HHS Public Access

Author manuscript

Oncogene. Author manuscript; available in PMC 2016 March 03.

Published in final edited form as:

Oncogene. 2016 March 3; 35(9): 1090–1098. doi:10.1038/onc.2015.174.

Role of HOXA9 in leukemia: dysregulation, cofactors and essential targets

Cailin T. Collins[1] and **Jay L. Hess**[2]

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

[2]Indiana University School of Medicine, Indianapolis, IN 46202

Abstract

HOXA9 is a homeodomain-containing transcription factor that plays an important role in hematopoietic stem cell expansion and is commonly deregulated in acute leukemias. A variety of upstream genetic alterations in acute myeloid leukemia (AML) lead to overexpression of *HOXA9*, which is a strong predictor of poor prognosis. In many cases, *HOXA9* has been shown to be necessary for maintaining leukemic transformation, however the molecular mechanisms through which it promotes leukemogenesis remain elusive. Recent work has established that HOXA9 regulates downstream gene expression through binding at promoter distal enhancers along with a subset of cell-specific cofactor and collaborator proteins. Increasing efforts are being made to identify both the critical cofactors and target genes required for maintaining transformation in *HOXA9*-overexpressing leukemias. With continued advances in understanding *HOXA9*-mediated transformation, there is a wealth of opportunity for developing novel therapeutics that would be applicable for the greater than 50% of AML with overexpression of *HOXA9*.

Keywords

HOXA9; leukemia; cofactor; enhancer

INTRODUCTION

HOX proteins are a family of homeodomain containing transcription factors that were first described in *Drosophila* for their ability to produce homeotic transformations – that is, changing one section of the body into another – when misexpressed during development (1, 2). Since this early discovery, an entire field has been devoted to studying these master regulators of developmental processes and their role in disease. The 39 mammalian *HOX* genes are arranged into four parologous clusters on separate chromosomes, allowing for the tight transcriptional control required to establish the anterior-posterior body plan and assign tissue fate (3, 4). As such, dysregulation of *HOX* genes results in a variety of developmental disorders and malignancies.

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.

HOXA9 is of particular interest as it has been shown to be over expressed in more than 50% of acute myeloid leukemias and is highly associated with poor prognosis (5–9). A variety of upstream genetic alterations can lead to dysregulation of *HOXA9*, including *MLL*-translocations, NUP98-fusions, *NPM1c* mutations, *CDX* dysregulation and MOZ-fusions. One challenge to defining the mechanisms through which HOXA9 over expression contributes to AML is the relative lack of understanding of how HOX proteins regulate gene expression. Recent work suggests that HOXA9 binding specificity is achieved through a combination of motif affinity, interactions with cofactor and collaborating proteins, and context-specific chromatin accessibility (10–12). In addition, multiple studies have established that HOXA9 can both activate and repress downstream gene expression, though the mechanisms for these actions are relatively unknown. Finally, increasing efforts are being made towards identifying the critical downstream targets of HOXA9 required for transformation in AML. In this review, we will highlight recent advances in understanding the role of HOXA9 in leukemia and discuss important questions that remain in the field.

REGULATION OF *HOX* GENE EXPRESSION

During development, *HOX* genes follow both a temporal and spatial pattern of expression, such that 3' *HOX* genes are expressed earliest in the embryo and in the anterior regions, while 5' *HOX* genes are expressed at later stages and more posteriorly (3, 4). The tight regulation of *HOX* expression is the coordinated effort of a variety of factors including epigenetic regulators, early developmental transcription factors, and long non-coding RNAs (13–15). Additionally, it is becoming clear that the 3D localization of the *HOX* loci within the nucleus also plays an important role in coordinating expression (16, 17).

The two master epigenetic regulators of *HOX* gene expression, including *HOXA9*, belong to the Trithorax and the Polycomb group histone methyltransferases, which activate and repress transcription respectively (18). The mixed lineage leukemia (MLL) methyltransferase positively regulates *HOXA9* expression by trimethylating histone 3 lysine 4 (H3K4me3) at its promoter (19). This activity is directly antagonized by the sequential activity of polycomb repressive complexes PRC1 and PRC2, responsible for trimethylating histone 3 lysine 27 (H3K27me3) (18). Studies in both *Drosophila* and mice have found that, similar to mutations in individual HOX proteins, mutations in trithorax proteins/MLL can lead to homeotic transformations (20). In addition, loss of MLL in mouse models leads to profound impairment of hematopoiesis (21, 22). As such, alterations in the activity or expression of *MLL* or PRCs can lead to a variety of both developmental disorders and hematopoietic malignancies (23, 24).

Along with MLL and PRC methyltransferases, the CDX family of transcription factors also play an important role in regulating *HOXA9* expression during embryonic hematopoiesis (25). CDX1, 2 and 4 are members of the unclustered ParaHox class of homeobox genes that, like HOX proteins, contain a DNA-binding homeodomain (26). Studies in various model systems show that CDX proteins activate expression of *HOX* genes primarily in the A and B clusters, though the mechanisms for this regulation are unknown (27–29). In addition, studies in zebrafish have established a requirement for CDX4 in maintaining *HOX* gene expression during embryonic hematopoiesis (27, 30).

Along with epigenetic modifiers and early transcription factors, *HOX* gene expression is also regulated by long non-coding RNAs (lncRNAs), though direct regulation of *HOXA9* by lncRNAs has yet to be established. LncRNAs can activate or repress *HOX* genes through the interaction and recruitment of trithorax and polycomb histone modifying complexes. Both HOTTIP (HOXA transcript at the distal tip) and Hoxb5b6as, lncRNAs expressed from the 5' region of HOXA13 and the Hoxb5/6 locus respectively, can interact with trithorax group proteins to maintain active transcription of their corresponding gene clusters (15, 31). Conversely, HOTAIR (HOX antisense intergenic RNA) is a lncRNA that is transcribed from the HOXC locus that functions to maintain repression of the HOXD locus through interaction with PRC2 and histone demethylase LSD1 (32). While there are currently no studies of HOX-specific lncRNAs in leukemia, misexpression of these lncRNAs has been observed in a variety of solid tumors, suggesting a possible role in hematopoietic malignancies as well (33–42).

As technologies for identifying long range chromatin interactions and mapping genome-wide chromosome conformation continue to improve, it is becoming clear that the chromosomal conformation and physical location of genes in the nucleus contributes greatly to the regulation of global gene expression (43). Recent work has established that the 3D localization of the *HOX* genes within the nucleus plays an important role in their regulation. Studies in *Drosophila* have shown that subsets of *HOX* genes frequently colocalize in distinct nuclear foci, called Polycomb repressive bodies, leading to coordinated repression of these targets (16, 17). DNA regulatory elements that contribute the physical interaction and colocalization of these loci are required for effective expression silencing, however these interactions are also topographically constrained by chromatin architecture (17).

ROLE OF HOXA9 IN HEMATOPOIESIS

Upon completion of development, most *HOX* genes are transcriptionally silenced, however certain members of the A, B and C clusters are important regulators of adult hematopoiesis (44, 45). Expression of *HOX* genes in hematopoiesis follows a pattern similar to that during development such that anterior *HOX* genes (*HOX1–6*) are expressed in early uncommitted progenitors while posterior *HOX* genes (*Hox7–13*) are expressed in myeloid and erythroid-committed CD34+ cells (45). As cells become fully mature and lose CD34 positivity, *HOX* gene expression is silenced. The functional redundancy of many of the HOX proteins is such that knockout models of many *HOX* genes result in only mild hematopoietic phenotypes. Loss of individual *HoxB* genes and even the entire *HoxB* locus leads to only slight reduction in bone marrow cellularity, without significantly affecting the ability of HSCs to repopulate bone marrow (46–50). Similarly, loss of *HOX* genes in the A and C cluster leads to mild lineage skewing affecting primarily the erythroid compartment (51–55). *HOXA9* is the most highly expressed *HOX* gene in the hematopoietic compartment, and as such *Hoxa9*$^{-/-}$ mice display the most dramatic hematopoietic phenotype in knockout mouse models (56, 57). While loss of *Hoxa9* in murine models leads to only mild pancytopenia, competitive repopulation assays uncover a significant reduction in *Hoxa9*$^{-/-}$ fetal liver HSC repopulation capacity compared to normal HSCs (58). In addition, over expression of *HOXA9* leads to expansion of HSCs and early progenitors, leading to myeloproliferative phenotypes in mice (59). It should be noted that this myeloproliferation will not progress to

AML in the absence of additional genetic factors, such as the co-expression of its cofactor *MEIS1* or fusion to nucleoporin protein Nup98. In the case of *MEIS1*, which is almost always expressed at high levels along with *HOXA9* in human disease, co-expression leads to a rapidly fatal leukemia in mice with an average latency of 40–60 days (50, 60, 61).

DYSREGULATION OF HOXA9 IN ACUTE LEUKEMIA

The most broadly studied diseases with dysregulation of *HOX* genes are acute leukemias (59, 62, 63). In most cases, *HOX* genes are expressed at high levels in acute leukemias, with *HOXA9* in particular having a 2–8 fold higher expression in AML compared to healthy controls in about 50% of cases (6, 64). High expression levels of *HOXA and B* genes has been associated with an intermediate to unfavorable prognosis in acute leukemias (5, 7, 9, 65). In one study, HOXA9 was found to be the single strongest predictor of poor prognosis in acute myeloid leukemia (8). It should be noted that high expression of *HOXA9* often coincides with upstream genetic alterations that themselves have negative prognostic values in AML. As such it is difficult to determine if *HOXA9* is a predictor of poor prognosis independently of concurrent genetic alterations. On the other hand, HOXA9 has been shown to directly regulate critical downstream genes such as *Bcl-2* and *Ink4a/ARF/Ink4b*, which themselves are linked to poor outcomes, providing plausible evidence for a direct role for HOXA9 in determining prognosis in AML (66, 67). Additionally, the wide variety of upstream genetic alterations that lead to over expression of *HOXA9* suggests that it serves as a common pathway for leukemic transformation.

MLL-Fusion Proteins

About 10% of acute leukemias harbor chromosomal translocations at the 11q23 locus involving *MLL*, that are associated with an aggressive clinical course (68). There have been over 60 different fusion partners of MLL identified, though 90% of these translocations involve one of nine partners: AF1P (EPS15), AF4 (AFF1), AF6 (MLLT4), AF9 (MLLT3), AF10 (MLLT10), AF17 (MLLT6), ENL (MLLT1), ELL, and SEPT6 (69). In addition, a partial tandem duplication event can occur within the N-terminus of MLL, which is observed in about 10% of cytogenically normal AML (70). MLL fusion proteins constitutively up regulate *HOXA9* expression, which is both required and sufficient for maintaining leukemic transformation (71, 72). The up regulation of *HOXA9* is directly linked to histone 3 lysine 4 trimethylation at promoters by MLL-fusion proteins, however there has also been documentation of DNA hypomethylation at various HOX promoters in MLL-fusion leukemias (73).

NUP98-Fusion Proteins

NUP98 is a member of the nucleoporin family of proteins that coassociate to form multisubunit channels in nuclear membranes. These nuclear pore complexes (NPCs) were first described for their role in facilitating transfer of metabolites and molecules between the cytoplasm and nucleus (74). Recent work has found that NPCs also play a critical role in defining the chromatin landscape in the nucleus and facilitating gene transcription from euchromatic regions of the genome (75). Nucleoporins are involved in chromosomal translocations that can lead to acute leukemias, most commonly involving *NUP98* (reviewed

in (76)). The most potent *NUP98* oncogenes are those fused to one of eight homeobox partners, including HOXA9 (77). These fusions in turn lead to general up regulation of additional *HOX* genes including *HOXA5, HOXA7, HOXA9* and *HOXA10,* which contribute to leukemogenesis (78). In addition, fusions with *NSD1* and *JARID1A* upregulate *HOXA* and *HOXB* in AML and AMKL (79, 80). It is noteworthy that, aside from increases in *HOX* genes, these leukemias have an expression signature distinct of that from *MLL*-rearranged leukemias (80).

NPM1c

One of the most common genetic abnormalities in adult AML is mutation in the chaperone protein Nucleophosmin1 (81). While under normal conditions NPM1 resides primarily in the nucleus, mutations seen in AML result in cytoplasmic localization of NPM1 (82). Cytoplasmic NPM1 (NPM1c) up regulates the expression of *HOXA9, HOXA10* and *MEIS1,* though the precise mechanism is currently unknown (83). One possible mechanism is that *HOXA9* is up regulated as a result of the cytoplasmic sequestration of HEXIM1 by NPM1c, leading to the activation of the MLL transcriptional partner P-TEFb (84–86). Studies in mice have also established that NPM1c can collaborate with *Flt3*, *Csf2* and *Rasgrp1* in vivo to produce leukemias with long latency (87).

Other mechanisms of HOXA9 dysregulation

Many additional upstream genetic alterations lead to *HOXA9* dysregulation in acute leukemia. Deletions or decreased expression of polycomb protein EZH2 leads to leukemia with up regulation of *HOXA9* (88). Conversely over expression of Cdx proteins, in collaboration with Meis1, leads to leukemias with high levels of *Hox* expression (89, 90). Monocytic leukemia zinc finger (MOZ) fusion proteins can directly up regulate *HOXA9/10* and *MEIS1* in AML by colocalizing at promoters with the histone acetyltransferase, BRPF1 (91). Chromosomal translocations generating the CALM-AF10 fusion protein, as well as those involving the T-cell receptor promoter and the HOXA locus, lead to *HOX* up regulation in T-ALL (78, 92). *Hoxa9* also collaborates with *E2A–PBX1* in murine B cell leukemia to repress B-cell genes and activate *Flt3* (93). Finally, mutations in *ASXL1* are common in myelodysplastic syndromes and are associated with high expression of HOXA9, mediated by inhibition of the PRC2 (94).

MECHANISMS OF HOXA9-REGULATED GENE TRANSCRIPTION

It is becoming clear *HOX* genes carry out their highly specialized function through association at promoter distal, lineage specific cis-regulatory elements, however understanding how HOXA9 and other HOX proteins are targeted these sites has been challenging (12, 95). As discussed below all HOX proteins share a highly homologous DNA binding homeodomain, which because of its short recognition sequence alone cannot account for the distinct subpopulations of target genes seen in development and hematopoiesis. Additional sequence specificity is likely achieved through association with other DNA-binding cofactors and collaborator proteins. These proteins may also function to establish areas of chromatin accessibility in a given cell type and recruit and stabilize HOX proteins at various loci. Furthermore, the downstream activity of HOXA9 to activate or

repress target gene expression may be modulated these cofactors and collaborators. Below we will discuss what is known about DNA binding properties of HOX proteins and known binding partners that confer specificity to HOX proteins, with a focus on recent advances in the field.

HOXA9 regulates gene expression through enhancer binding

The homeobox family of transcription factors is defined by the presence of a DNA binding homeodomain, which is highly homologous within the 39 mammalian HOX proteins and conserved across species. Early studies have found that this 60-amino acid region makes direct contact with DNA via 4 critical amino acids - aa47, 50, 51, and 54 - within the third alpha helix of the homeodomain (96). Interestingly, nearly all homeodomains contain the same residues in these critical positions (97). In addition, comprehensive work has established that all HOX homeodomains bind highly similar AT-rich DNA motifs (98–100). In Drosophila, this TAATNA motif occurs over 100,000 times throughout the genome, and thus cannot explain the distinct subsets of target genes for each HOX protein (99). Conversely, the presence of this recognition sequence seems critically important for DNA binding as a ChIP-seq study of genome-wide HOXA9 binding sites in transformed myeloblasts found that >98% of sites contain a HOX motif (101).

Studies have found that the small differences in homeodomains themselves can confer unique properties to HOX proteins (102, 103). For example, swapping the homeodomains of Hoxa1 and Hoxa9 conferred leukemogenic properties to Hoxa1 while abolishing those of Hoxa9 (104). This phenomenon required the presence of the N-terminal region and PBX cofactor interaction motif, though these regions were interchangeable between Hoxa1 and Hoxa9. There are additional examples of this phenomenon in HoxD proteins with respect to motor neuron fate and rib development (105, 106). Interestingly, the contributions of the homeodomain to specific phenotypes may also be the result of interaction with different cofactors, as this region has been found to mediate protein-protein interactions in addition to DNA-binding. For example, Cdx1 and Foxo1a have been shown to interact with the homeodomain regions of HOX proteins (107).

In addition to motif affinity of a particular homeodomain, gene regulation specific to a single HOX protein likely results from the combination of chromatin accessibility and the subset of cofactors and collaborators expressed in the specific cellular context. Chromatin immunoprecipitation (ChIP) of the *Drosophila* HOX protein ultrabithorax (Ubx) across various stages of development indicates that binding is strongly influenced by chromatin accessibility (108, 109). In the hematopoietic system, early factors such as PU.1 and C/EBPα are known to establish areas of relaxed chromatin that allow for signaling dependent recruitment of various transcription factors, likely mediated by SWI/SNF chromatin remodelers (110, 111). Interestingly, both C/EBPα and SWI/SNF factor Brg1 colocalize with HOXA9 at hundreds of promoter distal regulatory regions throughout the genome of HOXA9/MEIS1 transformed myeloblasts, suggesting that chromatin accessibility likely plays a key role in the targeting of HOXA9 to specific genomic loci (6, 101). This targeting is then further honed through specific protein-protein interactions with cofactors and collaborator proteins that are expressed along with HOXA9 in a particular cellular milieu.

HOXA9 interacting partners

It is well established that HOXA9 and other HOX proteins bind DNA and regulate downstream gene expression along with a small subset of cofactor proteins (112). The most well characterized cofactors are members of the Three-amino-acid-loop-extension (TALE) family of proteins (113) including Pbx1–4, Meis1–3 and Prep1–2. In addition, HOX proteins can homo and heterodimerize to aid in diversity and specificity of binding (114). Whether HOX proteins co-bind with Meis or with Prep proteins subdivides clusters of binding sites (115). There is also evidence that binding may be sequential such that TALE factors initially bind at regulatory elements to promote the deposition of poised chromatin marks, whereby subsequent recruitment of HOX proteins results in transcriptional activation (116). Indeed, the majority of sites co-bound by HOX and PBX proteins show histone H3 K27 acetylation and not trimethylation, suggesting that complexes containing HOX/PBX may be primarily transcriptional activators (99). Additional studies have established that interactions with TALE cofactors are not required at some loci and HOX proteins themselves may homo or heterodimerize at these sites (117). Furthermore, there is new evidence of antagonism between TALE proteins and HOX proteins at specific genomic regions (118).

In the setting of leukemia, the most critical cofactor of HOXA9 is the TALE protein, MEIS1. *MEIS1* expression parallels that of *HOXA9* during hematopoiesis, where it is highly expressed in early progenitors and subsequently downregulated during terminal differentiation (44). Like *HOXA9*, *MEIS1* is directly upregulated by MLL-fusion proteins in both acute myeloid and acute lymphoblastic leukemias and is required for maintaining transformation (19, 119, 120). Futhermore, *MEIS1* is almost always expressed at high levels along with *HOXA9* in non-MLL-translocated leukemias, where high expression correlates with poor prognosis (60, 121, 122). Multiple studies have implicated that HOXA9 and MEIS1 play both a synergistic and causative role in acute leukemias. More than 90% of leukemias that arise in the BZH2 murine retroviral mutagenesis model have independent viral integrations that result in upregulation of both *Hoxa9* and *Meis1* (123). In addition, murine models of *HOXA9*-mediated leukemia require co-expression of *MEIS1* to produce an aggressive disease (50, 61). This requirement is likely secondary to cooperation between HOXA9 and MEIS1 at enhancers on the genome-wide level. Indeed, nearly half of HOXA9 binding sites in *HOXA9/MEIS1*-transformed myeloblasts are co-bound by MEIS1, including sites associated with pro-leukemic target genes (101). At these co-bound sites, MEIS1 helps to recruit transcription regulatory machinery. Indeed MEIS1 has been shown to associate with CREB and CBP in a GSK-3-dependent manner, which is required for maintaining the MLL leukemia stem cell transcriptional program (124). This interaction can be targeted using GSK-3 inhibitors, leading to inhibition of cells transformed by MLL-fusion proteins or *HOXA9/MEIS1*, thus presenting a novel therapeutic target for leukemias with high expression of HOXA9 (124–126). More recent work has also established PBX3 as a critical cofactor required for cytogenetically abnormal AML, presenting an additional target for future therapies (127). Results are promising as the small molecule inhibitor HXR9, which targets the HOX/PBX interaction, was shown to inhibit cell growth and promote apoptosis in AML cell lines that expressed high levels of HOXA9 and PBX3 (127, 128).

It has been proposed by Mann and colleagues that context specific collaborator proteins provide a final level of binding specificity to HOX complexes to allow for their specific actions on gene expression (99). These tissue specific interactors bind along with HOX proteins and TALE cofactors to establish areas of chromatin accessibility, provide stability in DNA binding and help modulate the downstream activity of HOX complexes (129). Recent studies have focused on identifying potential collaborator proteins in a variety of systems. Yeast two hybrid approaches have been used to identify binding partners for Hoxa1 and Hoxa9 ((130) and unpublished). In addition, our group has identified interactors of Hoxa9 in transformed myeloblastic cell lines using co-immunoprecipitation with mass-spectrometry followed by western blot confirmation (101). The transcription factors C/ebpα and Stat5b were both identified in this binding partner screen, along with the chromatin-remodeling enzyme Brg1 and multiple other members of the SWI/SNF complex. Interestingly, each of these putative collaborators are known to be mutated or otherwise dysregulated in leukemia, providing further basis for studying their functional interplay with HOXA9 (131–133). In addition, recent work has shown that C/EBPα is required for HOXA9-mediated leukemogenesis *in vitro* and *in vivo* (6). Multiple other proteins that physically interact with HOXA9 have been identified using various techniques, as summarized in Table 1. With these approaches some themes in collaborator proteins are surfacing. Many are lineage specific factors known for general priming of enhancer regions of the genome, while others are involved in signal transduction.

Following targeting to specific sites, HOX complexes most likely control downstream gene expression through the interaction with histone modifying machinery. Both Hoxa9 and Meis1 have been shown to recruit the histone acetyltransferase p300/CBP to mediate activation of downstream targets (124, 134, 135). Recent work also established that Hoxa9 interacts with the histone methyltransferase G9a, and that this interaction is required for aggressive disease in mouse models of leukemia (136). Similarly, both activation and repression domains have been defined in Hoxa10 (and other Hox10 proteins) that facilitate interaction with CBP and HDAC2 respectively (137, 138). HOX proteins can also interact with other enzymes and machinery leading to their own modification (139). HOXA9 is phosphorylated by protein kinase C in the N-terminal region of it's homeodomain, leading to decreased DNA binding and promoting myeloid differentiation (140). In addition, HOXA9 can be methylated by PRMT5 in a TNFa-dependant manner, which promotes downstream expression of E-selectin and VCAM-1 (141).

TRANSCRIPTIONAL TARGETS OF HOXA9

In addition to characterizing the mechanisms through which HOXA9 regulates downstream gene expression, identifying the downstream targets that mediate leukemic transformation is critically important. Many efforts have been made using both genome-wide approaches and site-specific experiments for identifying these important targets in both development and disease (Table 2).

Targets in Leukemia

Considerable progress has been made towards understanding HOXA9-mediated leukemogenesis through the identification of the genome-wide binding sites of HOXA9 and

MEIS1 in transformed myeloblastic cells. ChIP-seq experiments using murine bone marrow transduced with HOXA9 and MEIS1 identified thousands genomic regions that bind HOXA9, MEIS1 or both, and these regions showed a high degree of evolutionary conservation (6, 101). Over 90% of the binding sites are located in distal intergenic regions (>10kb from transcriptional start sites) or gene introns, while less than 3% are located within 3kb of promoter regions. These studies also identified multiple proleukemic targets with cis-regulatory regions bound by HOXA9, including *Erg, Flt3, Lmo2 and Myb*. In addition, both microarray and RNA-seq studies in models with inducible expression of *HOXA9* have identified hundreds of genes with significant changes in expression following the loss of *HOXA9* (6, 101). Interestingly, near equal numbers of genes are activated and repressed by *HOXA9*, suggesting that *HOXA9* may play an important role in both activating and inhibitory transcriptional regulation complexes. Consistent with its role as a proto-oncogene, HOXA9 generally up-regulates proliferative genes, while suppressing expression of myeloid differentiation and inflammatory genes.

Many HOXA9 targets have been studied individually and found to play important roles in HOXA9-mediated leukemogenesis. Knockdown of Lmo2 impairs growth of leukemic cells and high levels of Lmo2 predict poor prognosis in patients (142). Hoxa9 activates *Bcl-2* expression, which is required for transformation by Hoxa9, Nup98-Hoxa9 and MLL. Furthermore, loss of Bcl-2 leads to improved survival in mouse model of Hoxa9/Meis1-transformed leukemia (143). MLL-ELL up regulates *Fgf2* expression in a Hoxa9/a10 dependent fashion, leading to increased proliferation and cytokine hypersensitivity (88, 91). Hoxa1 and Hoxa9 regulate Rac1 activity by directly upregulating Vav2 expression (144). In very recent work, *Igf1* has also been identified as a direct target of HOXA9 required for leukemic transformation (145). Finally, 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 (146). The *Inka/b* locus is commonly deleted or silenced in acute lymphoid leukemias (147–149). Interestingly Hoxa9 has been shown to repress *Ink4a* expression to overcome oncogene-induced senescence during transformation by AML1-ETO in Bmi1−/− cells, as well as in Hoxa9/Meis1 transformed cells (6, 150).

Currently genome-wide studies of HOXA9 binding have been constrained to over expression models due to lack of ChIP-grade antibodies for endogenous HOXA9 in either human or murine cells. While studies in transformed cells have led to significant advances in our understanding of the role of HOXA9 in leukemia, questions remain with regards to the function of HOXA9 in normal hematopoiesis. One of the more interesting unanswered questions is whether HOXA9 binding sites are shared in normal and transformed cells or if HOXA9-mediated transformation represents a true gain of function with activation/repression of novel leukemogenic target genes. In addition, fully characterizing HOXA9 binding sites in the setting of various upstream transforming oncogenes can help determine if there is a common HOXA9 target gene set in leukemia. The continued improvement of ChIP reagents and technology will help to answer these questions and others to allow for further advances in the understanding of HOXA9 biology.

Non-transcriptional roles of Hoxa9

In addition to acting as a classical transcription factor regulating downstream gene expression, HOXA9 may also have non-transcriptional functions that are critical for its role in malignancy (151). For example, Hoxa9 can act as an E3 ligase for DNA replication inhibitor Geminin, leading to its degradation, which contributes to Hoxa9-mediated transformation (152, 153). Conflicting reports however also find that Hoxa9-Geminin binding can sequester Hoxa9 thereby inhibiting its transcriptional activity (154). Alternate mechanisms have been described for other HOX proteins as well. For example, Hoxa2 can indirectly stabilize p53 by binding to p53's E3 ubiquitin ligase, RCHY1, leading to the degradation of RCHY1 (155). Hoxa7 and Hoxa14 can bind to the initiation factor eIF4E in liver cancer, potentially affecting the nuclear transport of eIF4E-dependent transcripts like *c-myc*, *fgf2*, *vegf*, ornithine decarboxylase and cyclin-D1 (156). Finally, the yeast-two-hybrid screen of Hoxa1 interactors identified many putative binding partners involved in signal transduction, cell adhesion and vesicular trafficking, pointing to additional non-transcriptional roles for this and other HOX proteins (130).

CONCLUSIONS

As more and more malignancies involving dysregulation of *HOX* genes are identified, it is clear that the mechanisms through which HOX proteins exert their function need to be better defined. HOXA9 is of particular interest as it is overexpressed in over 50% of acute myeloid leukemias, as well as B and T cell leukemias, and its high level of expression is associate with poor prognosis. Research to date suggests that HOXA9 acts to modulate the activity of distal regulatory elements through recruitment of histone modifying and transcriptional machinery that likely act at promoters via long-range chromatin interactions, thereby up regulating a set of proleukemogenic target genes while repressing others involved in processes such as cellular senescence. Identifying new posttranslational modifications and protein-protein interactions required for HOX function is likely to be a promising avenue for identifying new therapeutic targets along with the identification of drug-amenable HOX targets that are essential for leukemia.

References

1. Goodman FR. Limb malformations and the human HOX genes. American journal of medical genetics. 2002; 112(3):256–265. [PubMed: 12357469]
2. Lewis EB. A gene complex controlling segmentation in Drosophila. Nature. 1978; 276(5688):565–570. [PubMed: 103000]
3. Krumlauf R. Hox genes in vertebrate development. Cell. 1994; 78:191–201. [PubMed: 7913880]
4. Duboule D, Dolle P. The structural and functional organization of the murine HOX gene family resembles that of Drosophila homeotic genes. The EMBO journal. 1989; 8(5):1497–1505. [PubMed: 2569969]
5. Andreeff M, Ruvolo V, Gadgil S, Zeng C, Coombes K, Chen W, et al. HOX expression patterns identify a common signature for favorable AML. Leukemia. 2008; 22(11):2041–2047. [PubMed: 18668134]
6. Collins C, Wang J, Miao H, Bronstein J, Nawer H, Xu T, et al. C/EBPalpha is an essential collaborator in Hoxa9/Meis1-mediated leukemogenesis. Proceedings of the National Academy of Sciences of the United States of America. 2014; 111(27):9899–9904. Epub 2014/06/25. [PubMed: 24958854]

7. Drabkin HA, Parsy C, Ferguson K, Guilhot F, Lacotte L, Roy L, et al. Quantitative HOX expression in chromosomally defined subsets of acute myelogenous leukemia. Leukemia. 2002; 16(2):186–195. Epub 2002/02/13. [PubMed: 11840284]

8. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science. 1999; 286(5439):531–537. Epub 1999/10/16. [PubMed: 10521349]

9. Tholouli E, MacDermott S, Hoyland J, Yin JL, Byers R. Quantitative multiplex quantum dot in-situ hybridisation based gene expression profiling in tissue microarrays identifies prognostic genes in acute myeloid leukaemia. Biochemical and biophysical research communications. 2012; 425(2): 333–339. Epub 2012/07/31. [PubMed: 22842570]

10. Choo SW, Russell S. Genomic approaches to understanding Hox gene function. Advances in genetics. 2011; 76:55–91. Epub 2011/11/22. [PubMed: 22099692]

11. Slattery M, Riley T, Liu P, Abe N, Gomez-Alcala P, Dror I, et al. Cofactor binding evokes latent differences in DNA binding specificity between Hox proteins. Cell. 2011; 147(6):1270–1282. Epub 2011/12/14. [PubMed: 22153072]

12. Sorge S, Ha N, Polychronidou M, Friedrich J, Bezdan D, Kaspar P, et al. The cis-regulatory code of Hox function in Drosophila. The EMBO journal. 2012; 31(15):3323–3333. Epub 2012/07/12. [PubMed: 22781127]

13. Min H, Lee JY, Kim MH. Hoxc gene collinear expression and epigenetic modifications established during embryogenesis are maintained until after birth. International journal of biological sciences. 2013; 9(9):960–965. Epub 2013/10/25. [PubMed: 24155669]

14. Sheth R, Bastida MF, Kmita M, Ros M. “Self-regulation,” a new facet of Hox genes’ function. Developmental dynamics : an official publication of the American Association of Anatomists. 2014; 243(1):182–191. Epub 2013/08/06. [PubMed: 23913823]

15. Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature. 2011; 472(7341):120–124. Epub 2011/03/23. [PubMed: 21423168]

16. Bantignies F, Roure V, Comet I, Leblanc B, Schuettengruber B, Bonnet J, et al. Polycomb-dependent regulatory contacts between distant Hox loci in Drosophila. Cell. 2011; 144(2):214–226. [PubMed: 21241892]

17. Tolhuis B, Blom M, Kerkhoven RM, Pagie L, Teunissen H, Nieuwland M, et al. Interactions among Polycomb domains are guided by chromosome architecture. PLoS genetics. 2011; 7(3):e1001343. [PubMed: 21455484]

18. Schuettengruber B, Chourrout D, Vervoort M, Leblanc B, Cavalli G. Genome regulation by polycomb and trithorax proteins. Cell. 2007; 128(4):735–745. [PubMed: 17320510]

19. Milne TA, Briggs SD, Brock HW, Martin ME, Gibbs D, Allis CD, et al. MLL targets SET domain methyltransferase activity to Hox gene promoters. Molecular cell. 2002; 10(5):1107–1117. [PubMed: 12453418]

20. Yu BD, Hess JL, Horning SE, Brown GA, Korsmeyer SJ. Altered Hox expression and segmental identity in Mll-mutant mice. Nature. 1995; 378(6556):505–508. [PubMed: 7477409]

21. Hess JL, Yu BD, Li B, Hanson R, Korsmeyer SJ. Defects in yolk sac hematopoiesis in Mll-null embryos. Blood. 1997; 90(5):1799–1806. [PubMed: 9292512]

22. Jude CD, Climer L, Xu D, Artinger E, Fisher JK, Ernst P. Unique and independent roles for MLL in adult hematopoietic stem cells and progenitors. Cell stem cell. 2007; 1(3):324–337. [PubMed: 18371366]

23. Muntean AG, Hess JL. The pathogenesis of mixed-lineage leukemia. Annual review of pathology. 2012; 7:283–301.

24. Radulovic V, de Haan G, Klauke K. Polycomb-group proteins in hematopoietic stem cell regulation and hematopoietic neoplasms. Leukemia. 2013; 27(3):523–533. [PubMed: 23257781]

25. Rawat VP, Humphries RK, Buske C. Beyond Hox: the role of ParaHox genes in normal and malignant hematopoiesis. Blood. 2012; 120(3):519–527. [PubMed: 22547580]

26. Brooke NM, Garcia-Fernandez J, Holland PW. The ParaHox gene cluster is an evolutionary sister of the Hox gene cluster. Nature. 1998; 392(6679):920–922. [PubMed: 9582071]

27. Davidson AJ, Zon LI. The caudal-related homeobox genes cdx1a and cdx4 act redundantly to regulate hox gene expression and the formation of putative hematopoietic stem cells during zebrafish embryogenesis. Developmental biology. 2006; 292(2):506–518. [PubMed: 16457800]
28. McKinney-Freeman SL, Lengerke C, Jang IH, Schmitt S, Wang Y, Philitas M, et al. Modulation of murine embryonic stem cell-derived CD41+c-kit+ hematopoietic progenitors by ectopic expression of Cdx genes. Blood. 2008; 111(10):4944–4953. [PubMed: 18252864]
29. Wang Y, Yabuuchi A, McKinney-Freeman S, Ducharme DM, Ray MK, Chawengsaksophak K, et al. Cdx gene deficiency compromises embryonic hematopoiesis in the mouse. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(22):7756–7761. [PubMed: 18511567]
30. Davidson AJ, Ernst P, Wang Y, Dekens MP, Kingsley PD, Palis J, et al. cdx4 mutants fail to specify blood progenitors and can be rescued by multiple hox genes. Nature. 2003; 425(6955): 300–306. [PubMed: 13679919]
31. Dinger ME, Amaral PP, Mercer TR, Pang KC, Bruce SJ, Gardiner BB, et al. Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Genome research. 2008; 18(9):1433–1445. Epub 2008/06/20. [PubMed: 18562676]
32. Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, et al. Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res. 2011; 71(20):6320–6326. Epub 2011/08/25. [PubMed: 21862635]
33. Chen FJ, Sun M, Li SQ, Wu QQ, Ji L, Liu ZL, et al. Upregulation of the long non-coding RNA HOTAIR promotes esophageal squamous cell carcinoma metastasis and poor prognosis. Mol Carcinog. 2013; 52(11):908–915. Epub 2013/10/24. [PubMed: 24151120]
34. Cui L, Xie XY, Wang H, Chen XL, Liu SL, Hu LN. [Expression of long non-coding RNA HOTAIR mRNA in ovarian cancer]. Sichuan Da Xue Xue Bao Yi Xue Ban. 2013; 44(1):57–59. Epub 2013/04/23. [PubMed: 23600210]
35. Ge XS, Ma HJ, Zheng XH, Ruan HL, Liao XY, Xue WQ, et al. HOTAIR, a prognostic factor in esophageal squamous cell carcinoma, inhibits WIF-1 expression and activates Wnt pathway. Cancer Sci. 2013; 104(12):1675–1682. Epub 2013/10/15. [PubMed: 24118380]
36. He X, Bao W, Li X, Chen Z, Che Q, Wang H, et al. The long non-coding RNA HOTAIR is upregulated in endometrial carcinoma and correlates with poor prognosis. Int J Mol Med. 2014; 33(2):325–332. Epub 2013/11/29. [PubMed: 24285342]
37. Liu XH, Liu ZL, Sun M, Liu J, Wang ZX, De W. The long non-coding RNA HOTAIR indicates a poor prognosis and promotes metastasis in non-small cell lung cancer. BMC Cancer. 2013; 13:464. Epub 2013/10/10. [PubMed: 24103700]
38. Nakayama I, Shibazaki M, Yashima-Abo A, Miura F, Sugiyama T, Masuda T, et al. Loss of HOXD10 expression induced by upregulation of miR-10b accelerates the migration and invasion activities of ovarian cancer cells. International journal of oncology. 2013; 43(1):63–71. Epub 2013/05/15. [PubMed: 23670532]
39. Shi X, Sun M, Liu H, Yao Y, Song Y. Long non-coding RNAs: a new frontier in the study of human diseases. Cancer letters. 2013; 339(2):159–166. [PubMed: 23791884]
40. Sorensen KP, Thomassen M, Tan Q, Bak M, Cold S, Burton M, et al. Long non-coding RNA HOTAIR is an independent prognostic marker of metastasis in estrogen receptor-positive primary breast cancer. Breast Cancer Res Treat. 2013; 142(3):529–536. Epub 2013/11/22. [PubMed: 24258260]
41. Zhang JX, Han L, Bao ZS, Wang YY, Chen LY, Yan W, et al. HOTAIR, a cell cycle-associated long noncoding RNA and a strong predictor of survival, is preferentially expressed in classical and mesenchymal glioma. Neuro Oncol. 2013; 15(12):1595–1603. Epub 2013/11/10. [PubMed: 24203894]
42. Zhuang Y, Wang X, Nguyen HT, Zhuo Y, Cui X, Fewell C, et al. Induction of long intergenic non-coding RNA HOTAIR in lung cancer cells by type I collagen. J Hematol Oncol. 2013; 6:35. Epub 2013/05/15. [PubMed: 23668363]
43. Pombo A, Dillon N. Three-dimensional genome architecture: players and mechanisms. Nature reviews Molecular cell biology. 2015 Epub 2015/03/12.

44. Pineault N, Helgason CD, Lawrence HJ, Humphries RK. Differential expression of Hox, Meis1, and Pbx1 genes in primitive cells throughout murine hematopoietic ontogeny. Experimental hematology. 2002; 30(1):49–57. [PubMed: 11823037]
45. Sauvageau G, Lansdorp PM, Eaves CJ, Hogge DE, Dragowska WH, Reid DS, et al. Differential expression of homeobox genes in functionally distinct CD34+ subpopulations of human bone marrow cells. Proceedings of the National Academy of Sciences of the United States of America. 1994; 91(25):12223–12227. [PubMed: 7527557]
46. Bijl J, Thompson A, Ramirez-Solis R, Krosl J, Grier DG, Lawrence HJ, et al. Analysis of HSC activity and compensatory Hox gene expression profile in Hoxb cluster mutant fetal liver cells. Blood. 2006; 108(1):116–122. Epub 2005/12/13. [PubMed: 16339407]
47. Bjornsson JM, Larsson N, Brun AC, Magnusson M, Andersson E, Lundstrom P, et al. Reduced proliferative capacity of hematopoietic stem cells deficient in Hoxb3 and Hoxb4. Molecular and cellular biology. 2003; 23(11):3872–3883. Epub 2003/05/16. [PubMed: 12748289]
48. Brun AC, Bjornsson JM, Magnusson M, Larsson N, Leveen P, Ehinger M, et al. Hoxb4-deficient mice undergo normal hematopoietic development but exhibit a mild proliferation defect in hematopoietic stem cells. Blood. 2004; 103(11):4126–4133. Epub 2004/02/14. [PubMed: 14962901]
49. Ko KH, Lam QL, Zhang M, Wong CK, Lo CK, Kahmeyer-Gabbe M, et al. Hoxb3 deficiency impairs B lymphopoiesis in mouse bone marrow. Experimental hematology. 2007; 35(3):465–475. Epub 2007/02/21. [PubMed: 17309827]
50. Thorsteinsdottir U, Mamo A, Kroon E, Jerome L, Bijl J, Lawrence HJ, et al. Overexpression of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion. Blood. 2002; 99(1):121–129. [PubMed: 11756161]
51. Crooks GM, Fuller J, Petersen D, Izadi P, Malik P, Pattengale PK, et al. Constitutive HOXA5 expression inhibits erythropoiesis and increases myelopoiesis from human hematopoietic progenitors. Blood. 1999; 94(2):519–528. Epub 1999/07/09. [PubMed: 10397719]
52. Fuller JF, McAdara J, Yaron Y, Sakaguchi M, Fraser JK, Gasson JC. Characterization of HOX gene expression during myelopoiesis: role of HOX A5 in lineage commitment and maturation. Blood. 1999; 93(10):3391–3400. Epub 1999/05/11. [PubMed: 10233891]
53. Shimamoto T, Tang Y, Naot Y, Nardi M, Brulet P, Bieberich CJ, et al. Hematopoietic progenitor cell abnormalities in Hoxc-8 null mutant mice. The Journal of experimental zoology. 1999; 283(2):186–193. Epub 1999/01/27. [PubMed: 9919689]
54. So CW, Karsunky H, Wong P, Weissman IL, Cleary ML. Leukemic transformation of hematopoietic progenitors by MLL-GAS7 in the absence of Hoxa7 or Hoxa9. Blood. 2004; 103(8):3192–3199. Epub 2004/04/09. [PubMed: 15070702]
55. Takeshita K, Bollekens JA, Hijiya N, Ratajczak M, Ruddle FH, Gewirtz AM. A homeobox gene of the Antennapedia class is required for human adult erythropoiesis. Proceedings of the National Academy of Sciences of the United States of America. 1993; 90(8):3535–3538. Epub 1993/04/15. [PubMed: 8097318]
56. Izon DJ, Rozenfeld S, Fong ST, Komuves L, Largman C, Lawrence HJ. Loss of function of the homeobox gene Hoxa-9 perturbs early T-cell development and induces apoptosis in primitive thymocytes. Blood. 1998; 92(2):383–393. [PubMed: 9657735]
57. Lawrence HJ, Helgason CD, Sauvageau G, Fong S, Izon DJ, Humphries RK, et al. Mice bearing a targeted interruption of the homeobox gene HOXA9 have defects in myeloid, erythroid, and lymphoid hematopoiesis. Blood. 1997; 89(6):1922–1930. [PubMed: 9058712]
58. Lawrence HJ, Christensen J, Fong S, Hu YL, Weissman I, Sauvageau G, et al. Loss of expression of the Hoxa-9 homeobox gene impairs the proliferation and repopulating ability of hematopoietic stem cells. Blood. 2005; 106(12):3988–3994. [PubMed: 16091451]
59. Alharbi RA, Pettengell R, Pandha HS, Morgan R. The role of HOX genes in normal hematopoiesis and acute leukemia. Leukemia. 2013; 27(5):1000–1008. Epub 2012/12/06. [PubMed: 23212154]
60. Lawrence HJ, Rozenfeld S, Cruz C, Matsukuma K, Kwong A, Komuves L, et al. Frequent co-expression of the HOXA9 and MEIS1 homeobox genes in human myeloid leukemias. Leukemia. 1999; 13(12):1993–1999. Epub 1999/12/22. [PubMed: 10602420]

61. Kroon E, Krosl J, Thorsteinsdottir U, Baban S, Buchberg AM, Sauvageau G. Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b. The EMBO journal. 1998; 17(13):3714–3725. Epub 1998/07/03. [PubMed: 9649441]

62. Eklund E. The role of Hox proteins in leukemogenesis: insights into key regulatory events in hematopoiesis. Crit Rev Oncog. 2011; 16(1–2):65–76. Epub 2011/12/14. [PubMed: 22150308]

63. Sitwala K, Dandekar M, Hess J. HOX Proteins and Leukemia. Int J Clin Exp Pathol. 2008; 1:461–474. [PubMed: 18787682]

64. Figueroa ME, Lugthart S, Li Y, Erpelinck-Verschueren C, Deng X, Christos PJ, et al. DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. Cancer Cell. 2010; 17(1):13–27. Epub 2010/01/12. [PubMed: 20060365]

65. Li Z, Huang H, Li Y, Jiang X, Chen P, Arnovitz S, et al. Up-regulation of a HOXA-PBX3 homeobox-gene signature following down-regulation of miR-181 is associated with adverse prognosis in patients with cytogenetically abnormal AML. Blood. 2012; 119(10):2314–2324. Epub 2012/01/19. [PubMed: 22251480]

66. Faderl S, Kantarjian HM, Estey E, Manshouri T, Chan CY, Rahman Elsaied A, et al. The prognostic significance of p16(INK4a)/p14(ARF) locus deletion and MDM-2 protein expression in adult acute myelogenous leukemia. Cancer. 2000; 89(9):1976–1982. Epub 2000/11/07. [PubMed: 11064355]

67. Karakas T, Maurer U, Weidmann E, Miething CC, Hoelzer D, Bergmann L. High expression of bcl-2 mRNA as a determinant of poor prognosis in acute myeloid leukemia. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 1998; 9(2):159–165. Epub 1998/04/29. [PubMed: 9553660]

68. Krivtsov AV, Armstrong SA. MLL translocations, histone modifications and leukaemia stem-cell development. Nature reviews Cancer. 2007; 7(11):823–833. [PubMed: 17957188]

69. Meyer C, Kowarz E, Hofmann J, Renneville A, Zuna J, Trka J, et al. New insights to the MLL recombinome of acute leukemias. Leukemia. 2009; 23(8):1490–1499. [PubMed: 19262598]

70. Hess JL. MLL: a histone methyltransferase disrupted in leukemia. Trends in molecular medicine. 2004; 10(10):500–507. [PubMed: 15464450]

71. Ayton PM, Cleary ML. Transformation of myeloid progenitors by MLL oncoproteins is dependent on Hoxa7 and Hoxa9. Genes & development. 2003; 17(18):2298–2307. [PubMed: 12952893]

72. Faber J, Krivtsov AV, Stubbs MC, Wright R, Davis TN, van den Heuvel-Eibrink M, et al. HOXA9 is required for survival in human MLL-rearranged acute leukemias. Blood. 2009; 113(11):2375–2385. Epub 2008/12/06. [PubMed: 19056693]

73. Ng RK, Kong CT, So CC, Lui WC, Chan YF, Leung KC, et al. Epigenetic dysregulation of leukaemic HOX code in MLL-rearranged leukaemia mouse model. J Pathol. 2014; 232(1):65–74. Epub 2013/10/15. [PubMed: 24122813]

74. Hoelz A, Debler EW, Blobel G. The structure of the nuclear pore complex. Annual review of biochemistry. 2011; 80:613–643.

75. Dieppois G, Stutz F. Connecting the transcription site to the nuclear pore: a multi-tether process that regulates gene expression. Journal of cell science. 2010; 123(Pt 12):1989–1999. [PubMed: 20519581]

76. Gough SM, Slape CI, Aplan PD. NUP98 gene fusions and hematopoietic malignancies: common themes and new biologic insights. Blood. 2011; 118(24):6247–6257. Epub 2011/09/29. [PubMed: 21948299]

77. Saw J, Curtis DJ, Hussey DJ, Dobrovic A, Aplan PD, Slape CI. The fusion partner specifies the oncogenic potential of NUP98 fusion proteins. Leuk Res. 2013; 37(12):1668–1673. Epub 2013/10/05. [PubMed: 24090997]

78. Novak RL, Harper DP, Caudell D, Slape C, Beachy SH, Aplan PD. Gene expression profiling and candidate gene resequencing identifies pathways and mutations important for malignant transformation caused by leukemogenic fusion genes. Experimental hematology. 2012; 40(12): 1016–1027. Epub 2012/08/14. [PubMed: 22885519]

79. de Rooij JD, Hollink IH, Arentsen-Peters ST, van Galen JF, Berna Beverloo H, Baruchel A, et al. NUP98/JARID1A is a novel recurrent abnormality in pediatric acute megakaryoblastic leukemia

with a distinct HOX gene expression pattern. Leukemia. 2013; 27(12):2280–2288. Epub 2013/03/28. [PubMed: 23531517]

80. Hollink IH, van den Heuvel-Eibrink MM, Arentsen-Peters ST, Pratcorona M, Abbas S, Kuipers JE, et al. NUP98/NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. Blood. 2011; 118(13):3645–3656. Epub 2011/08/05. [PubMed: 21813447]
81. Falini B, Sportoletti P, Martelli MP. Acute myeloid leukemia with mutated NPM1: diagnosis, prognosis and therapeutic perspectives. Current opinion in oncology. 2009; 21(6):573–581. [PubMed: 19770764]
82. Falini B, Bolli N, Liso A, Martelli MP, Mannucci R, Pileri S, et al. Altered nucleophosmin transport in acute myeloid leukaemia with mutated NPM1: molecular basis and clinical implications. Leukemia. 2009; 23(10):1731–1743. [PubMed: 19516275]
83. Mullighan CG, Kennedy A, Zhou X, Radtke I, Phillips LA, Shurtleff SA, et al. Pediatric acute myeloid leukemia with NPM1 mutations is characterized by a gene expression profile with dysregulated HOX gene expression distinct from MLL-rearranged leukemias. Leukemia. 2007; 21(9):2000–2009. [PubMed: 17597811]
84. Gurumurthy M, Tan CH, Ng R, Zeiger L, Lau J, Lee J, et al. Nucleophosmin interacts with HEXIM1 and regulates RNA polymerase II transcription. Journal of molecular biology. 2008; 378(2):302–317. Epub 2008/03/29. [PubMed: 18371977]
85. Monroe SC, Jo SY, Sanders DS, Basrur V, Elenitoba-Johnson KS, Slany RK, et al. MLL-AF9 and MLL-ENL alter the dynamic association of transcriptional regulators with genes critical for leukemia. Experimental hematology. 2011; 39(1):77–86. e1–5 Epub 2010/09/22. [PubMed: 20854876]
86. Mueller D, Bach C, Zeisig D, Garcia-Cuellar MP, Monroe S, Sreekumar A, et al. A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. Blood. 2007; 110(13):4445–4454. Epub 2007/09/15. [PubMed: 17855633]
87. Vassiliou GS, Cooper JL, Rad R, Li J, Rice S, Uren A, et al. Mutant nucleophosmin and cooperating pathways drive leukemia initiation and progression in mice. Nat Genet. 2011; 43(5): 470–475. Epub 2011/03/29. [PubMed: 21441929]
88. Khan SN, Jankowska AM, Mahfouz R, Dunbar AJ, Sugimoto Y, Hosono N, et al. Multiple mechanisms deregulate EZH2 and histone H3 lysine 27 epigenetic changes in myeloid malignancies. Leukemia. 2013; 27(6):1301–1309. Epub 2013/03/15. [PubMed: 23486531]
89. Bansal D, Scholl C, Frohling S, McDowell E, Lee BH, Dohner K, et al. Cdx4 dysregulates Hox gene expression and generates acute myeloid leukemia alone and in cooperation with Meis1a in a murine model. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103(45):16924–16929. [PubMed: 17068127]
90. Rawat VP, Cusan M, Deshpande A, Hiddemann W, Quintanilla-Martinez L, Humphries RK, et al. Ectopic expression of the homeobox gene Cdx2 is the transforming event in a mouse model of t(12;13)(p13;q12) acute myeloid leukemia. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101(3):817–822. [PubMed: 14718672]
91. Shima H, Yamagata K, Aikawa Y, Shino M, Koseki H, Shimada H, et al. Bromodomain-PHD finger protein 1 is critical for leukemogenesis associated with MOZ-TIF2 fusion. Int J Hematol. 2014; 99(1):21–31. Epub 2013/11/22. [PubMed: 24258712]
92. Speleman F, Cauwelier B, Dastugue N, Cools J, Verhasselt B, Poppe B, et al. A new recurrent inversion, inv(7)(p15q34), leads to transcriptional activation of HOXA10 and HOXA11 in a subset of T-cell acute lymphoblastic leukemias. Leukemia. 2005; 19(3):358–366. Epub 2005/01/28. [PubMed: 15674412]
93. Hassawi M, Shestakova EA, Fournier M, Lebert-Ghali CE, Vaisson G, Frison H, et al. Hoxa9 collaborates with E2A–PBX1 in mouse B cell leukemia in association with Flt3 activation and decrease of B cell gene expression. Developmental dynamics : an official publication of the American Association of Anatomists. 2014; 243(1):145–158. Epub 2013/09/03. [PubMed: 23996689]
94. Inoue D, Kitaura J, Togami K, Nishimura K, Enomoto Y, Uchida T, et al. Myelodysplastic syndromes are induced by histone methylation-altering ASXL1 mutations. J Clin Invest. 2013; 123(11):4627–4640. Epub 2013/11/13. [PubMed: 24216483]

95. Polychronidou M, Lohmann I. Cell-type specific cis-regulatory networks: insights from Hox transcription factors. Fly. 2013; 7(1):13–17. [PubMed: 23221502]

96. Gehring WJ, Qian YQ, Billeter M, Furukubo-Tokunaga K, Schier AF, Resendez-Perez D, et al. Homeodomain-DNA recognition. Cell. 1994; 78(2):211–223. [PubMed: 8044836]

97. Mann RS. The specificity of homeotic gene function. BioEssays : news and reviews in molecular, cellular and developmental biology. 1995; 17(10):855–863.

98. Berger MF, Badis G, Gehrke AR, Talukder S, Philippakis AA, Pena-Castillo L, et al. Variation in homeodomain DNA binding revealed by high-resolution analysis of sequence preferences. Cell. 2008; 133(7):1266–1276. [PubMed: 18585359]

99. Mann, RS.; Lelli, KM.; Joshi, R. Current Topics in Developmental Biology. Academic Press; 2009. Chapter 3 Hox Specificity: Unique Roles for Cofactors and Collaborators; p. 63-101.

100. Noyes MB, Christensen RG, Wakabayashi A, Stormo GD, Brodsky MH, Wolfe SA. Analysis of homeodomain specificities allows the family-wide prediction of preferred recognition sites. Cell. 2008; 133(7):1277–1289. [PubMed: 18585360]

101. Huang Y, Sitwala K, Bronstein J, Sanders D, Dandekar M, Collins C, et al. Identification and characterization of Hoxa9 binding sites in hematopoietic cells. Blood. 2012; 119(2):388–398. Epub 2011/11/11. [PubMed: 22072553]

102. Busser BW, Gisselbrecht SS, Shokri L, Tansey TR, Gamble CE, Bulyk ML, et al. Contribution of distinct homeodomain DNA binding specificities to Drosophila embryonic mesodermal cell-specific gene expression programs. PloS one. 2013; 8(7):e69385. Epub 2013/08/08. [PubMed: 23922708]

103. Lelli KM, Noro B, Mann RS. Variable motif utilization in homeotic selector (Hox)-cofactor complex formation controls specificity. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(52):21122–21127. Epub 2011/12/14. [PubMed: 22160705]

104. Breitinger C, Maethner E, Garcia-Cuellar MP, Slany RK. The homeodomain region controls the phenotype of HOX-induced murine leukemia. Blood. 2012; 120(19):4018–40127. Epub 2012/09/20. [PubMed: 22990017]

105. Misra M, Sours E, Lance-Jones C. Hox transcription factors influence motoneuron identity through the integrated actions of both homeodomain and non-homeodomain regions. Developmental dynamics : an official publication of the American Association of Anatomists. 2012; 241(4):718–731. [PubMed: 22411553]

106. Guerreiro I, Casaca A, Nunes A, Monteiro S, Novoa A, Ferreira RB, et al. Regulatory role for a conserved motif adjacent to the homeodomain of Hox10 proteins. Development. 2012; 139(15): 2703–2710. Epub 2012/06/23. [PubMed: 22721778]

107. Brayer KJ, Lynch VJ, Wagner GP. Evolution of a derived protein-protein interaction between HoxA11 and Foxo1a in mammals caused by changes in intramolecular regulation. Proceedings of the National Academy of Sciences of the United States of America. 2011; 108(32):E414–E420. Epub 2011/07/27. [PubMed: 21788518]

108. Slattery M, Ma L, Negre N, White KP, Mann RS. Genome-wide tissue-specific occupancy of the Hox protein Ultrabithorax and Hox cofactor Homothorax in Drosophila. PloS one. 2011; 6(4):e14686. Epub 2011/04/13. [PubMed: 21483663]

109. Choo SW, White R, Russell S. Genome-wide analysis of the binding of the Hox protein Ultrabithorax and the Hox cofactor Homothorax in Drosophila. PloS one. 2011; 6(4):e14778. Epub 2011/04/13. [PubMed: 21483667]

110. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. Molecular cell. 2010; 38(4):576–589. Epub 2010/06/02. [PubMed: 20513432]

111. Salma N, Xiao H, Mueller E, Imbalzano AN. Temporal recruitment of transcription factors and SWI/SNF chromatin-remodeling enzymes during adipogenic induction of the peroxisome proliferator-activated receptor gamma nuclear hormone receptor. Molecular and cellular biology. 2004; 24(11):4651–4663. [PubMed: 15143161]

112. Ladam F, Sagerstrom CG. Hox regulation of transcription: more complex(es). Developmental dynamics : an official publication of the American Association of Anatomists. 2014; 243(1):4–15. Epub 2013/06/15. [PubMed: 23765878]
113. Longobardi E, Penkov D, Mateos D, De Florian G, Torres M, Blasi F. Biochemistry of the tale transcription factors PREP, MEIS, and PBX in vertebrates. Developmental dynamics : an official publication of the American Association of Anatomists. 2014; 243(1):59–75. Epub 2013/07/23. [PubMed: 23873833]
114. Papadopoulos DK, Skouloudaki K, Adachi Y, Samakovlis C, Gehring WJ. Dimer formation via the homeodomain is required for function and specificity of Sex combs reduced in Drosophila. Developmental biology. 2012; 367(1):78–89. Epub 2012/05/09. [PubMed: 22564794]
115. Penkov D, Mateos San Martin D, Fernandez-Diaz LC, Rossello CA, Torroja C, Sanchez-Cabo F, et al. Analysis of the DNA-binding profile and function of TALE homeoproteins reveals their specialization and specific interactions with Hox genes/proteins. Cell reports. 2013; 3(4):1321–1333. Epub 2013/04/23. [PubMed: 23602564]
116. Choe SK, Ladam F, Sagerstrom CG. TALE factors poise promoters for activation by Hox proteins. Developmental cell. 2014; 28(2):203–211. Epub 2014/02/01. [PubMed: 24480644]
117. Sambrani N, Hudry B, Maurel-Zaffran C, Zouaz A, Mishra R, Merabet S, et al. Distinct molecular strategies for Hox-mediated limb suppression in Drosophila: from cooperativity to dispensability/antagonism in TALE partnership. PLoS genetics. 2013; 9(3):e1003307. Epub 2013/03/19. [PubMed: 23505377]
118. Rivas ML, Espinosa-Vazquez JM, Sambrani N, Greig S, Merabet S, Graba Y, et al. Antagonism versus cooperativity with TALE cofactors at the base of the functional diversification of Hox protein function. PLoS genetics. 2013; 9(2):e1003252. Epub 2013/02/15. [PubMed: 23408901]
119. Zeisig BB, Milne T, Garcia-Cuellar MP, Schreiner S, Martin ME, Fuchs U, et al. Hoxa9 and Meis1 are key targets for MLL-ENL-mediated cellular immortalization. Molecular and cellular biology. 2004; 24(2):617–628. Epub 2004/01/01. [PubMed: 14701735]
120. Rozovskaia T, Ravid-Amir O, Tillib S, Getz G, Feinstein E, Agrawal H, et al. Expression profiles of acute lymphoblastic and myeloblastic leukemias with ALL-1 rearrangements. Proceedings of the National Academy of Sciences of the United States of America. 2003; 100(13):7853–7858. Epub 2003/06/05. [PubMed: 12782787]
121. Serrano E, Lasa A, Perea G, Carnicer MJ, Brunet S, Aventin A, et al. Acute myeloid leukemia subgroups identified by pathway-restricted gene expression signatures. Acta haematologica. 2006; 116(2):77–89. Epub 2006/08/18. [PubMed: 16914901]
122. Afonja O, Smith JE Jr, Cheng DM, Goldenberg AS, Amorosi E, Shimamoto T, et al. MEIS1 and HOXA7 genes in human acute myeloid leukemia. Leuk Res. 2000; 24(10):849–855. Epub 2000/09/21. [PubMed: 10996203]
123. Nakamura T, Largaespada DA, Shaughnessy JD Jr, Jenkins NA, Copeland NG. Cooperative activation of Hoxa and Pbx1-related genes in murine myeloid leukaemias. Nat Genet. 1996; 12(2):149–153. Epub 1996/02/01. [PubMed: 8563752]
124. Wang Z, Iwasaki M, Ficara F, Lin C, Matheny C, Wong SH, et al. GSK-3 promotes conditional association of CREB and its coactivators with MEIS1 to facilitate HOX-mediated transcription and oncogenesis. Cancer Cell. 2010; 17(6):597–608. [PubMed: 20541704]
125. Fung TK, Gandillet A, So CW. Selective treatment of mixed-lineage leukemia leukemic stem cells through targeting glycogen synthase kinase 3 and the canonical Wnt/beta-catenin pathway. Current opinion in hematology. 2012; 19(4):280–286. Epub 2012/04/25. [PubMed: 22525581]
126. Wang Z, Smith KS, Murphy M, Piloto O, Somervaille TC, Cleary ML. Glycogen synthase kinase 3 in MLL leukaemia maintenance and targeted therapy. Nature. 2008; 455(7217):1205–1209. Epub 2008/09/23. [PubMed: 18806775]
127. Li Z, Zhang Z, Li Y, Arnovitz S, Chen P, Huang H, et al. PBX3 is an important cofactor of HOXA9 in leukemogenesis. Blood. 2013; 121(8):1422–1431. Epub 2012/12/25. [PubMed: 23264595]
128. Morgan R, Pirard PM, Shears L, Sohal J, Pettengell R, Pandha HS. Antagonism of HOX/PBX dimer formation blocks the in vivo proliferation of melanoma. Cancer Res. 2007; 67(12):5806–5813. Epub 2007/06/19. [PubMed: 17575148]

129. Merabet S, Dard A. Tracking context-specific transcription factors regulating hox activity. Developmental dynamics : an official publication of the American Association of Anatomists. 2014; 243(1):16–23. Epub 2013/06/25. [PubMed: 23794379]

130. Lambert B, Vandeputte J, Remacle S, Bergiers I, Simonis N, Twizere JC, et al. Protein interactions of the transcription factor Hoxa1. BMC developmental biology. 2012; 12:29. Epub 2012/10/24. [PubMed: 23088713]

131. Ferbeyre G, Moriggl R. The role of Stat5 transcription factors as tumor suppressors or oncogenes. Biochimica et biophysica acta. 2011; 1815(1):104–114. [PubMed: 20969928]

132. Nerlov C. C/EBPalpha mutations in acute myeloid leukaemias. Nature reviews Cancer. 2004; 4(5):394–400. Epub 2004/05/04. [PubMed: 15122210]

133. Reisman D, Glaros S, Thompson EA. The SWI/SNF complex and cancer. Oncogene. 2009; 28(14):1653–1668. [PubMed: 19234488]

134. Dintilhac A, Bihan R, Guerrier D, Deschamps S, Pellerin I. A conserved non-homeodomain Hoxa9 isoform interacting with CBP is co-expressed with the 'typical' Hoxa9 protein during embryogenesis. Gene expression patterns : GEP. 2004; 4(2):215–222. Epub 2004/05/27. [PubMed: 15161102]

135. Shen WF, Krishnan K, Lawrence HJ, Largman C. The HOX homeodomain proteins block CBP histone acetyltransferase activity. Molecular and cellular biology. 2001; 21(21):7509–7522. Epub 2001/10/05. [PubMed: 11585930]

136. Lehnertz B, Pabst C, Su L, Miller M, Liu F, Yi L, et al. The methyltransferase G9a regulates HoxA9-dependent transcription in AML. Genes & development. 2014; 28(4):317–327. Epub 2014/02/18. [PubMed: 24532712]

137. Bei L, Lu Y, Bellis SL, Zhou W, Horvath E, Eklund EA. Identification of a HoxA10 activation domain necessary for transcription of the gene encoding beta3 integrin during myeloid differentiation. The Journal of biological chemistry. 2007; 282(23):16846–16859. [PubMed: 17439948]

138. Lu Y, Goldenberg I, Bei L, Andrejic J, Eklund EA. HoxA10 represses gene transcription in undifferentiated myeloid cells by interaction with histone deacetylase 2. The Journal of biological chemistry. 2003; 278(48):47792–47802. [PubMed: 14512427]

139. Wu X, Ellmann S, Rubin E, Gil M, Jin K, Han L, et al. ADP ribosylation by PARP-1 suppresses HOXB7 transcriptional activity. PloS one. 2012; 7(7):e40644. [PubMed: 22844406]

140. Vijapurkar U, Fischbach N, Shen W, Brandts C, Stokoe D, Lawrence HJ, et al. Protein kinase C-mediated phosphorylation of the leukemia-associated HOXA9 protein impairs its DNA binding ability and induces myeloid differentiation. Molecular and cellular biology. 2004; 24(9):3827–3837. Epub 2004/04/15. [PubMed: 15082777]

141. Bandyopadhyay S, Harris DP, Adams GN, Lause GE, McHugh A, Tillmaand EG, et al. HOXA9 methylation by PRMT5 is essential for endothelial cell expression of leukocyte adhesion molecules. Molecular and cellular biology. 2012; 32(7):1202–1213. Epub 2012/01/25. [PubMed: 22269951]

142. Calero-Nieto FJ, Joshi A, Bonadies N, Kinston S, Chan WI, Gudgin E, et al. HOX-mediated LMO2 expression in embryonic mesoderm is recapitulated in acute leukaemias. Oncogene. 2013; 32(48):5471–5480. [PubMed: 23708655]

143. Brumatti G, Salmanidis M, Kok CH, Bilardi RA, Sandow JJ, Silke N, et al. HoxA9 regulated Bcl-2 expression mediates survival of myeloid progenitors and the severity of HoxA9-dependent leukemia. Oncotarget. 2013; 4(11):1933–1947. [PubMed: 24177192]

144. Breitinger C, Maethner E, Garcia-Cuellar MP, Schambony A, Fischer KD, Schilling K, et al. HOX genes regulate Rac1 activity in hematopoietic cells through control of Vav2 expression. Leukemia. 2013; 27(1):236–238. [PubMed: 22713647]

145. Steger J, Fuller E, Garcia-Cuellar MP, Hetzner K, Slany RK. Insulin-like growth factor 1 is a direct HOXA9 target important for hematopoietic transformation. Leukemia. 2014 Epub 2014/09/26.

146. Ortega S, Malumbres M, Barbacid M. Cyclin D-dependent kinases, INK4 inhibitors and cancer. Biochimica et biophysica acta. 2002; 1602(1):73–87. Epub 2002/04/19. [PubMed: 11960696]

147. Drexler HG. Review of alterations of the cyclin-dependent kinase inhibitor INK4 family genes p15, p16, p18 and p19 in human leukemia-lymphoma cells. Leukemia. 1998; 12(6):845–859. Epub 1998/06/25. [PubMed: 9639410]

148. Williams RT, Sherr CJ. The INK4-ARF (CDKN2A/B) locus in hematopoiesis and BCR-ABL-induced leukemias. Cold Spring Harb Symp Quant Biol. 2008; 73:461–467. Epub 2008/11/26. [PubMed: 19028987]

149. Wolff L, Bies J. p15Ink4b Functions in determining hematopoietic cell fates: implications for its role as a tumor suppressor. Blood Cells Mol Dis. 2013; 50(4):227–231. Epub 2013/02/14. [PubMed: 23403260]

150. Smith LL, Yeung J, Zeisig BB, Popov N, Huijbers I, Barnes J, et al. Functional crosstalk between Bmi1 and MLL/Hoxa9 axis in establishment of normal hematopoietic and leukemic stem cells. Cell stem cell. 2011; 8(6):649–662. Epub 2011/06/01. [PubMed: 21624810]

151. Rezsohazy R. Non-transcriptional interactions of Hox proteins: inventory, facts, and future directions. Developmental dynamics : an official publication of the American Association of Anatomists. 2014; 243(1):117–131. [PubMed: 24115586]

152. Ohno Y, Yasunaga S, Janmohamed S, Ohtsubo M, Saeki K, Kurogi T, et al. Hoxa9 transduction induces hematopoietic stem and progenitor cell activity through direct down-regulation of geminin protein. PloS one. 2013; 8(1):e53161. [PubMed: 23326393]

153. Yasunaga S, Ohtsubo M, Ohno Y, Saeki K, Kurogi T, Tanaka-Okamoto M, et al. Scmh1 has E3 ubiquitin ligase activity for geminin and histone H2A and regulates geminin stability directly or indirectly via transcriptional repression of Hoxa9 and Hoxb4. Molecular and cellular biology. 2013; 33(4):644–660. [PubMed: 23207902]

154. Zhou B, Liu C, Xu Z, Zhu G. Structural basis for homeodomain recognition by the cell-cycle regulator Geminin. Proceedings of the National Academy of Sciences of the United States of America. 2012; 109(23):8931–8936. [PubMed: 22615398]

155. Bergiers I, Bridoux L, Nguyen N, Twizere JC, Rezsohazy R. The homeodomain transcription factor Hoxa2 interacts with and promotes the proteasomal degradation of the E3 ubiquitin protein ligase RCHY1. PloS one. 2013; 8(11):e80387. [PubMed: 24244684]

156. Topisirovic I, Kentsis A, Perez JM, Guzman ML, Jordan CT, Borden KL. Eukaryotic translation initiation factor 4E activity is modulated by HOXA9 at multiple levels. Molecular and cellular biology. 2005; 25(3):1100–1112. [PubMed: 15657436]

157. Zhang Y, Morrone G, Zhang J, Chen X, Lu X, Ma L, et al. CUL-4A stimulates ubiquitylation and degradation of the HOXA9 homeodomain protein. The EMBO journal. 2003; 22(22):6057–6067. Epub 2003/11/12. [PubMed: 14609952]

158. Schaefer LK, Wang S, Schaefer TS. Functional interaction of Jun and homeodomain proteins. The Journal of biological chemistry. 2001; 276(46):43074–43082. Epub 2001/09/12. [PubMed: 11551904]

159. Shen WF, Montgomery JC, Rozenfeld S, Moskow JJ, Lawrence HJ, Buchberg AM, et al. AbdB-like Hox proteins stabilize DNA binding by the Meis1 homeodomain proteins. Molecular and cellular biology. 1997; 17(11):6448–6458. Epub 1997/10/29. [PubMed: 9343407]

160. Shen WF, Rozenfeld S, Kwong A, Kom ves LG, Lawrence HJ, Largman C. HOXA9 forms triple complexes with PBX2 and MEIS1 in myeloid cells. Molecular and cellular biology. 1999; 19(4): 3051–3061. Epub 1999/03/19. [PubMed: 10082572]

161. Shi X, Bai S, Li L, Cao X. Hoxa-9 represses transforming growth factor-beta-induced osteopontin gene transcription. The Journal of biological chemistry. 2001; 276(1):850–855. Epub 2000/10/24. [PubMed: 11042172]

162. Dasse E, Volpe G, Walton DS, Wilson N, Del Pozzo W, O'Neill LP, et al. Distinct regulation of c-myb gene expression by HoxA9, Meis1 and Pbx proteins in normal hematopoietic progenitors and transformed myeloid cells. Blood cancer journal. 2012; 2(6):e76. Epub 2012/07/26. [PubMed: 22829978]

163. Hess JL, Bittner CB, Zeisig DT, Bach C, Fuchs U, Borkhardt A, et al. c-Myb is an essential downstream target for homeobox-mediated transformation of hematopoietic cells. Blood. 2006; 108(1):297–304. Epub 2006/03/02. [PubMed: 16507773]

164. Wang GG, Pasillas MP, Kamps MP. Persistent transactivation by meis1 replaces hox function in myeloid leukemogenesis models: evidence for co-occupancy of meis1-pbx and hox-pbx complexes on promoters of leukemia-associated genes. Molecular and cellular biology. 2006; 26(10):3902–3916. Epub 2006/05/02. [PubMed: 16648484]

165. Gwin K, Frank E, Bossou A, Medina KL. Hoxa9 regulates Flt3 in lymphohematopoietic progenitors. Journal of immunology. 2010; 185(11):6572–6583. Epub 2010/10/26.

166. Whelan JT, Ludwig DL, Bertrand FE. HoxA9 induces insulin-like growth factor-1 receptor expression in B-lineage acute lymphoblastic leukemia. Leukemia. 2008; 22(6):1161–1169. Epub 2008/03/14. [PubMed: 18337761]

167. Hu YL, Passegue E, Fong S, Largman C, Lawrence HJ. Evidence that the Pim1 kinase gene is a direct target of HOXA9. Blood. 2007; 109(11):4732–4738. Epub 2007/03/01. [PubMed: 17327400]

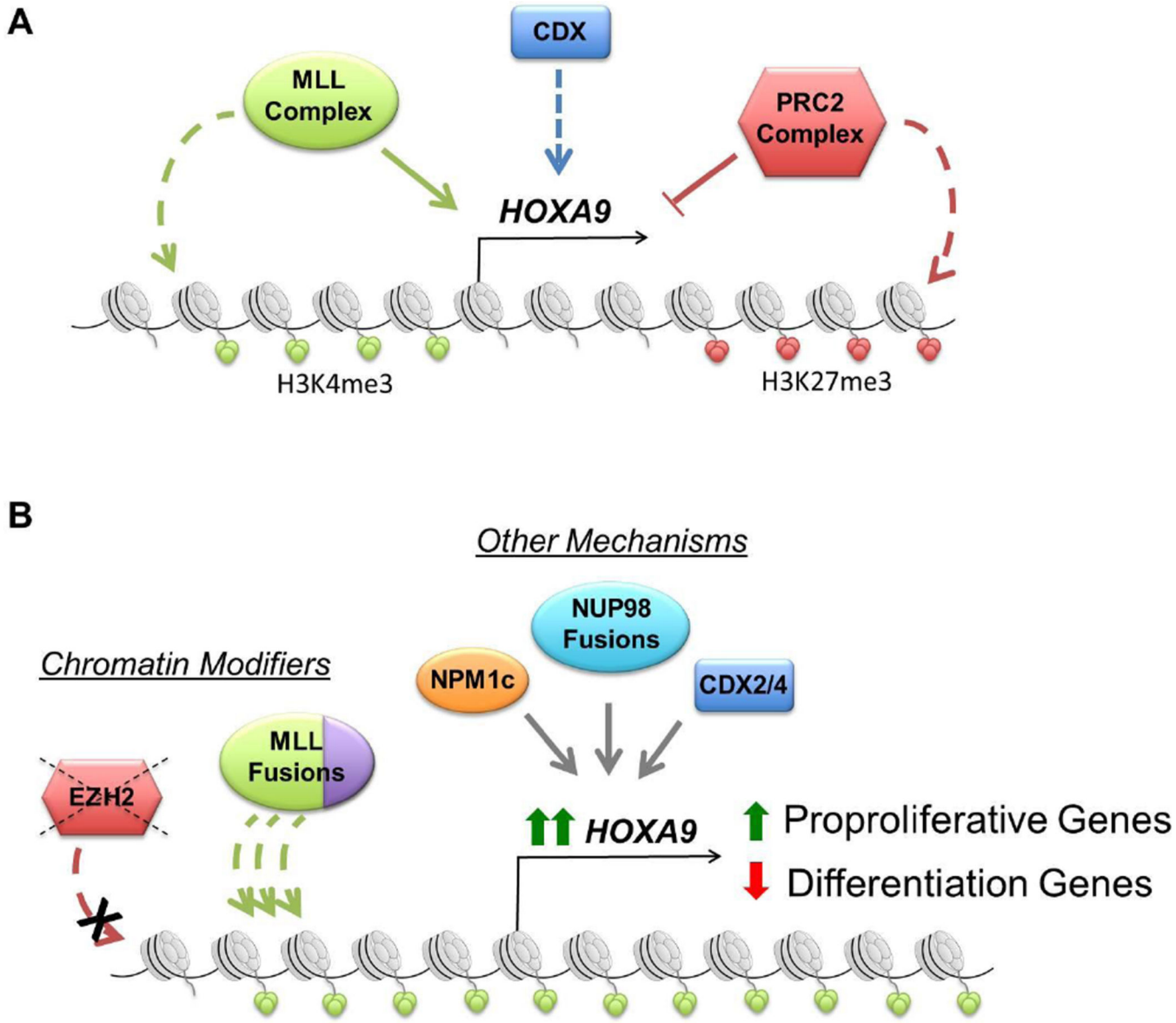

Figure 1.

HOXA9 regulation in normal hematopoiesis and leukemia. (A) During development and hematopoiesis, expression of *HOXA9* is primarily regulated by the antagonistic actions of the MLL complex and polycomb repressive complex. These histone methytransferases deposit the activating H3K4me3 and repressive H3K27me3 marks respectively. CDX proteins also play a role in *HOXA9* regulation, through mechanisms that are not well defined. (B) A variety of upstream genetic alterations lead to the up regulation of HOXA9, which is essential for the acute leukemias that result from these alterations. Decreased expression of *EZH2* and chromosomal translocations leading to MLL-fusion proteins result in activation of HOXA9 expression through dysregulated chromatin modification. Cytoplasmic mutations of *NPM1*, fusion proteins with NUP98 and overexpression of *CDX2* and *CDX4* also leads to up regulation of HOXA9 through mechanisms that remain to be completely defined. HOXA9 likely goes on to promote transformation through the

activation of proproliferative genes and the repression of genes required for cellular differentiation.

Table 1

HOXA9 Interacting Partners

Protein	Method	Reference
CEBPA	Affinity capture-MS Affinity capture-western	(101)
CEBPB	Affinity capture-MS	(101)
CEBPE	Affinity capture-MS	(101)
CREB1	Affinity capture-MS Affinity capture-western	(101)
CREBBP	Affinity capture-western Far western Reconstituted complex	(134, 135)
CUL4A	Affinity capture-western Reconstituted complex	(157)
G9A	Affinity capture-western	(136)
JUN	Yeast two-hybrid	(158)
MEIS1	Affinity capture-MS Affinity capture-western Reconstituted complex	(101, 134, 159, 160)
MEIS2	Affinity capture-western	(134)
NFKBIA	Affinity capture-western Far western	(134)
PBX1	Reconstituted complex	(134, 160)
PBX2	Affinity capture-western Reconstituted complex	(160)
PBX3	Reconstituted complex Affinity capture-MS	(101, 160)
PRMT5	Affinity capture-MS Affinity capture-western	(141)
SMAD4	Affinity capture-western	(161)
STAT1	Affinity capture-MS	(101)
STAT5	Affinity capture-MS Affinity capture-western	(101)

Table 2

HOXA9 Target Genes

Gene	Reference
Bcl-2	(143)
c-Myb	(162, 163)
Erg	(101)
Fgf-2	(88, 91)
Flt3	(101, 164, 165)
Fos	(124)
Igf1	(145)
Igf1R	(166)
Ink4a/ARF/Ink4b	(6, 150)
Lmo2	(142)
Pim1	(167)
Sox4	(101)
Vav2	(144)