

Deep Insight Section

MLL: Deep Insight

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Introduction

The mixed lineage leukemia gene, MLL (also known as MLL1), is disrupted in a variety of aggressive human B and T lymphoid as well as myeloid leukemias (Hess et al., 1997; Brock and van Lohuizen, 2001) (Fig. 1, Table 1). MLL is homologous to *Drosophila* Trithorax, a positive regulator of transcription during development. The best understood target genes of MLL are the clustered homeobox or Hox genes, which are transcription factors that are involved in the specification of cell fate during development. MLL is widely expressed during development and is expressed in most adult tissues including myeloid and lymphoid cells and is required for definitive hematopoiesis (Hess et al., 1997; Ernst et al., 2001). Previous studies showed that Mll (Mll = murine MLL) plays an important role in maintenance of Hox gene expression during development because heterozygous Mll knockout mice showed posterior shifts in Hox gene expression, and Mll knockout mice, which are embryonic, show loss of Hox gene expression after embryonic day 9.5 following normal initiation of expression (Yu et al., 1995).

MLL structure

MLL (3968 aa) is rapidly proteolytically cleaved into two fragments before entering the nucleus (Nakamura et al., 2002; Yokoyama et al., 2002; Hsieh et al., 2003a; Hsieh et al., 2003b) (Fig. 1). The amino terminus of (MLLN) is a 320 kD protein that targets MLL to specific chromosomal sites (i.e. Hox genes). These sequences span the three N-terminal AT hooks, which have been shown to bind the minor groove of DNA. A second region with [40347] homology to DNA methyltransferases (DNMT1) and methyl CpG [41308|41668] binding proteins (MBD1/PCM1) termed RD1 contains a CXXC zinc finger domain that binds CpG rich DNA (Birke et al., 2002). Both the RD1

domain and a C terminal RD2 domain act as independent repressors of transcription (Xia et al., 2003). MLL contains two short domains SNL-1 and SNL-2 conserved with *Drosophila* trithorax (TRX) required for its punctate subnuclear localization (Yano et al., 1997). The C terminus of MLLN contains a region with high homology to trx. This region contains four cysteine-rich zinc finger domains (termed PHD for plant homeodomain) that flank a divergent bromodomain, a domain implicated in binding to acetylated histones (Aasland et al., 1995) (Fig. 1). This region of MLL homodimerizes and is non-covalently associated with the C terminal MLL fragment (Fair et al., 2001; Nakamura et al., 2002; Yokoyama et al., 2002; Hsieh et al., 2003a). The PHD finger region also interact with Cyp33, which may result in transcriptional repression through enhancing binding of histone deacetylases HDAC 1 and 2 to the RD1 and RD2 domains (Xia et al., 2003).

MLL is cleaved C terminal to the PHD fingers at an evolutionarily conserved site by a novel endoprotease termed taspase (Hsieh et al., 2003a) into a 180 kD fragment, (MLLC) with potent transcriptional activating activity (Yokoyama et al., 2002). In addition inhibition of cleavage by taspase knockdown results in inhibition of 3' Hox expression (Hsieh et al., 2003a). Once cleaved the two halves of MLL remain non-covalently associated through an interaction between the FYRN domain of MLLN and the FYRC and SET domain of MLLC. Part of the transcriptional activation by MLLC appears to be the result of recruitment of the histone acetyltransferases CBP and MDF (Ernst et al., 2001) resulting in acetylation of histones H3 and H4 at target genes. In addition the evolutionarily conserved SET domain has intrinsic histone methyltransferase activity specific for histone H3 lysine 4 (Milne et al., 2002; Yokoyama et al., 2002). The SET methyltransferase preferentially methylates histone tails

that are already acetylated. The MLL SET domain homodimerizes and also interacts with INI1, a core component of the SWI/SNF chromatin remodeling complex (Rozenblatt-Rosen et al., 1998), which may also contribute to transcriptional activation.

In addition to its multiple domains involved in transcriptional regulation MLL has been reported to associate with more than 30 different proteins including core components of the SWI/SNF chromatin remodeling complex (BAF170, BAF155, INI1) that are likely involved in transcriptional activation, including [42481] basal transcription factors (TBP, TAFII250, TAFII80, TAFII31, TAFII20), which may be involved in targeting MLL to promoters or else MLL delivers to promoters and two different histone deacetylase (HDAC) containing corepressor [40802][40803] complexes NuRD (Mi2, MTA1-L1, HDAC1, HDAC2, [41310][42298][42202][42201] RbAp46, RbAp48, MBD3, KIAA0601) and Sin3A (Sin3A, SAP30, SAP18, HDAC1, HDAC2, RbAp48, RbAp46 (Nakamura et al., 2002). The MLL complex reported is somewhat controversial in that the complex differs dramatically from other, more recently reported MLL complexes which include MLL complex members WDR5 and Rbpbp5 as well as other mammalian homologs of the proteins in the yeast Set1 complex (Cui et al., 1998; Ernst et al., 2001; Petruk et al., 2001). Surprisingly this core complex also contains menin, the protein mutated in multiple endocrine neoplasia syndrome type I (MEN1), which was required for Hox gene activation (Hughes et al., 2004). Finally the PHD fingers of MLL interact with the cyclophilin Cyp33, an RNA binding protein that may modulate the recruitment of corepressors to the RD2 domains of MLLN (Fair et al., 2001; Xia et al., 2003).

MLL regulates Hox transcription through direct promoter binding and histone modifications

MLL directly binds to Hox promoters such as Hox c8 and Hox a9 promoters and regulates their transcription through both acetylation of histones H3 and H4 at Hox promoter and enhancer sequences as well as histone H3 lysine 4 di- and trimethylation (Milne et al., 2002; Nakamura et al., 2002). Because no known histone demethylases have been identified lysine methylation appears to be a long-term epigenetic mark for sustained transcription (Lachner and Jenuwein, 2002). The repressive PcG proteins are involved in Lys 9 and Lys 27 methylation strongly suggesting that histone methylation accounts for the antagonist effects of trx

and PcG proteins on transcription (Cao et al., 2002; Czermin et al., 2002; Kuzmichev et al., 2002; Muller et al., 2002).

It is unclear whether H3 Lys 4 trimethylation is an absolute requirement for transcription and what the mediators are of this mechanism. Lys 4 methylation might block corepressor recruitment as it is for the histone deacetylase containing NuRD complex (Zegerman et al., 2002). A second possibility is that Lys4 di- or trimethylation creates a docking site for a transcriptional coactivator. The DNA dependent ATPase yeast protein ISW1p and its mammalian homolog hSNF2H, core components of the ISW1 chromatin remodeling complex preferentially bind to di- or trimethylated histone H3 lysine 4 (Santos-Rosa et al., 2003). ISW1p exists in two complexes, one of which ISW1b is required for promoting early stages of transcriptional elongation as well as termination by RNA polymerase II. In yeast both SET1, a lysine 4 methyltransferase homologous to MLL and Isw1p are required for full activation of target genes. Interestingly hSNF2 was also in the MLL complex (Nakamura et al., 2002). These findings suggest that MLL is intimately involved in a Lys 4 methylation and ATPase dependent mechanism to promote transcription (Krogan et al., 2003). Recent work indicates that MLL interacts with RNA polymerase II and acts to promote transcriptional elongation (Lachner and Jenuwein, 2002).

MLL fusion proteins

Leukemogenic MLL rearrangements take a variety of forms including balanced translocations, partial tandem duplications of internal coding regions as well as amplification of apparently unrearranged forms of MLL (Fig. 1, Table 1). In rare cases of T-ALL exon 8 sequences are deleted resulting in disruption of the first PHD finger of MLL (Ayton and Cleary, 2001). However the most common MLL rearrangements are balanced translocations. The most frequent translocations in ALL are the t(4;11) and t(11;19) translocations, associated with expression of MLL-AF4 and MLL-ENL respectively, and pro-B cell or mixed lineage phenotype. Rarely MLL rearrangements such as MLL-ENL are seen in T-ALL. MLL translocations are also common in acute myelogenous leukemia, occurring in about 10% of cases. These leukemias are associated with a different set of translocations including the t(9;11) and t(6;11), which express MLL-AF9 and MLL-AF6 respectively. MLL rearrangements are also common in secondary acute leukemias arising following therapy with topoisomerase inhibitors such as etoposide (Felix, 1998).

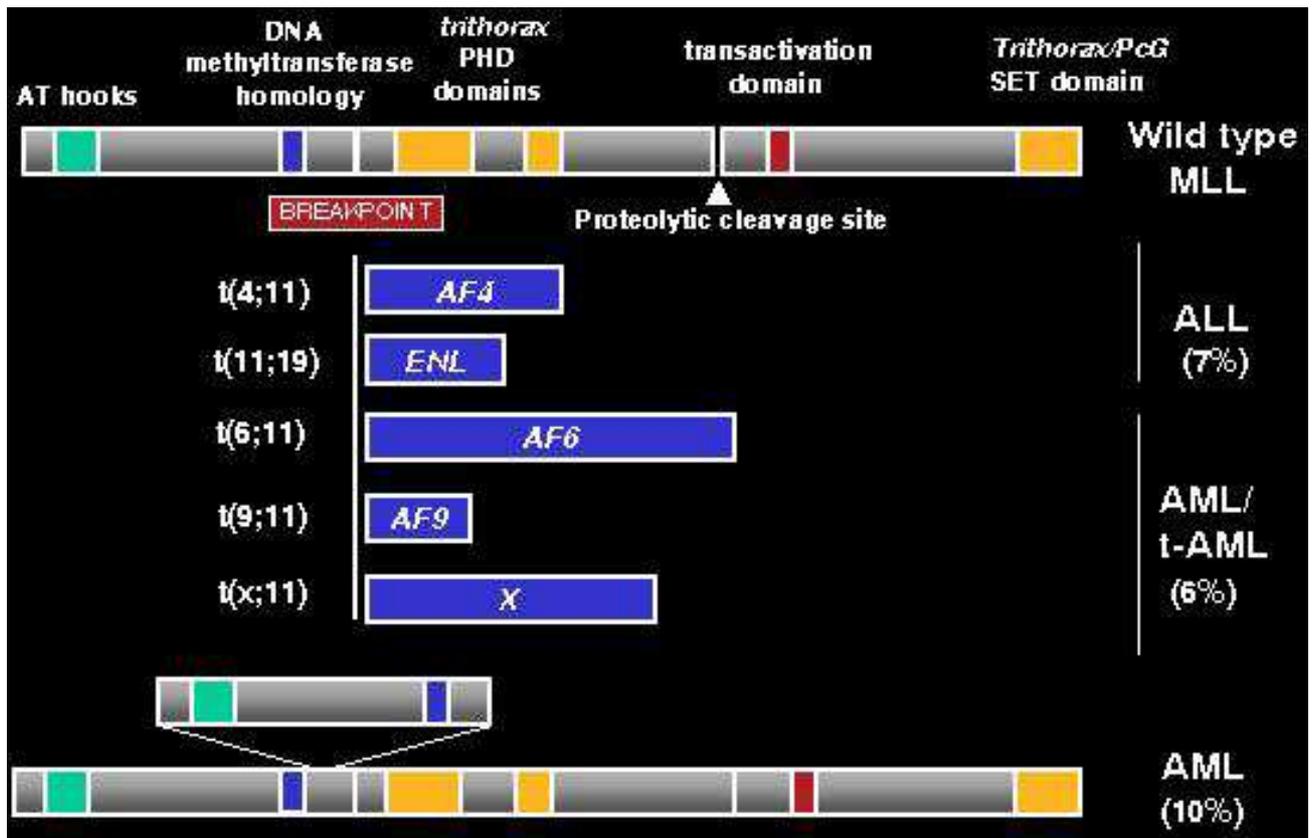


Fig. 1- MLL rearrangements associated with acute lymphoid and myeloid leukemias. MLL is homologous to *Drosophila trithorax (trx)* in a central PHD finger domain as well as in the C terminal SET domain (yellow). The AT hook and DNA methyltransferase homology regions are retained in all MLL rearrangements and are thought to mediate binding to specific chromosomal regions. Balanced translocations disrupt MLL in a breakpoint cluster region that spans exons 8 through 14 thereby deleting the sequences conserved with *trx* and replacing them with one of over 40 different translocation partners. Some such as MLL-AF4 and MLL-ENL are characteristically associated with ALL. Others such as AF6, AF9 and the majority of other translocations (X) are associated with AML, myelodysplasia or etoposide therapy related acute leukemia. In addition about 10% of AML cases without cytogenetic evidence of 11q23 rearrangements show internal tandem duplications of MLL, sometimes in association with trisomy 11.

| | | | |
|------------------|-------------------|---|-------------------|
| AF4 related | AF4 (FEL) 4q21 | Central transcriptional activation domain | pro-B ALL |
| | AF5q31 5q31.1 | Central transcriptional activation domain | pro-B ALL |
| | LAF4 2q11.2~q12 | Central transcriptional activation domain | pro-B ALL |
| AF9 related | AF9 9p22 | Central transcriptional activation domain | AML |
| | ENL 19p13.3 | Anc1 homology C-terminal transcriptional activation 1/2 domain Anc1 homology | AML, pro-B ALL |
| ELL related | ELL (MEN) 19p13.1 | C-terminal transcriptional activation domain RNA polymerase II elongation factor | AML |
| AF10 related | AF10 10p12 | N-terminal PHD finder domain, leucine zipper SWI/SNF interaction, transcriptional activation | AML |
| | AF17 17q21 | N-terminal PHD finder domain, leucine zipper | AML |
| CBP related | CBP 16p13.3 | Transcriptional activation, bromodomain Histone acetyltransferase | AML |
| | P300 22q13 | Transcriptional activation, bromodomain Histone acetyltransferase | AML |
| Forkhead related | AFX Xq13 | C-terminal transcriptional activation domain, forkhead DNA binding[1374] | T-ALL |
| | FKHR-L1 6q21 | C-terminal transcriptional activation domain, forkhead DNA binding domain | AML |

Table 1-Common MLL translocation partners occurring in leukemia

Balanced translocations involving MLL cluster between exons 8 and 14 resulting in deletion of the

PHD and distal domains and in frame fusion to one of many different translocation partners (Fig. 2). The

fusion proteins apparently do not interact with MLLC (Yokoyama et al., 2002). Several lines of evidence suggest that MLL fusion proteins transform by a gain of function rather than loss of function mechanism.

First growth of MLL null hematopoietic progenitors is impaired rather than enhanced (Hess et al., 1997; Yagi et al., 1998; Ernst et al., 2001).

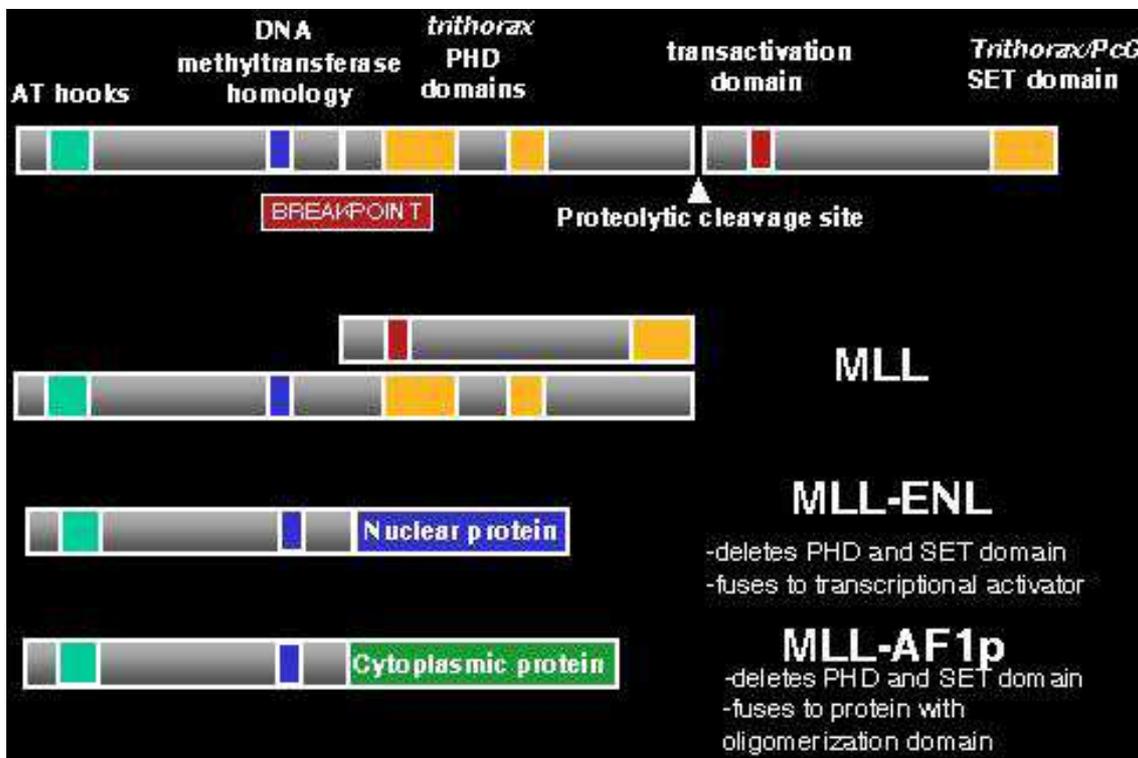


Fig 2. Possible mechanisms of transcriptional activation by MLL fusion proteins. Wild type MLL is composed of a heterodimers of MLLN and MLLC. MLLN directs the MLL complex to specific chromosomal sites including a subset of Hox genes where the complex serves as a regulatable activator of Hox genes. All MLL translocations result in loss of the conserved PHD fingers and SET domain and in frame fusion of MLL to one of more than 40 different translocation partners. The most common MLL fusion proteins involve nuclear translocation partners that act as potent transcriptional coactivators. Recent work suggests that some of the most common translocation partners, such as AF4 and AF9, interact and possibly recruit CDK9 and cyclin T1 to target promoters MLL [Zeisig, D. T., et al., 2005]. This potentially could promote transcriptional elongation through phosphorylation of RNA pol II 6 terminal domain by CDK9. Less commonly, MLL is fused to cytoplasmic translocation partners such as GAS7 and AF1p. These dimerize the truncated MLL molecule. This may involve recruitment of yet to be identified coactivators through MLLN sequences. This does not exclude the possibility that the translocation partner is also involved in coactivator recruitment.

Heterozygous MLL knockout mice do not have an increased incidence of leukemia nor do knock-in mice in which murine Mll is truncated by a Myc epitope tag (Corral et al., 1996). However, if the murine Mll is fused in frame to AF9 so that an Mll-AF9 fusion protein is expressed all of the mice develop first an expansion of myeloid progenitors and ultimately acute myeloid leukemias after a several month latency period (Dobson et al., 1999). These studies suggest that transformation by MLL-AF9 (and presumably other MLL fusions) is mediated by gain of function and in addition that additional genetic “hits” are likely to be required for full transformation. Retroviral transduction of MLL fusion proteins such as MLL-ENL has shown similar findings (Lavau et al., 1997). Studies of conditional forms of MLL fusion proteins suggest this is mediated by imposition of a reversible block on myeloid differentiation (Martin et al., 2003; Zeisig et al., 2004)

The domains in MLL fusion proteins required for transformation includes a serine threonine-rich sequence of unknown function at the extreme amino terminal of MLL as well as the DNMT homology region of MLL that is likely to be important for DNA binding (Slany et al., 1998; Birke et al., 2002; So et al., 2003b). The end result of these fusions is to convert the truncated MLL into a potent transcriptional activator (Schreiner et al., 1999; Zeisig et al., 2003). In most cases this is the result of fusion of MLL to translocation partners that are strong transcriptional activators. For example, both ENL and AF9 are nuclear proteins that activate transcription when tethered to heterologous promoters. An 84 amino acid C terminal domain of ENL conserved with AF9 is all that is required for transformation. Recently ENL has been found to be present in an SWI/SNF like complex termed EBAF, which has nucleosomal remodeling activity (Nie et al., 2003). The complex includes at least 8 proteins found

in other SWI/SNF complexes including INI1 and the Brg1 DNA-dependent ATPase.

The AF4 translocation partner is homologous to two other MLL translocation partners AF5q31 and LAF4, both of which are associated with pro-B cell leukemia. AF4 is also a potent activator of transcription when tethered to target promoters but less is known about its mechanism of activation in part because MLL-AF4 is unable to transform murine bone marrow in standard assays (Ayton and Cleary, 2001). AF4 and AF9 physically interact and colocalize at subnuclear foci suggesting that AF4 activation of transcription involves AF9 and SWI/SNF recruitment. The colocalization of AF4 and AF9 has prompted the "MLL Web hypothesis" that many MLL translocation partners are components of a larger supercomplex containing AF5q31, which itself interacts with CDK9 and cyclin T1 (Debernardi et al., 2002; Erfurth et al., 2004). This is of potential significance because this complex phosphorylates the c terminal domain of RNA polymerase II and therefore may promote transcriptional elongation (Zeisig et al., 2005).

MLL is fused to the known histone acetyltransferases CBP or p300 in some leukemias suggesting that abnormal histone acetyltransferase (HAT) activity may also play a role in MLL-mediated leukemogenesis. Structure function analysis of the MLL-CBP fusion protein shows that both the CBP HAT domain as well as an adjacent bromodomain are required for transformation (Lavau et al., 2000). Given that CBP also interacts with wild type MLL the question arises why when fused to MLL the protein becomes leukemogenic. One possibility is that deletion of potential negative regulatory sequences in CBP results in its abnormal activation. Alternatively covalent fusion may be important because this precludes regulated interactions with CBP that might be required for proper transcriptional regulation. CBP has also been implicated in transformation by other MLL fusion proteins. The MLL translocation partners AFX and FKHRL1 share two domains CR2 and CR3 that have been shown to cooperate in transcriptional activation and transformation (So and Cleary, 2003). The CR3 domain of interacts with CBP (So and Cleary, 2002). Like other forkhead transcription factors AFX induces cell cycle arrest and apoptosis by upregulating expression of p27Kip-1 (Medema et al., 2000). Interestingly MLL-AFX acts as a dominant negative inhibitor that suppresses FKHRL1 mediated apoptosis in myeloid cells suggesting that disruption of the normal activity of the translocation partner may play a role in the pathogenesis of the leukemia (So and Cleary, 2002).

ELL is another translocation partner of MLL that was originally biochemically isolated as an elongation factor for RNA polymerase II (Shilatfard et al., 1996). The central R2 domain of ELL promotes elongation by suppressing polymerase pausing. However structure-

function studies show that a carboxyl terminal R4 domain with transcriptional activating activity and not the R2 domain is necessary and sufficient for myeloid transformation (DiMartino et al., 2000; Luo et al., 2001). This domain has transcriptional activating activity, possibly as a result of recruitment of an ELL associated protein EAF1, which contains a serine/aspartic acid/glutamic acid rich transactivation domain conserved with AF4, LAF4, and AF5q31 (Luo et al., 2001). EAF1 fused to MLL also is transforming in retroviral transformation assays suggesting EAF1 is the critical transcriptional activator required for transformation (Luo et al., 2001). Recent work suggests that menin is required for transformation by multiple MLL fusion proteins. The protein interacts with amino acid sequences in an evolutionarily conserved domain at the extreme amino terminus of MLL, which is shared by both MLL and MLL fusion proteins (Dobson et al., 2000). Deletion of this domain blocks transformation. In addition, genetic ablation of menin blocks transformation and reduces Hox gene expression thought to be pivotal for leukemogenesis.

Despite similarities between some of the more common MLL translocations, many MLL translocation partners are not transcription factors. Some MLL translocation partners have self-association motifs, suggesting that dimerization of MLL is transforming. Further evidence for this is that fusion of MLL to Beta galactosidase, which is a tetramer in solution (Dobson et al., 2000). Subsequent experiments have conclusively shown that dimerization of MLL contributes to transformation by some MLL fusion proteins (Martin et al., 2003; So et al., 2003b).

The mechanisms by which dimerization of MLL converts it into an oncogene are unknown. Dimerization converts truncated MLL into an extremely potent transcriptional transactivator that has increased binding affinity for Hox promoters and that increases the amount of wild type MLL at target loci (Milne et al., 2005b). Whether the wild type MLL is required for transformation is not known. The end result of dimerization of fusion proteins, however, is upregulation of Hox a7, hox a9, and Meis1 expression to levels similar to those seen in leukemias with MLL fused to nuclear translocation partners (Martin et al., 2003) (Fig. 3, Fig. 4).

Partial tandem duplication of MLL

About 10% of AML with normal cytogenetics harbor internal tandem duplications of MLL (Fig. 1), an important finding because these cases are associated with a worse prognosis than those without MLL rearrangements (Schichman et al., 1994). Microarray studies on human leukemias with MLL PTD do not show the characteristic "signature" of Hox gene deregulation as leukemias with MLL translocations, leaving the mechanism of transformation unclear (Ross et al., 2004).

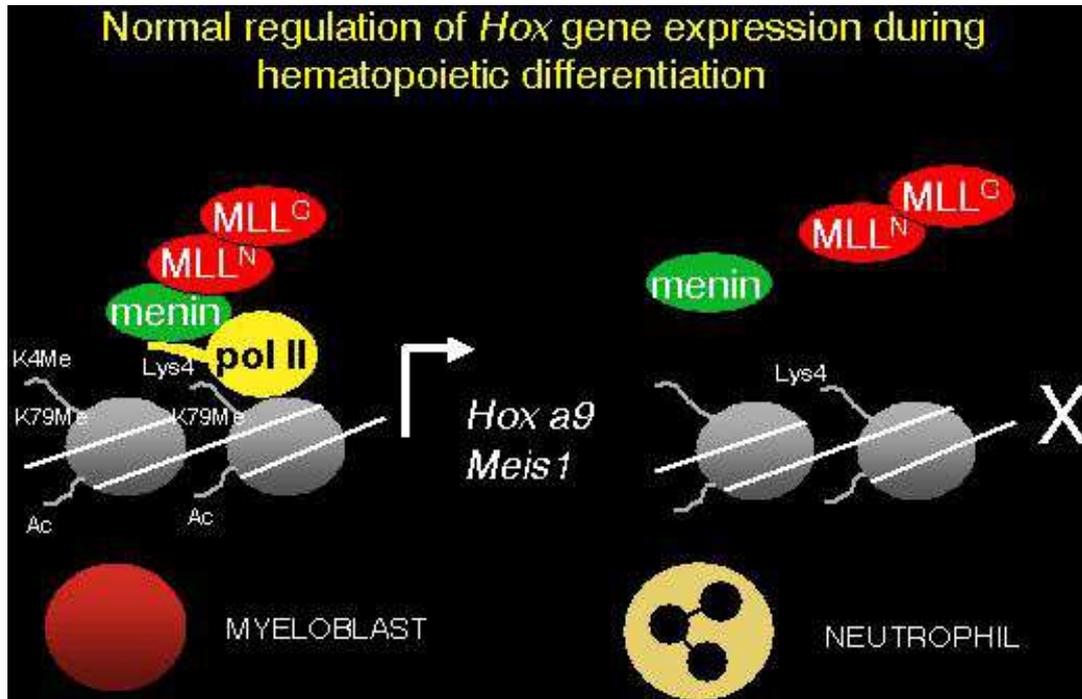


Fig 3. MLL and menin cooperatively regulate expression of Hox genes including Hoxa9 and Meis1. They are normally expressed on early hematopoietic cells. During hematopoietic differentiation, both menin and MLL dissociate from target loci accompanied by a loss of histone modification, including histone H3 and H4 acetylation and H3 lysine 4 and lysine 79 methylation.

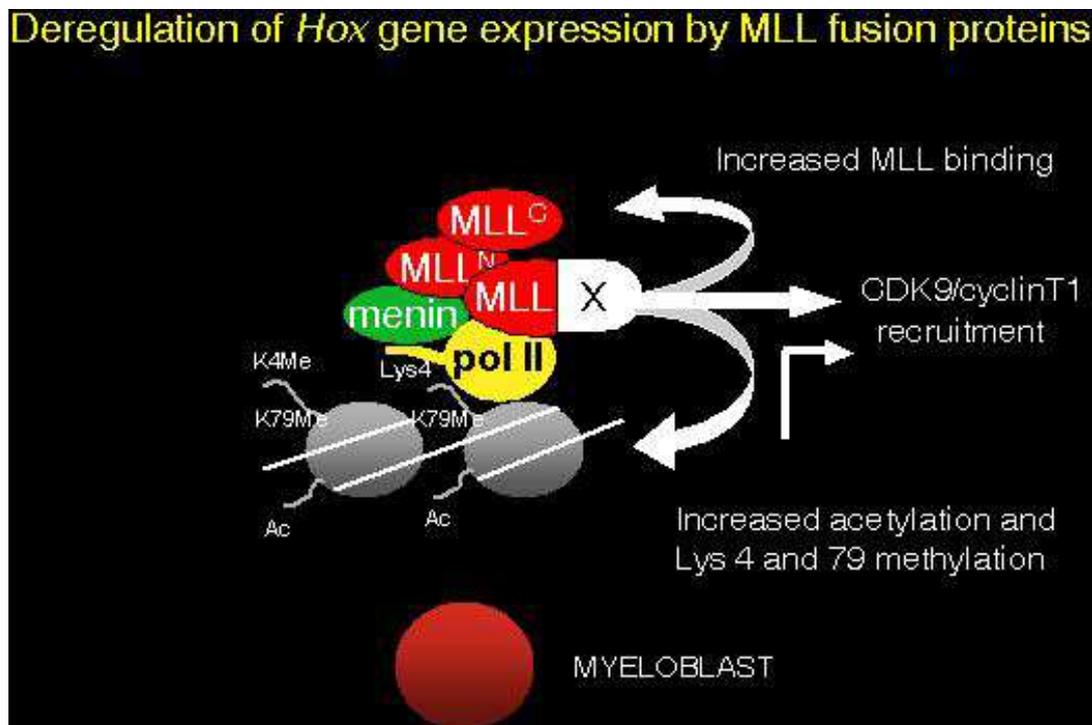


Fig 4. MLL fusion proteins potentially deregulate Hox gene expression through multiple mechanisms. The MLL fusion proteins increase the amount of wild type MLL at target loci (Milne, et al., 2005a). Some MLL translocation partners such as AF5q31 interact with CDK9/cyclin T1 and therefore may influence transcriptional elongation via phosphorylation of the C-terminal domain of RNA polymerase II (Zeisig et al., 2005; Lin, 2005). Either directly or indirectly, MLL fusion proteins induce increased histone acetylation, lysine 4 methylation and, for some fusion proteins such as MLL-ENL, histone H3 lysine 79 methylation (Zeisig et al., 2005).

MLL amplification

In some cases of MDS and AML MLL is present in increased copy number either as the result of additional copies of chromosome 11 or MLL amplification, either cytogenetically undetectable or in high copy number cases where MLL is present in homogeneous staining regions or double minute chromosomes (Thorsteinsdottir et al., 2002; Poppe et al., 2004). The coding region of MLL is apparently unaltered but overexpressed in these cases. Amplification of MLL is also associated with upregulation of at least some of the genes consistently expressed in leukemias with MLL rearrangements including HOX A9, MEIS1, PROM1, ADAM10, NKG2D and ITPA expression, suggesting similar mechanisms of pathogenesis (Poppe et al., 2004).

Hox genes are critical targets of leukemogenic forms of MLL

Hox genes appear to be the critical targets of MLL fusion proteins required for transformation. Hox genes such as Hox a9 are important regulators of hematopoiesis that act in part by promoting stem cell renewal (Sauvageau et al., 2001; Thorsteinsdottir et al., 2002). The A cluster Hox genes including Hox a7 and a9 and the Hox cofactor Meis 1 are normally only expressed in early Sca1+Lin-hematopoietic stem cells and then their expression is rapidly downregulated (Pineault et al., 2002) (Fig. 3). Studies of revealed that Hox a7 and Hox a9 are commonly overexpressed as a result of retroviral integration in leukemias spontaneously arising in BXH2 mice (Moskow et al., 1995; Nakamura et al., 1996). Mice transplanted with murine bone marrow cells transduced with Hox a9 alone resulted in AML with a long latency of up to 6 months (Kroon et al., 1998). The Hox proteins bind to DNA as heterodimers and trimers with members of the TALE (three amino acid loop extension proteins) family members including PBX1, [41647][41648] PBX2, and PBX3 as well as Meis 1 (Schnabel et al., 2000). Notably overexpression of Meis 1 is commonly associated with Hox a7 and a9 expression in BXH2 leukemias, and cotransduction of Hox a9 and Meis 1 rapidly accelerates leukemia development in transplanted mice (Kroon et al., 1998). Human ALL with MLL rearrangements consistently express HOX A7, HOX A9 and MEIS1 in sharp contrast to morphologically and immunophenotypically similar leukemias that lack MLL rearrangements (Rozovskaia et al., 2001; Armstrong et al., 2002; Yeoh et al., 2002; Ferrando et al., 2003). Recent experiments with a conditionally transforming version of MLL-ENL show that expression of Hox a9 and Meis1 can replace the gain of function activity of the fusion proteins indicating these are critical MLL fusion protein targets (Zeisig et al., 2004) and in addition retroviral transformation assays showing transformation by

MLL-ENL requires functional Hox a7 and Hox a9 (Ayton and Cleary, 2003).

Recently some questions have arisen regarding the role of Hox genes in MLL leukemogenesis. MLL-GAS7 was recently shown to transform Hox a9 knock-out bone marrow (So et al., 2003a). In addition Mll-AF9 knock-in mice develop leukemia with the same incidence and latency on either a Hox a9 null or wild type background (Kumar et al., 2003). One possible explanation is that MLL fusion proteins upregulate [40846] multiple other Hox genes including Hox a7, Hox a10 and Meis1 as well as non Hox targets such as Lmo2, N-Myc, and Flt3 (Kumar et al., 2003) and that under certain conditions this is sufficient for transformation.

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