Chapter 2

Hox Genes in Adult Tissues and Their Role in Endothelial Cell Differentiation and Angiogenesis

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Abstract

HOX genes belong to a family of transcription factors characterized by a 183 bp DNA sequence called homeobox, which code for a 61-amino-acid domain defined as the homeodomain. These genes play a central role during embryonic development by controlling body organization, organogenesis, and stem cell differentiation. They can also play a role in adult processes such as embryo implantation, hematopoiesis, and endothelial differentiation. Since endothelial cell differentiation is one of the main steps to initiate vasculogenesis and angiogenesis, we analyzed the role of several Hox genes in the regulation of these two processes. In this chapter, we summarized the evidence to support the function of Hox genes in adult tissues, specifically in endothelial cell differentiation, by studying their mechanism of action and how their target genes regulate vasculogenesis and angiogenesis. Understanding the cellular and molecular mechanisms triggered by Hox biological effects is pivotal for designing new drugs or therapies for high prevalent pathologies, such as cardiovascular diseases.

Keywords: Hox genes, endothelial cell differentiation, angiogenesis, vasculogenesis, embryonic development

1. Overview

Hox genes are responsible for the expression of a large family of transcriptional factors that play a key role in embryonic development, organogenesis, and anteroposterior body orientation

[1, 2]. Even though the main function of these genes is well known during embryogenesis, their role in adults remains under investigation. Several studies have linked Hox genes with adult processes such as vascularization, hematopoiesis, tumor angiogenesis, and cell differentiation [3]. In this chapter, we will focus our attention on the origin and main role of Hox genes in adult tissues, especially on endothelial cell differentiation, neovasculogenesis, and angiogenesis.

2. Origin of the Hox gene cluster

The Hox genes were discovered in 1915 by Calvin Bridges in a mutant *Drosophila melanogaster* named Bithorax, which showed a partial duplication of the thorax [4]. Years later, another mutation in the Hox genes was identified resulting in a mutant fly exhibiting legs instead of antenna named Antennapedia [5]. The Hox genes were then grouped into these two complexes (Bithorax and Antennapedia), which are located on chromosome 3 and play a key role in conferring the identity along the anteroposterior axis of the body. The role of these genes in establishing the anteroposterior axis is highly conserved in vertebrates [5, 6]; however, the Hox gene cluster has changed during its evolution, evidenced by different numbers of clusters between species (**Figure 1**). For example, whereas invertebrates typically possess a single cluster, vertebrates such as mice and humans possess four gene clusters coding for the three different axes: cervical, thoracic, and lumbosacral [2, 6]. Despite these differences, Hox genes have been identified in all species, which reflects the important role of these genes in the regulation of body structure [1, 7]. In humans, the 39 mammalian Hox genes are grouped into four chromosomal clusters named *HOXA*, *HOXB*, *HOXC*, and *HOXD*, located on chromosomes 7p14, 17q21, 12q13, and 2q31, respectively [8]. This large family encodes homeodomain transcription factors that share highly conserved DNA sequence formed by 183 bp called "homeobox," which encodes a polypeptide core of 61 amino acids formed by three alpha helices known as the homeodomain. Most homeodomains recognize highly conserved DNA elements that

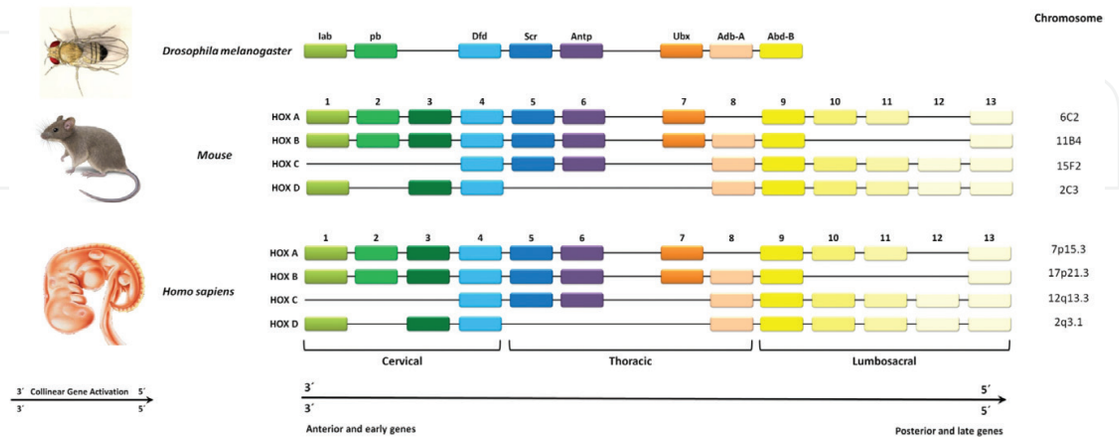

Figure 1. The composition and evolutionary differences of the HOX gene cluster between *Drosophila melanogaster*, mouse, and *Homo sapiens*. The HOX gene clusters and their chromosomal location were compared between *Drosophila melanogaster*, mouse, and *Homo sapiens*. Genes were grouped according to the distribution of the three axes corresponding to the anteroposterior part of the body (cervical, thoracic, lumbosacral).

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serve as a promoter for many genes (motif TAAT) being a T in the direction 5′ determinant for this coupling acknowledgment [9]. Hox transcription factors are well known for playing a key role during cell and tissue differentiation in developing embryos; however, other studies have shown that these homeotic genes also play a role in adult process such as hematopoiesis and embryo implantation by promoting neovasculogenesis and angiogenesis [10].

3. Hox genes in adult-related processes

3.1. Endometrial tissue

Hox genes are crucial during endometrium redevelopment and corpus luteum formation because they regulate cell growth and differentiation during each reproductive cycle [10]. Expression of HoxA10 in human epithelial and stromal endometrial cells has been significantly higher in the intermediate and late phase of the menstrual cycle, suggesting that it could favor the implantation of the embryo [11–13]. Mechanistically, the protein encoded by this gene regulates the expression of several proteins related to endometrial development such as Emx2/EMX2, integrin β3, insulin-like growth factor-binding protein-1 (IGFBP-1), cyclin inhibitors, Wnt family genes, and the prostaglandin receptors EP-3 and EP-4 [14, 15].

Endometrium development is regulated by estrogen and progesterone; thus, any regulation of Hox genes by these hormones suggests that these genes play a role in the growth and development of the endometrium. For example, 17β-estradiol and progesterone significantly increased the expression of HoxA10 in endometrial cells [16] and primary culture of stromal endometrial cells, respectively, with a higher response induced by progesterone compared to 17β-estradiol [17] and even higher when both hormones were used in combination [17, 18].

HOXA11 is another hox gene from the A cluster that has been closely associated with morphological alterations [19]. During the development of the female reproductive tract, *HOXA11* is normally expressed in the cervix and lower uterine segment. When the expression of this gene is impaired, it promotes aberrant epithelial cell differentiation leading to epithelial ovarian neoplasia [20, 21]. In addition, *HOXA11*$^{-/-}$ mice exhibit reduced development of the stroma in the glandular tissue and decidua during pregnancy [18, 22], suggesting a role in myometrium preparation to implantation.

More recently, Yim et al. suggested that *HOXA11* promotes metastasis by regulating the expression of gene coding for metastasis-related proteins [23]. These findings indicate that *HOXA11* plays a role in the aggressive nature of ovarian cancer cells through *HOXA11*-mediated expression of target genes such as matrix metalloproteinase (MMP) and VEGF.

3.2. Implantation

Implantation is a series of sequential biological events triggered after fertilization in which the blastocyst migrates from the fallopian tube into the uterus. The fertilized egg is then attached to the uterine wall and subsequently implanted in the endometrium. Implantation occurs only in a very specific time period and place during the mid-secretory phase of the uterine cycle [24]. During this period, the uterus becomes more receptive by promoting a series of cellular and

molecular events favoring the implantation of the embryo. In this stage, the role of several intercellular mediators has been implicated, which include specific cytokines, growth factors, adhesion molecules, lipid mediators, steroid hormones, and Hox transcription factors [25]. Like in endometrial tissue, *HOXA10* also plays a role during embryo implantation as it has been shown that despite the fact that *HOXA10*-deficient mice (*HOXA10*$^{-/-}$) exhibited normal ovulation cycle, the implantation did not occur. Interestingly, implantation was restored when embryos from *HOXA10*$^{-/-}$ were transferred to wild-type mice; however, wild-type embryos were not implanted in *HOXA10*$^{-/-}$ female mice [18], suggesting that *HOXA10* is required to have an adequate implantation environment. Moreover, *HOXA10*$^{-/-}$ and *HOXA11*$^{-/-}$ mice also exhibit poor implantation due to insufficient development of stromal glandular tissue and decidua during pregnancy [26]. In humans, the expression of both *HOXA10* and *HOXA11* genes rises gradually during the proliferative phase of the menstrual cycle, showing a peak of expression in mid-cycle, when implantation typically occurs [13, 27]. Interestingly, this peak of expression was not observed in women with endometriosis or in mice with induced endometriosis [13, 27], suggesting that HoxA10 and HoxA11 peaks require a healthy endometrium to support and continue with the implantation process. Several studies have shown that Hox10 not only promotes implantation directly but also inhibits detrimental factors such as empty spiracles homeobox 2 (EMX2), P300/CBP-associated factor (P/CAF), and gamma-aminobutyric acid (GABA). Studies by Taylor and colleagues demonstrated that HoxA10 repressed EMX2 expression, which in turn inhibited the proliferation of endometrial cells [28], suggesting that HoxA10 is a pro-proliferative and pro-implantation factor in these cells. Zhu and colleagues demonstrated that HoxA10 repressed the promoter activity of P/CAF, which impairs endometrial receptivity and embryo implantation by downregulating integrin β3 [29]. Recent studies have also shown that HoxA10 decreased mRNA levels and protein translocation of GABA receptor [30], which plays a role in the generation of uterine contractions and labor [31]. Thus, the quiescent uterus is required for adequate implantation and embryo development, along with reduced expression or activity of GABA receptor.

3.3. Hematopoiesis

Hox genes are highly expressed in hematopoietic stem cells (HSC) and immature progenitor cells [32]; however, this expression is gradually decreased upon cell differentiation. Moreover, overexpression of genes from the *HOXA* cluster impairs B and T lymphocyte differentiation, affects erythropoiesis, and reduces stem cell bone marrow homing, favoring the induction of myeloproliferative disorders and leukemias [33]. In fact, overexpression of *HOXA1*, *HOXA4*, and *HOXA6* genes has been shown to favor the generation of permanent cell lines [34]. Studies by Wang et al. showed increased proliferation and higher self-growth and self-renewal of hematopoietic stem progenitor cells (HSC) (Line 9 and Line H1) when HoxA6 was overexpressed compared to normal conditions [34]. The authors observed that overexpression of this gene sustained HSC self-renewal and multipotency by promoting mature erythroid lineage cells and partial apoptosis of erythroid progenitors.

Another gene involved in this process is *HOXA5*. Overexpression of HoxA5 in HSC isolated from umbilical cord blood, bone marrow [35], or mice [36] promotes a significant shift toward myeloid differentiation in relation to erythroid differentiation when compared to respective control cells [35, 36]. Then, the authors evaluated genes affected by HoxA5, and they observed downregulation of several genes involved in cell proliferation, differentiation, and metabolism [35, 36].

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HOXA9 has also been associated with the regulation of myeloid cell differentiation. The activation of HoxA9 complex favors the recruitment of CREB-binding protein (CBP/p300), histone acetylation, and activation of a number of transcription factors and proto-oncogenes, including Erg, FLT3, and SOX4 Myb, which regulate hematopoiesis [37].

Another Hox gene family member linked to hematopoiesis is *HOXA10*. The expression of this gene is high in myeloid progenitor cells, and it decreases during cell maturation [38]. Bei et al. [39] studied the expression of HoxA10 in bone marrow from patients with human acute myeloid leukemia (AML), and they observed increased expression of this gene in patients with poor prognosis. Then, they developed a HoxA10-overexpresing mouse model identifying *CDX4*, a caudal gene that contain homeodomain and code for transcription factor that plays an important role in hematopoiesis, as a *HOXA10* target gene [39]. Overall, their results demonstrated that *HOXA10* was contributing to AML pathogenesis via CDX4-positive feedback. Other groups demonstrated that HoxA13 was associated with the development of monocytes and macrophages, and its expression was observed more often in monocytic leukemia cell lines in comparison with other types of leukemia [40]. Moreover, the expression of genes *HOXB3* and *HOXB4* has been found to be altered in patients with AML with poor prognosis [41].

4. Hox genes in vascularity and angiogenesis

The development of the vascular system involves two processes called vasculogenesis and angiogenesis [42]. During vasculogenesis, angioblasts derived from different sources, including mesodermal embryonic layer or bone marrow, differentiate into endothelial cells and subsequently form a primitive network of tubular structures called blood vessels [43]. Vasculogenesis occurs largely during embryonic development; however, the presence of a population of circulating endothelial progenitor cells (EPCs) derived from the bone marrow in adults strongly suggests that this process may occur in the postnatal period [44]. In contrast, angiogenesis refers to the formation of new blood vessels from preexisting vessels by cell migration and remodeling of the primitive vascular network [45]. Vasculogenesis and angiogenesis are involved in the development of the functional vascular system in the embryo and the formation of blood vessels in the postnatal period. Both vasculogenesis and angiogenesis are under the regulation of several growth factors, which include vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF2), platelet-derived growth factor (PDGF), and transforming growth factor β1 (TGF-β1), among others [45]. Interestingly, different research groups have found that Hox genes regulate the expression of these growth factors and, in turn, endothelial cell differentiation. In the next section, we will describe supporting evidence about the role of Hox genes in endothelial differentiation, vasculogenesis, and angiogenesis (**Figure 2**).

4.1. HOXA3

The *HOXA3* gene is required for modeling the anterior body plan during embryogenesis, but they can also play a role in promoting angiogenesis [46, 47]. It has been shown that activation of *HOXA3* favors the migration of endothelial cells and keratinocytes, associated with increased expression of urokinase-type plasminogen activator receptor (uPAR) in

Figure 2. HOX genes modulate the expression of crucial target genes to promote the differentiation of mature endothelial cells. Hox genes promote the differentiation of endothelial progenitor cells, which exhibit an immature phenotype ($CD70^{+}CD34^{+}Oct\text{-}4^{+}$), into mature endothelial cells that express endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor receptor 2 (VEGFR2 or KDR), CD31, von Willebrand factor (vWF), and the lectin-type oxidized LDL receptor 1 (LOX-1). To promote this phenotype, some Hox genes upregulate crucial genes such as fetal liver kinase 1 (Flk1), angiopoietin 2 (ANG2), ephrin type-B receptor 4 (EphB4), and FI3K receptor, whereas other Hox genes downregulate other factors such as hypoxia-induced factor type 1α (HIF1α), cyclooxygenase-2 (cox-2), ephrin type-a receptor 1 (EphwA1), and VEGFR2.

both in vitro and in vivo studies using mice [46, 47]. Similar results were demonstrated by Hansen et al. who confirmed that *HOXA3* is a potent inducer of angiogenesis in vivo and also promotes direct keratinocyte migration [48]. These results suggest that *HOXA3* potentiates two key processes involved in efficient wound repair: angiogenesis and reepithelialization [46, 48]. Gene transfer studies of *HOXA3* suggest that this gene also functions as a potent inducer of wound repair in genetically modified diabetic animals. A single application of protein HoxA3 resulted in complete healing of wounds after 42 days, while wounds treated with the control plasmid without *HOXA3* (β gal) required 77 days for complete tissue repair. In addition, it was demonstrated that secreted protein HoxA3 or HoxA5, coming from respective genes and derived from composite skin constructs, exhibits decreased expression of CCL-2 and CxCL-12 inflammatory mediators, which play a key role in the attraction of monocytes, macrophages, and other wound immune cells [48]. Thus, reduced recruitment of leukocytes mediated by *HOXA3* may contribute to the prolonged integrity and viability of the composite skin constructs expressing *HOXA3*, by reducing inflammation during wound healing process. Taken together, the combined actions of HoxA3 on endothelial cells and keratinocytes lead to increased angiogenesis, normal epidermal differentiation, reduced expression of inflammatory mediators, and reduced graft contraction. These effects suggest that HoxA3 may have therapeutic benefits in wound repair by improving the integrity of composite skin grafts.

4.2. HOXA9

The *HOXA9* gene code for two different proteins, HA-9A and HA-9B isoform A (HA-9A) and HoxA9 protein isoform B (HA-9B) [49] that share a common homeodomain [15]. The expression of HA-9A has been observed exclusively during fetal development, whereas the HA-9B has been found not only in fetal but also in adult organism and specifically in endothelial cells [49, 50].

In 2004, Bruhl et al. showed that *HOXA9* was able to regulate angiogenesis [51]. These authors using human umbilical vein endothelial cells (HUVECs) with sense/antisense oligonucleotides or siRNA for this gene observed that *HOXA9* expression was essential for endothelial cell migration and tube formation. Also, they evaluated the regulation of ephrin type-B (Eph) receptor B4

(EphB4) by *HOXA9*, since previous reports [52, 53] showed that Eph receptors were homeobox protein potential targets. Then, they decided to study EphB4 since it was specifically associated with angiogenesis and cell migration processes [54, 55]. After elegant experimentation and analysis, they conclude that HoxA9 regulated endothelial cell migration and tube formation by promoting the expression of EphB4. Later in 2012, Zhang and colleagues established that HoxA9 was essential for postnatal neovascularization in vivo. In addition, they found that HoxA9 was able to regulate the expression of endothelial genes such as endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor receptor 2 (VEGFR2), and VE-cadherin in vitro in mature endothelial cells exposed to "shear stress" [56]. Furthermore, the *HOXA9*$^{-/-}$ mouse model showed a reduced number of circulating endothelial progenitor cells (EPCs) as well as reduced overall postnatal neovascularization after ischemia compared to wild-type mice. Altogether, these results demonstrated that HoxA9 is critical for postnatal neovascularization [57].

4.3. HOXA13

The central function of the placenta is to allow the formation of a vascular labyrinth, a juxtaposed series of finely branched blood vessels and trophoblast that regulate the exchange of nutrients and residues while maintaining the separation of maternal and fetal blood supplies. The study by Shaut et al. showed a morphological alteration in the labyrinth endothelial cells, branching of the vessels, and in the integrity of the vessels when *HOXA13* was dysfunctional [58, 59]. These findings suggest that *HOXA13* regulates a number of genes in the vascular endothelium required for vessel adhesion and branching, providing a functional explanation of the mean gestational lethality exhibited by *HOXA13* mutant mice. The same authors identified that EphA6 and EphA7 were direct transcriptional targets of *HOXA13* in the genital tubercle vascular endothelia [59]. Altogether, these findings provide a new genetic pathway to consider when placental pathologies or placental evolutionary ontogeny are characterized. Evidence for this coordination is observed in the labyrinth endothelium, where the genes required for cell adhesion and vascular branching are affected concomitantly by the loss of *HOXA13* function, including Neuropilin-1, Enpp2, Lyve1, Caveolin-1, Foxf1, and Tie2, resulting in reduced levels of provascular factors required for the vascular development of the labyrinth [58].

Besides HoxA genes, the HoxB and HoxD loci have also been involved in endothelial and angiogenesis regulation processes [60]. HUVECs, for example, express several genes from these loci [7], and it has been shown that some of these genes inhibit in vitro proliferation of HUVECs, whereas others have been associated with increased capillary morphogenesis and vasculogenesis [61].

4.4. HOXB1

Previous studies have revealed an overlap between HoxA1 and HoxB1 functions during the specification of the rhombomeres, a transiently divided segment of the developing neural tube, from which neural crest cells emerge. It has been demonstrated that both HoxA1 and HoxB1 functions are required for the heart development [62, 63]. HoxB1$^{-/-}$ embryos were previously described as embryos with normal pharyngeal arch arteries and cardiac neural crest-derived tissue remodeling [64]. However, more recently, Roux et al. observed one HoxB1 mutant embryo with an aortic arch artery defect, which is characteristic of a developmental failure of the left pharyngeal arch arteries (PAA) [65]. These data suggest that *HOXB1* is

important for PAA formation, and the authors provide a novel model to study the molecular origin of great artery defects, which are often observed in human patients.

4.5. HOXB3

The function of the *HOXB3* gene was studied after finding the function of its paralogous gene, *HOXD3*. While *HOXD3* is required for mediating the invasive and migratory behavior of endothelial cells during the early stages of neovascularization, *HOXB3* is required for the morphogenesis of new capillary tubes, suggesting that these paralogous Hox genes may perform complementary functions [53]. The authors also found that the capillary morphogenesis induced by *HOXB3* was mediated by ephrin A1 ligand (EFNA1) [53].

4.6. HOXB5

The *HOXB5* gene, also known as Hox-2.1, codes for a potent transcriptional regulator present in several adult tissues. Similar to *HOXA9*, *HOXB5* has been associated with vascular alterations. In this regard, studies have shown that *HOXB5* homeobox protein regulates the expression of VEGFR2, the earliest marker of endothelial precursors, by direct binding to the *HOXB5*-binding element (HBE) in the VEGFR2 gene [66]. They also found that overexpression of HoxB5 increased the number of angioblasts during embryonic stem cell differentiation and the number of mature endothelial cells, which in turn have been associated with high expression of platelet endothelial cell adhesion molecule (PECAM) and the formation of primitive blood vessels [66]. Years later, the same research group investigated the in vivo role of HoxB5 in angiogenesis using the chick (*Gallus gallus*) chorioallantoic membrane assay. They concluded that HoxB5 exerted an activating effect on angiopoietin 2 (ANG2), which was essential for endothelial cell sprouting and vascular growth [60]. More recently, the same group investigated the role of HoxB5 overexpression during revascularization in ischemic disease using femoral artery ligation in C57BL/6 mice. They observed that HoxB5 enhanced perfusion restoration and increased capillary density in vivo via monocyte chemotactic protein-1 (MCP-1) and interleukin-6 (IL-6) upregulation and increased endothelial cell migration [67].

Furthermore, other studies have shown that HoxB5 is a transactivator of the promoter of VEGFR2, an early marker of endothelial precursors [66], which might be involved in the differentiation of mesoderm-derived precursors toward an endothelial phenotype [66, 68]. In fact, it has been described that overexpression of HoxB5 leads to differentiation of mesoderm-derived precursors toward the endothelial phenotype, which in turn lead to high expression of angiopoietin 2 (ANG2) and therefore enhance vascularization in a model of fertilized white Leghorn chicken eggs [68].

4.7. HOXB7

HOXB7 has been associated with tumor progression and angiogenesis [61]. Care et al. in 2001 provided evidence that HoxB7 promotes tumor-associated angiogenesis by increasing the expression of VEGF, melanoma growth stimulatory activity/growth-related oncogene alpha, interleukin-8, and angiopoietin 2 (ANG2) in SkBr3 cells [69]. The authors concluded that HoxB7 acted as a key factor in a tumor-associated angiogenic switch [69]. In 2008, Murthi et al. identified differences in

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the expression of HoxB7 between micro- and macrovascular endothelial cells [70]. They observed higher expression of HoxB7 in macrovascular HUVECs and placenta compared to microvascular endothelial cells such as human placental endothelial cell (HPEC) line, human microvascular endothelial cells (HMVEC), and freshly isolated placental microvascular endothelial cells (PLEC). Storti et al. found that HoxB7 was expressed in 10 out of 22 multiple myeloma patients analyzed at the diagnosis related to high bone marrow angiogenesis [61]. They also found that HoxB7 was overexpressed in about 40% of myeloma cell lines compared with normal plasma cells [61]. Furthermore, they observed that HoxB7 overexpression in multiple myeloma cells significantly modified their transcriptional and angiogenic profile by upregulating VEGF, fibroblast growth factor 2 (FGF2), metalloproteinase-2 (MMP-2), platelet-derived growth factor A (PDGFA), and WNT5a, while HoxB7 also downregulates thrombospondin-2, an inhibitor of angiogenesis [61]. Finally, the homeobox gene HoxB7 is overexpressed across a range of cancers and promotes tumorigenesis by inducing cell proliferation, survival, invasion, and tumor angiogenesis in pancreatic adenocarcinoma [71], cervical cancer [72], glioblastoma tumors [73], and breast cancer [74].

4.8. HOXD1

HOXD1 is specifically expressed in mature endothelial cells compared to early-stage EPC [62, 75]. However, not only HoxD1 is expressed in these cells, but also microarray studies have revealed that several Hox genes from the cluster on chromosome 2 such as *HOXD1*, *HOXD3*, *HOXD4*, *HOXD8*, and *HOXD9* were highly expressed in blood-derived endothelial cells [62]. In particular, *HOXD1* regulates endothelial cell migration and cell adhesion on fibronectin by targeting integrin β1 (ITGβ1) in mature endothelial cells [75].

4.9. HOXD3

HOXD3 is a member of the *HOXD* cluster on chromosome 2, and it can be induced by extracellular matrix protein, Del-1, and integrin alphavbeta5 interaction on resting endothelium. Del-1 is a protein that accumulates around angiogenic blood vessels and promotes angiogenesis in the absence of exogenous growth factors [76]. Zhong et al. showed that Del-1 initiates angiogenesis by binding to integrin alphavbeta5 on the resting endothelium, resulting in expression of HoxD3 [76]. HoxD3 was then promoting angiogenesis by inducing the expression of the pro-angiogenic molecule integrin alphavbeta3 (integrin β3) [76]. These findings provide evidence for an angiogenic switch that can be initiated in the absence of exogenous growth factors indicating that the angiogenic matrix protein Del-1 may be a useful tool for the therapy of ischemic disease [76]. A year later, Chen and Ruley demonstrated the role of HoxD3 expression in human brain vessels [52]. They showed that HoxD3 expression significantly induced cerebral angiogenesis, increased focal cerebral blood flow, and reduced vascular leakage by inducing integrin β3. These data suggest that HoxD3 plays an important role in regulating angiogenesis. Other studies reported that HoxD3 mediates the basic fibroblast growth factor (bFGF)-induced expression of integrin β3 and urokinase plasminogen activator (uPA) in HUVECs [77] and promotes angiogenesis in in vivo models [78, 79]. Furthermore, *HOXD3* has been shown to be involved in cerebral angiogenesis in mice [52].

4.10. Hox genes with anti-angiogenic effects

As previously described, several transcription factors encoded by Hox genes contribute to anti-angiogenic activity such as *HOXA5*, *HOXC9*, and *HOXD10* [79].

4.11. HOXA5

It has been shown that the presence of HoxA5 was associated with the upregulation of thrombospondin-2 (TSP-2), a naturally occurring inhibitor of angiogenesis. In addition, HoxA5 expression was also associated with downregulation of pro-angiogenic genes such as Ephrin A1 (Efna1), VEGFR2, hypoxia-inducible 1α (HIF1α), and cyclooxygenase-2 (COX-2) [80].

4.12. HOXC9

HOXC9 is a transcription factor expressed in blood vessels in mice [81] and in the cardinal vein of zebrafish [82]. Kroll's group investigated this transcription factor in human vascular endothelial cells and zebrafish, and they observed that this protein was a negative regulator of circulating endothelial cells. They found that HoxC9 was highly expressed in resting endothelial cells; however, its expression was downregulated under hypoxic conditions, and overexpression of this factor inhibited endothelial migration, tube formation, and endothelial cell proliferation by targeting IL-8 transcription [82]. Finally, using a zebrafish model, they observed in vivo that HoxC9 overexpression inhibited the development of their vascular structure; this defect was rescued with exogenous IL-8. This data suggests that HoxC9 plays a negative role in the induction of endothelial cell growth by inhibiting IL-8 production [81, 82].

4.13. HOXD10

HOXD10 is another negative regulator gene for angiogenesis as its overexpression inhibited dermal microvascular endothelial cell migration in vitro [53]. In addition, it has been shown that HoxD10 reduces the expression of GATA-binding protein transcription factor, a family of transcription factors that contain two zinc finger motifs and bind to the DNA sequence (A/T) GATA(A/G), from where it acquires its name. HoxD10 via those transcription factors is able to regulate expression of VEGFR1 and VEGFR2 in differentiated endothelial cells [83]. Therefore,

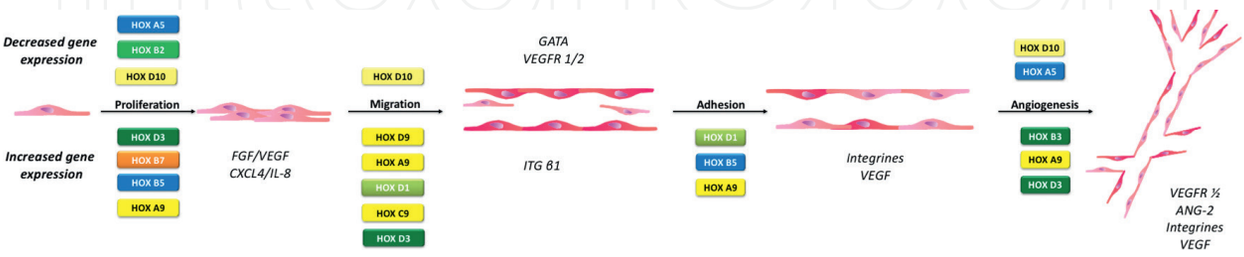

Figure 3. HOX genes regulate angiogenesis. Differential expression of Hox genes tightly regulates endothelial cell proliferation, migration, adhesion, and blood vessel formation (angiogenesis) by activating or silencing relevant target genes, such as fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), platelet factor 4 (PF4) or chemokine (C-X-C motif) ligand 4 (CXCL4), interleukin-8 (IL-8), integrin beta 1 (ITGβ1), and both vascular endothelial growth factor receptors 1 and 2 (VEGFR1/VEGFR2).

these observations suggest that there is an overlapping and complementary role between Hox genes to maintain a balance between pro-angiogenic and anti-angiogenic states (**Figure 3**).

5. Hox genes and adult stem cells

Hox genes act as transcriptional regulators, which have been involved in the differentiation of stem cells into several lineages and different cell types. One of the main steps to initiate vasculogenesis and angiogenesis is the differentiation to endothelial lineage from pluripotent stem cells. Studies have suggested that Hox genes contribute to the differentiation of EPCs into mature endothelial cells (**Table 1**). In the next section, we will present the evidence for the role of Hox genes in the differentiation of adult stem cell.

5.1. Endothelial progenitor cells

Several members of the Hox family play an important role in the embryonic development of the cardiovascular system and regulate angiogenesis in adults [84]. In addition, some Hox transcription factors such as HoxD3, HoxC6, and HoxC8 modulate the expression of proteins in mature endothelial cells, whereas HoxB5 appears to be involved in the in vitro differentiation of embryonic precursor cells toward endothelial lineage [66, 81]. *HoxA9* is important for myeloid, erythroid, and lymphoid hematopoiesis [88, 89] and stem cell expansion [90]. It is also essential for the migration and tube-forming capacity of mature endothelial cells [51] and could serve as a switch toward endothelial commitment during progenitor cell maturation. The *HOXD3* gene is also involved in the differentiation of EPC to endothelial cell. The expression of *HOXD3* retained endothelial cells in an invasive state and prevented vessel maturation leading to vascular malformations and vascular tumors. Therefore, HoxD3 regulates endothelial cell gene expression associated with the invasive stage of angiogenesis. The expression

Cellular type	Hox genes	Period of expression	Target gene	Regulation	Functions	Reference
Pro-angiogenic						
Endothelial cells of the human dermal microvasculature	HoxA3	Late embryogenesis and wound healing	uPAR	+	Endothelial cell migration	[47]
			MMP-14	+		
HUVECs	HoxA9	Post birth neovasculogenesis	EphrinB4	+	Angiogenesis	[51]
			eNOS	+	Endothelial cell proliferation	[84]
			VEGFR2	+	Endothelial cell activation	
Cellular line (MDA-MB-231, T47D, MTLn3)	HoxB2					
Endothelial cells of the human dermal microvasculature	HoxB3	Neovascularization	Ephrin A1	+	Endothelial cell vessel formation	[53]

Cellular type	Hox genes	Period of expression	Target gene	Regulation	Functions	Reference
Angioblasts (rat)	HoxB5	Neovascularization	VEGFR2	+	Endothelial cell activation	[66]
HUVECs	HoxD3	Neovascularization	Collagen1A1	+	Adhesion and migration of endothelial cells	[77]
Human microvasculature endothelial cells			Integrin-α	+		[78]
Murine embryonic stem cells	HoxA13	Postnatal neovascularization	EphA4	+	Organización células endoteliales y formación de vasos	[54]
			EphA7	+		
Vascular smooth muscle cells	Prx1	Late embryogenesis	TN-C	+	Proliferation of smooth muscle cells	[85]
			α-Actin	+		[65]
Vascular smooth muscle cells	Prx2	Late embryogenesis	TN-C	+	Proliferation of smooth muscle cells	[85]
Human pulmonary endothelial cells	Hhex	Vascular insult	Myh10	+	Plasticity smooth muscle cells	[84]
Human brain endothelial cells	Meox2	Postnatally	MLL77	—	Endothelial cell apoptosis	[66]
Anti-angiogenic						
HUVECs	HoxA5	Postnatally	VEGFR2	–	Endothelial cell activation	[86]
			Ephrin A1	–	Endothelial cell migration	[87]
Human endothelial cells	HoxD10	Postnatally	Integrin-α	—	Endothelial cell migration	[53]

uPAR, urokinase receptor; MMP-14, matrix metalloproteinase-14; EhB4, ephrin type-B receptor 4; eNOS, endothelial nitric oxide synthase; VEGFR2, vascular endothelial growth factor receptor 2; Myh10, myosin heavy chain 10; MLL, histone-lysine N-methyltransferase; HUVEC, human umbilical vein endothelial cell.

Table 1. Regulation of the Hox genes in vascular cells.

of HoxD genes has been shown to be temporally regulated as the expression of HoxD10 is maximal 3 days after stimulation with angiogenic factors, whereas the expression of HoxD3 increases after 3 days, indicating that the differentiation and maturation of endothelial cells work alongside with changes in the expression of Hox genes [90].

6. Conclusions

Hox genes have been traditionally recognized as genes involved in the embryonic development; however, further research showed that homeobox genes also play a role as master regulators of tissue and organ patterning in adults. These genes can regulate cell differentiation, proliferation, and migration to tissues exposed to constant turnover, such as vasculature,

endometrium, and bone marrow. Thus, it has been shown that Hox genes can play a role in defining an endothelial phenotype and/or promoting neovascularization; however, other genes from the Hox family can also play an anti-angiogenic role by preventing angiogenesis. These genes regulate different processes by targeting key proteins related to angiogenesis such as VEGF, IL-8, Efna1, and TSP-2 among other gene targets.

Since Hox genes play a role in the regulation of stem cell differentiation into endothelium, angiogenesis, and vasculogenesis, the manipulation of these genes could lead to a useful gene therapy in patients with vascular damage. A better understanding of the cellular and molecular mechanisms related to the biological effects of Hox genes is essential for designing new drugs and treatment to treat worldwide prevalent diseases such as cancer and cardiovascular disease.

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