Transcriptional addiction in mixed lineage leukemia: new avenues for target therapies

Ruijing Xiaoa,b, Honghong Wanga, Kaiwei Liangac,**

*Department of Pathophysiology, School of Basic Medical Sciences, Wuhan University, Wuhan, P.R. China; Department of Immunology, School of Basic Medical Sciences, Wuhan University, Wuhan, P.R. China; Research Center for Medicine and Structural Biology, School of Basic Medical Sciences, Wuhan University, Wuhan, P.R. China

Abstract

Mixed lineage leukemia (MLL) is an aggressive and refractory blood cancer that predominantly occurs in pediatric patients and is often associated with poor prognosis and dismal outcomes. Thus far, no effective target therapy for the treatment of MLL leukemia is available. MLL leukemia is caused by the rearrangement of MLL genes at 11q23, which generates various MLL chimeric proteins that promote leukemogenesis through transcriptional misregulation of MLL target genes. Biochemical studies on MLL chimeras have identified that the most common partners exist in the superelongation complex (SEC) and DOT1L complex, which activate or sustain MLL target gene expression through processive transcription elongation. The results of these studies indicate a transcription-related mechanism for MLL leukemogenesis and maintenance. In this study, we first review the history of MLL leukemia and its related clinical features. Then, we discuss the biological functions of MLL and MLL chimeras, significant cooperating events, and transcriptional addiction mechanisms in MLL leukemia with an emphasis on potential and rational therapy development. Collectively, we believe that targeting the transcriptional addiction mediated by SEC and the DOT1L complex will provide new avenues for target therapies in MLL leukemia and serve as a novel paradigm for targeting transcriptional addiction in other cancers.

Keywords: DOT1L complex, Leukemogenesis, Mixed lineage leukemia (MLL), MLL chimeras, Superelongation complex (SEC), Transcriptional addiction

1. HISTORICAL OVERVIEW OF MLL LEUKEMIA

Acute leukemia is a class of malignant blood cancers arising from the hematopoietic stem and progenitor cells (HSPCs); it is generally characterized by recurring chromosomal translocations, mutations, and epigenetic alternations. The diagnosis and classification of acute leukemia mainly relies on multidisciplinary approaches, such as bone marrow biopsy, karyotype analysis, molecular genetic testing, and immunophenotyping, for categorization into myeloid, B-lymphoid, or T-lymphoid lineage. In the 1980s, when monoclonal antibodies were applied to characterize the leukemic blasts, three acute myeloid leukemia (AML) patients were noted to co-express myeloperoxidase and terminal deoxynucleotidyl transferase, which had been discovered to be a biochemical marker for acute lymphoblastic leukemia (ALL).

Such findings indicate the presence of leukemic blasts with both lymphoid and myeloid markers.1 In 1985, St. Jude Children’s Research Hospital reported the clinicopathological features of a cohort of 123 children leukemia with lymphoid and myeloid characteristics using lymphoid-associated markers, such as anti-CD10, CD2, and CD5, and myeloid-associated markers, such as CD15, CD13, and CD11b,2 although these markers are no longer considered to be lineage specific. Based on these markers, a subset of these children leukemia (25/123) were characterized to have individual blasts expressing differentiation markers of more than one lineage; thus the term “mixed lineage leukemia” (MLL) was initially defined.

Since the 1980s, cytogenetic studies3–5 on acute leukemia have revealed that 11q23 translocations are the most common genetic alteration in infants with leukemia.6 These translocations are linked with the mixed-lineage characteristics by affecting progenitor cells capable of both myeloid and lymphoid differentiation at early stages.7–8 In 1991, Janet Rowley’s group first identified a gene spanning a breakpoint in the 11q23 translocations and named it MLL, meaning myeloid/lymphoid, or mixed-lineage, leukemia7; this gene was cloned and sequenced by another three groups,10–12 who also showed that the MLL (KMT2A) gene is the homolog of Drosophila trithorax. In 1995, the Mll+/- mice were reported to be embryonic lethal, whereas Mll-/- mice were found to be haploinsufficient with segmentation defects and hematopoietic abnormalities. These findings suggest that MLL plays a critical and nonredundant role in development and hematopoiesis.13 MLL has also been shown to be essential for adult hematopoietic stem cell self-renewal.14,15

Leukemia with 11q23 translocations has been demonstrated to feature MLL gene rearrangements,16 and multiple partner genes
were found to be fused with the MLL gene, resulting in diverse oncogenic MLL chimeras composed of an MLL N-terminal portion fused in-frame to a C-terminal fragment of the partners (Fig. 1A). Based on an updated recombinome of MLL in 2017, a total of 135 different MLL rearrangements have been identified and 94 fusion partner genes have been characterized at the molecular level. These fusion partners share few sequence or functional similarities, and the most common partner genes are AF9 (MLLT3), AF4 (AFF1), ENL (MLLT1), AF10 (MLLT10), ELL, AF6 (MLLT4), EPS15, and SEPT6. In 2002, Armstrong et al reported that MLL-rearranged leukemia is clearly different from conventional acute leukemia using gene expression profiles from microarrays; MLL leukemia has been reported to be a unique entity. Interestingly, partial tandem duplication (PTD) of the MLL gene, which produces an extra amino-terminus in-frame to the full-length MLL protein, has been found in AML and myelodysplastic syndrome (MDS) with unique characteristics. MLL-PTD has been thoroughly described in a recent study and, therefore, will not be discussed in this review.

2. CLINICAL FEATURES OF MLL LEUKEMIA

MLL translocations predominantly occur in pediatric AML and ALL, including 69%–79% of all infant ALL cases (aged < 1 year) and approximately 10% of all other ALL cases, >90% of all infants with congenital ALL (aged <1 month), and 35%–50% of infants with AML. MLL translocations also present less often in older children and adult patients with acute leukemia, especially AML, compared with their younger counterparts. Patients treated with topoisomerase inhibitors can develop therapy-related or secondary leukemia; of these patients, 10% carry MLL translocations similar to de novo ALL and AML. Considering the broad spectrum of the MLL recombione and the fact that molecular genetic tests do not cover all MLL fusion genes, the incidences of MLL translations may be underestimated.

Compared with other subsets of acute leukemia, MLL leukemia is more likely to be associated with certain phenotypic features such as hyperleukocytosis, central nervous system infiltration, and therapy refractoriness. MLL leukemia is generally associated with dismal prognosis due to the development of resistance and high chance of relapse with established therapies, including chemotherapy and hematopoietic stem cell transplantation. A recent study also showed that MLL leukemia could circumvent CD19-directed immunotherapies through lineage plasticity. Adults who survive childhood leukemia tend to suffer long-term and multiorgan-related health effects. As such, MLL leukemia remains a clinical challenge for which the development of more effective target therapies is urgently needed.

3. EPIGENETIC AND TRANSCRIPTIONAL REGULATION BY MLL PROTEINS

The MLL gene is conserved with Drosophila trithorax and yeast Set1; it encodes a member of the complex of proteins associated with Set1 (COMPASS) family of enzymes, which catalyze methylation of histone H3 lysine 4 (H3K4) via a conserved SET domain. MLL is a large, multidomain protein with approximately 4000 amino acids; it can be extensively cleaved by Taspase I to generate an N-terminal 320-kDa fragment (N320) and a C-terminal 180-kDa fragment (C180) (Fig. 1A). The N320 fragment contains three AT-hook domains near the N terminus of MLL, a CXXC domain that binds nonmethylated CpG islands, chromatin-binding PHD fingers and bromodomain, and a SET domain at the C-terminus of MLL. MLL breakpoints vary in different patients and locate mainly between exon 7 and exon 10, which was designated a region called "breakpoint cluster region". The resulting MLL fusion proteins retain the chromatin-binding CXXC module (Fig. 1B). Identification of SEC and DOT1L complexes containing the most common MLL fusion partners. Fusion of MLL and SEC/DOT1L subunits could aberrantly recruit SEC and DOT1L complexes to activate the MLL target genes and promote the transformation of HSPCs to leukemia.
4. COOPERATING EVENTS IN MLL LEUKEMIA

The retroviral transduction strategy and knock-in mouse models have demonstrated that MLL chimeras can induce acute leukemogenesis. However, the long latency in knock-in mice and monoclonal nature of the induced leukemia indicate that multiple cooperating events or signaling pathways may be required to promote the pathogenic processes, consistent with the “two-hit” or Knudson hypothesis in cancer. Since 2000s, multiple signaling pathways, mediated by FLT3; RAS/MEK/ERK; Integrin β3/5/7K; Wntβ-catenin; GSK3; NFκB; CEBPs and PU.1; chromatin-modifying complex; and the target genes of MLL chimeras such as HOXA9, MEIS1, EVI1, MEF2C, C-MYC, miR196b, and PBX proteins, have been reported to promote MLL leukemogenesis. Here, we describe the relevant cooperating events, including BRD4, CBP/P300, WDR5, MEN1, CDK6, MS21, JMJ1D1C, and CAF1 complex, and their potential use.

Bromodomain-containing protein 4 (BRD4) BRD4 interacts with acetylated histone 3 lysine 27 (H3K27) and promotes the transcription of MYC, and this interaction can be blocked by small-molecule BET domain inhibitors. In 2011, an unbiased RNA interference screening with chromatin modifiers identified BRD4 as a therapeutic target in MLL-AF9 leukemia through the downregulation of MYC expression by the BET domain inhibitor JQ-1. Two months later, Couzagui’s group reported another BET domain inhibitor, I-BET151, as a potential compound for both MLL-AF9 and MLL-AF4 leukemia through the induction of early cell cycle arrest and apoptosis. BET domain inhibitors have been demonstrated to suppress the function of hematopoietic transcription factors in AML. However, in 2015, two independent groups reported the recurring development of resistance to BRD4 inhibitors through increased Wnt/β-catenin signaling and rewiring of transcriptional programs, which highlights the potential therapeutic limitations of BET inhibitors in MLL leukemia.

CREB-binding protein (CBP) and E1A-binding protein (P300), which mediate the acetylation of H3K27, have been shown to be involved in GSK3-mediated MLL leukemia stem cell transcriptional programs through their association with MEIS1. Because CBP and P300 are fused to MLL in the MLL-CBP and MLL-P300, which retain active histone acetyltransferase activity, the recruitment of BRD4 to H3K27-acetylated chromatin is crucial for MLL leukemia. Histone acetyltransferase activity is clearly required for the maintenance of MLL; this requirement suggests that the chemical inhibition of CBP or P300 presents a therapeutic strategy for treating MLL leukemia. Indeed, in 2015, the selective CBP/P300 inhibitor I-CBP112 has been reported to inhibit MLL-AF9 leukemia in vitro and in vivo. In 2017, another potent and selective inhibitor, A-485, that showed inhibitory effects on MLL-AF9 leukemia cells was discovered.

As mentioned earlier, MLL translocations generally occur on one allele of MLL gene to produce the MLL chimeras, whereas the other MLL allele could generate the wild-type MLL protein. Early studies involving shRNAs targeting the wild-type copy of MLL have small molecules targeting the MLL methyltransferase activity by disrupting wild-type MLL-WDR5 interaction have showed that wild-type MLL and methyltransferase activity are required for the maintenance of MLL leukemia. However, a recent study featuring the genetic perturbation of the MLL or MLL SET domain demonstrated that the wild-type MLL and MLL methyltransferase activity are dispensable for leukemia maintenance. This finding is consistent with the existence of patient-derived MLL leukemia cell lines (such as ML2) with a deletion of the entire wild-type MLL locus. In addition, the methyltransferase activity of MLL is not necessary to maintain the expression of the direct target genes of MLL in hematopoietic populations or to facilitate MLL-AF9-mediated leukemogenesis, thus suggesting that MLL methyltransferase activity is not an ideal target for MLL leukemia. Indeed, despite the similar expressions of the wild-type and chimeric MLL alleles at the mRNA level, we found that the wild-type MLL protein is much less abundant than the MLL chimeras due to rapid protein turnover in MLL leukemia cells.

Menin (MEN1), as a common interacting protein between wild-type MLL and MLL chimeras, is an oncogenic cofactor for the initiation of MLL chimera-mediated leukemogenesis. The interaction of MEN1 with the N-terminus of MLL is required for leukemic transformation, and small molecules targeting the interaction show profound effects against MLL leukemia both in vitro and in vivo. However, MEN1 is ubiquitously expressed and widely recognized as a tumor suppressor in endocrine organs such as parathyroid glands, pancreatic islet cells, and anterior pituitary gland. Whether targeting MEN1 would impair its function as a tumor suppressor in endocrine organs remains unclear.

As the targets of MLL chimeras, cyclin-dependent kinase 6 (CDK6) and Junonji domain containing 1C (JMJ1D1C) were reported to be required for MLL leukemia. Placke et al. conducted an shRNA screening in MLL-AF9 AML cells and found that the CDK6 depletion or CDK4/6 inhibitor palbociclib preferentially inhibits leukemia cell proliferation and induces myeloid differentiation. Similarly, JMJ1D1C has been identified from an epigenetic shRNA library screening and found to maintain the growth and colony formation of MLL-AF9 AML cells in vitro and in vivo. Furthermore, JMJ1D1C has been
demonstrated to substantially maintain AML stem cell frequency and block differentiation of MLL-AF9 leukemia through direct interaction with HOXA9 in a conditional mouse model. As an RNA binding protein, Musashii2 (MS2) maintains the MLL leukemia sel-renewal program by directly interacting with and retaining the efficient translation of HOXA9, MYC, and IKZF2 mRNAs, while conditional deletion of Ms2 led to delayed leukemogenesis, reduced leukemia burden, and impairment of leukemia stem cell function in a murine MLL-AF9 leukemia model. We recently showed that the histone chaperone complex chromatin assembly factor (CAF1) and its subunit CHAF1B are required for hematopoiesis, whereas CHAF1B overexpression promotes MLL by blocking myeloid differentiation transcription factors such as CEBPA. Abrogation of CHAF1B inhibits the progression of MLL-AF9 leukemia in vivo.

5. TRANSCRIPTIONAL ADDICTION IN MLL LEUKEMIA

Since the discovery of the MLL gene and its translocation, numerous studies have attempted to uncover the molecular mechanism of underlying MLL pathogenesis despite the complexity of MLL fusion partners. Interestingly, an artificial MLL-βGal fusion protein in a knock-in mouse model was found to result in the development of lymphoid and myeloid leukemias with reduced penetrance and long latencies. However, the β-galactosidase possesses neither inherent transcriptional activity nor any homology to MLL partner genes. β-Galactosidase can form a tetramer, thus suggesting that MLL-βGal oligomerizes MLL to drive leukemia, albeit with a long latency period. Further studies with dimerized MLL-FKBP, MLL-AF1p, and MLL-GAS7 showed that homo-oligomerization of MLL is necessary and sufficient for MLL leukemogenesis and activation of the downstream HOX genes and MEIS1. However, the molecular mechanisms of dimerized MLL chimeras in transcriptional regulation and MLL leukemogenesis remain unknown.

The most common MLL translocation partners contain transcriptional activity and exist in two biochemically distinct complexes, namely, super elongation complex (SEC) and DOT1L complex, which cooperate to activate MLL target gene expression and provide insights into how MLL chimeras mediate the misregulation of transcription and leukemogenesis (Fig. 1B). In 2010, three groups individually identified a large complex containing AF4/FMR2 (AFF) family protein (AFFI-4), the YEATS domain protein family members ENL or AF9, the Pol II elongation factors 11 (ELL) proteins, ELL-associated factor 1 (EAF1) or EAF2, and P-TEFb (composed of CDK9 and CCNT1). P-TEFb is essential for the transition from transcription pausing to elongation through the phosphorylation of the Pol II C-terminal domain (CTD) and negative elongation factor (NELF). The DOT1L complex contains the subunits of DOT1L, which is a histone 3 lysine 79 (H3K79) methyltransferase, AF10, and ENL or AF9. Genetic perturbation of DOT1L in MLL-AF9 leukemia leads to decreased of H3K79 methylation, which may mediate the transcriptional elongation of MLL-AF9 targets. However, although the DOT1L catalytic inhibitor EPZ004777 showed prolonged survival in a mouse MLL xenograft model, phase I clinical trials suggest that the DOT1L inhibitors need to be used in combination with other therapies. These data suggest that DOT1L complex may have catalytic-independent functions in

...
To directly target SEC, we recently identified KL-1, a peptidomimetic lead compound, and its structural homolog, KL-2, through structure-based in silico screening and biochemical validation. These two small molecules disrupt the interaction between AF9 proteins and P-TEFb, leading to degradation of SEC complex in cells. Disruption of SEC impairs the release of Pol II from promoter-proximal pausing and reduces the transcription elongation rates for processive elongation. These two inhibitors are potent subjects in targeting multiple SEC-mediated biological processes and speculated to be promising compounds in targeting the transcriptional addiction mediated by MLL chimeras and SEC in MLL. 86

6. SUMMARY

Despite significant advances in the understanding of the pathogenesis of MLL, no effective clinical treatment for this type of aggressive leukemia is yet available (Fig. 2). Whether the mechanisms gained mostly from MLL-AF9, MLL-AF4, and MLL-AF4 could be generally applied to other MLL fusion proteins and MLL-PTD is unknown because of the diversity and complexity of the MLL recombine. Moreover, no systematic comparison of the functions and mechanisms of these molecules in MLL leukemogenesis and maintenance has been published. The exact targets and transcriptional programs mediated by various MLL fusions remain unclear because different MLL fusions in different lineages have different target genes and transcriptional circuits. How the cell of origin, microenvironment, and types of MLL fusion affect the lineage specification of MLL remains undetermined.

Although numerous cooperating events, including MLL target genes, downstream and parallel pathways, have been identified for MLL leukemia based on the two-hit hypothesis, they are not necessarily useful in translation if targeting of these events impairs the normal hematopoesis. Why MLL relies on these cooperating events more than normal hematopoesis is unclear. Moreover, because these events are cooperative and not the drivers of mutations for MLL, completely blocking the progression of MLL by targeting these events is insufficient; in fact, resistance may be expected to arise shortly. 49, 50 Such complexities are the major challenges impeding the development of effective therapeutic strategies for MLL leukemia. Thus far, none of these target therapies or drugs has been approved to specifically target the aggressive MLL leukemia. Targeting the transcriptional addiction mediated by MLL chimeras and their associated SEC and DOT1L complexes seems to be a better option for obtaining effective MLL therapies. Understanding the mechanisms underlying SEC and DOT1L complex recruitment to chromatin by MLL fusions, how these molecules promote MLL leukemogenesis through their target genes, and how they cooperate to drive transcriptional misregulation of the MLL target genes are important to reveal the transcriptional addiction in MLL leukemia and develop suitable treatment approaches. Thus, targeting the transcriptional addiction in MLL leukemia may serve as a novel paradigm for the target therapy of various cancers.

ACKNOWLEDGMENTS

We apologize for the work that could not be recognized here due to space constraints. We thank Edwin R. Smith for discussing and sharing his views. This work was supported by grant from the “Thousand Young Talent Program” awarded to K.L.

REFERENCES

[1] McGraw TP, Folds JD, Bollum FJ, Stass SA. Terminal deoxynucleotidyl transferase-positive acute myeloblastic leukemia. Am J Hematol 1981;10(3):251–258.
[2] Mirro J, Zipf TF, Pui CH, et al. Acute mixed lineage leukemia: clinicopathologic correlations and prognostic significance. Blood 1985;66(5):1115–1123.
[3] Kaneko Y, Shikano T, Maseki N, et al. Clinical and hematologic characteristics in acute leukemia with 11q23 translocations. Blood 1986;67(2):484–491.
[4] Rovigatti U, Watson DK, Yunis JJ. Amplification and rearrangement of Hu-ets-1 in leukemia and lymphoma with involvement of 11q23. Science 1986;232(4748):398–400.
[5] Rowley JD, Diaz MO, Espinosa R 3rd, et al. Mapping chromosome band 11q23 in human acute leukemia with biotinylated probes: identification of 11q23 translocation breakpoints with a yeast artificial chromosome. Proc Natl Acad Sci U S A 1990;87(23):9358–9362.
[6] Kaneko Y, Shikano T, Maseki N, et al. Clinical characteristics of infant acute leukemia with or without 11q23 translocations. Leukemia 1988;2(10):672–676.
[7] Childs CC, Hirsch-Ginsberg C, Walters RS, et al. Myeloid surface antigen-positive acute lymphoblastic leukemia (Mys-ALL): immuno-phenotypic, ultrastructural, cytogenetic, and molecular characteristics. Leukemia 1989;3(11):777–783.
[8] Altman AJ. Clinical features and biological implications of acute mixed lineage (hybrid) leukemias. Am J Pediatr Hematol Oncol 1990;12(2):123–133.
[9] Ziemin-van der Poel S, McCabe NR, Gill HJ, et al. Identification of a gene, MLL, that spans the breakpoint in 11q23 translocations associated with human leukemias. *Proc Natl Acad Sci U S A* 1991;88(23):10735–10739.

[10] Djabali M, Selleri L, Parry P, Bower M, Young BD, Evans GA. A trithorax-like gene is interrupted by chromosome 11q23 translocations in acute leukemias. *Nat Genet* 1992;2(11):113–118.

[11] Yu Y, Nakamura T, Alder H, et al. The t(4;11) chromosome translocation of human acute human leukemias fuses the ALL-1 gene, related to Drosophila trithorax, to the AF-4 gene. *Cell* 1992;71(4):701–708.

[12] Tomizawa D, Kohler S, Cleary ML. Involvement of a homolog of Drosophila trithorax by 11q23 chromosomal translocations in acute leukemias. *Cell* 1992;71(4):691–700.

[13] Yu BD, Hess JL, Horning SE, Brown GA, Korsmeyer SJ. Altered Hox expression and segmental identity in Mll-mutant mice. *Science* 1991;250(5000):10739.

[14] Jude CD, Climer L, Xu D, Artinger E, Fisher JK, Ernst P. Unique and shared characteristics of acute leukemias. *Cancer Cell* 2001;2(2):113–124.

[15] Thirman MJ, Gill HJ, Burnett RC, et al. Rearrangement of the MLL gene in acute lymphoblastic and acute myeloid leukemias with 11q23 chromosomal translocations. *Nat Eng Med* 1993;35(9):909–914.

[16] Meyer C, Burmeister T, Groger D, et al. The MLL recombination of acute leukemias in 2017. *Leukemia* 2018;32(2):273–284.

[17] Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemic subtype of childhood acute lymphoblastic leukemia. *Nat Genet* 2002;30(2):41–47.

[18] Choi SM, Dewar R, Burke PW, Shao L. Partial tandem duplication of KMT2A (MLL) may predict a subset of myelodysplastic syndrome with unique characteristics and poor outcome. *Haematologica* 2018;103(3):e131–e134.

[19] Mohan M, Lin C, Guest E, Shilatifard A. Licensed to elongate: a molecular mechanism for MLL-based leukaemogenesis. *Nat Rev Cancer* 2010;10(10):723–728.

[20] Raimondi EA, Chang MN, Ravindranath Y, et al. Chromosomal abnormalities in 478 children with acute myeloid leukemia: clinical characteristics and treatment outcome in a cooperative pediatric oncology group study-POG 8821. *Blood* 1999;94(11):3707–3716.

[21] Schoch C, Schnittger S, Klaas M, Kern W, Hiddemann W, Haferschl T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *EMBO J* 2011;30(12):3373–3383.

[22] Yokoyama A, Wang Z, Wysocka J, et al. Leukemia proto-oncoprotein MLL forms a SET1-like histone methyltransferase complex with menin to regulate Hox gene expression. *Mol Cell Biol* 2004;24(11):5639–5649.

[23] Le BE, Ernst P. Two decades of leukemia oncoprotein epistasis: the MLL1 paradigm for epigenetic deregulation in leukemia. *Exp Hematol* 2014;42(12):995–1012.

[24] Wang QF, Wu G, Mi S, et al. MLL fusion proteins preferentially regulate a subset of wild-type MLL target genes in the leukemic genome. *Blood* 2011;117(25):6895–6905.

[25] Kroon E, Krol J, Thorsteinsson U, Baban S, Buchberg AM, Sauvageau G. Hox9 transforms primary bone marrow cells through specific collaboration with Meis1 but not Pbx1b. *EMBO J* 1999;18(73):3714–3724.

[26] Ferrando AA, Armstrong SA, Neuberg DS, et al. Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: dominance of HOX dysregulation. *Blood* 2003;102(1):262–268.

[27] Kumar AR, Hudson WA, Chen W, Nishiuchi R, Yao Q, Kersey JH. Hox9 influences the phenotype but not the incidence of MLL-AF9 fusion gene leukemia. *Blood* 2004;103(5):1823–1828.

[28] Wang Y, Krivtsov AV, Sinha AU, et al. The Wnt/beta-catenin pathway is required for the development of leukemia stem cells in AML. *Science* 2010;327(5973):1650–1653.

[29] Lavau C, Szilvassy SJ, Slany R, Cleary ML. Immortalization and leukemic transformation of a myelomonocytic precursor by retrovirally transduced HRX-ENL. *EMBO J* 1997;16(14):4226–4237.

[30] Corral J, Lavenir I, Impney H, et al. An MLL-AF9 fusion gene made by homologous recombination causes acute leukemia in chimeric mice: a method to create fusion oncogenes. *Cell* 1996;85(6):833–861.

[31] Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. *Nat Rev Cancer* 2007;7(11):823–833.

[32] Knudso AG. Mutation and cancer: study of retinoblastoma. *Proc Natl Acad Sci U S A* 1971;68(4):820–823.

[33] Filippakopoulos P, Qi J, Picard S, et al. Selective inhibition of BET bromodomains. *Nature* 2010;468(7327):1067–1073.

[34] Zubler J, Shi J, Wang E, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukemia. *Nature* 2011;478(7370):524–528.

[35] Dawson MA, Prinjha RK, Dittmann A, et al. Inhibition of BET bromodomains blocks the association of CREB and its coactivators with MEIS1 to facilitate HOX-dependent transcription and oncogenesis. *Nat Rev Cancer* 2007;7(11):823–833.

[36] Wang Z, Iwasaki M, Ficara F, et al. GSK-3 promotes conditional association of CREB and its coactivators with MEIS1 to facilitate HOX-mediated transcription and oncogenesis. *Cancer Cell* 2010;17(6):597–608.

[37] Picard S, Fedorov O, Thanasopoulou A, et al. Discovery of a selective catalytic small molecule inhibitor of the CBP/p300 bromodomain for leukemia chemotherapy. *Cancer Res* 2010;70(10):4133–4143.

[38] Lasko LM, Jakob CG, Edalji RP, et al. Generation of a selective catalytic small molecule inhibitor of the CBP/p300 bromodomain for leukemia chemotherapy. *Cancer Res* 2010;70(10):4133–4143.

[39] Li BE, Ernst P. Unique characteristics and poor outcome. *Annu Rev Biochem* 2004;73:59–72.
Xiao et al.

[58] Yokoyama A, Somervaille TC, Smith KS, Rozenblatt-Rosen O, Meyerzon M, Cleary ML. The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. *Cell* 2005;123(2):207–218.

[59] Caslini C, Yang Z, El-Osta M, Milne TA, Slany RK, Hess JL. Interaction of MLL amino terminal sequences with menin is required for transformation. *Cancer Res* 2007;67(15):7275–7283.

[60] Borkin D, He S, Miao H, et al. Pharmacologic inhibition of the menin-MLL interaction blocks progression of MLL leukemia in vivo. *Cancer Cell* 2015;27(4):589–602.

[61] Grembecka J, He S, Shi A, et al. Menin-MLL inhibitors reverse oncogenic activity of MLL fusion proteins in leukemia. *Nat Chem Biol* 2012;8(3):277–284.

[62] Cierpicki T, Grembecka J. Challenges and opportunities in targeting the menin-MLL interaction. *Future Med Chem* 2014;6(4):447–462.

[63] Sroczynska P, Cruickshank VA, Bukowski JP, et al. shRNA screening identifies JMJ1D as being required for leukemia maintenance. *Blood* 2014;123(12):1870–1882.

[64] van der Linden MH, Willekes M, van Roon E, et al. MLL fusion-driven activation of CDK6 potentiates proliferation in MLL-rearranged infant ALL. *Cell Cycle* 2014;13(5):834–844.

[65] Placke T, Faber K, Nonami A, et al. Requirement for CDK6 in MLL-rearranged acute myeloid leukemia. *Blood* 2014;124(1):13–23.

[66] Zhu N, Chen M, Eng R, et al. MLL-AF9- and HOXA9-mediated acute myeloid leukemia stem cell self-renewal requires JMJ1D1. *J Clin Invest* 2016;126(3):997–1011.

[67] Park SM, Gonen M, Vu L, et al. Musashi2 sustains the mixed-lineage leukemia-driven stem cell regulatory program. *J Clin Invest* 2015;125(3):1286–1298.

[68] Volk A, Liang K, Suraneni P, et al. A CHAF1B-dependent molecular switch in leukemia pathogenesis. *Cancer Cell* 2018;34(5):707–723.e707.

[69] Dolbosc CL, Warren AJ, Pannell R, Forster A, Rabbits TH. Tumorigenesis in mice with a fusion of the leukaemia oncogene MLL and the bacterial lacZ gene. *EMBO J* 2000;19(5):843–851.

[70] Martin ME, Milne TA, Bloyer S, et al. Dimerization of MLL fusion proteins immortalizes hematopoietic cells. *Cancer Cell* 2003;4(3):197–207.

[71] So CW, Lin M, Ayton PM, Chen EH, Cleary ML. Dimerization contributes to oncogenic activation of MLL chimeras in acute leukemias. *Cancer Cell* 2003;4(2):99–110.

[72] Slany RK. The molecular biology of mixed lineage leukemia. *Haematologica* 2009;94(7):984–993.

[73] Okuda H, Stanoevich B, Kanai A, et al. Cooperative gene activation by AF4 and DOT1L drives MLL-rearranged leukemia. *J Clin Invest* 2017;127(5):1918–1931.

[74] Yokoyama A, Lin M, Naresh A, Kitabayashi I, Cleary ML. A higher-order complex containing AF4 and ENL family proteins with P-TEFb facilitates oncogenic and physiologic MLL-dependent transcription. *Cancer Cell* 2010;17(2):198–212.

[75] Lin C, Smith ER, Takahashi H, et al. AFF4, a component of the ELLP-TEFb elongation complex and a shared subunit of MLL chimeras, can link transcription elongation to leukemia. *Mol Cell* 2010;37(3):429–437.

[76] He N, Liu M, Hsu J, et al. HIV-1 Tat and host AFF4 recruit two transcription elongation factors into a bifunctional complex for coordinated activation of HIV-1 transcription. *Mol Cell* 2010;38(3):428–438.

[77] Liang K, Gao X, Gilmore JM, et al. Characterization of human cyclin-dependent kinase 12 (CDK12) and CDK13 complexes in C-terminal domain phosphorylation, gene transcription, and RNA processing. *Mol Cell Biol* 2015;35(6):928–938.

[78] Mohan M, Herz HM, Takahashi YH, et al. Linking H3K79 trimethylation to Wnt signaling through a novel Dot1-containing complex (DotCom). *Genes Dev* 2010;24(6):574–589.

[79] Bernt KM, Zhu N, Sinha AU, et al. MLL-rearranged leukemia is dependent on aberrant H3K79 methylation by DOT1L. *Cancer Cell* 2011;20(1):66–78.

[80] Nguyen AT, Taranova O, He J, Zhang Y. DOT1L, the H3K79 methyltransferase, is required for MLL-AF9-mediated leukemogenesis. *Blood* 2011;117(25):6912–6922.

[81] Daigle SR, Olhava EJ, Therkelsen CA, et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer Cell* 2011;20(1):53–65.

[82] Erb MA, Scott TG, Li BE, et al. Transcription control by the ENL YEATS domain in acute leukaemia. *Nature* 2017;543(7644):270–274.

[83] Jan L, Wen H, Li Y, et al. ENL links histone acetylation to oncogenic gene expression in acute myeloid leukemia. *Nature* 2017;543(7644):265–269.

[84] Weinstein IB, Joe AK. Mechanisms of disease: oncogene addiction – a rationale for molecular targeting in cancer therapy. *Nat Clin Pract Oncol* 2006;3(8):448–457.

[85] Bradner JE, Hnisz D, Young RA. Transcriptional addiction in cancer. *Mol Cell* 2015;60(3):435–457.

[86] Liang K, Smith ER, Aoi Y, et al. Targeting processive transcription elongation via SEC disruption for MYC-induced cancer therapy. *Cell* 2018;175(3):766–779.e717.

[87] Liang K, Woodfin DR, Slaughter BD, et al. Mitotic transcriptional activation: clearance of actively engaged Pol II via transcriptional elongation control in mitosis. *Mol Cell* 2015;59(3):435–445.

[88] Elmeyer CC, Erickson B, Allen RL, et al. Human TFIIH kinase CDK7 regulates transcription-associated chromatin modifications. *Cell Rep* 2017;20(5):1173–1186.

[89] Grembecka J, Cierpicki T. Stabilizing the mixed lineage leukemia protein. *N Engl J Med* 2017;376(17):1688–1689.

[90] Brien GL, Stegmaier K, Armstrong SA. Targeting chromatin complexes in fusion protein-driven malignancies. *Nat Rev Cancer* 2019;19(5):255–269.