Brief report

The *Mll* partial tandem duplication: differential, tissue-specific activity in the presence or absence of the wild-type allele

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The partial tandem duplication of *MLL* (*MLL*-PTD) is found in 5% to 10% of patients with acute myeloid leukemia (AML) and normal cytogenetics. Its expression in leukemic blasts is coincident with a silenced wild-type (WT) *MLL* allele. We therefore generated mice expressing the *Mll*-PTD in the absence of *Mll*-WT. These $Mll^{PTD/-}$ mice die at birth unlike the normal life expectancy of $Mll^{PTD/WT}$, $Mll^{WT/-}$, and $Mll^{WT/WT}$ mice. Using $Mll^{WT/WT}$ fetal liver cells (FLC) as baseline, we compared $Mll^{PTD/-}$ with $Mll^{PTD/WT}$ FLC and found both had increased *HoxA* gene expression and granulocyte-macrophage colony-forming progenitor cells (CFU-GM); in contrast, only $Mll^{PTD/WT}$ FLC had increased pluripotent hemopoietic progenitors (CFU-GEMM). The similarities between $Mll^{PTD/WT}$ and $Mll^{PTD/-}$ mice suggest that the *Mll*-PTD mutation can upregulate target genes in a dominant, gain-of-function fashion. The differences between these 2 genotypes suggest that in select tissues the *Mll*-PTD requires cooperation with the *Mll*-WT in the genesis of the observed abnormality. (Blood. 2008;112:2508-2511)

Introduction

Approximately 5% to 10% of patients with acute myeloid leukemia (AML) and normal cytogenetics present with rearrangement of the mixed-lineage leukemia (*MLL*, also known as *ALL1* or *HRX*) gene as the result of a partial tandem duplication (PTD) within a single *MLL* allele.[1,2] In AML blasts harboring the somatic *MLL* PTD mutation, the *MLL* wild-type (WT) allele is not expressed and, when reexpressed, leukemic cell death was observed.[3] We previously reported on the $Mll^{PTD/WT}$ knockin mice that are fully viable with modest developmental defects, have aberrant gene expression and altered hematopoiesis, but do not develop leukemia.[4] These mice express both the *Mll* PTD and WT *Mll* alleles. Thus, to partially recapitulate what is observed in human primary AML blasts regarding the *MLL*-PTD and absence of *MLL*-WT expression, we generated mice that harbor a *Mll*-PTD but lack *Mll*-WT ($Mll^{PTD/-}$) in the germ line. We then asked how loss of function of *Mll*-WT in the context of *Mll*-PTD would affect *HoxA* gene expression and hematologic abnormalities previously observed in $Mll^{PTD/WT}$ mice and, eventually, occurrence of leukemia.

Methods

Generation of $Mll^{PTD/-}$ mice

$Mll^{WT/-}$ mice[5] were generously provided by the late Dr Stanley Korsmeyer (Dana-Farber Cancer Institute, Boston, MA). These mice were maintained on a B6C3F1 background. To obtain $Mll^{PTD/-}$ mice, F1 offspring were obtained by crossing the $Mll^{WT/-}$ mice with the $Mll^{PTD/WT}$ mice[4] (maintained on a pure C57Bl/6J background). This work was performed with approval of The Ohio State University institution review board and under an Institutional Animal Care and Use Committee–approved proposal.

Comparative real-time RT-PCR

Total RNA was extracted from E17.5 fetal liver cells (FLC) from $Mll^{PTD/WT}$, $Mll^{PTD/-}$, $Mll^{WT/-}$, and $Mll^{WT/WT}$ embryos. Comparative real-time reverse transcription–polymerase chain reaction (RT-PCR) assays on whole fetal liver and c-kit$^+$- and CD11b$^+$-sorted populations were performed as previously described.[4,6]

Chromatin immunoprecipitation

H3 (Lys4) dimethylation has been shown to occur as a direct result of MLL's SET domain methyltransferase activity.[4,7] Therefore, chromatin immunoprecipitation (ChIP) assays were performed on 2×10^6 FLC using the EZ ChIP Assay Kit with the anti-dimethyl Histone H3 (Lys4) antibody (Millipore, Billerica, MA) according to the manufacturer's standard protocol. DNA was quantified using PCR and nested real-time quantitative PCR with SYBR green incorporation (Applied Biosystems, Foster City, CA) using previously described methods.[4]

Colony forming unit–progenitor assays

Single cell suspensions were plated at a density of 50 000 cells/dish in M3434 methylcellulose (StemCell Technologies, Vancouver, BC), and were performed according to the manufacturer's protocol (StemCell Technologies) and methods as previously described.[4]

Statistics

To evaluate whether significant differences in pluripotent hemopoietic progenitors (colony forming unit [CFU]–GEMM), CFU-GM, or burst forming unit-erythroid (BFU-E) existed between mouse genotypes as indicated in the Figure 1 legend, paired *t* tests were carried out using siblings.

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Table 1. Comparison of *Mll* genotypes

Characteristic	$Mll^{PTD/WT}$	$Mll^{PTD/-}$	$Mll^{WT/-}$	$Mll^{WT/WT}$
Premature death	no	yes*	no	no
HoxA expression	increased	increased	decreased but NS	normal
Increased H3 (Lys4)	yes	yes	no	no
Increased BFU-E	yes	yes	no	—
Increased CFU-GEMM	yes	no	no	—
Increased CFU-GM	yes	yes	no	—

NS indicates not significant; BFU-E, erythroid progenitors; and —, not applicable.
*Pups died by postpartum day 1.

Results and discussion

Results of the comparative analysis among the $Mll^{WT/WT}$, $Mll^{WT/-}$, $Mll^{PTD/WT}$ and $Mll^{PTD/-}$ mice are summarized in Table 1. In terms of survival, the first 3 genotypes were all viable and born at expected Mendelian ratios, however, although present at normal Mendelian ratios, 100% of the pups having the $Mll^{PTD/-}$ genotype died by postpartum day 1 (P1). Interestingly, $Mll^{-/-}$ mice are also nonviable, but die around embryonic day 10.5 (E10.5).[5] These results indicate the *Mll* PTD itself provides some, albeit insufficient, compensation for embryonic development in the absence of both *Mll* WT alleles.

With regards to *HoxA* gene expression, we found that the *Mll* PTD is required for aberrant overexpression of *HoxA* genes in E17.5 FLC. $Mll^{PTD/WT}$ and $Mll^{PTD/-}$ mice showed nearly equivalent levels of *HoxA* over-expression in unsorted FLC, while $Mll^{WT/-}$ cells expressed *HoxA* levels that were consistently lower but not significantly different from the expression levels found in $Mll^{WT/WT}$ FLC (Figure 1A). To determine whether the overexpression of *Hoxa9* was on a per cell basis rather than an increase in a *Hox*-expressing subpopulation within the unsorted FLC, we sorted out equivalent numbers of c-kit$^+$ (Figure 1B) and CD11b$^+$ (Figure 1C) cells from $Mll^{PTD/WT}$, $Mll^{PTD/-}$, $Mll^{WT/WT}$ and $Mll^{WT/-}$ E17.5 fetal livers. Significantly increased levels of *Hoxa9* were found in the $Mll^{PTD/WT}$- and $Mll^{PTD/-}$-sorted FLC but not in $Mll^{WT/WT}$- or $Mll^{WT/-}$-sorted FLC. In fact, $Mll^{WT/-}$ CD11b$^+$ cells had no detectable levels of *Hoxa9* transcript. These results support the notion that the overexpression of *HoxA* is occurring on a per cell basis within the fetal liver. Furthermore, ChIP assays demonstrate that the presence of the *Mll* PTD is associated with increased H3 (Lys4) methylation at the *Hoxa9* promoter in the presence or absence of the *Mll*-WT allele (Figure 1D). This increased H3 (Lys4) methylation likely accounts for the observed *HoxA* gene overexpression in both $Mll^{PTD/WT}$ and $Mll^{PTD/-}$ FLC.

Although premature death of the $Mll^{PTD/-}$ mice precluded assessment of leukemia development, we did examine fetal livers for any alterations in normal hematopoiesis. We performed CFU assays to assess fetal hematopoietic liver function in vitro using E17.5 FLC obtained from each of the 4 genotypes. Significant increases in the CFU-GM, BFU-E, and the more immature CFU-GEMM were seen in cells obtained from the $Mll^{PTD/WT}$ mice compared with $Mll^{WT/WT}$ mice ($P < .01$, $P < .01$, and $P < .05$, respectively), suggesting that the *Mll* PTD may cooperate with the *Mll* WT allele at an early stage of hematopoiesis such as the common myeloid progenitor cell. In contrast, FLC obtained from $Mll^{PTD/-}$ mice had increases in the CFU-GM and BFU-E populations compared with $Mll^{WT/WT}$ mice ($P < .05$ and $P < .05$, respectively), but not in the CFU-GEMM population (Figure 1E). Finally, FLC obtained from $Mll^{PTD/-}$ mice had a significantly lower number of BFU-E progenitors compared with $Mll^{PTD/WT}$ FLC. Together these results are consistent with the notion that the *Mll* PTD itself is required and sufficient for abnormal expansion at some stages of progenitor cell differentiation but may not be at other stages. These results also support 2 recent reports that showed the role of *Mll* WT in hematopoietic stem cells is distinct from its role in hematopoietic progenitor cells.[8,9]

Similar phenotypic abnormalities observed in $Mll^{PTD/-}$ and $Mll^{PTD/WT}$ genotypes support the notion that the *Mll* PTD by itself is capable of dysregulating downstream targets and can therefore behave as a dominant gain-of-function mutation in the absence of the Mll WT. Although these studies suggest a direct role for the Mll PTD, it is important to note that a more stable (lacZ fused) Mll protein containing several important N-terminal Mll functional motifs exists in the knockout model. Because our heterozygous animals exhibit a more severe phenotype than other (non-lacZ fused) *Mll* heterozygous knockout mice,[8,9] we cannot exclude the possibility that the knockout allele acts in an interfering manner.

Mll has now been shown to have very different functions in different subpopulations in the hematopoietic compartment.[10] Our results suggest that in some cases Mll function may have been lost and cannot be replaced by the *Mll* PTD allele, as in the case of $Mll^{PTD/-}$ early lethality at P1. However, in some cases such as *HoxA* gene overexpression and CFU-GM expansion, the Mll PTD appears to behave more as a dominant gain-of-function mutation because the quantifications performed for these experiments were similar between the $Mll^{PTD/WT}$ and $Mll^{PTD/-}$ genotypes, ie, in the presence and absence of Mll WT, respectively. In contrast, differences in the number of BFU-E and CFU-GEMM progenitors seen between the $Mll^{PTD/WT}$ and $Mll^{PTD/-}$ genotypes reveal the lack of dominant activity by the *Mll* PTD allele. In this tissue-specific context, the *Mll* PTD may require interaction with the *Mll* WT in order to achieve the maximum manifestation of the abnormality.

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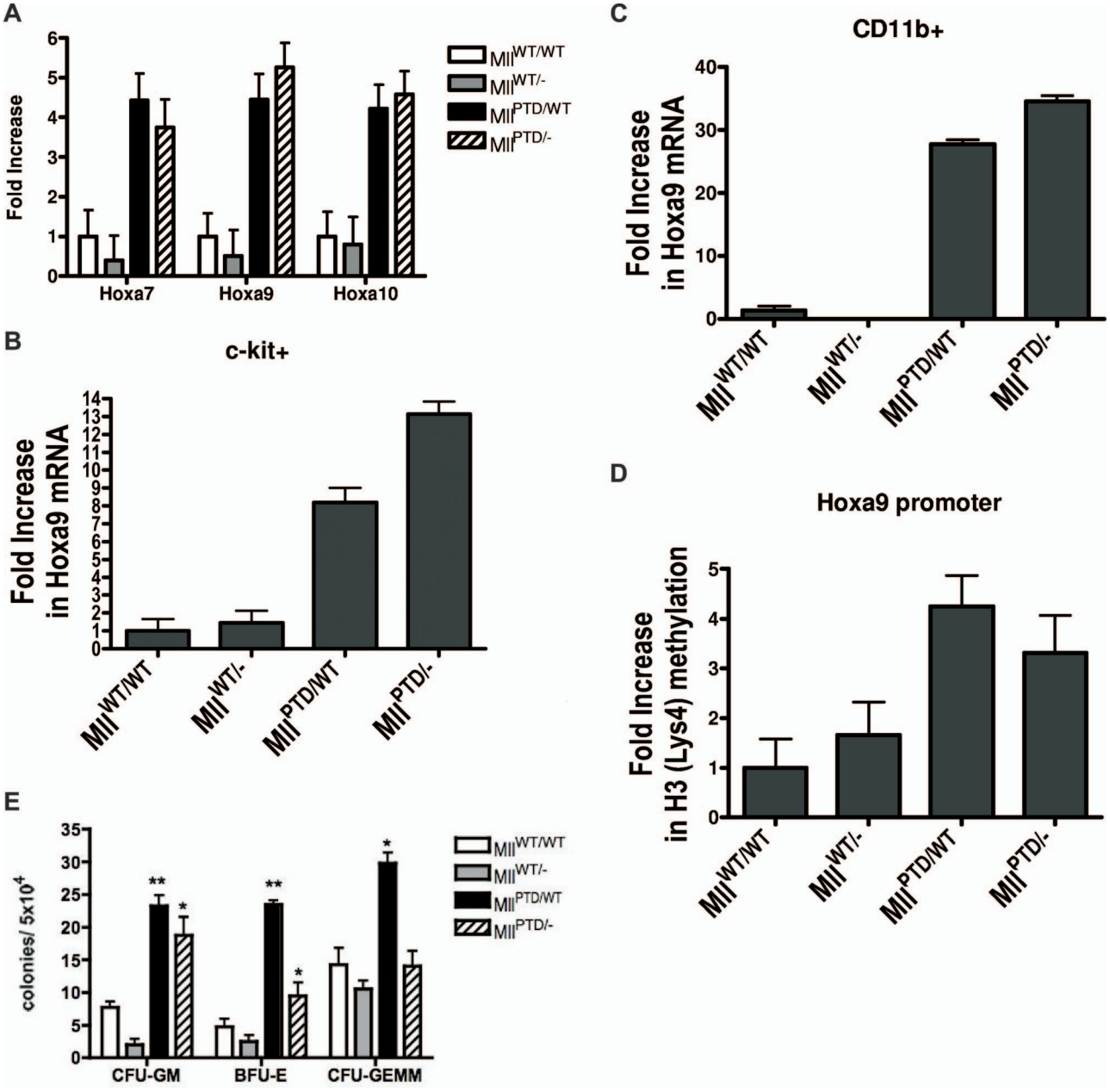

Figure 1. Phenotypic characterization of the *Mll* $^{PTD/-}$ mice. (A) Increased *HoxA* gene expression in *Mll*$^{PTD/-}$ E17.5 FLC. Using real-time RT-PCR, *Hoxa7, Hoxa9,* and *Hoxa10* were shown to be overexpressed in E17.5 FLC from *Mll*$^{PTD/-}$ and *Mll*$^{PTD/WT}$ embryos compared with both *Mll*$^{WT/-}$ and *Mll*$^{WT/WT}$ littermate controls. Error bars show standard deviation. Equivalent numbers of (B) c-kit^{+} and (C) CD11b^{+} FLC were sorted with more than 95% purity from *Mll*$^{WT/WT}$, *Mll*$^{WT/-}$, *Mll*$^{PTD/WT,}$ and *Mll*$^{PTD/-}$ E17.5 embryos. Using real-time RT-PCR, increases in *Hoxa9* were measured in *Mll*$^{PTD/WT}$ and *Mll*$^{PTD/-}$ cells but not in *Mll*$^{WT/WT}$ and *Mll*$^{WT/-}$ cells. Error bars show standard deviation. (D) ChIP experiments using an anti-H3 dimethylated antibody showed increases in the levels of H3 (Lys4) methylation at the *Hoxa9* promoter in both *Mll*$^{PTD/WT}$ and *Mll*$^{PTD/-}$ FLC compared with *Mll*$^{WT/WT}$ and *Mll*$^{WT/-}$ controls. Error bars show standard deviation. (E) E17.5 fetal liver hematopoietic progenitor populations were assessed using CFU assays. *Mll*$^{PTD/WT}$ mice showed increases in CFU-GM, BFU-E, and CFU-GEMM compared with *Mll*$^{WT/WT}$, while *Mll*$^{PTD/-}$ mice showed increases in CFU-GM and BFU-E compared with *Mll*$^{WT/WT}$. Notably, *Mll*$^{PTD/-}$ mice showed substantially lower increases in BFU-E compared with *Mll*$^{PTD/WT}$ mice. Error bars represent standard error of the means. $^{*}P < .05$, $^{**}P < .01$.

Authorship

Contribution: A.M.D. and M.A.C. designed experiments, analyzed and interpreted data, and cowrote the manuscript; A.M.D., S.L., A.C., B.R.P., D.N., M.G., W.Y., and D.C. performed experiments; and S.P.W. and G.M. provided intellectual expertise and careful review and editing of the manuscript. All authors agreed on the final text version.

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