[Frontiers in Bioscience 13, 4297-4308, May 1, 2008]

Grappling with the *HOX* network in hematopoiesis and leukemia

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1. ABSTRACT

The mammalian *HOX* gene network encodes a family of proteins which act as master regulators of developmental processes such as embryogenesis and hematopoiesis. The complex arrangement, regulation and co-factor association of HOX has been an area of intense research, particularly in cancer biology, for over a decade. The concept of redeployment of embryonic regulators in the neoplastic arena has received support from many quarters. Observations of altered *HOX* gene expression in various solid tumours and leukemia appear to support the thesis that 'oncology recapitulates ontogeny' but the identification of critical *HOX* subsets and their functional role in cancer onset and maintenance requires further investigation. The application of novel techniques and model systems will continue to enhance our understanding of the *HOX* network in the years to come. Better understanding of the intricacy of the complex as well as identification of functional pathways and direct targets of the encoded proteins will permit harnessing of this family of genes for clinical application.

2. THE *HOX* NETWORK

The mammalian class I homeobox (*HOX*) gene network consists of 39 genes organized in four clusters (A-D) on four separate chromosomes (Human; 7p14-15, 17q21-22, 12q12-13 and 2q31-37 and Mouse; 6C2, 11B4, 15F2 and 2C3 respectively) (1). Of particular interest to developmental biologists, the position of the gene within the cluster appears to be correlated with both the boundary of expression in the developing embryo and its temporal expression (2). The genes within each cluster have been further characterised and assigned to paralog groups 1-13 based on homeobox sequence similarity and position within the cluster. Each cluster contains up to eleven paralogous members.

2.1. Evolution and conservation

The term homeobox gene was derived from the Greek word 'homeosis' the basis for a term 'homeotic mutation' used by *Drosophila melanogaster* geneticists over 112 years ago to define the transformation of a body part 'into the likeness of something else' (3). Landmark

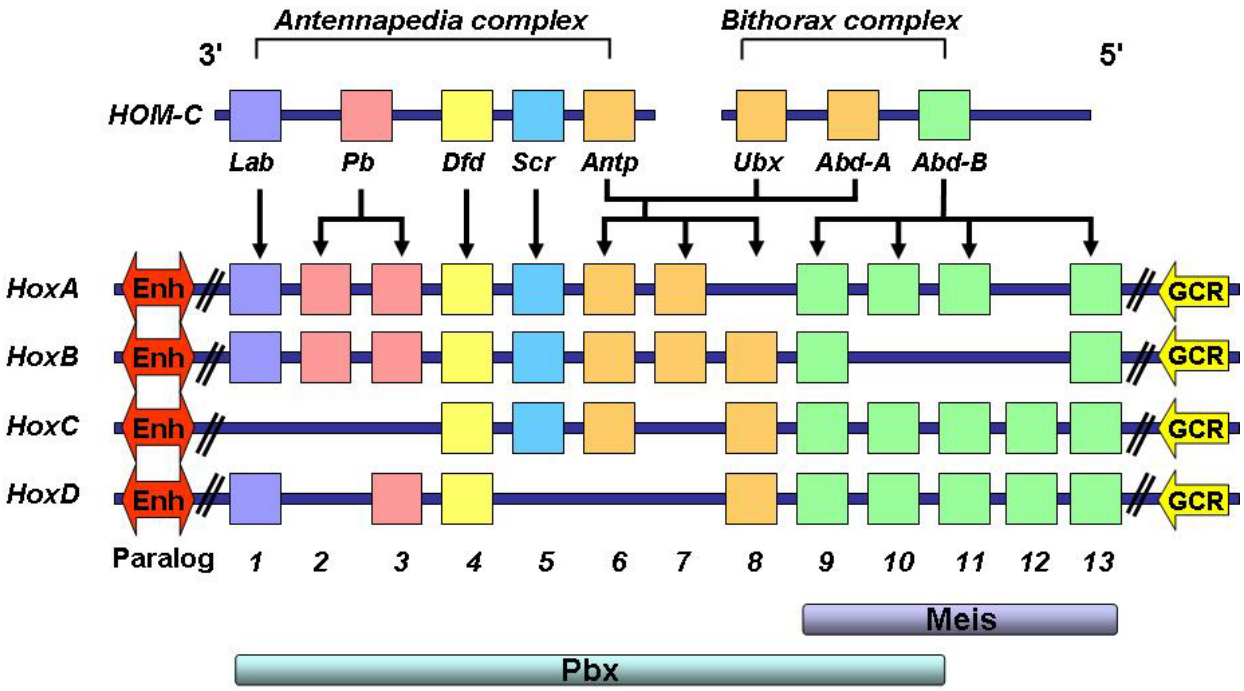

Figure 1. Schematic representation of conservation of the mammalian HOX gene network from *Drosophila Hom-C* depicting preferential binding of Pbx to paralog groups 1-10 and Meis to paralog groups 9-13. Downstream Enh (Enhancer) sequences and upstream GCR (global control region) elements thought to control global expression of individual clusters are represented.

discoveries within the field included the identification of a gene complex (*Bithorax*) that controlled development of both the middle and posterior regions of *Drosophila* (4) and subsequently another complex (*Antennapedia*) that regulated the formation of anterior structures within the fruit fly. Both discoveries arose from mutational analyses that resulted in the generation of dramatic phenotypes. In the gain-of-function *Antennapedia* (*Antp*) mutation the antennae are changed into legs (5), whereas the loss-of-function *Ultrabithorax* (*Ubx*) mutation results in transformation of the haltere (balancing organ on the third thoracic segment) into a wing. Subsequent studies revealed that the *Bithorax* complex (*Bx*-C) consists of 3 genes *Ultrabithorax (Ubx)*, *Abdominal-A (AbdA)*, and *Abdominal-B (AbdB)*. The *Antennapedia* complex (*Ant*-C), located on the right arm of chromosome 3 comprises 5 genes *Labial* (*Lab*), *Proboscipedia (Pb)*, *Deformed (Dfd)*, *Sex combs reduced (Scr)*, and *Antennapedia (Antp)* (6). The combination of the *Ant-C* and *Bx-C* results in production of the Homeotic selector complex (*HOM-C*) a term devised to encapsulate the idea of a master regulatory region that could control the development of each segment of the fly (7). *HOX* genes are descendants of the eight-member *HOM-C* complex and retain both the cluster organization and relative chromosomal positioning indicative of a high degree of conservation presumably from a primordial common ancestral gene.

2.2. Structure and co-factors

The canonical *HOX* gene consists of two exons with one intron that ranges in size from 200 bases to several kilobases. The 180 bp homeobox sequence is located within a GC-rich region of the second exon and encodes a 60 amino acid DNA-binding motif, the homeodomain. Early studies indicated that *Drosophila* and mammalian HOX proteins bind consensus DNA sequences weakly *in vitro* suggesting that associated co-factors may provide the level of specificity *in vivo* required for the regulation of distinct target genes for organogenesis. The best characterised co-factors are *Drosophila* extradenticle (Exd) (8), Homothorax (Hth) (9), mammalian pre-B cell leukemia homeobox (Pbx) (8), and myeloid ecotropic viral insertion site (Meis) (10) family members. Interestingly all are members of the TALE (three amino acid loop extension) family of homeodomain proteins encoded by Class II non-clustered homeobox genes (11). The founding members of the two best characterised TALE subfamilies, namely Pbx1 and Meis1 interact preferentially with 3' or 5' Hox proteins respectively ((12) and Figure 1), however trimeric Hox-Pbx-Meis complexes have also been reported (13). Both Pbx/Hox and exd/Hom-C heterodimers bind to the consensus TGAT (T/G)NA (T/C) with the Hox or Hom-C proteins binding to (T/G)NA (T/C) and the Pbx or exd binding to the 5′ TGAT. The Meis1 consensus binding site (TGACAG) is followed by a AbdB-like Hox binding site TTA (C/T)GAC (12).

It is predicted that Hox, Pbx and Meis1 proteins interact within multiprotein complexes that afford the highest level of specificity in terms of DNA binding and co-ordination of activation or repression events (14). The discovery and manipulation of specific protein:protein interacting domains has identified functional determinants within the Hox:TALE interactions and key regulatory

regions of the mammalian proteins (15-17). The recent finding of a novel zinc-finger protein that appears to associate with PBX1 in a HOX-independent manner and inhibit HOXA9:PBX-DNA binding within embryonic tissue (18) reinforces the concept of competition for binding partners within the active site of genes and raises the question of HOX:TALE as to which is the primary factor and which is the co-factor? Indeed a network of zinc finger proteins appears to contribute to body patterning specificity in *Drosophila* by functioning in a permissive capacity in parallel with Hom-C members (19, 20).

2.3. Extracellular signals and targets

Transcription networks integrate extracellular signals with modulation of target gene expression. In the case of the HOX network communication between *HOX* genes and signal transduction pathways has recently been demonstrated in hematopoietic cells. *HOX* expression was modulated during expansion of cord blood-derived stem/ progenitor cells in the presence of the early acting cytokines c-Kit-ligand, Flt-3 ligand and thrombopoietin (TPO) (21). Genes associated with uncommitted hematopoietic cells (*HOXB3*, *B4* and *A9*) were upregulated while expression levels of *HOXB8* and *A10* (associated with myeloid committed cells) were reduced. Interestingly Flt-3 has recently been identified as a downstream target of Hoxa9 or Meis1 enforced expression, suggesting a degree of autoregulation of Flt-3 via the Hoxa9/meis1 axis (15). TPO acting through the p38 MAPK pathway activates the *Hoxb4* promoter (22, 23). Furthermore *Hoxb4* was identified as a target of the wingless (Wnt) signaling pathway in HSCs (24). Post-translational modifications of Hox proteins following activation of signal transduction pathways have also been demonstrated. In myeloid progenitors DNA binding capacity and transcriptional activity of HOXA10 and HOXA9 was impaired by SHP1-mediated tyrosine phosphorylation (25) and PKC-mediated phosphorylation (26) resulting in reduced myeloid differentiation.

The identification and characterisation of *bona fide* HOX targets is a rapidly advancing field, due in part to increased accessibility to microarray techniques, including ChIP on CHIP. It is vital that microarray data are appropriately validated to harness the full power of this methodology. A recent study in *Drosophila* may act as a template for determining key mechanisms of the mammalian *HOX* network (27). The combination of microarray-based gene expression profiling followed by functional analyses recently applied to an ES-derived model of hematopoiesis indicates a dominant negative link between fibroblast growth factor (FGF) signaling and HOXB4 activity (28) and identified HOXB4 target genes known to play distinct roles in cell cycle regulation, differentiation and apoptosis. The hematopoietic system appears to be the model of choice for identifying HOX target genes and several HOXA9 targets including Trib1 and Evi1 (29), E-selectin (30), Pim1 kinase (31), gp91Phox (32) and EphB4 receptor (33) have been identified using various approaches including microarray and retroviral insertional mutagenesis. However, their functional significance remains to be fully explored.

3. HEMATOPOIETIC *HOX* GENES

HOX expression in hematopoietic cells was initially demonstrated using immortalised cell lines of human and murine origin (34-40). In addition these studies suggested that *HOX* genes were expressed in a lineage-restricted manner with *Hoxb* expression being associated with erythroid cells, *Hoxc* expression with lymphoid cells and *Hoxa* expression with myeloid cells. *Hoxd* genes did not appear to be well-expressed in the hematopoietic compartment. The application of more sensitive and specific technologies such as real-time quantitative PCR (Q-PCR) in conjunction with isolation of purer cell populations by advanced sorting methods reinforced and extended these early findings. In addition, these recent approaches have permitted larger scale analyses which highlight the oversimplification of earlier models that suggested individual HOX clusters directed formation of individual hematopoietic lineages. *HOX* genes are indeed expressed in primary hematopoietic stem cells (HSCs) and in hematopoietic progenitors (HPCs) in a pattern characteristic of both lineage and stage of differentiation (41-43). Similar expression profiles have been observed in cells originating from both fetal and adult tissues and in an embryonic stem cell model of hematopoietic differentiation (44). Self-renewal of HSCs and HPCs may be HOX-dependent while inappropriate *HOX* expression may underlie leukemic stem cell maintenance (45).

3.1. Overexpression models

Following up on the gene expression profiling of enriched HPCs, Humphries and colleagues selected three genes that showed a similar expression pattern for overexpression studies, *HOXB3*, *HOXB4* and *HOXA10*. Retroviral transduction and transplantation assays showed distinct phenotypes that were *HOX*-dependent (46-48). Briefly *HOXB3* overexpression resulted in hematological anomalies that included reduced B and T-cell differentiation and a delayed increase in myeloid progenitor numbers. *HOXA10* overexpression also resulted in reduced B-cell differentiation but without affecting the T-cell compartment. In addition *HOXA10* overexpression resulted in a marked increase in megakaryocyte blast colony-forming progenitor production at the expense of macrophage colony formation. The majority of *HOXA10* recipient mice succumbed to leukemia after a long latency period (19-50 weeks). Perhaps the most surprising finding of the three phenotypes was that observed for overexpression of *HOXB4* as in this case no overt hematological anomalies were observed. Instead the authors reported enhanced HSC regeneration (~50-fold) as demonstrated by serial transplantation and compared to control transplants. Increased HSC self-renewal is a common feature of several overexpressed *HOX* genes; however

lack of leukemic transformation appears to be unique to HOXB4.

3.2. Knockout models

Compared to murine models, loss-of-function *Hox* mutations in lower organisms such as *Bithorax* in *Drosophila* are very striking, presumably due to lack of compensatory mechanisms. The retention of up to four paralogs within the mammalian *Hox* complex suggests a high degree of redundancy that underscores the importance of this particular network. *Hox*-deficient mouse models display embryonic phenotypes that reflect both unique and redundant functions of these clustered genes. Phenotypic analyses of individual and compound knockout models indicate the importance of overall gene dosage of the network (49-54). *Hox* members also display overlapping and redundant functions in hematopoiesis and knockout models have been used in attempts to unravel the more critical roles for these molecules in blood cell development. The primary focus of these studies has been on genes previously shown to have a role in normal hematopoiesis *e.g. Hoxb6* mutant mice display increased numbers of erythroid progenitors in the developing fetal liver and bone marrow yet have normal mature hematopoietic cell types and immunity (55). In terms of *Hoxb4*, three separate knockout mouse models have been generated and examined for hematological defects. The single *Hoxb4*$^{-/-}$ mouse model exhibited subtle reductions in HSC/HPC numbers and associated decrease in repopulating ability (56). The phenotype of the compound *Hoxb3/b4*$^{-/-}$ model was similar but slightly more pronounced than *Hoxb4*$^{-/-}$ alone (57). Together these studies suggest that *Hoxb4* is not critical for HSC function and indicate the potential for complementary mechanisms. These findings were reinforced by examination of the hematopoietic compartment of a compound *Hoxb1-Hoxb9*$^{-/-}$ mouse model which clearly demonstrated that *Hoxb4* and indeed the complete expressed *Hoxb* cluster was dispensable for both primitive and definitive hematopoiesis. Further evaluation of the *Hoxb1-b9*$^{-/-}$ progenitor cells identified genetic interaction between the *Hoxb* cluster, *Hoxc4*, *Hoxa4* and *Hoxa11* with a potential compensatory role for *Hoxc4* (58). Together these three models highlight the difficulty and complexity of generating and assessing appropriate models of *Hox* loss-of-function in the mammalian system.

Hoxa9 is highly expressed in hematopoietic cells primarily within the HSC/HPC compartment. Overexpression of *Hoxa9* causes increased HSC self-renewal, myelopoiesis and results in leukemia with long latency. Co-overexpression with *Meis1* in mouse results in the rapid onset of an aggressive, fatal and transplantable leukemia (59). *Hoxa9*-deficient mice display the most dramatic hematopoietic phenotype for a single *Hox* gene to date, indicative of lack of redundancy for this particular member of the *Hox* network. *Hoxa9*$^{-/-}$ mice exhibit multiple lineage abnormalities (60, 61) due to an almost complete lack of repopulation ability by the HSCs (62). The authors suggest that the striking phenotype observed may be due to the preponderance of *Hoxa9* within the HSC pool, indicative of a critical role for the molecule in normal hematopoiesis. In fact the authors propose that, due to its preponderance, Hoxa9 may be the major determinant of physiologic HSC self-renewal which warrants further investigation.

Several other individual *Hox* gene knockout models result in hematopoietic phenotypes generally leading to impaired HSC self-renewal, myelopoiesis or lymphopoiesis. *Hoxa7*-deficient mice display reduction in committed progenitors, particularly of the megakaryocyte/erythroid lineage with resultant reticulocytosis and thrombocytopenia without anaemia (63). *Hoxc8*-deficient mice have reduced erythroid, granulocyte and macrophage colony formation potential yet peripheral blood levels are normal (64) and *Hoxb3*-deficient mice exhibit impaired B-lymphopoiesis (65). Although insights have been gained from these traditional knockdown approaches, the phenotypes tend to be subtle, requiring intricate competition and repopulating experiments to be fully observed. One issue with the traditional approaches is that compensatory mechanisms are primed within the developing embryo and may be retained in the adult, thereby diminishing the importance of individual Hox members within the process. One way of amplifying the potential loss of Hox is to knockout the co-factors which are required for normal function. Pbx1 deficient mice (Pbx1$^{-/-}$) are embryonic lethal between e15.5 and e17.5 and have severe anemia and hypoplasia of multiple organs (66). These mice have fewer progenitors residing within the fetal liver which show decreased colony-forming activity and are unable to establish multilineage hematopoiesis in competitive reconstitution experiments (67). The mice also have defects of the axial skeleton, especially the cervical and thoracic vertebrae and malformations of the proximal fore- and hind-limbs. Meis1 deficient mice (Meis1$^{-/-}$) have eye abnormalities, fail to produce megakaryocytes and die by e14.5 with extensive hemorrhaging (68) and Meis1 inactivation results in loss of definitive hematopoiesis and altered vascular patterning (69). Meis1$^{-/-}$ mice do not develop the skeletal abnormalities seen with Pbx1$^{-/-}$ mice, but fetal liver cells taken from Meis$^{-/-}$ or Pbx1$^{-/-}$ mice perform poorly in repopulation studies. The application of more refined conditional knockout models with tissue-specific disruption of individual genes or appropriately defined subsets will help elucidate the criticality of *Hox* family members in hematopoiesis.

4. *HOX* GENES IN ACUTE LEUKEMIA

The evidence for a functional role of *Hox* subsets in normal hematopoiesis as examined by overexpression or gene knockout studies remains primarily circumstantial and limited to a relatively small subset of genes. Examination of gene expression levels in the leukemic state (primarily acute leukemia) shows global dysregulation of the *HOX* network in hematological malignancy (70-74). Within these large-

scale studies a subset of *HOX* genes has emerged as candidate prognostic indicators (70, 71).

4.1. Direct involvement of *HOX* in leukemia

Non-random chromosomal rearrangements are observed in over 60% of all acute leukemias and generally involve genes with a critical role in normal blood development (75). The discovery of recurrent chromosomal translocations involving members of the *HOX* network in both acute myeloid leukemia (AML) and acute lymphoid leukemia (ALL) therefore provides compelling evidence that *HOX* genes are involved in normal hematopoiesis. Currently eight Abd-B *HOX* genes (*HOXA9*, *A11*, *A13*, *B9*, *C11*, *C13*, *D11* and *D13*) have been identified as fusion partners with the NUP98 nucleoporin gene in human AML and chronic myeloid leukemia (76-79). In addition *HOXA13* has been shown to be dramatically upregulated in two cases of human T-ALL presumably due to an in-frame fusion with a strong promoter element as part of a novel translocation (80). Such translocations provide a good rationale for determining the functional consequences of the particular dysregulated *HOX*. Overexpression of *NUP98-HOXA9* or *NUP98-HOXD13* generated either by standard bone marrow transplantation (81, 82) or by transgenic knock-in (83, 84) caused myeloproliferative disease that ultimately progressed to AML. Further analyses of these models indicated that leukemic potential within the *NUP98-HOX* context is both *HOX* gene specific (78) and reliant on *HOX*-dependent pathways such as *Meis1* (81, 82, 85).

5. INVOLVEMENT OF *HOX* REGULATORS IN LEUKEMIA

Indirect evidence for a role of *HOX* in both normal hematopoiesis and leukemia is provided by a growing list of *HOX* regulators that are either directly associated with chromosomal rearrangements (translocations and inversions) or whose expression is dramatically increased within the leukemic setting. It is likely that distinct 'leukemic *HOX* codes' are established by various oncogenes such as that for *MYST3-CREBBP* (86). The main *Drosophila* regulators of *HOM-C* are encoded by *Trithorax*, *Polycomb group* and *Caudal* genes. The human orthologs have also been shown to direct *HOX* expression in leukemia. Particular *HOX* signatures are also associated with mutations in the nucleophosmin (NPM) and FLT3 genes, implicating these molecules as *HOX* regulators.

5.1. MLL

The best characterised regulator of *HOX* is the *Trithorax* ortholog *Mixed-Lineage Leukemia* (*MLL*) gene product which appears to act as a surrogate locus control region for the *ABD HOXA* genes (87). MLL acts as a histone methyltransferase and catalyses trimethylation of histone H3 Lys 4 (H3K4) associated with transcriptional activation. In the case of *HOX*, association of MLL with promoter regions appears to result in transcription maintenance rather than initiation (88). The MLL gene is located on chromosome 11q23 and it is rearranged in approximately 10% of all human leukemias (89) but more strikingly in >70% of infant leukemias (90). Although over 50 different translocation fusion partners have been identified for MLL the five most frequent rearrangements account for approximately 80% of all associated leukemias. The translocations and fusions are classified as t (4;11) (q21;q23) or MLL–AF4; t (9;11) (p22;q23) or MLL–AF9; t (11;19) (q23;p13.3) or MLL–ENL; t (10;11) (p12;q23) or MLL–AF10; and t (6;11) (q27;q23) or MLL–AF6 (89, 91). There is much interest in MLL-associated leukemias due to poor clinical outcome for infants and children. MLL fusion partners appear to regulate transcription through complex association with chromatin remodelling factors (92). Leukemic transformation afforded by MLL-ENL appears to be associated with histone modification of *HOXA9* and *MEIS1* promoters (93). A murine model of MLL-EEN-induced leukemia that showed recruitment of a protein arginine methyltransferase to MLL target genes suggests that histone modification is a common and important aspect of MLL-fusion-mediated transformation (94). Large-scale microarray studies show *HOX* dysregulation in MLL fusion-associated T- and B-cell ALL suggestive of a specific *HOX* signature associated with disrupted MLL (71, 72, 95, 96). Murine models of MLL-ENL showed reliance on *Hox* and Hox co-factor expression for both the initiation and maintenance of the leukemic phenotype (97-99). This reliance on *HOX* may be fusion partner dependent as enforced expression of MLL-AF9 in a *Hoxa9*$^{-/-}$ background still resulted in a leukemia, albeit with a slightly different phenotype (100). Such models demonstrate the complexity of the *HOX* network that results in unique and overlapping functions which are highly context dependent, yet reinforce the concept of a leukemic *HOX* code which may or may not be MLL fusion dependent.

5.2. Polycomb group proteins

The Polycomb group (PcG) proteins were first identified as transcription repressors in *Drosophila* that are vital for the temporal and spatial regulation of *HOM-C* genes during development (101) and catalyse the methylation of histone 3 at position lysine 27 (H3K27me) (102). In association with DNA methyltransferases PcG proteins control cellular transcription programmes and play an essential role in the maintenance of epigenetic memory. They exist in at least two separate large protein complexes termed Polycomb repressor complex 1 (PRC1) and Polycomb repressor complexes 2 to 4 (PRC2/3/4). The PRC2 complex contains the protein Ezh2 which is a key molecule for initiation of gene silencing by methylation of H3K27. A recent study has demonstrated that the aberrant silencing of genes by PML-RAR-alpha is due in part to association with the PRC2 complex (103). The oncogenic PML-RAR-alpha fusion generated by the t (15;17) translocation accounts for 99% of cases of acute promyelocytic leukemia and is associated with global downregulation of *HOX* gene expression (104).

5.3. The CDX family

The vertebrate *Cdx* gene family (*Cdx1*, *2* and *4*) are homologues of the *Drosophila Caudal* homeobox

gene that encode proteins which bind within *Hox* promoter regions at established consensus sites (105, 106). *Cdx*-deficient mice and zebrafish exhibit homeotic phenotypes and both *Cdx4* and *Cdx1* act redundantly to specify HSC formation in zebrafish (107-109). Several recent studies suggest a pivotal role for CDX proteins in both normal hematopoiesis and *HOX*-associated AML. *CDX2* expression was reported in 153 of 170 AML patients but not seen in HSC/HPCs obtained from normal individuals. In addition, enforced expression of *CDX2* resulted in dysregulated *HOX* expression *in vitro* and *in vivo* with associated colony serial replating ability and generation of a transplantable leukemia with medium to long latency (110). *CDX4* was also expressed in AML patient samples but to a lesser degree than *CDX2* (25% compared to 90%). Enforced expression and bone marrow transplantation of *CDX4* transduced cells resulted in a transplantable long latency leukemia with lower penetrance than *CDX2* (50%) and associated *HOX* dysregulation. In addition the leukemic potential of *CDX4* was shown to be dependent on intact transcription factor function (111). CDX4 has also been implicated with menin in a complex that modulates early transformation events of MLL *via* occupancy of *Hoxa* cluster gene regulatory elements (112). These reports certainly support a role for CDX proteins in regulating *HOX* expression in the leukemic setting and may explain in part the high level of *HOX* expression observed in the absence of overt chromosomal rearrangements.

5.4. Nucleophosmin 1 and FLT3

Nucleophosmin 1 (NPM1) is a chaperone protein that facilitates ribosomal protein transport through the nuclear membrane (113). Disruption of *NPM1*, either by translocation or mutation, may be an early event in leukemogenesis. The discovery of high frequency *NPM1* mutations in adult AML (35%) with otherwise normal karyotype (AML-NK) associated with a more favourable outcome has afforded additional classification of this disease subtype (114). Of particular interest a distinct *HOX* signature was associated with *NPM1* and *FLT3* status. The frequency of the *NPM1* mutations in pediatric AML is significantly lower (6%) than adult AML perhaps indicative of acquired somatic mutation. Microarray analyses of the pediatric samples confirmed increased expression of multiple *HOX* genes which were significantly different from those associated with MLL rearrangements within the same cohort (115). It is important to note however that the increased *HOX* expression associated with aberrant cytoplasmic expression of NPM in AML ($NPMc^+AML$) occurs as part of an overall increased stem cell molecular signature that includes expression of *Notch* and *Jag1* (116). The molecular mechanism of NPM1 regulation of *HOX* genes and indeed the 'stem cell signature' is unknown and warrants investigation.

6. POST-TRANSCRIPTIONAL REGULATION OF *HOX*

As with many mammalian genes, regulation of the *HOX* cluster genes includes alternative splicing and promoter usage. The vast majority of published work has focused on individual expressed genes. The preponderance of noncoding RNA (ncRNA) species and their significance in controlling the overall transcriptional output is well documented, and has recently been reviewed by Costa (117). MicroRNAs (miRNAs) are ncRNA molecules that downregulate expression of their mRNA targets through direct binding within the 3'-untranslated regions of specific mRNA sequences and targeting them for enzymatic degradation (118). Each miRNA, of which there are over 300 in the human genome, may regulate multiple genes implicating a major role for miRNAs in controlling protein levels (119). Both miRNAs and transcript stability are important determinants of HOX protein production. Two specific microRNAs have recently been identified embedded within the mouse *Hoxb* and *Hoxd* clusters, designated miR10a and miR10b respectively. Significant similarity in the expression domains of *Hoxb4* and miR10a suggests this miRNA may affect Hoxb4 translation (120). A further miRNA designated miR-196 encoded at paralogous regions within the *Hoxa*, *b* and *c* clusters represses the translation of *Hoxb8*, *c8*, *d8* and *a7* and actively cleaves *Hoxb8 (*121, 122). Recent investigation of the intergenic regions of *HOX* clusters identified Retinoic Acid (RA) regulated opposite strand transcription of ncRNAs which may be important for initiation and maintenance of the active state within the human *HOXA* cluster (123). Proper expression levels of *HOX* genes are very important for normal function, *e.g.* the level of *HOXA10* on HSC self-renewal (124). The recent discovery of various 'non-annotated' HOXA9 transcripts (125) indicates greater complexity within the locus region than previously reported. The application of high-throughput analyses has identified at least seven polycistronic regions (up to 30 kb) within the *HOX* clusters and ten ncRNAs (>40 bp) that overlap *Hox* transcripts in both human and mouse, suggesting functionality (126).

Together these findings highlight the complexity within the *HOX* gene cluster network and between the *HOXOME* regulatory network within the hematopoietic compartment (Figure 2) and supports the value of current approaches aimed at deciphering it.

7. SUMMARY AND PERSPECTIVE

Although *HOX* genes and their encoded proteins have been around for over 1,000 million years it has taken until the last couple of decades for scientists to classify and identify functional mechanisms of these highly conserved molecules. That altered *HOX* expression is involved in cancer and leukemia is unequivocal, the challenge now is to understand at the fundamental level the role of critical members of the network. The fact may be that the fundamental role of the *HOX* network is to 'initiate and maintain a state of self-renewal' within the HSC that is necessary but not sufficient for

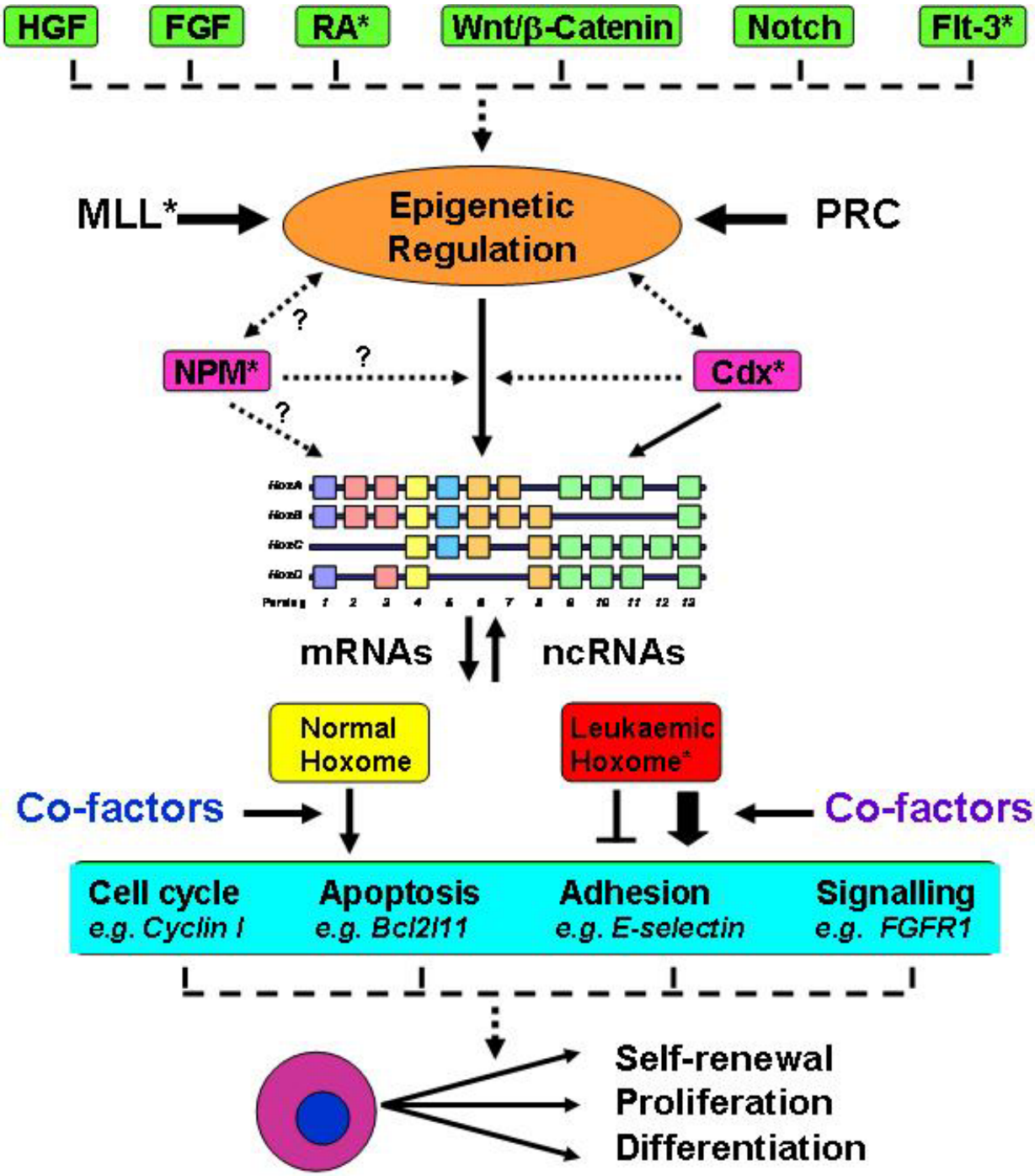

Figure 2. Diagrammatic representation for a proposed central position played by the HOXOME in regulating hematopoietic cell fate. Integration of multiple signals via global epigenetic events results in production of a specific HOX signature which regulates the expression of target genes involved in regulating key cellular processes. Identified and novel co-factors of HOX are predicted to provide a high level of target gene specificity within this network. Disruption of upstream regulators (marked *) results in an altered HOXOME which subsequently leads to activation of a different subset of target genes that contribute to the leukemic phenotype. ncRNA: non-coding RNA, MLL: Mixed Lineage Leukemia, PRC: Polycomb repressor complex.

leukemia development as recently reviewed and proposed (79). Elucidation of the cellular pathways involved and determination of non-TALE protein complexes in which HOX members play a crucial functional role is vital for better understanding of the molecular mechanisms underlying HOX function. This knowledge can then provide the basis for therapeutic strategies for diseases with a dysfunctional HOX network.

8. ACKNOWLEDGEMENTS

G.J.McG is funded by the Leukemia Research Fund

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Abbreviations: HOX: Human Class I homeobox protein (gene in italics throughout), Hox: Non-Human Class I homeobox, Antp: Antennapedia, Ubx: Ultrabithorax, Bx: Bithorax, HOM-C: Homeotic selector complex, Lab: labial, Pb: proboscipedia , Dfd: Deformed, Scr: Sex combs reduced, Abd: Abdominal, Exd: extradenticle, Hth: Homothorax, Pbx: pre-B cell leukemia homeobox, (PBX: Human) Meis: myeloid ecotropic viral insertion site, (MEIS: Human) TALE: three amino acid loop extension, TPO: Thrombopoietin, FGF: fibroblast growth factor, HSC: hematopoietic stem cell, HPC hematopoietic progenitor, NUP98: nucleoporin-98, AML: acute myeloid leukemia, ALL: acute lymphoid leukemia, MLL: Mixed-Lineage Leukemia, PcG: Polcomb group, PRC: Polycomb repressor complex, CDX: Caudal-type homeobox, NPM1: Nucleophosmin 1, ncRNA: noncoding RNA, miRNA: MicroRNAs, HOXOME: tissue-specific expressed HOX proteins (genes in italics).

Key Words: HOX, Co-factors and Regulators, Hematopoiesis, Leukemia, Transcription Network, Target Genes, Review

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