A PRC2-Kdm5b axis sustains tumorigenicity of acute myeloid leukemia

Zhihong Ren,1,2 Arum Kim,1,3 Yu-Ting Huang,1,3 Wen-Chieh Pi,1,3 Weida Gong,1 Xufen Yu,1 Jun Qi,1 Jian Jin,1 Ling Cai,1,4 Robert G. Roeder,4,5 Wei-Yi Chen,1,4,6 Gang Greg Wang1,4

1Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599; 2Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599; 3Department of Biochemistry and Molecular Biology, National Yang Ming Chiao Tung University, Taipei 112, Taiwan; 4Mount Sinai Center for Therapeutics Discovery, Departments of Pharmacological Sciences and Oncological Sciences, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029; 5Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA 02215; 6Department of Genetics, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599; 7Laboratory of Biochemistry and Molecular Biology, The Rockefeller University, New York, NY 10065; 8Cancer Progression Research Center, National Yang Ming Chiao Tung University, Taipei 112, Taiwan; and 9Department of Pharmacology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC 27599

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Acute myeloid leukemias (AMLs) with the NUP98-NSD1 or mixed lineage leukemia (MLL) rearrangement (MLL-r) share transcriptional profiles associated with stemness-related gene signatures and display poor prognosis. The molecular underpinnings of AML aggressiveness and stemness remain far from clear. Studies with EZH2 enzymatic inhibitors show that polycomb repressive complex 2 (PRC2) is crucial for tumorigenicity in NUP98-NSD1+ AML, whereas transcriptional analysis reveals that Kdm5b, a lysine demethylase gene carrying “bivalent” chromatin domains, is directly repressed by PRC2. While ectopic expression of Kdm5b suppressed AML growth, its depletion not only promoted tumorigenicity but also attenuated anti-AML effects of PRC2 inhibitors, indicating a PRC2–Kdm5b axis for AML oncogenesis. Integrated RNA sequencing (RNA-seq), chromatin immunoprecipitation followed by sequencing (ChIP-seq), and Cleavage Under Targets & Release Using Nuclease (CUT&RUN) profiling also showed that Kdm5b directly binds and represses AML stemness genes. The anti-AML effect of Kdm5b relies on its chromatin association and/or scaffold functions rather than its demethylase activity. Collectively, this study describes a molecular axis that involves histone modifiers (PRC2–Kdm5b) for sustaining AML oncogenesis.

Significance

Acute myeloid leukemias (AMLs) with NUP98-NSD1 or MLL abnormality are generally aggressive, demanding a better understanding of the underlying oncogenic mechanisms. We show that these AMLs rely on a regulatory axis involving PRC2–Kdm5b to sustain stemness genes for sustaining an oncogenic program. The H3K27 methylase activity of polycomb repressive complex 2 (PRC2) is crucial for repressing Kdm5b, a co-repressor carrying a H3K4me3 reader domain, that antagonizes the AML oncoproteins by directly binding to and down-regulating the AML stemness genes, thereby suppressing acute leukemogenesis. Such an AML-suppressing role of Kdm5b is not dependent on its intrinsic demethylase activity but requires its scaffold and/or chromatin association functions. The findings of this study shall aid in potential therapeutics of the affected AML patients.

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1Z.R. and A.K. contributed equally to this work.
2Present address: Division of Hematologic Malignancies, Johns Hopkins University School of Medicine, Baltimore, MD 21287.
3Y.-T.H. and W.-C.P. contributed equally to this work.
4To whom correspondence may be addressed. Email: roeder@rockefeller.edu, chenwy@nycu.edu.tw, or greg_wang@med.unc.edu.

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critical downstream target repressed by PRC2. Depletion of Kdm5b not only promoted AML development but also significantly desensitized AML cells to the PRC2 inhibitor, thus uncovering an involvement of the PRC2–Kdm5b axis for AML tumorigenesis. Genetic profiling by chromatin immunoprecipitation followed by sequencing (ChIP-seq), Cleavage Under Targets & Release Using Nuclease (CUT&RUN), and RNA-seq further showed that both Kdm5b and NUP98-NSD1 are targeted to stemness- and proliferation-related genes and that Kdm5b functions to suppress a suite of AML proto-oncogenes. Systematic mutagenesis showed Kdm5b's intrinsic demethylase activity to be dispensable for AML suppression, whereas its chromatin-binding functions are essential, indicating a scaffolding role of Kdm5b in assembling the chromatin-bound complex for transcriptional repression. Together, this study unveils a PRC2–Kdm5b–stemness axis that operates to promote AML oncogenesis and aggressiveness, which sheds light on potential therapeutic means.

**Results**

**NUP98-NSD1+ AML Cells Are Sensitive to Enzymatic Inhibitor of PRC2.** Previously, it has been shown that UNC1999, a selective inhibitor of the PRC2 catalytic subunit (EZH2 or EZH1), suppressed growth of MLL-r AML cells (17). Here, we treated murine AML cells established by NUP98-NSD1 (7) with UNC1999. Following the depletion of global H3K27me3 (Fig. 1, Insert), we observed a robust response and sensitivity to UNC1999, comparable to what was seen with MLL-r AML (17). Relative to mock treatment, UNC1999 dramatically suppressed the in vitro proliferation (Fig. 1A) and colony formation of NUP98-NSD1+ AML cells (Fig. 1B). Treatment with UNC1999 also resulted in enhanced cell differentiation (Fig. 1C), increased apoptosis (Fig. 1D and SI Appendix, Fig. S1), and slower cell cycle progression (Fig. 1E) in AML cells. Furthermore, when compared to vehicle treatment, UNC1999 treatment also significantly delayed tumor progression in the murine NUP98-NSD1+ AML model (Fig. 1F). Taken together, these results demonstrate a PRC2 dependency for the NUP98-NSD1–associated AML growth.

**Transcriptomic Profiling Identified Kdm5b to Be a PRC2-Depressed Gene in AML with NUP98-NSD1 or MLL-r.** In order to dissect downstream mediators through which PRC2 sustains AML oncogenesis, we conducted transcriptomic profiling of NUP98-NSD1+ AML cells posttreatment with PRC2 inhibitors. To rule out a potential off-target effect, we treated NUP98-NSD1+ AML cells either with UNC1999 or with a second PRC2 inhibitor, JQEZ5 (18, 19), and then performed RNA-seq analysis (Dataset S1 and SI Appendix, Fig. S2A). Replicated RNA-seq profiles within the same treatment group were highly consistent (SI Appendix, Fig. S2B), and transcripts showing the increased expression after treatment with either of the two inhibitors also overlapped significantly (Fig. 2A and SI Appendix, Fig. S2C), although the overall effect of JQEZ5 was somewhat milder (please note a milder overall change in gene expression caused by JQEZ5, compared to UNC1999, as revealed by principal component analysis, PC1 of SI Appendix, Fig. S2B). Gene set enrichment analysis (GSEA) showed the blockade of PRC2's enzymatic activity by either inhibitor to be correlated with up-regulation of transcripts repressed by PRC2 or those related either to leukocyte differentiation or apoptosis (Fig. 2B and SI Appendix, Fig. S2 D and E), consistent with the observed cell phenotypes posttreatment (Fig. 1). To further identify genes commonly repressed by PRC2 in NUP98-NSD1+ and MLL-r AMLs, we compared UNC1999–derepressed transcripts identified in the two AMLs and, to further enhance the rigor, also included our previous transcriptomic data for knockdown of the essential component of PRC2, Eed (17) (Fig. 2C). This analysis identified a 31-gene signature that is commonly up-regulated among all three comparisons (Fig. 2C and SI Appendix, Table S1) and included Cdkn2a, a cell cycle inhibitor gene known to be directly repressed by PRC2 (17). In addition, Kdm5b, encoding a histone demethylase shown to have a tumor-suppressive role in MLL-r AML (20), was also found to be significantly activated upon PRC2 inhibition (Fig. 2C). The qRT-PCR assays verified the marked up-regulation of Kdm5b following either PRC2 enzymatic inhibition (Fig. 2D) or EZH2 depletion (SI Appendix, Fig. S2F) in independent AML cell lines.

To assess whether Kdm5b is a direct target of PRC2 and H3K27me3, we examined H3K27me3 and H3K4me3 ChIP-seq data that we previously generated in MLL-AF9–transformed murine AML cells (17) and found the Kdm5b promoter to be demarcated with both histone marks (Fig. 2E), which is a characteristic of bivalent domains in chromatin. We also conducted ChiP-seq in MV4;11 cells, a human AML line harboring MLL-AF4, and also found KDM5B to be directly bound by EZH2 and H3K4me3 (Fig. 2E). Interrogation of multiple clinical outcome datasets of human AML patients, including the TCGA AML cohort, consistently displayed a positive correlation between the lower KDM5B expression and the significantly poorer prognosis (Fig. 2F). Collectively, these observations support the view that KDM5B is dynamically regulated, with PRC2-catalyzed H3K27me3 serving as a critical repressor, and that KDM5B expression is a predictive marker of AML prognosis.

**Kdm5b Depletion not only Promotes AML Oncogenesis but also Significantly Desensitizes AML Cell Response to PRC2 Inhibition.** Kdm5b depletion in the NUP98-NSD1+ AML cells resulted in the significantly accelerated proliferation (SI Appendix, Fig. S3A) and increased colony formation in the in vitro assays (Fig. 3 A and B; see DMSO, Right versus Left). Compared to mock treatment, Kdm5b depletion also significantly accelerated AML development in syngeneic mice (Fig. 3C; see black lines, dashed versus solid), consistent with what was previously observed with MLL-r AML (20). Importantly, Kdm5b depletion significantly attenuated the AML cell sensitivity to UNC1999 in both liquid cultures (SI Appendix, Fig. S2B) and colony formation assays (Fig. 3A). Relative to mock treatment, Kdm5b depletion completely abrogated the UNC1999–mediated suppression of apoptosis (Fig. 3F, left; compared to UNC1999, as revealed by principal component analysis, PC1 of SI Appendix, Fig. S2B), consistent with the previously observed delayed tumor progression in the murine NUP98-NSD1+ AML model (Fig. 1F). These results demonstrated a tumor-suppressive role for Kdm5b and an oncogenic axis involving PRC2–Kdm5b in NUP98-NSD1+ AMLs.

**RNA-seq profiling showed a role of Kdm5b in suppressing a stemness-related gene-expression program in AML.** To examine the gene-regulatory role for Kdm5b in NUP98-NSD1+ AMLs, we carried out RNA-seq profiling after Kdm5b depletion, which revealed transcripts showing the altered expression due to Kdm5b loss (Fig. 3D and Dataset S2). GSEA using RNA-seq profiles revealed a negative correlation between Kdm5b depletion and the differentiation-related gene sets (Fig. 3E). Furthermore, GSEA of RNA-seq profiles obtained after ectopic Kdm5b expression in MLL–AF9+ AML cells (Dataset S3) revealed a positive correlation between Kdm5b expression and gene sets related to differentiation, apoptosis, or slowed cell proliferation (Fig. 3F and SI Appendix, Fig. S3 D and E). Despite the cell background difference, there is a significant overlap between the Kdm5b-depressed transcripts in the two AML models, including genes, such as Sox4, Id2, Myb, Brd3, Hmga2, and Hmgb3, known to be essential for AML stemness or differentiation arrest (Fig. 3G and SI Appendix, Table S2). Interestingly, oncogenes such as Kras and cell cycle–related genes that include Ccnd1, Ccnd2, and Ccne2 were also found to be repressed by Kdm5b (Fig. 3G). These results thus link Kdm5b to AML-related gene-expression programs.
ChIP-seq Data Establish Direct Cobinding of Kdm5b and NUP98-NSD1 to AML-Related Target Genes, Thus Providing a Molecular Explanation for Antagonism between KDM5B and AML Oncoproteins. To further address whether Kdm5b directly regulates the AML-related gene program, we conducted ChIP-seq for Kdm5b, NUP98-NSD1 and also transcriptionally repressed AML cells (Datasets S4 and S5). Notably, our results revealed a significant coexistence of the three factors at gene promoters (Fig. 4 A and B), which is in good agreement with previous reports that the C-terminal PHD finger domain of Kdm5b directly binds H3K4me3 (21, 22) and that Kdm5b binding sites overlap H3K4me3 in different cell models (23, 24); also, chimeric NUP98 fusion oncoproteins (NUP98-NSD1 or NUP98-PHD) were found targeted to promoters for oncogene activation (7, 25–27). Integrated analyses of our ChIP-seq and RNA-seq datasets in NUP98-NSD1+ AML cells further defined genes that are cotargeted by both Kdm5b and NUP98-NSD1 and also transcriptionally repressed by Kdm5b (Fig. 4B and SI Appendix, Table S3), which again included a suite of AML proliferative and stemness genes such as Brd3, Ccnd3, Kras, Sox4, and Hoxa7 (Fig. 4 C–F and SI Appendix, Fig. S4). These results demonstrate a direct suppressing effect by Kdm5b on the oncogenic gene-expression program, thereby antagonizing AML-promoting effects by oncoproteins.

Kdm5b’s Chromatin Association Domains Are Essential for Its AML Suppressive Role, whereas Its Demethylase Activity Is Dispensable. Kdm5b is a large protein with multiple functional motifs. Besides a Jumonji (Jmj) domain, essential for lysine demethylation, Kdm5b contains three PHD fingers, with the first and third ones specifically binding nonmodified (H3K4me0) and highly methylated (e.g., H3K4me3) H3K4, respectively, as well as an ARID domain previously shown to bind CG-rich DNA (28) (Fig. 5A). To examine potential involvement of these functional domains in Kdm5b-mediated AML suppression, we ectopically expressed Kdm5b in the NUP98-NSD1+ AML cells. Here, we employed a set of Kdm5b mutants exhibiting the abolished activity in either chromatin binding or demethylation (Fig. 5A). These mutants included K164E/S168D (in the Arid domain) previously shown to bind CG-rich DNA (29, 30), ΔH2 (PHD2 deletion), and W150A (in PHD3) (22, 26). Wild-type (WT) and mutant forms of Kdm5b showed comparable levels of expression (Fig. 5B). Compared to mock, WT Kdm5b robustly suppressed AML growth in vitro (Fig. 5C; see WT) and in mice, as assessed by assessment of the in vivo expansion of luciferase/GFP-labeled AML cells via live animal imaging and fluorescence-activated cell sorting (FACS) with the isolated cells from killed mice (Fig. 5 D and E; see

**Fig. 1.** PRC2 sustains tumorigenesis in the NUP98-NSD1+ AML. (A) Proliferation of NUP98-NSD1-transformed murine AML cells after treatment with UNC1999, an enzymatic inhibitor of EZH2/1, for the indicated duration. The y-axis represents the relative percentage of cell numbers in cultures, normalized to dimethyl sulfoxide (DMSO) treatment (n = 3 independent experiments; shown as mean ± SD). Inset shows immunoblotting of global H3K27me3 and H3K27ac after a 24-h treatment with 3 μM of UNC1999, relative to DMSO. (B) Quantification of CFUs (Left) and images of representative single-cell colonies (Right) formed by NUP98-NSD1+ AML cells, cultured in the semisolid medium containing DMSO or 3 μM of UNC1999 for 10 d (n = 3 independent experiments; mean ± SD; unpaired two-tailed Student’s t test). ***P < 0.001. (Scale bar, 2 mm.) (C) Wright–Giemsa staining images showing the differentiation status of NUP98-NSD1+ AML cells, treated with DMSO or the indicated concentration of UNC1999 for 4 d. (Black bar, 10 μm.) (D and E) Summary of apoptotic cells, analyzed by propidium iodide (PI), and annexin-V staining (F) Kaplan–Meier curve showing kinetics of NUP98-NSD1-induced AML in a transplantation model using syngeneic mice. Starting from day 7 posttransplantation, mice received oral administration of either vehicle (blue) or 100 mg/kg UNC1999 (red) once per day. n, cohort size. Statistical significance was determined by log-rank test.
Fig. 2. **Kdm5b**, encoding a tumor suppressor of AML, is a direct target of PRC2 and H3K27me3 in AML. (A) Venn diagram showing the overlap between DEGs, as determined by RNA-seq to be up-regulated in NUP98-NSD1+ AML cells after a 4-d treatment with 3 μM of independent PRC2 inhibitors, either UNC1999 (Left) or JQEZ5 (Right), relative to DMSO (n = 3 replicated samples). Threshold of DEG is set as adjusted DESeq calculated probability value (adj.p) < 0.05, the indicated fold-change (FC) cutoff, and mean tag counts > 10. (B) GSEA revealing that enzymatic inhibition of PRC2 by UNC1999 is positively correlated to activation of the indicated PRC2-repressed, myeloid differentiation–related, or apoptosis-related gene signature in the NUP98-NSD1+ AML cells. NES, normalized enrichment score; FDR, false discovery rate. (C) Venn diagram showing overlap among the DEGs up-regulated posttreatment of NUP98-NSD1+ (black) or MLL-AF9+ (red) AML cells with UNC1999, or postknockdown of Eed, an essential PRC2 component, in the latter cells, relative to the respective controls. (D) qRT-PCR for **Kdm5b** in MLL-AF9+ murine AML cells treated with 3 μM of UNC1999 for the indicated duration, relative to DMSO (n = 3 independent experiments; shown as mean ± SD). The y-axis represents PCR signals relative to DMSO-treated cells after normalization to GAPDH. (E) ChIP-seq profiles of H3K27me3 or EZH2 and H3K4me3 at the **Kdm5b** gene in MLL-AF9+ murine AML (mAML) cells (Upper) and MV4;11 cells, an MLL-AF4+ human AML line (Lower). (F) Kaplan–Meier survival curve for **KDM5B** expression in the indicated AML patients, including the TCGA cohort (Left). Statistical significance was determined by log-rank test. n, cohort size.
WT). As a result, the typical splenomegaly seen with the NUP98-NSD1+ murine AML cells, transduced with either EV (shEV) or Kdm5b-targeting shRNA (shKdm5b), after a 10-d cultivation in the semisolid medium containing either DMSO or 3 μM of UNC1999. **P < 0.01; n.s., not significant. (B) Immunoblotting of Kdm5b after Kdm5b depletion. (C) Survival curve showing the kinetics of murine AMLs induced by NUP98-NSD1+ cells, which were stably transduced with vector (shEV; solid lines) or Kdm5b-targeting shRNA (shKdm5b; dashed lines), in syngeneic mice (n = 5) treated with either vehicle (black) or UNC1999 (red). Statistical significance was determined by log-rank test. *P < 0.05; NS, not significant. (D) Heatmap of RNA-seq results showing relative expression of DEGs in NUP98-NSD1+ murine AML cells after Kdm5b knockdown (KD), relative to mock (EV). Threshold of DEG is indicated. n = 3 biological replicates (rep.). (E) GSEA shows a negative correlation between Kdm5b knockdown (Kdm5b_KD) and the indicated differentiation-related gene sets in NUP98-NSD1+ murine AML cells (n = 3 replicated samples). (F) GSEA shows a positive correlation between ectopic expression of Kdm5b (Kdm5b_exp.) and the indicated differentiation-related gene sets in MLL-AF9+ murine AML cells (n = 2 replicated samples). (G) Venn diagram showing overlap between the up-regulated transcripts after Kdm5b KD (Left) and the down-regulated transcripts after ectopic Kdm5b expression (Kdm5b exp.) (Right) relative to their respective controls, as determined by RNA-seq in AML cells. AML-related oncogenes and the DEG threshold are labeled. padj, adjusted P values.

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requirements for Kdm5b’s chromatin association and/or scaffolding functions for suppressing leukemogenesis, whereas its intrinsic demethylase activity is dispensable.

To further assess the role of the Kdm5b demethylase activity during leukemogenesis, we employed MLL-AF9–transformed AML as a second model and confirmed comparable suppressive effects by WT Kdm5b and its H499A mutant on AML cell proliferation when compared to mock (Fig. 6 A and B). It has been reported that Kdm5b or related Kdm5a associates with the nucleosome remodeling and deacetylase (NuRD) complex, which has histone deacetylase (such as HDAC1) and gene-repressive activities (22, 31, 32). Coimmunoprecipitation (CoIP) showed that both WT and H499A-mutated Kdm5b associated with HDAC1 (Fig. 6C), a strong transcriptional corepressor. These results indicate a scaffolding role, rather than an intrinsic demethylase function, for Kdm5b in suppressing AML. Furthermore, we compared the genome-targeting and gene-modulatory effects by WT Kdm5b versus an enzymatic-dead mutant (H499A). First, we introduced either WT or mutant forms of Kdm5b into HEK293 cells because this cell line is generally insensitive to Kdm5b expression and thus allows comparison of WT versus mutant Kdm5b without being affected by cell status changes (such as differentiation and proliferation seen with AML). CUT&RUN results for WT and H499A-mutated Kdm5b revealed comparable binding at target genes (Fig. 6 D and E), as exemplified by Kdm5b peaks at AML-related genes such as Sox4, Ccnd2, and Hmgb3 (Fig. 6F). Equally repressive effects of WT and H499A-mutated Kdm5b on the tested AML oncogenes (Sox4, Ccnd2, and Hmgb3) were also verified by qRT-PCR (Fig. 6I).

Taken together, our results demonstrate a striking similarity between WT Kdm5b and its demethylase-dead mutant in terms of AML growth suppression, chromatin binding, cofactor interaction (such as HDAC1), and gene-expression modulation.
AML is an aggressive disease, demanding a better appreciation of the underlying oncogenic mechanisms and improved therapy. Expression of the stem-like gene-expression program in AML is known to be well correlated with poor prognosis (33, 34). How such a transcriptomic feature is maintained in AML has remained unclear. Chromatin modulation is increasingly appreciated as a major molecular determinant for the cell biology of AML.

**Fig. 5.** The chromatin association and/or scaffolding function of Kdm5b is essential for its tumor-suppressive effect in AML, whereas its intrinsic demethylase activity is dispensable. (A) Protein architecture of Kdm5b, with its functional domains and used mutations labeled on the Top and Bottom, respectively. del, deletion. (B) Anti-Flag immunoblotting of WT and mutant Flag-tagged Kdm5b after stable expression in cells. (C) Proliferation of NUP98-NSD1 murine AML cells after stable transduction of WT or mutant Kdm5b, relative to EV. The y-axis represents relative percentage of cell numbers normalized to EV-expressing controls (n = 3 independent experiments; presented as mean ± SD). Star indicates the domain mutation. (D–G) Effect of ectopically expressed Kdm5b, either WT or mutant, on AML leukemogenesis. NUP98-NSD1 murine AML cells carrying a luciferase-IRES-GFP reporter were stably transduced with the indicated Kdm5b and then transplanted intravenously into syngeneic mice. Leukemogenesis was monitored by chemiluminescence imaging of live animals (30 d posttransplantation; D), FACS (E) of the isolated bone marrow cells (cKit^+GFP^+ indicates in vivo expansion of GFP-labeled NUP98-NSD1 AML cells), measurement (F) of spleen size (50 d posttransplantation when leukemic mice were close to their terminal stage), and survival curves (G) of mice transplanted with the indicated NUP98-NSD1 AML cells (n = 5 per cohort). ***P < 0.001. KD, knockdown.

**Discussion**

AML is a heterogeneous disease, demanding a better appreciation of the underlying oncogenic mechanisms and improved therapy. Expression of the stem-like gene-expression program in AML is known to be well correlated with poor prognosis (33, 34). How such a transcriptomic feature is maintained in AML has remained unclear. Chromatin modulation is increasingly appreciated as a major molecular determinant for the cell biology of AML.

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Fig. 6. Kdm5b suppresses AML-related gene targets in a demethylase-independent manner. (A) Proliferation of MLL-AF9+ murine AML cells transduced with EV or Flag-tagged Kdm5b, either WT or H499A-mutated. The y-axis represents accumulative cell numbers (n = 3 independent experiments; mean ± SD). ****P < 0.0001. (B) Immunoblotting for exogenously expressed Kdm5b using cells in (A). (C) CoIP for HDAC1 interaction with GFP-tagged Kdm5b, WT, or H499A-mutated, in HEK293 cells. IP, immunoprecipitation. (D and E) Heatmap (D) and averaged plotting (E) of GFP CUT&RUN signals for WT and H499A-mutated Kdm5b (GFP-tagged) at the peak-centered genomic regions (± 5 kb) in the HEK293 stable expression cells. Non–GFP-tagged cells (EV) act as a background control. (F) IGV views showing WT and H499A-mutated Kdm5b CUT&RUN signals at the indicated gene in the HEK293 stable expression cells. (G) Venn diagram showing the overlap of DEGs, as determined by RNA-seq to be down-regulated in MLL-AF9+ murine AML cells posttransduction of WT (Left) or H499A-mutated (Right) Flag-Kdm5b, relative to EV (n = 2 replicated samples). AML-related oncogenes and the DEG threshold are indicated. (H) GSEA reveals that relative to EV, expression of WT (Left) or H499A-mutated (Right) Kdm5b is negatively correlated to activation of proliferation-related gene sets in MLL-AF9+ murine AML cells. NOM p, normalized p-values. (I) qRT-PCR of the indicated AML-related genes in MLL-AF9+ murine AML cells after ectopic expression of WT or H499A-mutated Kdm5b. The qRT-PCR signals are relative to EV-transduced cells after being normalized to GAPDH (n = 3 independent experiments; presented as the mean ± SD). **P < 0.01; ***P < 0.001; ****P < 0.0001. FC, fold-change.
growth. In this scenario, Kdm5b assembles a transcriptional repressive machinery (with HDAC1 and others), leading to the down-regulation of stemness genes and suppression of AML genesis initiated by NUP98-NSD1 and MLL-r. Upon treatment with PRC2 inhibitors (PRC2-i) such as UNC1999, the Kdm5b level is elevated. Then, Kdm5b assembles a transcriptional repressive machinery (with HDAC1 and others), leading to the down-regulation of stemness genes and suppression of AML growth. In this scenario, Kdm5b’s gene-repressing and anti-AML effects are largely demethylase-independent.

Fig. 7. Scheme showing a role for the PRC2-Kdm5b axis in mediating AML tumorigenesis. Kdm5b is subject to epigenetic repression by PRC2 and H3K27me3, and such a Kdm5b repression is required for sustaining the expression of AML-causing and stemness-related genes, promoting the leukemogenesis initiated by NUP98-NSD1 and MLL-r. Upon treatment with PRC2 inhibitors (PRC2-i) such as UNC1999, the Kdm5b level is elevated. Then, Kdm5b assembles a transcriptional repressive machinery (with HDAC1 and others), leading to the down-regulation of stemness genes and suppression of AML growth. In this scenario, Kdm5b’s gene-repressing and anti-AML effects are largely demethylase-independent.
can be oncogenic in solid tumors, including breast cancer and melanoma (47–49). As an example, KDM5B can silence tumor immunity-associated pathways (45). On the other hand, tumor-suppressing roles for KDM5 have been demonstrated in solid tumors such as breast cancer (22, 31), consistent with its AML-suppressing function shown in this work. The functions of KDM5 proteins are multifaceted and subject to further investigation.

**Materials and Methods**

Plasmid construction, tissue culture and cell lines, antibodies and Western blotting, chemicals, phenotypic assays of hematological cells, such as colony-forming units (CFUs), Wright-Giemsa staining and proliferation assays, assays of cell cycle progression and apoptosis, in the vivo leukemogenic studies, RNA sequencing and data analysis, ChIP-seq and data analysis, CUTC&RUN and data analysis, analysis of publicly available patient datasets, qRT-PCR, and statistics and reproducibility can be found in SI Appendix, Materials and Methods.

All animal experiments were performed according to the protocol approved by Institutional Animal Care and Use Committee, University of North Carolina (UNC).

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**Data Availability.** All study data are included in the article and/or supporting information. RNA-seq, ChIP-seq, and CUT&RUN datasets related to this work have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) under accession number GSE179826.

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