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*Review Article*

# HOX Proteins and Leukemia

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**Abstract**: HOX and three amino acid loop extension (TALE) proteins cooperate to induce transformation in mouse leukemia models, and are dysregulated in a variety of human leukemias. Despite decades of research, the mechanism of action for Hox proteins in embryogenesis and hematopoiesis remains unclear. Recent studies on the roles of Hoxa9 and Meis1 in leukemia has led to a wealth of new data, but their molecular mechanisms of action and synergy remain obscure. Advances in genome-wide technologies offer new avenues for understanding how homeodomain-containing transcription factors exert their programs in normal and neoplastic development.
**Key Words**: HOX, leukemia

## Introduction

HOX proteins are an evolutionarily conserved family of homeodomain-containing DNA binding transcription factors with significant homology to the HOM-C factors in *Drosophila melanogaster* that are involved in the specification of segmental body pattern during development [1, 2]. In mammals, 39 *HOX* genes are arranged in four paralogous clusters (A-D) that map to four different chromosomes. The genes are arranged contiguously within each locus so that their 3' to 5' position parallels their temporal and spatial distribution; in the embryo 3' factors are expressed anteriorly and 5' factors are expressed posteriorly (**Figure 1**).

The homeodomain is a DNA-binding motif consisting of 60 amino acids configured in a helix-turn-helix motif with three alpha helices connected by short loops. Whereas the first two helices are anti-parallel, the C-terminal helix is longer, resting perpendicularly to the others, and binding directly to DNA in the major groove [3]. The N-terminus of the homeodomain is unstructured and interacts with the adjacent minor groove of DNA. Hox protein homeodomains share considerable homology and by themselves are unlikely to contribute significant DNA sequence specificity beyond a conserved TTNAT motif, which has been defined primarily through *in vitro* binding assays [4-6]. Additional target selectivity *in vivo* is likely conferred by associated proteins including TALE (three amino acid loop extension) cofactors such as Meis1 [5-7].

## HOX Proteins in Hematopoiesis

Whereas body pattern development occurs only once at the beginning of life, hematopoietic maturation continues indefinitely, allowing for life-long renewal of blood and immune cells. Hox proteins are important regulators of hematopoiesis. *Hox a, b*, and *c* cluster genes are expressed early in hematopoiesis and are generally downregulated during differentiation [8-10]. Mouse models provide evidence of Hox protein involvement at various stages of hematopoiesis. *MLL* (mixed lineage leukemia)-null murine embryonic stem cells exhibit downregulation of many *Hox a*, *b*, and *c* cluster genes with significant impairment of normal hematopoiesis [11]. Hoxa7 appears to be involved in erythropoiesis and megakaryopoiesis [12]. Hoxb4 is a potent stimulator of hematopoietic stem cell (HSC) expansion [13-16]. However, Hoxb4 is not necessary for normal hematopoiesis as Hoxb4 deficient mice show only a mild proliferative defect in HSCs [17]. Hoxb3 deficiency causes impaired B lymphopoiesis [18]. Hoxa10 also appears to be involved in lymphopoiesis, erythropoiesis and megakaryopoiesis [19, 20].

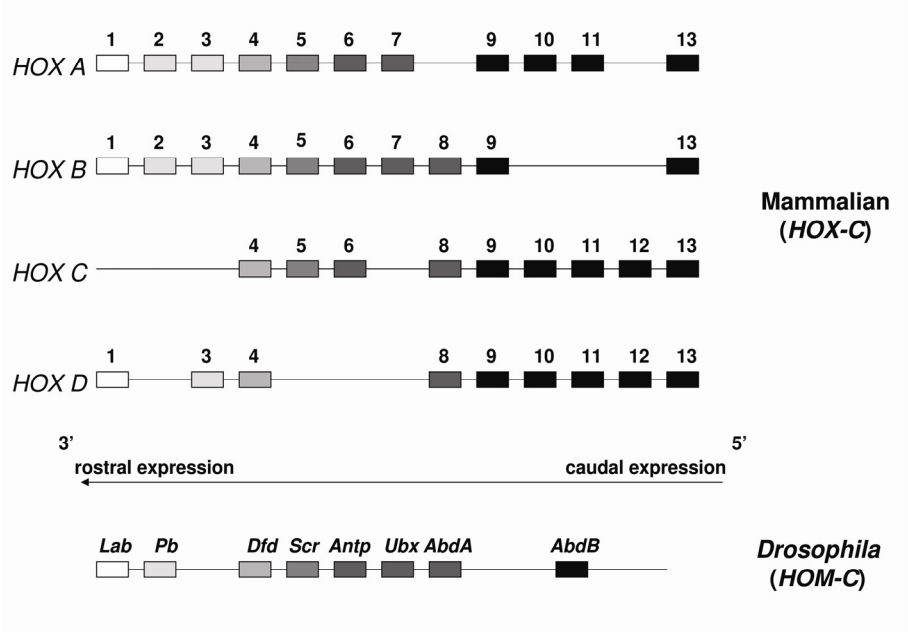


**Figure 1** Organization of the clustered mammalian HOX genes. HOX genes form linear arrays on 4 different chromosomes and exhibit significant homology to the Drosophila HOM-C clustered homeotic genes.

Notably, *Hoxa9* is the most highly expressed *Hox* gene in the HSC compartment. It is expressed in early hematopoietic progenitors (HPs), downregulated during differentiation and is important in HSC expansion [13, 21]. Among single *Hox* knockouts, Hoxa9 deficiency yields the most severe phenotype; however even in these animals, multilineage hematopoietic defects are mild (**Table 1**) [22, 23]. The most significant impairment exhibited by *Hoxa9*-null HSCs is the poor repopulation in bone marrow transplantation experiments [24]. Compound *Hox* knockouts show similarly mild hematopoietic phenotypes, most likely because of considerable redundancy of function among and between paralog groups [25].

## TALE Proteins – HOX Partners and Beyond

TALE proteins are homeodomain-containing transcription factors that are thought to enhance DNA binding specificity of Hox proteins through heterodimerization [5, 7]. Within this category, subgroups include the Pbx and Meis families, which have been shown to assemble in trimeric complexes composed of Hox, Pbx and Meis subunits upon a single DNA sequence [6]. Although the crystal structure of a Hoxa9/Pbx heterodimer bound to DNA has been reported [4], no structural data is available for a Hoxa9-Meis1 dimer or Pbx-containing trimer.

Like Hox proteins, TALE proteins are involved in embryogenesis as well as hematopoiesis [26]. *Meis1* expression during hematopoiesis parallels *Hox* expression, with levels high in HPs and increasingly downregulated with further differentiation [10]. Meis1 plays an important role in limb and eye development, as evidenced by the severe phenotypes seen in *Meis1*-null embryos (*Table 1*). However, lethality results from its hematopoietic contribution, as embryos succumb from amegakaryopoiesis by day E14 [26]. This broader phenotype compared to Hox knockout animals may result from hetero-dimerization of Meis1 with several Hox proteins; alternatively the phenotype may be the result of non-Hox related contributions to embryogenesis. A recent study revealed linkage of the neurological disorder Restless Legs Syndrome to the *MEIS1* locus, underscoring the broad scope of physiologic processes impacted by TALE factors [27].

## HOX and TALE Proteins in MLL-Rearranged Leukemias

Acute leukemias with rearrangements of the *MLL* gene (11q23) have an intermediate to

**Table 1** Characteristics of Hoxa9 and Meis1 in mouse models.

| Experimental model | Hoxa9 | Meis1 |
|---|---|---|
| Loss of expression (null mice) | Viable, morphologically normal, normal number of HSCs [22] | Fatal by E14.5 – bleeding from amegakaryopoiesis |
| | Mild leucopenia/blunted G-CSF response [22] | Additional defects in angiogenesis, eye development |
| | Decreased number of committed myeloid and lymphoid progenitors [22, 23] | Reduction in myeloerythroid colony-forming units |
| | BMT repopulation defect with decreased HSC proliferation [24] | Heterozygotes viable; no phenotypic defects [26] |
| Enforced expression (HSC transduction) | Immortalization, differentiation block [100] | Pro-apoptotic |
| | Non-transforming when transplanted [60] | Does not promote immortalization |
| Lack of expression in MLL-fusion cells | Can be tolerated [71] | Required for transformation [63] |

poor prognosis compared to cytogenetically favorable types [28-31]. *MLL* rearrangements comprise 5-6% of all acute myeloid leukemias and about 20% of acute lymphoblastic leukemias [32, 33]. In development and hematopoiesis, wild-type MLL activates *Hox* gene transcription by methylation of histone H3 at lysine 4 through its intrinsic histone methyltransferase activity [34]. Additionally, fusion proteins involving MLL deregulate *Hoxa9* and *Meis1* expression [34-37]. Acute lymphoblastic leukemias with *MLL* rearrangements show high expression of *HOXA7* and *HOXA9* as well as *MEIS1* [38-40]. These findings suggest that HOXA9 and MEIS1 are key mediators of transformation by *MLL* rearrangements.

## Clinical Evidence for HOX Involvement in Other Leukemias

*HOX* genes are overexpressed or rearranged in a variety of experimental and human leukemias [38-45]. In acute lymphoblastic leukemias, *HOXA9* overexpression appears to be largely limited to those with *MLL* translocations, whereas a large fraction of myeloid leukemias show dysregulated *HOXA9* expression [38]. *HOXA9* is associated with refractory AML and poor prognosis [46]. High level *HOXA9* expression is implicated in T-cell ALL cases, including subtypes harboring *MLL*, *CALM-AF10* t(10;11), and *TCRβ-HOXA* locus (inv(7)(p15q34)) rearrangements [47, 48]. A recent study identified a correlation between *HOXA9* expression in T-ALL and a prognostically distinct subgroup with relatively primitive phenotype [49]. Given that the prevalence of leukemia increases with age, it is interesting that *HOXA9* levels tend to be higher in marrow from elderly donors [50]. Direct involvement of HOX proteins in oncogenic fusions with NUP98, such as NUP98-HOXA9 t(7;11) and NUP98-HOXD13 t(2;11)(q31;p15) in AML has been well reviewed [51, 52].

Like *HOXA9*, *MEIS1* expression appears to correlate with poor prognosis in AML. Two patient series showed decreased *MEIS1* expression in AML patients with good outcome [53, 54].

## Role of HOX and TALE Proteins in Experimental Models of Transformation

Enforced retroviral expression of certain *Hox* genes (for example *Hoxa9*, *Hoxa10*, *Hoxb3*, or *Hoxb6*) in murine bone marrow cells results in differentiation block with continuous self-renewal in culture [21, 55-58]. The stage at which maturation arrest occurs varies with the

particular Hox protein that is overexpressed; for example, *Hoxa9* overexpression causes arrest with an early myelomonocytic phenotype [59]. Transplantation of *Hoxa9*-transduced bone marrow into mice does not result in a fully penetrant leukemia; instead, transformation to leukemia is seen in a small subset of animals after prolonged latency, suggesting that additional "hits" are required [56, 57, 60]. Co-expression of *Meis1* greatly increases the transformation efficiency of *Hoxa9*; HSCs transduced with both *Hoxa9* and *Meis1* confer a rapidly fatal leukemia in transplanted animals [60]. Meis1 has also been shown to accelerate Nup98-Hox fusion mediated leukemogenesis [61, 62]. These findings imply synergy between *Hox* genes and *Meis1* in leukemic transformation.

The role of Pbx proteins in transformation process is unclear. MLL fusion proteins have been shown to upregulate *Pbx3* expression [36, 63]. In addition, joint reduction of Pbx2 and Pbx3 levels interferes with MLL-driven leukemogenesis [63]. Furthermore, the Pbx interaction domain of Meis1 is required for MLL-fusion mediated transformation and for promoting leukemia in Hoxa9-immortalized cells [63, 64]. Hoxa9 also requires its Pbx-interaction domain for its myeloid expansion phenotype [65].

Despite its critical role in promoting leukemic transformation when expressed with Hox proteins, enforced expression of Meis1 in hematopoietic progenitors does not lead to immortalization and is in fact pro-apoptotic [66]. Two groups have shown that addition of a VP16 transactivating domain to the N-terminus of Meis1 results in a chimeric factor capable of immortalizing HPs; however VP16-Meis1 leukemias show much longer latency than seen with Hoxa9+Meis1 coexpression [67, 68]. The implications of this observation are discussed later in the review.

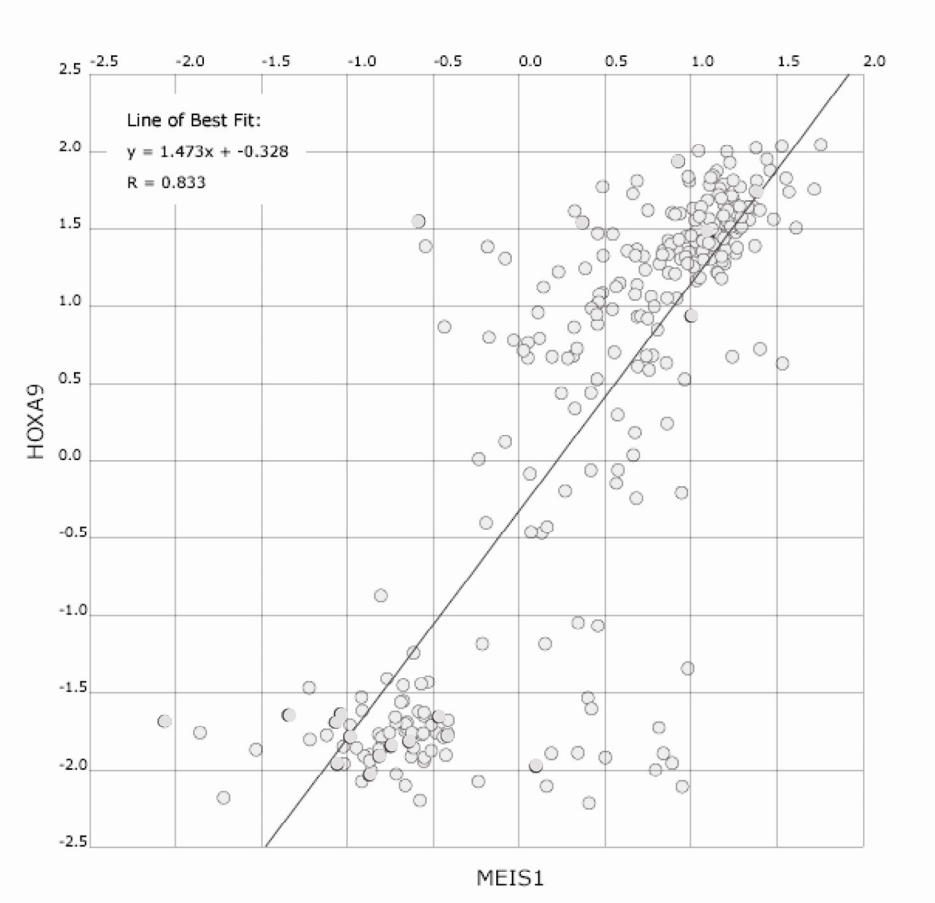


**Figure 2** HOXA9 and MEIS1 expression levels in AML patient samples. Expression data from 285 leukemias [104] was analyzed using the co-expression module at oncomine.org and shows the correlation between HOXA9 and MEIS1 expression [70]. MLL-fusion cases represent only 7% of cases in this study [104].

### Cooperation between HOX and TALE Proteins in Leukemia

In addition to their parallel up-regulation by MLL fusion proteins, several lines of evidence indicate a strong synergy between Hox and TALE proteins. In the BXH2 murine retroviral mutagenesis model of AML, *Hoxa9* and *Meis1* loci are nearly always activated in tandem through independent viral integrations [69]. In addition, analysis of human AML samples by microarray (www.oncomine.org) shows strong correlation between *MEIS1* and *HOXA9* levels (**Figure 2**)[70]. The most convincing evidence comes from the phenotype of murine HSCs coexpressing *Hoxa9* and *Meis1* when compared to overexpression of either gene alone: as discussed above, the combination is potently leukemogenic [60, 64]. Since *Meis1* has the opposite phenotype when expressed by itself (**Table 1**), the nature of synergy between Hoxa9 and Meis1 is unlikely to be simply additive.

### Are HOXA9 and MEIS1 Dispensible in Leukemogenesis?

Studies using HSCs purified from *Meis1*-null embryos prior to demise indicate that Meis1 is necessary for MLL-fusion protein mediated transformation [63]. This was found to be the case for 12 different MLL fusion proteins (but not for the unrelated translocation E2A-HLF), and was linked to both induction and maintenance of leukemic proliferation. The report also showed acceleration of MLL-fusion leukemias through addition of ectopically expressed Meis1. The combination of these two findings lends strong support to Meis1 dose dependency, since the baseline Meis1 concentration induced by MLL-fusion expression is necessary, but higher level expression of Meis1 has a potentiating effect [63].

The necessity of Hoxa9 for MLL fusion protein mediated leukemogenesis is less clear-cut. Initial data offers conflicting results, as a single group found loss of transformation by *MLL-ENL* in *Hoxa7*- or *Hoxa9*-null mice but retention of leukemic potential with *MLL-GAS7* expression in a *Hoxa9*$^{-/-}$ background [12, 37]. At the same time, others found Hoxa9 to be dispensible in generation of leukemias from transgenically expressed *MLL-AF9*; in fact, *Hoxa9*-null HSCs exhibited less myeloid differentiation than wild-type counterparts [71]. These observations may be due to experimental design: in the *MLL-ENL* study, Lin$^-$ cells (HSCs) were chosen for retroviral transduction, while the *MLL-GAS7* report used c-Kit-selected precursors which include common myeloid progenitors [the *MLL-AF9* study involved a germline transgene and did not require a selection step] [72]. One potential explanation is that Hoxa9 freezes cells in a particular stage of differentiation that permits MLL-fusion mediated transformation. Kit-selected cells represent a spectrum between HSCs and later myeloid progenitors, while Lin$^-$ HSCs are homogeneous. Indeed, these observations have been attributed to the concept that the committed myeloid progenitor is the "leukemic stem cell" for MLL fusions [73].

More recent data suggests that continued *HOXA9* expression is essential for maintenance of immortalization by MLL-fusion proteins; shRNA knockdown of HOXA9 in a t(9;11) human leukemia cell line (MOLM-14) inhibits proliferation as early as 48 hours after transduction [74]. In addition HOXA9 reduction reverses the *in vivo* transformation phenotype in mice transplanted with SEMK2, a t(4;11) leukemia line. Whereas proliferation/viability of non-*MLL*-rearranged leukemic cell lines is also affected by HOXA9 knockdown, there is a statistically significant difference in magnitude when compared to human AML cells with *MLL* translocations. Not surprisingly, the greatest effects are seen in leukemic cells with the highest baseline expression levels of HOXA9 [74]. These findings suggest an ongoing role for HOXA9 in AML cells, rather than a "hit-and-run" contribution to leukemogenesis.

### Why is HOXA9 so Commonly Implicated in Leukemogenesis?

Although HOXA9 is the most commonly deregulated HOX factor in leukemia, abundant evidence suggests that HOX proteins play redundant roles in leukemogenesis. The ability of MLL fusion proteins to transform in a *Hoxa9*-null background (discussed above) points to this: In addition, Hoxb6, Hoxa7, Hoxa10, and others show similar properties in HSC immortalization/transformation studies [12, 56, 57, 67]. Gene expression profiling of MLL-fusion mouse models shows overexpression of *Hoxa5*, *Hoxa6*, *Hoxa7*, *Hoxa9*, and *Hoxa10*, and a subset of human leukemias show coordinated upregulation of

*HOXA5-10* along with *HOXA9* [38, 75]. However, it is *HOXA9* that emerges as the single most prognostic gene in clinical leukemias, and *Hoxa9* and *Hoxa7* are the most common insertion sites (along with *Meis1*) in BXH2 insertion-mediated mouse leukemias [46, 69]. Although viable, *Hoxa9*-null mice have a more profound hematopoietic phenotype than other characterized *Hox* knockouts [52]. Additionally, comparison of *NPM1*-mutated and *MLL*-rearranged human leukemias shows a shared *HOX* dysregulation profile including the posterior *HOXA* cluster and *MEIS1*, while other *HOX* genes show differential regulation [42]. Finally, t(7;11)/*NUP98-HOXA9* is the most common recurring cytogenetic abnormality involving a HOX protein [76, 77]. The precise state of myeloid differentiation specified by HOXA9 may be a key contributor to its relative leukemogenic potential. In this context it may be relevant that *HOXA9* is the most highly predictive transcript for AML vs ALL [38]. Overall, although current data supports considerable redundancy among HOX paralogs, human and mouse studies indicate a particularly important role for HOXA9 in leukemogenesis.

## Which HOXA9 and MEIS1 Targets are Essential for Leukemogenesis?

When ectopically expressed in hematopoietic progenitors, Hoxa9 induces two main phenotypic effects: differentiation arrest (at an early myeloid progenitor phase) and indefinite self-renewal (immortalization) [64]. The strongest support for the latter are the repopulation defects that are seen in *Hoxa9*-null mice (see **Table 1**) [24]. The long latency required to reach transformation in animals, and the elimination of this latency by coexpression of *Meis1*, imply that by themselves Hox proteins do not confer sufficient proliferative capacity for leukemogenesis. There is *in vitro* support for a Meis1 contribution to proliferation. Cells transduced with *Hoxa9* and *Meis1* outgrow *Hoxa9*-transduced cells in culture, and also allow for selective growth in particular cytokine combinations [64, 67]. The genes that mediate these effects are not well defined.

Putative downstream effectors of HOXA9 were first reported from microarray experiments using transient overexpression of *HOXA9* in cultured cell lines in 2004 [78]. Cells included Jurkat T lymphoblasts, K562 lymphoblasts, and U937 promonocytes. These lines were established from human leukemias and are functionally independent of exogenous *HOXA9* expression. Many identified targets were cell line-specific, but a small subset of genes (14/220) was found to correlate with *HOXA9* expression in published AML tumor profiling data. In a second study from the same laboratory, *HOXA9* was transiently overexpressed in human CD34+ cord blood cells. Surprisingly this resulted in a distinct target set with minimal overlap [79].

A second group of investigators published two reports measuring gene expression differences between cells immortalized with Hoxa9 and Hoxa9+Meis1 [64, 67]. Acute leukemias often arise as the result of activation of a nuclear protooncogene, which blocks differentiation, along with a cytoplasmic receptor tyrosine kinase, which promotes proliferation [80]. One Hoxa9/Meis1 target that fits this model is the tyrosine kinase Flt3, which is the most common signal transduction "hit" in AML [81] Increased expression of Flt3 is seen in *Hoxa9/Meis1*-transduced HPs when compared to HPs immortalized by Hoxa9 alone; in addition, chromatin immuno-precipitation (ChIP) demonstrated binding of the *Flt3* promoter by both Hoxa9 and Meis1 [67]. However, HPs from *Flt3*-null mice can be transformed by Hoxa9+Meis1 with efficiency comparable to wild-type cells, so Flt3 cannot be considered the single, necessary mediator of leukemogenesis [82]. Wild-type Flt3 is insufficient for transformation of hematopoietic precursors on its own; Flt3 can promote leukemogenesis with Nup98-Hoxa9, but the latency in this setting is longer than with co-expression of Meis1 [83]. Other notable targets include the stem-cell associated surface marker CD34 and the proleukemic transcription factor Sox4.

A third laboratory in collaboration with our own took a different approach to identify targets of joint Hoxa9 and Meis1 regulation: inducible expression of MLL-ENL was used to immortalize hematopoietic progenitors; the effects of retroviral introduction of *Hoxa9* and *Meis1* were observed after withdrawal of MLL-ENL support [84]. The oncogene *c-Myb* was found to be necessary but not sufficient for transformation by Hoxa9+Meis1.

Several reports at the 2007 American Society

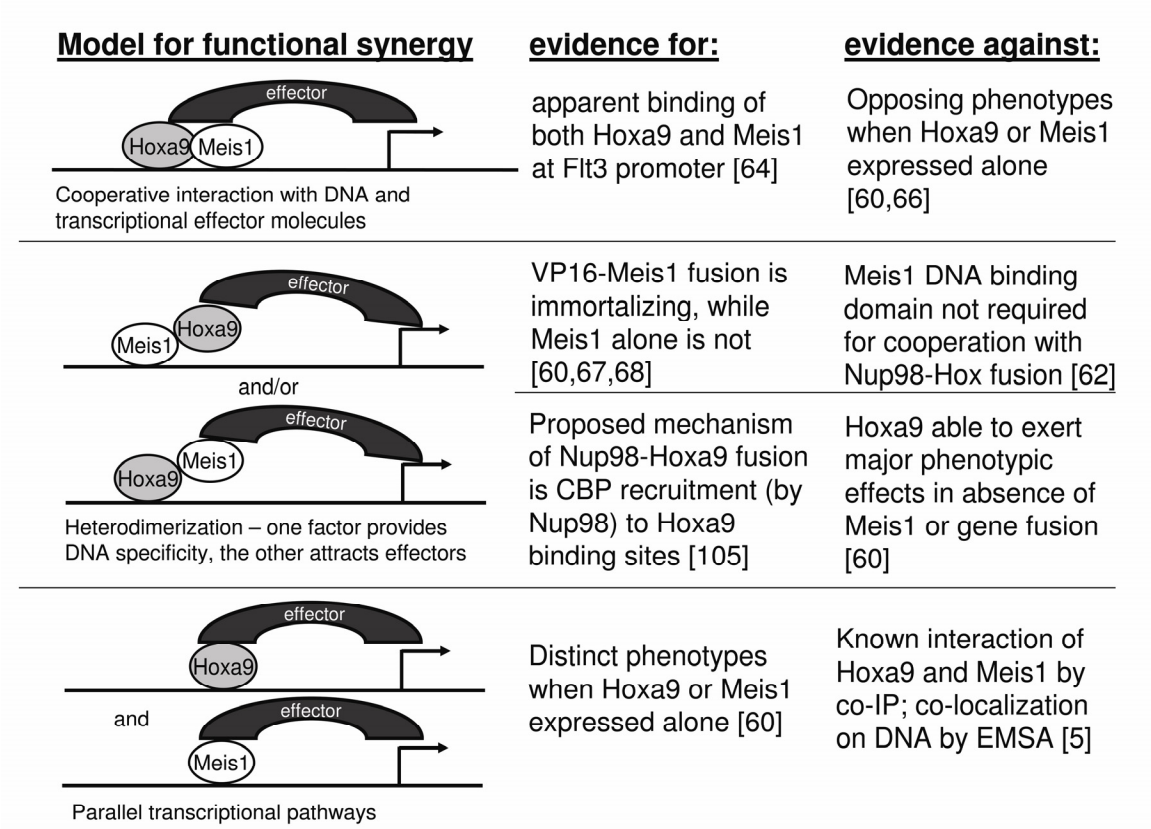


**Figure 3** Possible modes of cooperation between Hoxa9 and Meis1. No single model is completely supported by current evidence; rather it is likely that targets of Hoxa9 and Meis1 are regulated by a combination of these mechanisms.

of Hematology meeting highlighted Hoxa9 targets identified through RNA interference knock-down, including Bim and Mef2c [85, 86]. The latter factor is of interest as it is part of an 11-gene minimal "immediate" gene expression signal in *MLL-AF9* transformed HSCs which includes *Hoxa9* and *Meis1* [87]. More recently, Insulin-like growth factor-1 receptor (*IGF-1R)* was shown to be upregulated in the setting of *HOXA9* overexpression suggesting a possible downstream mechanism for HOXA9 induced cell proliferation [88].

## Molecular Functions of HOXA9 and MEIS1 in Leukemia

Canonical transcription factors merge a sequence-specific DNA binding domain with an interaction motif capable of recruiting enzymatic activities. Although evidence for both functions has been described for individual HOX proteins, the mechanisms by which HOX proteins regulate transcription remain unclear. For example, interaction with CREB binding protein (CBP) has been demonstrated for multiple HOX proteins including HOXA9 [89]. Although the most straightforward explanation is that HOX proteins function by recruiting histone acetyltransferase activity, another report suggests that CBP prevents DNA binding by HOX proteins and that the HOX interaction inhibits CBP acetylase activity [90].

Moreover, it remains an open question whether HOX proteins act predominantly as activators, repressors, or both. It is likely that this is highly dependent on cellular and specific promoter context. It is appealing to propose that the unique synergy between HOXA9 and MEIS1 results from an altered property of the heterodimer. As mentioned above, addition of the VP16 activating moiety

to Meis1 reverses its pro-death phenotype and promotes immortalization [67]. Notably, the Meis1-VP16 fusion retains cooperativity with Hoxa9, arguing against a straightforward "replacement" of Hoxa9 function by VP16. Hoxa9 shows transcriptional activation in reporter gene assays using a Hox-responsive enhancer element; this activity is retained when the N-terminus of Hoxa9 is replaced by VP16 [65]. In a more physiologic model employing the *CYBB* (gp91$^{Phox}$) proximal promoter, HOXA9 was shown to activate transcription in a PBX-dependent manner; however addition of MEIS1B blocked transcriptional activation [91]. Occupancy experiments suggest that HOXA9 is not prevented from binding to DNA but that MEIS1 forms a tripartite complex with PBX1.

Another unresolved topic is the molecular mechanism for the synergy between HOXA9 and MEIS1 in transformation. **Figure 3** illustrates possible models of cooperation, including data supporting and refuting each theory. For example, the finding that VP16-Meis1 has immortalizing activity in HPs, suggests that the DNA binding specificity of Meis1 is crucial to its function [64]. However, the homeodomain of Meis1 is not necessary for acceleration of transformation by the Nup98-Hoxd13 fusion protein, suggesting that Meis1 may play an important role independent of DNA binding [62].

The contribution of PBX proteins to transformation and their role in HOXA9+MEIS1 transcriptional modulation is similarly unclear. TALE factors were traditionally thought to contribute to additional binding specificity rather than enzyme recruitment potential [7]. However, there is evidence for direct interaction between Pbx factors and chromatin remodeling machinery, and also between Pbx1 and histone deacetylase-containing corepressor complexes [92]. Additionally Pbx2 and Pbx3 appear to be involved in MLL-mediated transformation [63].

The solution to these unresolved questions is likely to come from more rigorous biochemical approaches to identifying HOX protein interactors as well as improved approaches to identify *in vivo* binding sites. The assumption that HOX proteins function as transcription factors is well supported, although there are intriguing reports of Hoxa9 modulating gene expression through potentiation of translational efficiency via eIF4E [93]. The Hoxa9-Pbx2-Meis1 trimer, characterized by electrophoretic mobility shift assays using "consensus" binding motifs, has not yet translated into a rigorous demonstration of triple occupancy at *in vivo* promoters using ChIP. In fact, the empiric nature of ChIP data is squandered when putative targets are selected or verified based on the presence of the TTNAT core (which mathematically recurs by chance within 300 bp, compared to an average ChIP DNA fragment size of 500 bp). Finally, the assumption of proximal promoter-mediated function, which has been repeatedly disproven for many transcription factors, continues to drive investigation of target gene regulation for HOX and other homeodomain families. With the advent of truly genomic screening capabilities, these entrenched constraints can be put to the test in an unbiased fashion.

## Additional Insights from HOX Protein Expression in Non-Hematopoietic Tissues

In angiogenesis, Hoxa9 is a positive regulator of EphB4, which stimulates endothelial cell migration and tube forming activity [94]. A recent report demonstrates that Hoxa9 mediates TNFα induction of the pro-inflammatory marker E-selectin in endothelial cells [95]. As in hematopoiesis, Hoxa9 regulation in endothelial development is controlled by MLL [96]. The common hemangioblast theory would predict that the functions of Hoxa9 in hematopoiesis and angiogenesis may be quite similar, with possible overlap of target genes.

Expression of HOX proteins in other non-hematopoietic tissues and solid tumors lends strong support for context-dependent function. HOXA9 in particular appears to have tumor suppressor activity in ectodermally-derived tissue. The Hox 9 paralog group is required for mammary development during pregnancy in mice – lactation does not occur in knockout mice, and foster mothers are required for pup survival [97]. Interestingly, *HOXA9* is frequently epigenetically silenced in human breast cancer [98]. In addition, *HOXA9* CpG islands are frequent DNA methylation targets in stage I primary squamous carcinomas of the lung and there is evidence for HOXA9 dysregulation in human lung cancer cell lines compared to normal lung tissue [99, 100]. *HOXA9* repression by promoter hyper-

methylation is also seen in testicular germ cell tumors [101]. Recently, the *HOXA9* promoter was found to be hypermethylated in over 50% of ovarian cancer samples [102]; interestingly, MEIS1 (as well as MEIS2 and PBX3) show downregulation in a cisplatin-resistant ovarian tumor cell line when compared with chemosensitive parent cells [103]. Assuming that hypermethylation of HOX factors is not an epiphenomenon in solid tumors, the opposing phenotypes of HOXA9 in leukemia versus carcinoma emphasize the role of cellular context and argue against a general pro-proliferation or anti-apoptotic role for HOX proteins.

## Current Approaches to Open Questions in HOX/TALE Biology

Recent advances in genome-wide technologies show promise for better defining the effector mechanisms employed by HOX proteins in leukemia. Multiple platforms are now available for unbiased identification of DNA binding sites on a genome-wide level, including ChIP-chip, ChIP-SAGE (serial analysis of gene expression), and ChIP-Sequencing. Comparing direct binding sites of Hoxa9 and Meis1 (when expressed individually and together) to the gene expression changes induced by each factor can help unravel the cascade of events leading to leukemic transformation. This approach will potentially resolve important molecular questions such as the mechanism of cooperation between Hoxa9 and Meis1, the transcriptional function of the two proteins, and the precise DNA motifs responsible for HOX recruitment.

## Conclusion

Recent improvements in therapy for leukemia subsets (such as acute promyelocytic leukemia) rested upon advances in molecular pathogenesis. Characterizing downstream pathways involved in HOX-mediated leukemias, which are among the poorest prognosis AMLs and ALLs, will hopefully contribute to ameliorating the dismal outcomes seen in these groups. Whereas the topic of HOX and TALE factors in leukemia represents a newcomer to the decades-old field of HOX function in development, contemporary genomic approaches may further an understanding of HOX biology in embryogenesis and normal hematopoiesis as well as in neoplasia.

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