Loss of MLL PHD Finger 3 Is Necessary for MLL-ENL–Induced Hematopoietic Stem Cell Immortalization

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Abstract

Reciprocal chromosomal translocations at the MLL gene locus result in expression of novel fusion proteins, such as MLL-ENL, associated with leukemia. The three PHD finger cassette, one of the highly conserved domains in MLL, is absent in all fusion proteins. This domain has been shown to interact with Cyp33, a cyclophilin which enhances the recruitment of histone deacetylases (HDAC) to the MLL repression domain and mediates HOX gene repression. Insertion of the third PHD finger of MLL into MLL-ENL allows the recruitment of Cyp33 and, subsequently, HDAC1 to the fusion protein. Furthermore, expression of the fusion protein with the PHD finger insertion mediates the down-regulation of the HOXC8 gene expression in a Cyp33-dependent manner. Finally, the addition of the PHD finger domain or the third PHD finger alone into MLL-ENL blocks the hematopoietic stem cell immortalization potential of the fusion protein in serial plating colony assays. Insertion of only the first and second PHD fingers has no such effect. These data support the hypothesis that the binding of Cyp33 to the MLL third PHD finger switches the MLL function from transactivation to repression. In the immortalizing MLL fusion protein, the loss of the PHD fingers, in combination with the gain of the activation domain of ENL or of other partner proteins, makes the fusion protein a constitutive transactivator. This leads to constitutive overexpression of MLL target genes that block stem cell commitment and promote stem cell renewal, probably the first step in MLL-related leukemogenesis. [Cancer Res 2008;68(15):6199–207]

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Introduction

The MLL (HTRX, HRX, ALL-1) gene is frequently rearranged in infant acute leukemia and both de novo and therapy-related leukemia in adults. Reciprocal translocations with a breakpoint at chromosomal band 11q23 generate various MLL fusion genes, encoding chimeric proteins in which MLL contributes its NH2 terminal DNA-binding domains and its repression domain. In the translocations, MLL loses the sequences encoding its highly conserved PHD finger domain, activation domain, and SET domain and fuses in frame with one of up to 40 different partner genes (1). The MLL fusion proteins are postulated to disrupt normal gene expression patterns maintained by wild-type MLL, which contributes to malignancy.

MLL-ENL is a fusion protein encoded by the fusion gene generated by the translocation t(11;19)(q23;p13.3) (2). The fusion partner ENL has been shown to be a transcriptional activator in human lymphoid and myeloid cells (3). Transduction with retroviral MLL-ENL expression constructs immortalizes murine hematopoietic progenitor cells in vitro, and mice adoptively transferred with these immortalized cells develop myeloid leukemia (4, 5). The immortalization depends on the abnormal activation of Hoxa7, Hoxa9, Meis1, and c-Myc (6–8).

Soon after translation, MLL is proteolytically split in two fragments, MLL-N and MLL-C, by the taspase enzyme (9, 10). MLL-N contains the DNA and chromatin-targeting domains of MLL: the three AT hook motifs, several nuclear localization domains, and a repression domain (11), which recruits repression complexes both dependent and independent of histone deacetylase (HDAC) activity (12). MLL-C contains a CBP-binding activation domain and the SET domain (13), which has histone methyltransferase activity with specificity for trimethylation of lysine 4 of histone H3 (14). The two MLL fragments associate in complexes that include other proteins. The presence of both transcriptional repression and activation potentials within the same protein suggests that the normal MLL may switch between these two opposite functions in response to cellular signals.

The PHD finger cassette is one of the highly conserved domains in MLL and is located between the repression domain and the activation domain. PHD fingers, represented by a highly conserved Cys4HisCys3 zinc finger motif, have been found in >300 eukaryotic nuclear proteins and are involved in protein-protein interaction and chromatin-mediated transcriptional regulation (15, 16).

We have previously shown that the third PHD finger of MLL interacts with Cyp33/PPIE (17, 18), an RNA-binding nuclear cyclophilin (19). Cyp33 enhances the recruitment of HDAC1 to the MLL repression domain and down-regulates expression of the HOXC8 gene in an MLL-dependent manner (12, 17). This indicates that Cyp33 may contribute to the intrinsic repression potential of MLL. The MLL repression domain Cyp33 and the PHD finger domain form a repression unit that contributes to the repression potential of MLL-N and balances the activation potential of MLL-C. In MLL-ENL, the loss of the PHD fingers disrupts this repression unit. Therefore, the intrinsic repression potential is compromised, and in the presence of an activation domain on the partner protein, the MLL-ENL fusion functions as a dominant constitutive activator of MLL target HOX genes.

To test the above hypothesis and also to investigate the function of the PHD finger domain in the context of an MLL fusion protein, the DNA sequences encoding either the third PHD finger or the first three PHD fingers of MLL were reinserted into MLL-ENL. Then, we tested the functionality of the restored “repression domain-Cyp33-PHD finger” repression unit and the cellular effects (HOX gene regulation and progenitor cell immortalization) of
expressing this modified fusion protein in human cell lines and mouse primary hematopoietic progenitor stem cells.

Materials and Methods

Constructs. The ePHDf3 (aa1531-1636) or PHDf1-3 (aa1405-1636) were amplified by PCR using full-length MLL cDNA (pcDNA3-MLL-F from M. Seto) as the template, the common reverse primer R 5'-GCTAGGACGGGAGCTGGACATTGCGCTACCTGCT-3' with forward primers 5'-AATGTCGACGGTCTAGGTGAGAAGAGGAGTGG-3' and 5'-AATGTCGACGGTGACATGTGAGAAGAGGAGTGG-3', respectively. The ePHDf3 sequence was then subcloned into pGEMT vector and recovered as Sall-ePHDf3-Nhel fragment. This fragment was then inserted into SalI/NotI-PHDf1-3-NheI fragment. Both fragments were then inserted into SalI/PstI- and SalI/SalI-PHDf1-3-NheI fragment. The ePHDf3 sequence was then subcloned into the pGEMT vector and recovered as Xbal-ePHDf3-Nhel fragment. This fragment was then inserted into XbaI site in MLL exons 1-8. The MScvneo-MLL-ENL construct was used to generate the ePHDf3 insertion mutant (MSCvneo-MLL-ePHDf3-ENL) and the PHDf1-3 insertion mutant (MSCvneo-MLL-PHDf1-3-ENL), respectively. Both constructs were transfected into 293T cells.
correct folding of the motif, we included this 5’ extension sequence as part of the third PHD finger domain (ePHD3, aa1531-1636). The ePHD3 encoding sequence was then inserted in frame into MLL-ENL to form the MLL-ENL insertion mutant (MLL-ePHD3-ENL). We also inserted the DNA sequences encoding the first three PHD fingers (aa1405-1636) into MLL-ENL to form MLL-PHDf1-3-ENL (Fig. 1).

**MLL-ENL with a PHD finger 3 insertion recruits both Cyp33 and HDAC1.** To determine if the insertion of the PHD finger sequence into the MLL-ENL fusion protein restores the interaction between the fusion protein and Cyp33, FLAG-tagged MLL-ePHD3-ENL or FLAG-tagged MLL-PHDf1-3-ENL expression vectors were cotransfected with an HA-tagged Cyp33 expression construct into 293 cells. The fusion proteins were then precipitated with anti-FLAG antibody, and the bound Cyp33 was detected by anti-HA antibody.

All the fusion proteins which included either ePHD3 (Fig. 2A, lane 3) or PHDf1-3 (Fig. 2A, lane 4), were able to coprecipitate Cyp33 whereas their PHD finger-lacking counterpart, MLL-ENL, could not (Fig. 2A, lane 2). A construct expressing a FLAG-tagged 5’ MLL fragment that contains NH2 terminal sequences, including the AT-hooks, and nuclear localization signals but no PHD finger 3, was used as a negative control (Fig. 2A, lane 1). These data suggest that the insertion of the PHD finger sequence into MLL-ENL allows the latter to interact with Cyp33 similar to wild-type MLL.

Because Cyp33 can enhance HDAC1 recruitment by the repression domain of MLL (15), insertion of the PHD finger into MLL-ENL may enhance HDAC1 binding to the fusion protein. To test this possibility, the level of endogenous HDAC1 bound to the fusion proteins was also determined. The results showed that MLL-ePHD3-ENL or MLL-PHDf1-3-ENL coprecipitate more HDAC1 than MLL-ENL (Fig. 2A, lane 3, lane 4 versus lane 2). The 5’-MLL fragment also coprecipitated a small amount of HDAC1 (Fig. 2A, lane 1) similar to that coprecipitated by a FLAG-GFP protein (data not shown). It is noticeable that although two forms of HDAC1 with different mobility were found in the input, the slower form was preferentially coprecipitated.

**The MLL RD2 domain is not required for the recruitment of HDAC1 to MLL-ENL with PHD finger insertion.** The repression domain of MLL can be split into two subdomains, RD1 and RD2, each one of which can mediate repression of reporter genes (11). Both of these domains recruit HDACs (12). To investigate the role that the MLL RD1 and RD2 domains played in the above interactions, the RD2 domain was deleted from MLL-ENL to generate MLL-ΔRD2-ENL. The ePHD3 was then introduced into this RD2-deleted MLL-ENL to form MLL-ΔRD2-ePHD3-ENL (Fig. 1).

As expected, MLL-ΔRD2-ENL did not interact with Cyp33 and could not recruit more HDAC1 than the background level (Fig. 2B, lane 2), but the insertion of ePHD3 restored the interaction (Fig. 2B, lane 3). Addition of the ePHD3 into MLL-ΔRD2-ENL enhanced HDAC1 recruitment (Fig. 2B, lane 3), suggesting that Cyp33 may facilitate the binding of HDAC1 to RD1, as well as to the complete RD1 + RD2 repression domain.

**The PHD finger domain, when inserted into MLL-ENL, allows Cyp33-mediated HOX8 gene repression in human cells.** The next question we addressed was if the insertion of the PHD finger sequences into MLL-ENL has any effects on the regulation of a target gene of these fusion proteins. MLL fusion proteins have been proposed to act as transcription activators that constitutively activate MLL target genes. This idea has been supported by the fact that HOX genes are abnormally up-regulated in primary leukemia cells carrying MLL translocations (22–24) in a leukemia cell line harboring MLL fusion proteins and in MLL-ENL.
The HOXC8 gene is one of the genes that have been thoroughly characterized as a direct target of MLL (14, 25); MLL-AF9 directly activates the HOXC8 promoter in an in vitro reporter gene assay (14). We expected MLL-ENL to have the same effect as MLL-AF9 on the HOXC8 gene. MLL and Cyp33 bind the HOXC8 promoter in HEK293 cells (Supplementary Fig. S1), and Cyp33 overexpression results in reduced acetylation of histones H3 and H4 at this promoter (Supplementary Fig. S2).

High levels of Cyp33 down-regulate HOXC8 gene expression in cells expressing only wild-type MLL, but not in cells that express MLL fusion proteins (17) indicating that Cyp33 may regulate HOXC8 gene expression indirectly through its interaction with MLL. The MLL-ENL protein with the PHD finger sequence insertion may recruit Cyp33 to the HOXC8 gene locus and restore this second layer of regulation.

To test the effect of the different MLL-ENL constructs, described above, on HOXC8 expression, we used a Cyp33-inducible system in the HEK293 cell line. Different pCMV2 plasmid constructs expressing each one of the fusion proteins were introduced into the system, and the expression of Flag-Cyp33 (Fig. 3A and B) and of the Flag-MLL fusion proteins (Fig. 3C and D) was confirmed by Western blot; the HOXC8 mRNA levels were compared in samples with or without Cyp33 induction by RT-PCR (Fig. 4). When Cyp33 is induced in HEK293 cells, the HOXC8 gene is down-regulated, as previously described, for leukemia cells with wild-type MLL (17).

To minimize the variation in transfection efficiency in different experiments, a plasmid expressing a truncated human CD2 cell surface marker was cotransfected with the fusion protein constructs into Cyp33-induced or uninduced HEK293 cells. CD2+ cells, representing the cells that were transfected, were enriched by immunoselection using antibody-conjugated magnetic beads. Twenty-four hours post-co-transfection, Cyp33 overexpression was induced by tetracycline for 36 hours before the samples were harvested. In parallel, Cyp33-uninduced samples were collected 60 hours after cotransfection.

At 36 hours postaddition of tetracycline, Cyp33 was expressed at high levels (Fig. 3) and the HOXC8 gene was down-regulated (Fig. 4). Both uninduced and induced HEK293 cells expressed comparable levels of the fusion proteins, and Flag-tagged Cyp33 was induced at similar levels in tetracycline-treated HEK293 cells (Fig. 3).

The HOXC8 mRNA level in all samples was measured using semiquantitative RT-PCR. Different numbers of PCR cycles were used to amplify the HOXC8 mRNA.
used to insure that the signal was not saturated. The band intensity (at cycle 28 for \textit{HOXC8} signal) was quantified and normalized to that of the \textit{GAPDH} control (at 18 cycles). All samples were then compared with the empty vector–transfected, Cyp33-uninduced HEK293 cells.

In the uninduced HEK293 cells, the expression of all fusion proteins had little effect on the endogenous \textit{HOXC8} gene expression (Fig. 4A and B). MLL-ENL did not up-regulate \textit{HOXC8} in the absence of Cyp33, and this result is consistent with what has been found in a MLL-ENL immortalized murine cell line (7). Upon induction of Cyp33 in the presence of the fusion protein without PHD finger insertion, a relatively high \textit{HOXC8} mRNA level equivalent to that in the uninduced 293 cells was observed (Fig. 4A and B). On the other hand, overexpression of Cyp33, in the presence of the fusion proteins with PHD fingers, resulted in down-regulation of the \textit{HOXC8} mRNA level (Fig. 4A and B).

The RD2 domain of MLL is not necessary for repression of \textit{HOXC8} by Cyp33. We tested if the deletion of RD2 impairs the regulation of \textit{HOXC8} by Cyp33. Expression of the RD2 deleted MLL-ENL fusion protein did suppress the Cyp33-dependent down-regulation of \textit{HOXC8} in HEK293 cells. Expression of the RD2 deleted fusion protein with the included third PHD finger instead did support repression of the \textit{HOXC8} gene after Cyp33 overexpression (Fig. 4C and D).

The insertion of PHD fingers into MLL-ENL suppresses MLL-ENL–induced immortalization of murine bone marrow progenitor cells. Reestablishment of the Cyp33-MLL interaction in the MLL fusion protein by introduction of the PHD fingers may antagonize the hematopoietic stem cell immortalization potential of the fusion protein. To test this hypothesis, we used the methylcellulose colony-forming assay to evaluate the mouse hematopoietic progenitor immortalization potential of various MLL-ENL fusion proteins with or without PHD finger insertion.

Each one of the fusion protein-coding genes with or without PHD and RD domain mutations (expressed from MSCVneo vectors) was introduced into enriched murine bone marrow progenitor cells by retroviral transduction. The expression level of these fusion proteins was monitored by Western blot in the transiently transfected ecotropic packaging Phoenix cells (Fig. 5A). The replating potential of the infected progenitor cells was then evaluated by serial methylcellulose colony-forming assays. Figure 5B shows the detection of the mRNA from various fusion genes by RT-PCR in the neomycin-resistant bone marrow progenitor cells after the primary plating.

![Figure 4. Modulation of the HOXC8 gene expression by the fusion proteins with or without Cyp33 induction. Primers specific to HOXC8 transcripts were used to amplify HOXC8 mRNA from different cell samples, and GAPDH was used as a loading control. A and C, representative gel images of ethidium bromide–stained gels after electrophoretic separation of the RT-PCR products (MLL and ENL are sometimes abbreviated as M and E, respectively). B and D, the relative expression level of HOXC8 in Cyp33 uninduced or induced 293 cells overexpressing various fusion proteins as determined by RT-PCR and estimation of the PCR product amount from image analysis. Columns, mean of the samples in three independent experiments; bars, SD. All samples are compared with empty vector–transfected Cyp33-uninduced 293 cells.](http://cancerres.aacrjournals.org/doi/10.1158/0008-5472.CAN-08-0017)
Expression of the MLL-ENL fusion protein, as previously shown (4, 5), extended the proliferation potential of the progenitor cells, and the cells continued forming colonies after the third serial plating (Fig. 5C). The MLL-ENL–infected cells formed big and compact colonies (Fig. 5C) and maintained an immature phenotype (Fig. 5D). In contrast, empty vector–infected cells stopped proliferating after the second serial plating and generated small and dispersed cell clusters on the plate, indicating these cells are undergoing myeloid differentiation and become quiescent (Fig. 5D).

When the ePHDf3 or PHDf1-3 were inserted into MLL-ENL, the modified proteins could no longer immortalize murine bone marrow progenitor cells, as indicated by the absence of colonies after the third or fourth serial plating; the few colonies formed at the tertiary plating were similar to the cell clusters formed by empty vector–infected cells (Fig. 5D). In similar experiments, in which the MLL PHD fingers 1 and 2 only were inserted into the MLL-ENL fusion protein, the replating potential was conserved through the third plating with average colony counts of 186 (SD, 64) in the third plating in four independent experiments; these colonies were of the same type observed in MLL-ENL–transduced cells.

An MLL-ENL fusion protein without the RD2 domain loses the ability to immortalize hematopoietic progenitors. When we used a MLL-ENL fusion gene without the RD2 domain expressed from a retroviral vector to transduce mouse bone marrow cells and tested these in a serial plating colony essay, the cells behaved as wild-type mouse cells, extinguishing their proliferation potential after two platings. This was independent of the inclusion or not of the third PHD finger coding sequences in the construct. So, the RD2 domain is essential for HSC immortalization.

Discussion

The MLL fusion proteins associated with leukemia conserve the NH₂ terminal sequences of MLL and lose the COOH terminal sequences, including the highly conserved PHD fingers and SET domain and an activation domain that interacts with the histone

Figure 5. Serial colony assays of mouse bone marrow progenitor cells (BMPC) transduced with retroviral constructs. A, expression of various fusion protein constructs (in MSCVneo vector) at the protein level in transiently transfected Phoenix cells detected by immunoprecipitation–Western blot. B, expression of the various fusion genes in the MSCVneo vector (in the same order as in A) at the mRNA level detected by RT-PCR (+RT) in bone marrow progenitor cells after primary plating on methylcellulose medium supplemented with interleukin 3 (IL-3), IL-6, granulocyte macrophage colony-stimulating factor, SCF, and G418. The primers used in the PCR reaction amplify the junction region between 5’ MLL and the ENL portion as shown in the diagram. C, number of primary, secondary, tertiary, and quaternary colonies generated per 10⁴ plated cells after methylcellulose culture of bone marrow progenitor cells transduced with MLL-ENL and its PHD finger insertion mutants. Columns, mean of three independent assays; bars, SD. D, the colonies that appeared on the methylcellulose plates after the tertiary plating were photographed. Cells collected after the tertiary plating were prepared by cytocentrifuging and stained with Wright-Giemsa (Hema3 Stain Set, Fisher Scientific).
acetyltransferase CBP (1, 26). These COOH terminal sequences are replaced by sequences from the partner protein that, in the case of ENL and other partner proteins, has a transcription activation domain.

We have identified Cyp33 as a ligand of the third PHD finger of MLL (17). Upon binding to MLL, Cyp33 mediates a switch from transcriptional activation to repression. This switch to repression is mediated in part by an increased recruitment of HDACs to the repression domain of MLL. Concordantly, the HDAC inhibitor trichostatin A inhibits the switching to repression function (12). The HDAC activity may directly antagonize histone acetylation by CBP, but it may also inhibit the CBP enzymatic activity that is dependent on autoacetylation (Fig. 6A; ref. 27). We postulate that loss of the third PHD finger of MLL from the MLL fusion proteins associated with leukemia eliminates this switch to repression, converting the MLL fusion protein into a constitutive activator (Fig. 6B).

Reinsertion of the MLL PHD fingers 1 through 3 or of the third PHD finger alone in an MLL-ENL fusion protein and expression of this modified protein in HEK293 cells resulted in recruitment of Cyp33 and increased recruitment of HDAC1 to the fusion protein upon Cyp33 overexpression. These results confirm that the repression domain of MLL can recruit HDAC1 in the context of the fusion protein, as has been shown before for the wild-type MLL protein (12), and shows that such recruitment depends on the presence of the third PHD finger (Fig. 6C).

We had previously shown that overexpression of Cyp33 results in repression of the HOXC8 gene in human leukemia cells that have wild-type MLL, but such repression did not occur in leukemia cells expressing MLL fusion genes (MLL-AF9 or MLL-AF6), even in the presence of the wild-type MLL allele (17). In our present experiments, the HOXC8 gene is repressed in HEK293 cells by overexpression of Cyp33, but such repression is inhibited after expression of an MLL-ENL fusion protein. Expression of MLL-ENL alone does not increase the levels of HOXC8 expression in HEK293 cells (Fig. 4A and B, second lane), because the gene is already maximally expressed in these cells. Nevertheless, expression of an MLL-ENL fusion protein with a reinserted third PHD finger again supports HOXC8 repression in this system. These results support the hypotheses that repression of HOX genes upon overexpression of Cyp33 is mediated by MLL, that the interaction of Cyp33 with the third PHD finger is necessary for this repression, and that the
MLL fusion proteins without the third PHD finger are constitutive activators that cannot respond to Cyp33 (Fig. 6).

The third PHD finger of MLL also has all of the features necessary to interact with methylated lysines on histone tails (28). Thus, the presence or absence of this PHD finger may affect the targeting of MLL to chromatin bearing certain histone modifications, whereas the absence of this PHD finger from the MLL fusion proteins may release them from targeting constraints, allowing them to interact with chromatin that normally would not be bound by wild-type MLL. The loss of these targeting constraints, with the loss of the PHD fingers, may contribute to the increased target gene activation by the MLL fusion proteins, but additional experiments will be needed to clarify its role.

Hematopoietic progenitor cells in a serial plating colony assay become exhausted after one or two passages. It has been shown previously that expression of an MLL-ENL fusion protein in mouse bone marrow cells results in dominant immortalization of progenitors, such that they survive for multiple passages without losing their self renewal potential (5). MLL-ENL proteins with the reinserted third PHD finger were not able to support hematopoietic progenitor immortalization. This suggests that the immortalization ability of the MLL fusion protein depends on its constitutive transactivating activity, which is lost with the reinsertion of the third PHD finger. This is not due simply to the separation of the MLL sequences from the ENL sequences by the PHD finger peptide, because a similar fusion with inserted PHD fingers 1 and 2 does immortalize hematopoietic progenitors.

The constitutive transactivating activity of MLL fusion proteins is thus explained by two features of the fusion protein: (a) the incorporation of a transcription activating domain from the fusion partner and (b) the loss of the third PHD finger that controls a repression function of MLL mediated by recruitment of HDACs to the repression domain (Fig. 6).

Nevertheless, this mechanism only applies to a subset of MLL fusion proteins. A different subset seems to form through the acquisition of a dimerization domain but lacks an activation domain (29, 30). It is possible that these MLL fusion proteins activate transcription through some other mechanism. We do not know if the loss of the third PHD finger is a relevant event for leukemogenicity in this subset of leukemia. Nevertheless, in the MLL partial tandem duplications, the third PHD finger is conserved, but only in one copy, whereas the repression domain is present in two copies. It is possible that the presence of an extra repression domain that cannot be targeted by Cyp33 results in constitutive transactivation function for the abnormal protein.

The MLL repression domain is contained in two subdomains, each one of which can repress a reporter gene when attached to a promoter (11). We tested the role of the RD2 subdomain by using MLL-ENL fusion proteins that had a deletion of this domain with or without the inclusion of the third PHD finger. The results showed that the RD2 subdomain is not necessary for the Cyp33-dependent recruitment of HDAC1 or for the Cyp33-dependent repression of the HOX8 gene. This means that the RD1 is sufficient to mediate these two activities and that it can be targeted by Cyp33. Nevertheless, we also found that the RD2 subdomain is necessary for the immortalization activity of the MLL-ENL fusion protein, independently of the inclusion of the third PHD finger in the protein. This observation suggests that RD2 has a function that is nonredundant with RD1 and is independent of Cyp33.

References


Disclosures of Potential Conflicts of Interest

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