Cancer Cell
Article

MLL-AF9-Induced Leukemogenesis Requires Coexpression of the Wild-Type *Mll* Allele

Austin T. Thiel,[1,5] Peter Blessington,[1,5] Tao Zou,[2] Danielle Feather,[1] Xinjiang Wu,[1] Jizhou Yan,[1,6] Hui Zhang,[1] Zuguo Liu,[3] Patricia Ernst,[4] Gary A. Koretzky,[2] and Xianxin Hua[1,*]

[1]Abramson Family Cancer Research Institute, Department of Cancer Biology, University of Pennsylvania, Philadelphia, PA 19104, USA
[2]Abramson Family Cancer Research Institute, Department of Chemistry, Department of Bioengineering, Department of Medicine, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
[3]Eye Institute and Affiliated Xiamen Eye Center, Xiamen University, Xiamen, Fujian 361005, China
[4]Department of Genetics and Norris Cotton Cancer Center, Dartmouth Medical School, Hanover, NH 03755, USA
[5]These authors contributed equally to the work
[6]Present address: Shanghai Ocean University, Shanghai, 201306, China
*Correspondence: huax@mail.med.upenn.edu
DOI 10.1016/j.ccr.2009.12.034

SUMMARY

Oncogenic fusion proteins are capable of initiating tumorigenesis, but the role of their wild-type counterparts in this process is poorly understood. The mixed lineage leukemia (*MLL*) gene undergoes chromosomal translocations, resulting in the formation of oncogenic MLL fusion proteins (MLL-FPs). Here, we show that menin recruits both wild-type MLL and oncogenic MLL-AF9 fusion protein to the loci of *HOX* genes to activate their transcription. Wild-type MLL not only catalyzes histone methylation at key target genes but also controls distinct MLL-AF9-induced histone methylation. Notably, the wild-type *Mll* allele is required for MLL-AF9-induced leukemogenesis and maintenance of MLL-AF9-transformed cells. These findings suggest an essential cooperation between an oncogene and its wild-type counterpart in MLL-AF9-induced leukemogenesis.

INTRODUCTION

Multiple oncogenic fusion proteins resulting from chromosomal translocations are capable of initiating tumorigenesis, but little is known about the role of the remaining wild-type (WT) allele in this process. The mixed lineage leukemia gene (*MLL*) is fused with one of over 60 distinct partner genes through chromosomal translocations in various human acute leukemias, resulting in the formation of multiple MLL fusion proteins (MLL-FPs) (Hess, 2004; Krivtsov and Armstrong, 2007). MLL-FPs are capable of leukemic transformation and dysregulation of multiple *Hox* genes, including *Hoxa9*. In one well-characterized example, MLL-AF10 directly interacts with Dot1L, the only known H3K79-specific methyltransferase, via the AF10 moiety and recruits Dot1L to the *Hoxa9* locus to aberrantly increase H3K79 dimethylation (Okada et al., 2005). The H3K79 methyltransferase activity of Dot1L is required for enhanced transcription of certain *Hox* genes and for MLL-AF10-induced bone marrow (BM) transformation. MLL-AF4 also enhances Dot1L-mediated H3K79 methylation at *Hox* genes (Krivtsov et al., 2008) and the WT counterparts of additional MLL fusion partners such as AF4 and ENL have been shown to interact with Dot1L in a large protein complex (Bitoun et al., 2007; Mueller et al., 2007), illustrating one common mechanism for transformation.

WT *MLL* is homologous to the *Drosophila* trithorax gene, a positive regulator of gene expression. WT MLL is proteolytically cleaved into two parts, MLL-N and MLL-C, by the protease Taspase 1 (Hsieh et al., 2003). MLL-C contains a conserved SET domain (Suv3-9, *E*nhancer of *zeste* and *Trithorax*), which catalyzes histone H3 lysine 4 (H3K4) methylation and upregulates transcription of *HOX* genes in fibroblasts or epithelial cell lines (Milne et al., 2002; Nakamura et al., 2002). H3K4 trimethylation (H3K4m3) is associated with euchromatin and active genes and specifically recruits chromatin-remodeling proteins to stimulate gene expression (Berger, 2007; Flanagan et al., 2005; Li et al., 2006).

WT MLL forms a large complex with several proteins including menin (Hughes et al., 2004; Yokoyama et al., 2005), a nuclear

Significance

The potential role of wild-type (WT) MLL in the development of mixed lineage leukemia, which is highly aggressive and often refractory to therapy, has been elusive. We demonstrate a crucial role for WT MLL, the common WT precursor of over 60 distinct MLL-FPs, in MLL-AF9-induced leukemogenesis. WT MLL influences MLL-AF9-induced histone methylation and gene expression as well as growth and survival of MLL-AF9-transformed leukemia cells. These findings underscore the importance of WT MLL in the development of MLL-AF9-induced acute leukemia.

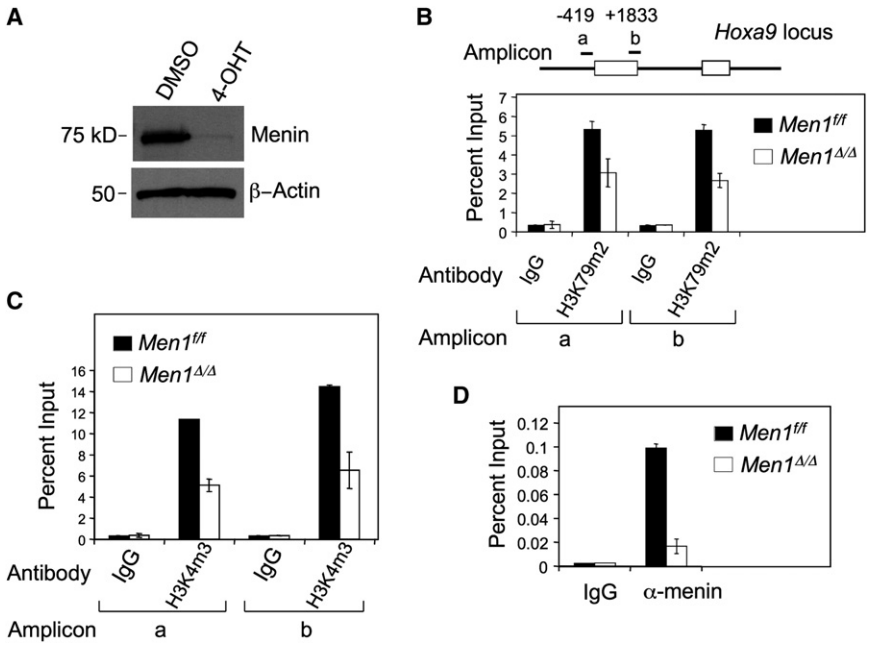

Figure 1. Menin Is Required for Both H3K4 Trimethylation and H3K79 Dimethylation at *Hoxa9* in MA9-Transformed Cells

(A) Western blot for menin in control or *Men1* excised MA9-transformed AT1 cells, which harbored *Men1*$^{f/f}$;*Cre-ER*. The cells were treated with either control DMSO (*Men1*$^{f/f}$) or 4-OHT (*Men1*$^{\Delta/\Delta}$) to excise the floxed *Men1*.

(B–D) ChIP assay, with two distinct amplicons, for detecting dimethylated H3K79 (B), trimethylated H3K4 (C), and menin binding (D) at *Hoxa9* in *Men1*$^{f/f}$ and *Men1*$^{\Delta/\Delta}$AT1 cells. Error bars denote ± SD.

DNA-binding protein that is mutated in an inherited human endocrine tumor syndrome (La et al., 2004). Menin interacts with the N terminus of both MLL and MLL-FPs (Yokoyama et al., 2005), increases H3K4 trimethylation (H3K4m3) at the *Hoxa9* locus, and upregulates its transcription in MLL-FP-transformed hematopoietic cells (Chen et al., 2006; Yokoyama et al., 2005). Moreover, menin is required for proliferation of cells transformed by MLL-AF9 fusion protein (MA9 hereafter) (Chen et al., 2006). However, little is known as to whether menin affects MA9-regulated H3K79 methylation and whether WT MLL is important for MA9-mediated leukemic transformation.

The potential role (or lack thereof) of WT MLL in MLL-FP-induced leukemogenesis has not been addressed. On the one hand, despite a lack of the WT MLL SET domain, MA9 remains capable of initiating leukemogenesis when introduced into WT murine or human hematopoietic progenitors (Barabe et al., 2007; Krivtsov et al., 2006; Somervaille and Cleary, 2006; Wei et al., 2008). Moreover, MLL-AF10 reduces H3K4 dimethylation at the *Hoxa9* locus (Okada et al., 2005), which is mediated at least partly by WT MLL. Furthermore, in MLL-FP-expressing human leukemia cells, which in theory lose one of the two WT *MLL* alleles in chromosomal translocation, expression of WT MLL target genes such as *Hoxa9* is even higher than in non-MLL-FP-leukemia cells (Armstrong et al., 2002). These studies raise the possibility that WT MLL is not crucial for oncogenic transformation by MLL-FPs. On the other hand, WT MLL is crucial for H3K4 methylation and expression of *HOX* genes in fibroblasts and HeLa cells (Milne et al., 2002; Nakamura et al., 2002). Moreover, WT *Mll* excision compromises the function of hematopoietic stem cells (HSCs) and expression of 5′ *Hoxa* genes, including *Hoxa9* (Jude et al., 2007; McMahon et al., 2007), yet these *Hox* genes are upregulated in an MA9-transformed leukemia stem cell (LSC)–enriched population (Krivtsov et al., 2006), raising the possibility that WT MLL is involved in MA9-induced leukemogenesis. Therefore, whether the WT *MLL* allele is crucial for MA9-induced leukemogenesis remains unresolved.

A long list of oncogenic fusion proteins resulting from chromosomal translocations has been identified in various leukemias and solid cancers (Nambiar et al., 2008). However, it is poorly understood whether the WT alleles influence tumorigenesis induced by the majority of the known oncogenic fusion proteins. A better understanding of the function of these WT alleles in tumorigenesis could yield insights into the mechanisms of transformation. Our earlier findings on the role of menin in proliferation and gene transcription of MA9-transformed cells prompted us to investigate the potential role of WT MLL in MA9-induced leukemogenesis.

RESULTS

Menin Is Required for Methylation of Both Histone H3 Lysine 4 and Histone H3 Lysine 79 at the *Hoxa9* Locus

The MLL-AF10 fusion protein has been reported to transform BM by increasing Dot1L-catalyzed H3K79 methylation but repressing histone H3 lysine 4 (H3K4) methylation at the *Hoxa9* locus, suggesting that histone H3 lysine 79 (H3K79)-methylating Dot1L, but not H3K4-methylating WT MLL, is crucial for MLL-FP-induced leukemic transformation (Okada et al., 2005). Although menin interacts with the N terminus of WT MLL and MLL fusion proteins (Yokoyama et al., 2005), little is known as to whether menin is crucial for H3K79 methylation at *Hoxa9* in MA9-transformed BM cells. To address this question, we excised the floxed *Men1* gene in MA9-transformed BM cells (AT1 cells), which harbor *Men1*$^{f/f}$;*Cre-ER*, using 4-hydroxyl tamoxifen (4-OHT) to induce Cre activity (Figure 1A, lane 2). We performed chromatin immunoprecipitation (ChIP) assays with the control and *Men1*-excised cells. Our results showed that *Men1* excision reduced H3K79 dimethylation in two separate locations at the *Hoxa9* locus, as shown by amplicons a and b (Figure 1B). As Dot1L is the only known H3K79 methyltransferase in mammals, this finding is consistent with the notion that menin is crucial for MA9-induced Dot1L recruitment to the *Hoxa9* locus and H3K79 methylation at the locus.

We also noted that *Men1* excision reduced H3K4 trimethylation at *Hoxa9* (Figure 1C), in agreement with our previous findings (Chen et al., 2006). However, this finding is different from the proposed role of MLL-AF10 in reducing H3K4 methylation at