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Deregulation of the HOXA9/MEIS1 Axis in Acute Leukemia

Cailin T. Collins¹ and Jay L. Hess²

¹Department of Pathology, University of Michigan, Ann Arbor, MI 48109

²Indiana University School of Medicine, Indianapolis, IN 46202

Abstract

Purpose of review—HOXA9 is a homeodomain transcription factor that plays an essential role in normal hematopoiesis and acute leukemia, where its over expression is strongly correlated with poor prognosis. This review highlights recent advances in the understanding of genetic alterations leading to deregulation of *HOXA9* and the downstream mechanisms of HOXA9-mediated transformation.

Recent findings—A variety of genetic alterations including *MLL*-translocations, *NUP98*-fusions, *NPM1* mutations, *CDX* deregulation, and *MOZ*-fusions lead to high level *HOXA9* expression in acute leukemias. The mechanisms resulting in *HOXA9* over expression are beginning to be defined and represent attractive therapeutic targets. Small molecules targeting *MLL*-fusion protein complex members, such as *DOT1L* and *menin*, have shown promising results in animal models, and a *DOT1L* inhibitor is currently being tested in clinical trials. Essential *HOXA9* cofactors and collaborators are also being identified, including transcription factors *PU.1* and *C/EBP α* , which are required for *HOXA9*-driven leukemia. *HOXA9* targets including *IGF1*, *CDX4*, *INK4A/INK4B/ARF*, *mir-21* and *mir-196b* and many others provide another avenue for potential drug development.

Summary—*HOXA9* deregulation underlies a large subset of aggressive acute leukemias. Understanding the mechanisms regulating the expression and activity of *HOXA9*, along with its critical downstream targets, shows promise for the development of more selective and effective leukemia therapies.

Keywords

HOXA9; *MEIS1*; *MLL*; AML

Corresponding author: Jay L. Hess M.D., Ph.D., M.H.S.A., Dean of the School of Medicine, Vice President for University Clinical Affairs, Indiana University, 340 West 10th Street, Fairbanks 6200, Indianapolis, IN 46202-3082, Ph: (317) 278-3048, F: (317) 274-8439, jayhess@iu.edu.

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INTRODUCTION

Homeobox proteins are a family of homeodomain-containing transcription factors, first identified in *Drosophila*, that control cell fate and segmental identity during development (1, 2). In mammals the 39 class 1 homeobox, or *HOX* genes, are arranged into four paralogous clusters (A, B, C and D) on separate chromosomes (3, 4). In addition to their role in regulating development, a subset of A and B cluster *HOX* genes also play crucial roles in regulating hematopoiesis and leukemogenesis (5–7). Of these, *HOXA9* has been most intensively studied as it is over expressed in more than 50% of acute myeloid leukemias and in a subset of B and T acute lymphoblastic leukemias (8–10). Understanding the *HOXA9* axis—how its expression is regulated in normal and neoplastic states, how it regulates transcription and which downstream targets are essential for transformation—may lead to new therapies in leukemia and other hematologic malignances.

DEREGULATION OF HOXA9 IN ACUTE LEUKEMIA

HOXA9 is expressed in high levels in hematopoietic stem cells (HSCs) and early progenitors, and its expression is down regulated with further differentiation (Figure 1a) (11). *Hoxa9*-deficient mice show mild pancytopenia and decreased spleen and thymus cellularity (12). Conversely, over expression of *Hoxa9* in mice leads to HSC expansion and a myeloproliferative disorder that progresses to AML (5). The onset of these leukemias is rapidly accelerated by coexpression of *HOX* cofactors, *MEIS1* and *PBX3*, which are often coexpressed at high levels with *HOXA9* in human leukemias (13–16).

A variety of upstream genetic alterations can lead to deregulation of *HOXA9*, including *MLL1*-translocations, *NUP98*-fusions, *NPM1* mutations, *CDX* deregulation, *MOZ*-fusions as well as translocations involving *HOXA9* itself (17). Regardless of the mechanism of deregulation, high-level *HOXA9* expression appears to be a strong independent adverse prognostic factor in acute leukemia (8, 9, 18).

MLL1-Fusion Proteins

Chromosomal translocations involving the mixed lineage leukemia gene *MLL1* at chromosome 11q23 occur in about 10% of AML (19, 20). *MLL1* (also known as *KMT2A*) is a large protein of nearly 4000 amino acids, which is one of five mammalian *MLL* proteins that are homologous to the *Drosophila* protein Trithorax, a positive regulator of homeobox gene expression in the fly. All *MLL* proteins share a C terminal SET domain that has methyltransferase activity specific for histone H3 lysine 4. *MLL* family members antagonize the action of Polycomb group proteins, which form multi-subunit complexes that have histone H3 lysine 27 methyltransferase activity. *MLL1* has been shown to be required for normal hematopoiesis at least in part due to its ability to regulate *HOX* gene expression (Figure 1b) (21). Increasing evidence suggest this involves not only histone methylation, but also recruitment of the histone acetyltransferase MOF (22).

Leukemia associated *MLL1*-translocations result in fusion of *MLL1* to one of over 60 different translocation partners and deletion of the coding regions for the central PHD domains and C terminal SET domain. Nine translocation partners AF1p, AF4, AF6, AF7,

AF10, AF17, ENL, ELL, and SEPT7 comprise 90% of *MLL* translocations (23). These include both nuclear factors (such as AF4, AF9 and ENL) and less common cytoplasmic partners, such as AF1p and SEPT7 (24). *MLL*-fusion proteins enforce high-level *HOXA9* expression, which is required for transformation in most experimental models (25, 26). This mechanism involves recruitment of at least two complexes important for transcriptional regulation. One, the super elongation complex (SEC), contains common *MLL*-translocation partners ELL, ENL or AF9, AF4 and positive transcription elongation factor b (P-TEFb), which includes the cyclin-dependent kinases CDK9 and Cyclin-T (27). The other complex includes the histone methyltransferase DOT1L, which specifically methylates histone H3 lysine 79, and the *MLL*-translocation partners AF10, AF17 AF9 and ENL. *MLL*-fusion proteins either directly or indirectly recruit the P-TEFb and DOT1L complexes to the *HOXA* locus, resulting in large increases in histone H3 lysine 79 methylation and high level transcription (Figure 1c) (28). DOT1L inhibitors have been shown to have efficacy for *MLL*-rearranged leukemias and are currently being tested in clinical trials (29, 30).

Partial tandem duplications involving the sequence encoding the N-terminus of *MLL* are observed in about 10% of cytogenetically normal AML and are also associated with *HOXA9* over expression (31). Interestingly, these have been shown to be sensitive to DOT1L inhibition, despite the fact that they do not involve fusion with partners that interact with DOT1L (32).

Another attractive therapeutic target for *MLL*-rearranged leukemias is menin, which interacts with the amino terminus of both *MLL* and *MLL*-fusion proteins and is required for their recruitment to specific chromosomal sites (Figure 1b). Small molecule inhibitors of the *MLL*-menin interaction, which are under active development, show considerable efficacy in killing AML cells *in vitro* as well as in animal models (33).

Nucleoporin-fusion proteins

Nucleoporins are members of the nuclear pore complex (NPC), which facilitates shuttling of metabolites and molecules between the cytoplasm and nucleus and also plays a role in promoting euchromatic transcription (34, 35). *NUP98* and less commonly *NUP214*, are involved in a number of chromosomal translocations in acute leukemia (reviewed in more detail in (36)). *NUP98*-translocations include fusions with clustered *HOX* genes (*A9*, *A11*, *A13*, *C11*, *C13*, *D11* and *D13*), non-clustered *HOX* genes (*HHEX*, *PRRX1*, *PRRX2*), and non-*HOX* genes such as *NSD1* and *JARID1A* (37). These fusions result in *HOX* gene up regulation, which contributes to leukemogenesis (38). AML and AMKL cases harboring *NUP98*-translocations show consistent *HOXA* and *HOXB* cluster up regulation, with an overall gene expression signature that is distinct from *MLL*-rearranged leukemias (39, 40).

***NPM1* mutation**

Mutations of the chaperone protein nucleophosmin1 (*NPM1*) are common, occurring in about 50–60% of AMLs (41, 42). Leukemia-associated *NPM1* mutations create an additional nuclear export signal resulting in relocalization of *NPM1* from the nucleus to the cytoplasm (43). Cytoplasmic *NPM1* (*NPM1c*) up regulates the expression of *HOXA9*,

HOXA10 and *MEIS1*, possibly as a result of cytoplasmic sequestration of HEXIM1 by NPM1c, which subsequently activates P-TEFb (44–46).

Other mechanisms of *HOXA9* deregulation

Many additional upstream genetic alterations lead to *HOXA9* deregulation in acute leukemia (17). Deletions or decreased expression of the polycomb protein *EZH2* leads to leukemia with up regulation of *HOXA9* (47). Similarly mutations in the polycomb gene *ASLX1* are common in myelodysplastic syndromes and are associated with high expression of *HOXA9* (48). In addition, fusion of *CDX2* and *ETV6* (*ETV6-CDX2*) is seen in rare cases of AML, resulting in high level *CDX2* expression, which promotes *HOXA9* expression (49, 50). Chromosomal translocations generating the *CALM-AF10* (*PICALM-MLLT10*) fusion leads to *HOXA* cluster up regulation in T-ALL and some AML cases (38, 51). The *MYST-CREBBP* (*MOZ-CBP*) is seen in *de novo* and therapy-related AML cases and is also associated with *HOXA9* and *MEIS1* up regulation (52). Finally, rare cases of T-ALL harbor translocations involving the *HOXA9* locus with the T cell receptor β locus, which results in high-level *HOXA9* expression (53).

TRANSCRIPTIONAL REGULATION AND TRANSFORMATION BY H/M

The mechanisms through which *HOXA9* regulates downstream gene expression and specific targets that are essential to transformation are areas of active investigation. It is clear that *HOXA9* is a member of both activating and repressive transcriptional regulatory complexes, which include cofactor and collaborator proteins that provide target specificity and stabilization on the DNA, as well as epigenetic modifiers and transcriptional machinery (Figure 2) (54–56). Early studies with small molecules disrupting protein interactions in *HOXA9*-complexes have been successful at killing leukemia cells both in vitro and in murine studies (16, 57–59). In addition, critical downstream targets of *HOXA9* in leukemia are being more rapidly identified, providing another avenue for targeted therapies in AML. Two comprehensive review articles were recently published discussing *HOX* protein function (60, 61). In the following section, we will highlight the latest publications related to *HOXA9* and its cofactors in leukemia.

Cofactors

HOX proteins regulate downstream gene expression through direct binding at cis-regulatory elements via their highly conserved homeodomains. These 60-amino acid domains are responsible for binding to DNA and for providing an interface mediating protein-protein interactions (62, 63). While homeodomains are highly conserved across the 39 mammalian *HOX* proteins, multiple studies suggest that homeodomains confer unique properties via their small differences (64, 65). For example, swapping the homeodomains of *HOXA9* and *HOXA1* abolishes the leukemogenic properties of *HOXA9* while conferring those properties to *HOXA1* (66).

HOXA9 binds DNA along with a small subset of cofactors, many of which are members of the Three-amino-acid-loop-extension (TALE) family. The best characterized of these cofactors is *MEIS1*, which plays a synergistic causative role in leukemia with *HOXA9* (14,

15). A recent study with Meis1 knockout/MLL-AF9 knockin murine model established that Meis1 is required for development of MLL-AF9 driven leukemias (67). This requirement is partially mediated through promoting a low oxidative environment established by direct regulation of HLF by Meis1. In addition, MEIS1 is responsible for recruiting CREB and CBP to HOXA9 binding sites in a GSK-3 dependent manner, which is required for maintaining the MLL leukemia stem cell transcriptional program (58). This interaction can be targeted using GSK-3 inhibitors, leading to growth inhibition of cells transformed by either HOXA9/MEIS1 or MLL-fusion proteins (57–59). The oncogenic properties of MEIS1 are antagonistically regulated by PREP1, another TALE family protein, through direct competition for binding sites (68). PREP1 also competitively heterodimerizes and sequesters PBX proteins, thereby decreasing stability of MEIS1 and preventing the MEIS1-DDX3x/DDX5 interactions that are required for tumorigenesis (69, 70). Furthermore, *in vivo* leukemogenesis studies with HOXA9/MEIS1 in PREP1-deficient cells showed more aggressive leukemias compared to wild-type (69).

Recent work has characterized the requirement of a second TALE cofactor, PBX3, in the setting of leukemia with high expression of *HOXA9*. PBX3, and not PBX1 or PBX2, was found to be essential for MLL-fusion protein mediated transformation. Disruption of the PBX3/HOXA9 interaction with the small molecule HXR9 selectively kills leukemic cells, providing a promising strategy for developing future therapies (16). PBX3 also dimerizes with MEIS1 and inhibits its ubiquitination and degradation, thereby increasing the half-life of MEIS1 and enhancing the proliferation and colony forming ability of primary cells transduced by *HOXA9* (71). This MEIS1/PBX3 dimerization is required for expression of HOXA9/MEIS1 target genes such as *FLT3* and *TRIB2* (71). Moreover, coexpression of MEIS1/PBX3 is sufficient to transform cells in culture and lead to formation of leukemia *in vivo* with similar latency to that of MLL-AF9 (72). Expression patterns in cells transformed by MEIS1/PBX3 are consistent with that of the MLL-AF9 core transcriptome, including upregulation of *HOXA9* (72).

Collaborators

It is likely that tissue specific collaborator proteins provide a final level of binding specificity to HOX complexes (73). Recent work has shown that collaborators establish areas of chromatin accessibility, provide stability in DNA binding and help modulate the downstream activity of HOX complexes (74). Two such collaborators are the lineage-specific transcription factors, PU.1 and C/EBP α , which are known to establish areas of relaxed chromatin and allow for signal-dependant recruitment of additional proteins (75). PU.1 was recently found to be essential for MLL-induced leukemias and was shown to directly regulate key genes in the HOX/MEIS signature, including *FLT3* and *c-KIT* (76). Taken together with previously published work, which found that HOXA9 and PU.1 physically interact and that the PU.1 binding motif is enriched at HOXA9 binding sites, these findings suggest PU.1 may be an essential member of a HOXA9 transcriptional regulatory complex (54). PU.1 also plays a role in leukemias without *MLL*-translocations, potentially through direct activation of *MEIS1* via binding at the MEIS1 promoter (77).

Multiple recent publications have established that C/EBP α is required for MLL-fusion protein and *HOXA9*-driven leukemias. Initial studies found that the initiation of MLL-ENL transformed leukemias required C/EBP α , whereas it was not required for maintenance of transformation (78). Loss of C/EBP α in MLL-ENL transformed cells also resulted in decreased expression of *HOXA9/MEIS1*, however expression of *HOXA9/MEIS1* could not rescue transformation in these cells. Work from our lab found that loss of C/EBP α significantly improved survival in murine *in vivo* leukemogenesis models of *HOXA9/MEIS1*-driven AML (10). We also established that C/EBP α and HOXA9 physically interact and colocalize at over 50% of HOXA9 binding sites, raising the possibility that C/EBP α is an essential member of the HOXA9-transcriptional regulatory complex. More recent work also suggests that C/EBP α -driven myeloid differentiation, rather than C/EBP α itself, is required for initiation of MLL-rearranged leukemia (79). The failed transformation of MLL-AF9 in C/EBP α deleted cells could be rescued with cytokine induction of GMP formation. It is possible that this requirement is due to the need for myeloid specific enhancers for proper downstream HOXA9 function. Similarly, it is likely that collaborators such as PU.1 and C/EBP α are cell type-specific and that HOXA9 would have different collaborators in B and T-cell leukemias.

Targets in leukemia

Considerable progress has been made towards understanding HOXA9-mediated leukemogenesis through the identification of proleukemic targets with cis-regulatory regions bound by HOXA9 and MEIS1 (54). Many of these targets have been studied individually and found to play important roles in leukemic transformation (comprehensively reviewed in (60)). Recently additional critical targets have been identified and mechanistically studied. HOXA9 was shown to regulate *IGF1* expression through binding at an upstream putative promoter and DNA hypersensitive region in intron 1 (80). *IGF1*-null cells transformed by *HOXA9* have reduced leukemogenic potential and increased apoptosis in response to serum starvation. HOXA9 is also involved in a feedback loop along with HOXA10 that directly regulates *CDX4* expression during normal hematopoietic differentiation (81). In the context of leukemia, MLL-ELL cooperates with constitutively activated SHP2 mutants to block the tyrosine phosphorylation of HOXA9/10 that is required for repression of *CDX4*, thereby contributing to the sustained expression of *CDX4* and leukemic transformation (81).

Multiple studies have implicated a role for HOXA9 in the regulation of *INK4A/B* expression, critical mediators of HSC self-renewal, apoptosis and oncogene-induced senescence whose expression leads to a block in cell cycle at the G1 phase (82). Hoxa9 represses *Ink4a* expression to overcome oncogene-induced senescence during transformation by AML1-ETO in *Bmi1*^{-/-} cells, as well as in *Hoxa9/Meis1* transformed cells (10, 83). This repression may be the result of direct recruitment of EZH2 by Hoxa9, however recent work suggests that EZH2 may also regulate *p16* in HOXA9-independent fashion in MLL-rearranged leukemias (84, 85).

Finally, HOXA9 is involved in antagonistic regulation of GFI-1 target microRNAs, *mir-21* and *mir-196b*, whereby direct binding of HOXA9 to cis-regulatory regions increases expression of these microRNAs (86). Targeting *mir-21* and *mir-196b* with antagomirs results

in specific inhibition of colony forming activity and leukemia-initiating cell activity in *HOXA9*, *NUP98-HOXA9*, and *MLL-AF9*, and also leads to leukemia-free survival in *MLL-AF9* murine leukemogenesis studies. Furthermore, samples from patients with *MLL*-translocated and *NPM1* mutant leukemias showed specific growth inhibition in colony forming assays when treated with the antagonomirs, providing a promising therapeutic approach for *HOXA9* driven AML (86).

CONCLUSION

While significant progress has been made towards our understanding of the genetic alterations leading to deregulation of *HOXA9* and the mechanisms of *HOXA9*-mediated transformation, many answered questions remain. It will be important to identify the cofactors, interactions and downstream activities of *HOXA9* that are essential for leukemogenesis. *HOXA9* is associated with both activation and repression of transcription, with the latter being particularly poorly understood. In addition, the downstream targets that are essential for leukemogenesis are only beginning to be defined using genome-wide screening. Given the central role of *HOXA9* proteins in development and oncogenesis, further work is warranted which may have broad applicability towards the development of more potent and selective therapies.

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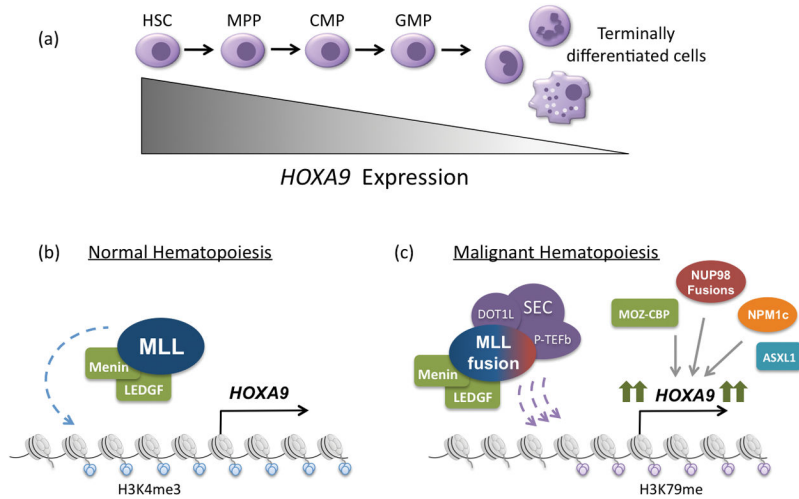
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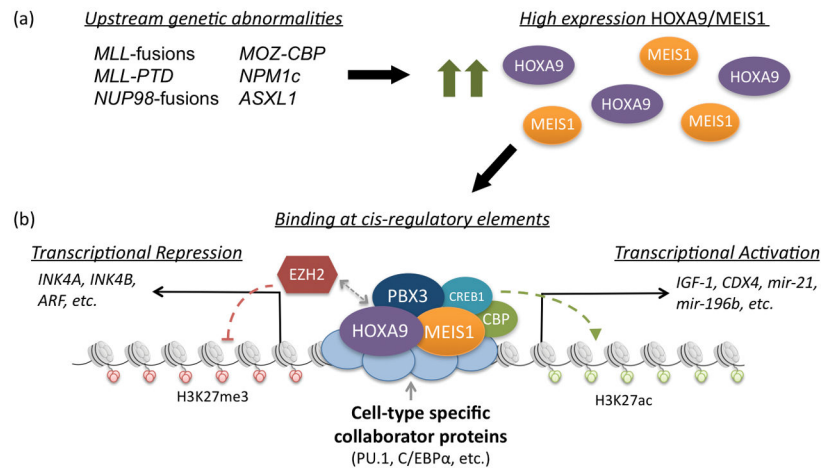
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KEY POINTS

- *HOXA9* is highly expressed in a variety of hematopoietic malignancies and is generally associated with poor prognosis.
- A variety of upstream genetic alterations lead to high expression of *HOXA9* including *MLL1*-translocations, *NUP98*-fusions, *NPM1c* mutations, *CDX* dysregulation, *MOZ*-fusions and translocations of *HOXA9* itself.
- Small molecule inhibitors targeting DOT1L and menin are showing efficacy in *HOXA9*-driven leukemia models.
- *HOXA9* regulates gene expression through binding at cis-regulatory elements with a subset of essential cofactors (MEIS1 and PBX3) and cell-type specific collaborators (PU.1, C/EBP α).
- Further study of *HOXA9*-mediated leukemogenesis is warranted given its central role in development and oncogenesis.

**Figure 1.**

Regulation of *HOXA9* expression in normal and malignant hematopoiesis. (a) During normal hematopoiesis, *HOXA9* is expressed most highly in early progenitor cells and its expression is subsequently down regulated as cells become terminally differentiated. (b) In normal hematopoiesis, *HOXA9* expression is regulated by the MLL histone methyltransferase, which deposits activating histone 3, lysine 4 trimethylation (H3K4me3) along the *HOXA9* locus. This process requires interaction with menin and its cofactor LEDGF. (c) In approximately 50% of acute leukemias, *HOXA9* is highly expressed as the result of a variety of upstream genetic alterations. These include *MLL1*-fusions, *NUP98*-fusions, *MOZ-CBP* fusions, *NPM1c* mutations and *ASXL1* mutations. In the case of *MLL1*-fusions, one of 60 translocations partners is fused to the C-terminus of MLL, resulting in recruitment of the SEC (including DOT1L) and PTEF-b complexes. DOT1L is responsible for depositing activating histone 3 lysine 79 methylation, leading to high *HOXA9* expression and malignant transformation. The other genetic abnormalities also result in high *HOXA9* expression, though the mechanisms are less well understood.

**Figure 2.**

Model for *HOXA9*-mediated leukemogenesis. (a) A variety of upstream genetic abnormalities are associated with high expression of *HOXA9* and its cofactor *MEIS1*, both of which are known to play an essential role in acute leukemias. (b) *HOXA9* and *MEIS1* promote malignant transformation through binding at cis-regulatory elements throughout the genome, whereby they activate and repress downstream gene expression. The targeting and stabilization of *HOXA9/MEIS1* at specific loci is likely mediated by cell-type specific collaborator proteins, such as PU.1 and C/EBP α . Upon binding at loci along with the additional cofactor PBX3, *HOXA9/MEIS1* recruit coactivating and corepressing histone modifying complexes, such as CREB1/CBP and EZH2 respectively. Recently established activated targets include *IGF-1*, *CDX4*, *mir-21* and *mir-196b*. Recently established repressed targets include *INK4A*, *INK4B* and *ARF*.