Temporal changes in Hox gene expression accompany endothelial cell differentiation of embryonic stem cells

S. Bahram Bahrami,1 Mandana Veiseh,2 Ashley A. Dunn1 and Nancy J. Boudreau1,*

1Department of Surgery; University of California San Francisco; San Francisco, CA USA; 2Life Science Division Lawrence Berkeley National Laboratory; Berkeley, CA USA

Key words: embryonic stem cell differentiation, endothelial cells, Hox gene expression

In pluripotent embryonic stem cells (ESCs), expression of the Hox master regulatory transcription factors that play essential roles in organogenesis, angiogenesis and maintenance of differentiated tissues is globally suppressed. We investigated whether differentiation of endothelial cells (ECs) from mouse ESCs was accompanied by activation of distinct Hox gene expression profiles. Differentiation was observed within three days, as indicated by the appearance of cells expressing specific endothelial marker genes (Flk-1/VE-Cadherin+). Expression of HoxA3 and HoxD3, which drive adult endothelial cell invasion and angiogenesis, peaked at day 3 and declined thereafter, whereas expression of HoxA5 and HoxD10, which maintain a mature quiescent EC phenotype, was low at day 3, but increased over time. The temporal and reciprocal changes in HoxD3 and HoxA5 expression were accompanied by corresponding changes in expression of established downstream target genes including integrin β3 and Thrombospondin-2. Our results indicate that differentiation and maturation of ECs derived from cultured ESCs mimic changes in Hox gene expression that accompany maturation of immature angiogenic endothelium into differentiated quiescent endothelium in vivo.

Introduction

During embryonic development, the first organ system to form is the cardiovascular system, which arises from mesoderm and later gives rise to the lymphatic vascular system.1,4 Hemangioblasts, the common pluripotent mesodermal precursor of endothelial and hematopoietic cells,5,6 generate blood islands, the peripheral part of which differentiates into endothelial cells (ECs).7,8 Vascularization happens through two separate but related processes of vasculogenesis (de novo vessel formation by ECs), and angiogenesis (formation and development of vessel from pre-existing vessels).9,10 Vascularization occurs prominently during embryonic development and postnatally is restricted to the menstrual cycle, and pathological conditions including wound healing, inflammatory disease and tumor growth and metastases.11 The embryonic stem cell (ESC) culture model offers a unique way to investigate the early development of hemangioblasts and the molecular mechanisms that contribute to subsequent endothelial cell commitment, differentiation and contribution to tissue regeneration.10-12 ESCs partially differentiate into embryoid bodies (EBs) that contain all three germ layers capable of self renewal and differentiation into different cell types.13,14 The development of differentiated endothelial cells from EBs has been documented and the corresponding gene expression patterns have been characterized.12,15 Large scale changes in gene expression accompany both the initial differentiation of ESCs into EBs, and subsequent changes in gene expression are linked to lineage specific differentiation of EBs along mesenchymal, epithelial, neural or hematopoietic lineages.16,17 The formation of hemangioblast cells, which can further differentiate into either hematopoietic or endothelial cells, has been widely used to study the expression of transcription factors that control EC lineage and recapitulate many aspects of vascular development in vivo.8 Moreover, as many of the same factors, including vascular endothelial growth factor (VEGF), which drive embryonic vasculogenesis also activate tumor-induced angiogenesis, investigating early EC development may also identify additional molecular mechanisms that may be therapeutically exploited to manage tumor growth and dissemination.

Our laboratory has focused on the Homeobox family of “master-regulatory” transcription factors and their role in regulating adult endothelial cell behavior.18-26 Hox genes, originally discovered in Drosophila melanogaster, are associated with anterior-posterior axis patterning during embryonic development.27 Mammalian Hox genes are distributed in four homologous Hox loci, termed HoxA, B, C and D, which are located at four different chromosomal locations and are comprised of 9–11 genes arranged in homologous sequence organizations referred to as paralog group.1,28 Homeobox genes play an essential role as transcriptional regulators, both for development of the blood and lymphatic vascular systems, as well as for their maintenance and remodeling during embryonic development and adult life.4
Results

Differentiation and characterization of endothelial cells derived from EBs. We partially differentiated ESCs to form EBs, and to induce EC differentiation, EBs were subsequently treated with a cocktail of the angiogenic growth factors VEGF, FGF and EGF for up to 18 days (Fig. 1A and B). To first confirm that adherent cells that emerged from the EBs were undergoing differentiation, we assessed expression of Oct-4, a transcription factor expressed by pluripotent ECs but lost during differentiation36,37 and confirmed that expression of Oct-4 was progressively lost consistent with acquisition of a more differentiated phenotype (Fig. 1C).

In ESCs, the entire Hox clusters are actively repressed by polycomb repressor complexes, which are key regulators of differentiation in ESCs and multipotent progenitors in developing tissues.33 Suppression of these complexes subsequently allows Hox gene expression to be activated as cells commit to a distinct lineage.34,35 Whether a unique Hox code regulates differentiation of multipotent progenitor cells into specific tissues (e.g., the vascular system), and/or then maintains those tissues is not known, but remains a fundamental question in modern biology.29,33 Moreover, while the roles of individual members of the Hox gene family in EC differentiation and vascular development has been investigated, less is known about the expression of Hox genes and their related downstream target genes during ESC differentiation to ECs. However, pattern of homeobox expression, rather than any one individual homeobox gene, likely plays a more important role in regulating the ultimate behavior of ECs during embryogenesis and vascular remodeling.29 To explore this possibility, we investigated the expression of Hox genes with established functions during pathological angiogenesis, during differentiation of mouse ESCs into ECs. We observed distinct temporal changes in Hox gene expression that mimicked the temporal changes in gene expression accompanying the maturation of angiogenic EC in adult vasculature.
We also assessed expression of the angiogenic HoxD3 gene. Similar to HoxA3, levels of Hox D3 peaked by day 3 in culture and then declined as cells matured (Fig. 5A). We also assessed expression of the HoxD3 target gene Integrin β3 and observed that its expression paralleled that of HoxD3 (Fig. 5B). To evaluate whether the expression of integrin β3 in differentiating ECs was dependent upon the appearance of HoxD3, we knocked down HoxD3 expression in Day 0 cultures using siRNA. RT-PCR analysis of Day3 cultures revealed a marked reduction in HoxD3 mRNA in siRNA treated cells and a corresponding loss of integrin β3 mRNA levels at this timepoint (Fig. 5C).

Finally, in unsorted, mixed cell populations, the expression levels of HoxD3 and HoxA3 peaked at later time points (Sup. Fig. 2), suggesting that the early temporal activation of these Hox genes is cell-type specific.

Expression of pro-angiogenic Hox genes coincides with onset of EC differentiation. Because Hox genes are globally repressed in pluripotent ESCs, and are known to be expressed in adult ECs, we evaluated whether they were temporally induced in ESCs undergoing differentiation along the EC lineage. We performed real-time PCR on sorted double-positive (Flk-1+/VE-Cadherin) cells harvested after 1, 3, 9 and 18 days. We assessed mRNA levels of HoxD3 and HoxA3, which are expressed by activated, angiogenic adult ECs. Expression of HoxA3 increased significantly during the first 3 days of culture and subsequently declined (Fig. 4A). We also assessed expression of the putative HoxA3 target genes, uPAR and CCL-2 (Fig. 4B and C). Whereas expression of uPAR continued to increase after levels of HoxA3 declined, levels of CCL-2 mimicked that of HoxA3 by peaking at Day3 then subsequently declining over time in culture.

We also assessed expression of the angiogenic HoxD3 gene. Similar to HoxA3, levels of Hox D3 peaked by day 3 in culture and then declined as cells matured (Fig. 5A). We also assessed expression of the HoxD3 target gene Integrin β3 and observed that its expression paralleled that of HoxD3 (Fig. 5B). To evaluate whether the expression of integrin β3 in differentiating ECs was dependent upon the appearance of HoxD3, we knocked down HoxD3 expression in Day 0 cultures using siRNA. RT-PCR analysis of Day3 cultures revealed a marked reduction in HoxD3 mRNA in siRNA treated cells and a corresponding loss of integrin β3 mRNA levels at this timepoint (Fig. 5C).

Finally, in unsorted, mixed cell populations, the expression levels of HoxD3 and HoxA3 peaked at later time points (Sup. Fig. 2), suggesting that the early temporal activation of these Hox genes is cell-type specific.

Expression of Hox genes linked to mature EC phenotype occurs at later stages of ESC differentiation. In contrast to angiogenic ECs, which express HoxD3 and HoxA3, mature quiescent adult ECs predominantly express HoxA5 and Hox D10, which help to maintain a differentiated, stable phenotype. To determine if expression levels of HoxA5 and/or HoxD10 increased over time in differentiating ECs, we performed real-time PCR in double-positive (Flk-1+/
VE-Cadherin⁺ cells at various time points. We observed that expression of both HoxD10 and HoxA5 (Fig. 6A and C) were significantly upregulated as early as day 3, but in contrast to HoxA3 and D3, expression continued to rise over time in culture and was dramatically increased at day 18 in culture. In unsorted, mixed cell populations, the expression levels of HoxD10 peaked at day 9 and declined at later time points (Sup. Fig. 3), again suggesting that later peak in expression of these Hox genes is cell-type specific.

We also examined expression of HoxD10 and HoxA5 downstream targets. We observed that as HoxD10 levels increased between Day 9 at Day 18, expression of MMP-14 which is downregulated by HoxD10 in mature EC²³ began to decline between Day 9 and Day 18 (p = 0.056) (Fig. 6B). As levels of HoxA5 increased, we noted a parallel increase in expression of its putative target, TSP-2 whose expression reached maximum levels at day 18 (Fig. 6D).

To determine whether the late increase in HoxA5 expression was required for the expression of TSP-2, we treated differentiating ECs with siRNA against HoxA5. On day 17, we observed that in contrast to control treated cells which express abundant HoxA5 at this time point, siRNA against HoxA5 expression dramatically reduced both HoxA5 and TSP-2 mRNA expression (Fig. 6E).

**EC differentiation of ESC mimics angiogenesis.** These results collectively show that the temporal sequence of Hox
inactivated and allow subsequent expression of the Hox cluster of morphoregulatory genes that drive organogenesis and maintain tissue differentiation.\(^{33-35}\) Whether expression of a unique Hox code specifies or directs differentiation of these multipotent progenitor cells along specific lineages has long been a subject of intense investigation, and numerous studies have noted organ- and tissue-specific Hox expression patterns.\(^4\)\(^{29}\) Our study shows that distinct temporal expression of Hox genes accompanies the differentiation of ESCs into endothelial cells. It also shows that the pattern of Hox genes expressed by differentiating and mature ECs derived from EBs is similar to Hox expression patterns that accompany and direct the angiogenic cycle of adult ECs; high

**Discussion**

When pluripotent ESCs are induced to differentiate along specific cell lineages in vitro, polycomb repressor complexes are inactivated and allow subsequent expression of the Hox cluster of morphoregulatory genes that drive organogenesis and maintain tissue differentiation.\(^{33-35}\) Whether expression of a unique Hox code specifies or directs differentiation of these multipotent progenitor cells along specific lineages has long been a subject of intense investigation, and numerous studies have noted organ- and tissue-specific Hox expression patterns.\(^4\)\(^^{29}\) Our study shows that distinct temporal expression of Hox genes accompanies the differentiation of ESCs into endothelial cells. It also shows that the pattern of Hox genes expressed by differentiating and mature ECs derived from EBs is similar to Hox expression patterns that accompany and direct the angiogenic cycle of adult ECs; high

---

**Figure 4.** Early expression of the pro-angiogenic HoxA3 during EC differentiation. Histograms show mRNA levels of HoxA3 (A) and putative target genes uPAR (B) and CCL-2 (C) in sorted cells that were double-positive for endothelial markers (Flk-1/Ve-Cadherin\(^+\)), as measured by real-time PCR. Results are normalized to the GusB (internal standard) and are expressed relative to day zero. Error bars indicate standard deviation. \(*\) denotes a statistical difference (p < 0.05) between each individual time point and day 0.

**Figure 5.** Early expression pro-angiogenic HoxD3 and integrin β3 in double-positive cells (Flk-1/Ve-Cadherin\(^+\)) during EC differentiation. Histograms show mRNA levels of HoxD3 (A) and its target gene integrin β3 (B) in sorted double positive (Flk-1/Ve-Cadherin\(^+\)) cells measured by real-time PCR. (C) Relative HoxD3 and Integrin β3 mRNA levels at day 3 following treatment with control (scrambled) or siRNA against HoxD3 and shows a marked reduction in HoxD3 mRNA and a corresponding loss of integrin β3 mRNA levels following knockdown of HoxD3. Results are normalized to the GusB (internal standard) and are expressed relative to day zero or control. Error bars indicate standard deviation. \(*\) and \(**\) denote a statistical difference (p < 0.05) and (p < 0.005) between each individual time point and day zero or control, respectively.
levels of HoxA3 and HoxD3, which promote EC growth and invasion (angiogenesis), are expressed within three days and subsequently decline, whereas expression of HoxA5 and Hox D10, which have been linked to stabilization of mature EC phenotype, begins at day 3 and continues to rise overtime as ECs continue to differentiate and mature.

Previous studies have documented the differentiation of vascular endothelial cells from EB via a mesodermal Flk-1+ cell population. Flk1+ cells can give rise to hemangioblasts with the potential to differentiate into either hematopoietic or endothelial cells. In our experiments, Flk-1 was upregulated between day 2–3 and Hox A3 and D3 genes were also increased beginning at day 3, but before expression of the distinct EC markers VE-Cadherin and PECAM-1 at day 4. It is not clear whether the early appearance of Hox A3 and Hox D3 genes in differentiating Flk-1+ cells play an inductive role in promoting the EC phenotype and/or restrict differentiation of Flk-1+ cells towards a non-EC phenotype, such as hematopoietic cells.

An earlier study showed that mesenchymal stem cells (MSCs) derived from bone marrow can be induced to differentiate along the EC lineage, and during this process, express a distinct profile of Hox genes including HoxB3, HoxA7 and HoxB13, and a similar high level of expression of HoxA3, which subsequently falls over time in culture as ECs mature. That study however did not establish what percentage of MSCs differentiated into ECs. We noted that only less than 2% of adherent cells that emerged from EB cultures were in fact exclusively endothelial, whereas abundant mesenchymal and epithelial lineage markers (BMPR and K14) indicative of heterogeneous cell populations, were detected. Thus, considering the relatively low percentage of purified ECs reported in differentiating stem cell culture models, it is difficult to determine whether the previously reported changes in Hox gene expression reflect that which occurs exclusively in the EC population or arises from changes in other cell types within the MSC cultures. Indeed, we observed that the temporal expression of several Hox genes was distinctly different in purified, sorted

---

**Figure 6.** Delayed expression of Hox genes linked to maturation of EC phenotype during EC differentiation. Histograms show relative mRNA expression of Hox D10 (A) and Hox A5 (C), and respective target genes MMP14 (B) and Thrombospondin-2 (D), in sorted cells double-positive for endothelial markers (Flk-1+/VE-Cadherin+), as measured by real-time PCR at various time point during EC differentiation. (E) Relative mRNA levels for HoxA5 and Thsp-2 in differentiating ECs treated with scrambled (control) or siRNA against HoxA5, at day 17 and corresponding reduction in HoxA5 and Thrombospondin-2 mRNA levels. Results are normalized to the GusB (internal standard) and are expressed relative to day zero or control. Error bars indicate standard deviation. **"** and ***" denote statistical differences (p < 0.05) and (p < 0.005) between each individual time point and day 0 or control, respectively.
ECs compared to the mixed EB populations that expressed markers of the EC, MSC and epidermal lineages.

Other studies have noted that MSCs derived from different organs can be distinguished by specific Hox codes. For example, while HoxA3 and HoxA5 were expressed in all MSC populations regardless of tissue origin, expression of HoxD3 and HoxD10 showed restricted expression. Thus, whether MSCs from different organs show similar Hox expression patterns when induced to undergo EC differentiation or express Hox genes that reflect the tissue of origin has not been established.

Members of the HoxA cluster are also highly expressed in HSCs, and HoxA5 in particular has been linked to increasing differentiation along myelopoietic lineages while inhibiting erythroid development. Moreover, HoxA5 is also expressed in adult mammary, lung and gut epithelial cells, as well in neurons and loss of HoxA5 impairs branching in each of these tissues. We previously showed that endothelioma cells, in contrast to normal endothelial cells, lack HoxA5 and fail to form branching capillary-like structures and restoring HoxA5 expression promotes branching. Thus, while the expression of HoxA5 is not restricted or unique to differentiating ECs, its expression increases over time and is consistent with a role in establishing and maintaining a differentiated phenotype.

Hematopoietic precursors or their myeloid and granulocytic derivatives do not express members of the entire HoxD cluster, whereas inappropriate activation of HoxD3 gives rise to leukemic cells. Hemangioblasts arising from EBs can be differentiated both into ECs and hematopoietic cells. Given that MSCs and EBs can both differentiate into ECs, and that HoxD genes have a more restricted expression pattern in both types of stem cell populations, induction of HoxD genes may either promote acquisition of the EC phenotype or restrict pluripotency. Commitment to the EC lineage in hemangioblasts has been linked to increased expression of Wnt2, but whether Wnt2 subsequently induces early HoxD gene expression to direct EC differentiation has not been investigated.

While it remains unclear whether unique Hox codes give rise to distinct cell lineages, simply provide positional information for differentiated cells, or both our studies indicate that the temporal expression patterns of Hox genes during EC differentiation and maturation mimic the temporal changes observed during the angiogenic cycle in adult ECs. Early expression of HoxA3 and HoxD3 by day 3 was noted in purified EC cultures, but expression of these genes peaked at later time points (day 9) in mixed populations. Thus, while different cell populations may express similar Hox genes, different cell lineages may be distinguished by distinct temporal patterns of Hox expression that reflect the early angiogenic and invasive stages.

Our study shows that the expression pattern of the downstream target of HoxD3, integrin β3, correlated with HoxD3, and this matches our previous finding that integrin β3 is a direct target for HoxD3 that binds directly to the promoter. Thus, the rapid induction of HoxD3 and HoxA3 within three days may be early markers of commitment to the EC lineage.

Finally, our study shows that Hox genes linked to more mature stages of endothelial development, namely Hox A5 and D10, are also induced early, but that expression continues to rise over time, possibly reflecting maintenance of mature, quiescent ECs. Indeed, we also observed that sustained expression of HoxA5 was followed by sustained expression of the anti-angiogenic TSP-2 gene.

In conclusion, the temporal changes in Hox gene expression during differentiation of endothelial cells from cultured ESCs mimic the changes in Hox gene expression that accompany maturation of immature angiogenic endothelium into differentiated quiescent endothelium in vivo. Whether these distinct temporal changes in Hox genes can be exploited to either expand production of angiogenic ECs for therapeutic application, or alternatively to promote maturation of immature endothelial progenitors recruited to the angiogenic tumor vasculature, has not been directly tested. However, considering the profound impact of Hox genes on cellular differentiation and/or maintenance of tissue phenotype, understanding the temporal and tissue-specific expression patterns of Hox genes may provide the necessary insight to therapeutically manage pathological angiogenesis.

**Materials and Methods**

**ESC culture and EB formation.** Mouse embryonic fibroblasts (MEFs) were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with β-mercaptoethanol, nonessential amino acids, sodium pyruvate, L-glutamine, Pen/Strep and 15% fetal calf serum (FBS), on gelatinized tissue culture dishes. 95% confluent MEF cells were treated with 10 μg/ml of mitomycin C in MEF media for 3 hrs at 37°C. The cells were then washed 3x with PBS and maintained in MEF media. Mouse ESCs were cultured on a feeder layer of MEFs treated with mitomycin C and maintained in DMEM supplemented with β-mercaptoethanol,
nonessential amino acids, sodium pyruvate, L-glutamine, Pen/Strep and 15% FBS, and 1,000 unit/ml recombinant ESGRO® (LIF) to maintain them in undifferentiating status.

**ECs derived from mouse ESCs.** Mouse ESCs were harvested using 0.1% collagenase IV in Knockout DMEM and cultured in Poly-HEMA-coated plates in MEF media to prepare EBs. To induce EC differentiation, the EBs were maintained in differentiation EGM-2 (Lonza) medium, 15% FBS, supplemented with an angiogenic “cocktail” of growth factors including VEGF, epidermal growth factor human recombinant (rh EGF) and recombinant human fibroblast growth factor-B (rhFGF-B), in a gelatin-coated plate. The media was replaced every other day for up to 18 days.

**TaqMan real-time PCR analysis.** Real-time PCR was carried out on the ABI PRISM® 7000 system (Applied Biosystems). Relative quantitation of gene expression levels was achieved using the comparative ΔΔCt method. The PCR reactions were carried out in a final volume of 50 μl in 96-well optical reaction plates. 30 μl of TaqMan universal master mix was used together with 10 μl of diluted cDNA from the RT step, and 10 μl of the appropriate concentration of primer/probe. The plates were sealed using optical adhesive covers and run on the ABI 7000 sequence detection system according to the recommended protocol and analyzed with the sequence detection software.

**siRNA transfection.** The delivery of the siRNA into EC-derived ESCs was performed using with the Nucleofector nucleoporation device and the basic endothelial transfection kit from Amaxa Inc., (Cologne, Germany). Transfections were performed according to the manufacturer’s instructions using M003 program for EC from day 0 and program T023 for EC at day 15. The annealed siRNA against HoxD3 and HoxA5 were purchased from Ambion (Austin, TX USA) with 5'-CCU GGA CAG UGA UAA CCC ATT-3’ sequence for HoxD3 and 5’-CCA GUU GCA UAA UUG ATT-3’ sequence for HoxA5. As additional controls, we used the murine endotheliolemma cell line (EOMA) treated with either HoxD3 and HoxA5 siRNAs and a control siRNA against pMAXGFP (Amaza, Inc.). After transfection cells were plated into gelatinized six-well plates and RNA extracted three days later and real-time PCR performed as described above.

**Flow cytometry.** The cells were dissociated by PBS/EDTA treatment (10 mM, 37°C, 10 min) and were passed through G25 needles and 100 μm mesh filter for single cell formation. After cells were washed with PBS/0.1% sodium azide/5% FBS solution, 1 x 10⁶ cells (viability 88%) were blocked in PBS/0.1% sodium azide/10% FBS solution for 30 min on ice. Phycocerythrin (PE)-conjugated PECAM-1 (CD31), (PE)-conjugated Flk-1 and Allophycocyanin (APC)-conjugated-VE-Cadherin antibodies (e-Bioscience) were used to stain single or double markers for 30 min on ice. Cells were washed three times with PBS/0.1% sodium azide/5% FBS. Isotype control and non-stained samples were used for specificity assessment and background signal adjustment. Live cells collected at different time points following differentiation were analyzed and sorted on a FACS Vantage instrument (BD Biosciences) operated by DIVA software. Cells at day zero of differentiation (EBs that transferred from poly-HEMA plate to gelatinized plated) were used as non-differentiated control cells and subject to similar sorting and staining protocols as outlined above for cells undergoing differentiation.

**Statistical analysis.** In all experiments, groups were compared using Student’s t-tests and significance was considered to be p < 0.05.

**Acknowledgements**
This work is supported by grants from NIH/NCI T32 grant (U54CA126552) to Nancy Boudreau and Mina J Bissell and US Department of Energy, Office of Biological and Environmental Research (DE-AC02-05CH1123), a Distinguished Fellow Award and Low Dose Radiation Program (03-765F00098) to Mina J. Bissell. Mandana Veiseh was supported by a postdoctoral fellowship from the NCI of the NIH (F32 CA132491A). We thank Pamela Derish in the Department of Surgery at UCSF for editorial review of the manuscript.

**Note**
Supplemental materials can be found at: [www.landesbioscience.com/journals/celladhesion/article/14373](http://www.landesbioscience.com/journals/celladhesion/article/14373)
Mace KA, Hansen SL, Myers C, Young DM, Boudreau N. HOXA3 induces cell migration in endothelial and epithelial cells promoting angiogenesis and wound repair. J Cell Sci 2005; 118:2567-77.

Uyeno IA, Newman-Keagle JA, Cheung I, Hunt TK, Young DM, Boudreau N. A role for Hox A5 in regulating angiogenesis and vascular patterning. Lymphat Res Biol 2005; 3:240-52.

Rhoads K, Arderiu G, Charbonneau A, Hansen SL, Hoffman W, Boudreau N. A role for Hox A5 in regulating angiogenesis and vascular patterning. J Cell Sci 2001; 100:46-56.

Carrio M, Arderiu G, Myers C, Boudreau NJ. Homeobox D10 induces phenotypic reversion of breast tumor cells in a three-dimensional culture model. Cancer Res 2005; 65:7177-85.

McGinnis W, Krumlauf R. Homeobox genes and axial patterning. Cell 1992; 68:283-302.

Scott MP. Vertebrate homeobox gene nomenclature. Cell 1992; 71:551-3.

Gorski DH, Walsh K. The role of homeobox genes in vascular remodeling and angiogenesis. Circ Res 2000; 87:865-72.

Botas J. Control of morphogenesis and differentiation by HOM/Hox genes. Curr Opin Cell Biol 1993; 5:1015-22.

Boudreau N, Andrews C, Srebro A, Ravanpay A, Chereis DA. Induction of the angiogenic phenotype by Hox D3. J Cell Biol 1997; 139:257-64.

Cheresh DA. Induction of the angiogenic phenotype of endothelial and smooth muscle like cells and form vascular networks in vivo. Circ Res 2007; 101:286-94.

Lawrence HJ, Christensen J, Fong S, Hu YL, Weissman I, Sausville G, et al. Loss of expression of the Hox-A9 homebox gene impairs the proliferation and repopulating ability of hematopoietic stem cells. Blood 2002; 99:1231-9.

Thorsteindottir U, Mamia A, Koon J, Erode L, Bilj L, Lawrence HJ, et al. Overexpression of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion. Blood 2002; 99:1231-9.

Lawrence HJ, Christensen J, Sonea IM, Jeannotte L, et al. Transgenic mice ectopically expressing HOX4A in the dorsal spinal cord show structural defects of the cervical spinal cord along with sensory and motor defects of the forelimb. Brain Res Dev Brain Res 2004; 150:125-39.

Mandeville J, Aubin J, LeBlanc M, Lalancette-Hebert M, Janelle MF, Tremblay GM, et al. Impact of the loss of Hoxa5 function on lung alveogenesis. Am J Pathol 2006; 169:1312-27.

Aubin J, Chailler P, Menard D, Jeannotte L. Loss of Hoxa5 gene function in mice perturbs intestinal maturation. Am J Physiol 1999; 277:965-73.

Thomson MA, Kalousek DK, Burdsall DR, Kehinde A, Mulligan RC, Thomson MA, et al. Human embryonic stem cell lines derived from eight-week-old embryos display pluripotency in vitro and in vivo. Nat Biotechnol 1998; 16:137-42.

Krieges KE, Abbott MA, Joksimovic M, Lueth PA, Sonea IM, Jeannotte L, et al. Transgenic mice ectopically expressing HOX4A in the dorsal spinal cord show structural defects of the cervical spinal cord along with sensory and motor defects of the forelimb. Brain Res Dev Brain Res 2004; 150:125-39.