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H3K79 Methylation Profiles Define Murine and Human MLL-AF4 Leukemias

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SUMMARY

We created a mouse model wherein conditional expression of an Mll-AF4 fusion oncogene induces B precursor acute lymphoblastic (ALL) or acute myeloid leukemias (AML). Gene expression profile analysis of the ALL cells demonstrated significant overlap with human *MLL*-rearranged ALL. ChIP-chip analysis demonstrated histone H3 lysine 79 (H3K79) methylation profiles that correlated with Mll-AF4-associated gene expression profiles in murine ALLs and in human *MLL*-rearranged leukemias. Human *MLL*-rearranged ALLs could be distinguished from other ALLs by their H3K79 profiles, and suppression of the H3K79 methyltransferase DOT1L inhibited expression of critical MLL-AF4 target genes. We thus demonstrate that ectopic H3K79 methylation is a distinguishing feature of murine and human MLL-AF4 ALLs and is important for maintenance of MLL-AF4-driven gene expression.

INTRODUCTION

Leukemias mediated by *MLL* rearrangements possess unique clinical and biological features. *MLL* rearrangements can be found in acute lymphoblastic leukemia (ALL), acute myeloid leukemias (AML), and acute biphenotypic or mixed-lineage leukemia (MLL). *MLL* translocations are present in over 70% of cases of infant leukemias (Biondi et al., 2000) and in general account for approximately 5% of ALLs, 5%–10% of AMLs, and a significant portion of acute biphenotypic or mixed-lineage leukemias (Huret et al., 2001). Patients with *MLL*-rearranged ALL have a particularly unfavorable prognosis as compared to patients with other forms of ALL (Chen et al., 1993). To date, more than 50 MLL fusion partner genes have been reported (Ayton and Cleary, 2001; Krivtsov and Armstrong, 2007). The t(4;11)(q21;q23) encodes MLL-AF4 and is the most frequent *MLL* translocation found in ALL.

Multiple mouse models have been developed that recapitulate *MLL*-rearranged AML (reviewed in Ayton and Cleary, 2001). However, development of models that faithfully recapitulate MLL fusion-mediated ALL has proven more difficult. Constitutive *Mll-AF4* knockin results in mixed lymphoid/myeloid hyperplasia and mature B cell neoplasms in mice (Chen et al., 2006). Conditional expression of *Mll-AF4* based on interchromosomal recombination in lymphoid lineages produces mature B cell lymphomas (Metzler et al., 2006). Thus, further development of murine models of *MLL*-rearranged ALL is needed.

MLL is a mammalian homolog of *Drosophila* trithorax and possesses multiple functional domains, including amino-terminal AT hooks that bind DNA and a carboxy-terminal Su(var)3-9, Enhancer-of-zeste, Trithorax (SET) domain that methylates lysine 4 of histone H3 (H3K4). H3K4 methylation is associated with transcriptional activation (reviewed in Shilatifard, 2006),

SIGNIFICANCE

The t(4;11) encodes an MLL-AF4 fusion protein and predicts a particularly poor prognosis when found in acute lymphoblastic leukemia (ALL). Recent studies suggest certain MLL fusion proteins enhance gene expression by recruitment of the histone H3 lysine79 (H3K79) methyltransferase DOT1L. We demonstrate that H3K79 methylation is enhanced at many loci in leukemia cells from a murine model of Mll-AF4 and in human MLL-AF4 leukemia cells, and that this elevation is correlated with enhanced gene expression. Furthermore, suppression of H3K79 methylation leads to inhibition of gene expression in MLL-AF4 cells. These data demonstrate that inhibition of DOT1L may be a therapeutic approach in this disease and that this mouse model should be useful for assessment of therapeutic approaches for *MLL*-rearranged ALL.

and MLL positively regulates expression of clustered homeobox (*HOX*) and other genes during development, at least in part via H3K4 methylation (Milne et al., 2002; Nakamura et al., 2002). Mll has important roles in development, including hematopoietic development (Hess et al., 1997; Jude et al., 2007).

MLL translocations invariably encode fusion proteins that have lost the H3K4 methyltransferase (SET) domain. However, MLL fusion proteins retain the ability to bind *HOX* genes and other promoter regions and are associated with enhanced gene expression (Armstrong et al., 2002; Guenther et al., 2005; Rozovskaia et al., 2001; Yeoh et al., 2002; Zeisig et al., 2004). Several mechanisms have been proposed as to how MLL fusions may deregulate gene expression, including recruitment of abnormal histone modification activities (Cheung et al., 2007; Krivtsov and Armstrong, 2007; Okada et al., 2005). For example, MLL-AF10 and MLL-ENL have been shown to recruit the non-SET domain methyltransferase DOT1L, which promotes methylation of histone H3 lysine 79 (H3K79) on the *HOXA9* promoter (Mueller et al., 2007; Okada et al., 2005; Zeisig et al., 2005). Since H3K79 methylation is linked to positive transcriptional regulation (Schubeler et al., 2004; Shilatifard, 2006), DOT1L-mediated methylation of H3K79 may contribute to increased expression of *HOXA9* in MLL-AF10- and MLL-ENL-induced leukemias. Furthermore, AF10, ENL, and other MLL fusion partners such as AF4 and AF9 are normally found in nuclear complexes associated with DOT1L (Bitoun et al., 2007; Mueller et al., 2007; Okada et al., 2005; Zeisig et al., 2005; Zhang et al., 2006). Thus, aberrant recruitment of DOT1L to the promoters of MLL target genes may be a common feature of many oncogenic MLL fusion proteins. However, the extent of H3K79 methylation changes and the specificity of these epigenetic changes for *MLL*-rearranged leukemias have not been defined.

Here, we report the development of a murine model in which conditional expression of MLL-AF4 induces both ALL and AML. Genome-wide assessment of gene expression and H3K79 methylation demonstrates that this model faithfully recapitulates human ALL resulting from *MLL-AF4* translocation and identifies ectopic H3K79 methylation as an important part of MLL-AF4-driven gene expression and transformation.

RESULTS

Generation of a Conditional *Mll-AF4* Knockin Mouse

We used a conditional expression approach that proved successful for development of an Mll-Cbp myelodysplasia/AML model to create a model in which the Mll-AF4 fusion product was conditionally expressed from the endogenous *Mll* locus. We engineered a conditional Mll-AF4^{stop} targeting construct by replacing the *Cbp* cDNA in the previously reported Mll-Cbp^{stop} targeting vector (Wang et al., 2005) with a cDNA encoding the C-terminal portion of human AF4 (Figure 1A). This generated a targeting construct that placed the human AF4 sequence in the murine *Mll* exon 8, downstream of a transcriptional stop site flanked by *LoxP* sites, which allows conditional expression of an *Mll-AF4* fusion RNA upon expression of Cre recombinase (Figure 1B). The construct was electroporated into CJ7 mouse embryonic stem cells (Swiatek and Gridley, 1993), and clones possessing the targeted allele were selected by Southern blot (Figure 1C) and used to achieve germline transmission of the knockin allele. Mice heterozygous for the *Mll-AF4*^{stop} conditional allele were born at slightly less than Mendelian frequency, presumably due to heterozygosity for *Mll* (Yu et al., 1995). Founder mice were backcrossed to C57BL6/129 F1 mice. We used Mll-AF4-specific primers to confirm the absence of the fusion RNA in bone marrow from mice heterozygous for the *Mll-AF4*^{stop} allele.

Mll-AF4 Expression Enhances Serial Replating of Lymphoid Progenitors

First, we determined whether expression of Mll-AF4 could transform lymphoid cells in vitro. We collected bone marrow from *Mll-AF4*^{stop} (MA4) heterozygous mice (three experiments, five mice each) 5 days after 5-fluorouracil (5-FU) treatment and transduced cells with retroviruses encoding either GFP control (MIG) or Cre-GFP (Cre) to initiate expression of Mll-AF4. Cells were then cultured in semisolid media supplemented with IL-7, SCF, and FLT3. After 14 days of culture, over 90% of cells in both Cre and MIG groups expressed CD19 and B220. Weekly replating of 1×10^4 MIG-transduced cells exhausted their colony-forming potential by the third week, while similar replating of Cre-transduced cells did not exhaust their replating potential for at least 6 weeks (see Figure S1A available online). To assess for gene expression changes associated with Mll-AF4 expression, we extracted RNA from 1×10^5 cells at the end of the second week of plating and amplified, labeled, and hybridized labeled RNA to Affymetrix mouse 430 A2.0 microarrays. Supervised analysis identified *HoxA5*, *HoxA9*, *Runx2*, *Meis1*, and *Mylk* among the 20 most upregulated genes in the cells expressing Mll-AF4 (Figure S1B). These genes are also found as central members of early gene expression changes associated with MLL-AF9 expression in myeloid cells and in human *MLL*-rearranged lymphoblastic leukemias (Armstrong et al., 2002; Krivtsov et al., 2006). Thus, conditional expression of Mll-AF4 in lymphoid cells leads to in vitro transformation and gene expression changes associated with human MLL fusion leukemias.

Mll-AF4 Expression Induces Acute Leukemias

The initial strategy we used to activate Mll-AF4 expression utilized a self-excising retrovirus that transiently expresses Cre recombinase in transduced cells (Silver and Livingston, 2001). We either collected bone marrow (BM) from heterozygous MA4 mice 5 days after 5-FU treatment or used 5-FU-untreated bone marrow depleted for cells expressing CD3, CD4, CD8α, F4/80, B220, Gr1, and TER119 (Lin$^-$). BM cells were transduced with retroviruses encoding either "hit-and-run Cre" (HR-Cre) or GFP (MIG) and transplanted into lethally or sublethally irradiated syngeneic recipients. The expression of Mll-AF4 in HR-Cre cells but not MIG-transduced cells was confirmed by RT-PCR (Figure 1D). Mice (n = 43) expressing the *Mll-AF4* allele as a result of retroviral transduction of Cre developed a fatal disease (Table 1; Figures S2A and S2B) consistent with acute leukemia including bone marrow replacement, splenomegaly, and variable lymphadenopathy (Table 1; Figure 2A). Mice transplanted with BM from MA4 mice (n = 20) transduced with MIG did not develop leukemia (Figure S2A).

In order to confirm leukemia development in a purely genetic model, we crossed MA4 mice with mice transgenic for *Mx1-Cre* (Kuhn et al., 1995). *Mx1-Cre* × MA4 mice and control littermates received three intraperitoneal injections of polyinosinic/polycytidylic