essential for setting up AP traps or utilizing synthetic lethality in the correct AML patient group, these studies collectively reveal the therapeutic potential of targeting PcG/TrxG proteins for AML treatment.

CONCLUSION

Our understanding of the composition and functions of PcG and TrxG proteins in normal and malignant cells has expanded considerably over the past few decades. Several tumor suppressor and oncogenic functions for PcG and TrxG proteins have been identified in AML. However, these properties are highly context dependent. Despite this complexity, with careful selection of patients according to their genetic mutations, stages of disease development, and/or AML subgroups, inhibition of PcG and TrxG emerges as a promising option in the therapeutic arsenal for treatment of AML. Clever design of synthetic lethal and AP targeting of PcG or TrxG can further expand the scope of application and enhance the efficacy of the treatments. Further research is needed to shed light on many outstanding key issues. These include canonical PRC1/noncanonical PRC1 and PRC2 relationships, their recruitment to the chromatin, the relative importance and contributions of H2AK119ub or H3K27me3 in gene repression, the canonical antagonism and noncanonical synergism of PcG and TrxG in normal and disease development, and their roles in DDR and outside of the classical PcG/TrxG gene regulatory functions. Since the discovery of these two classes of proteins half a century ago, we have been constantly surprised by their unexpected functions and potential to help fight against cancers.

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