The Role of Homeobox Genes in Vascular Remodeling and Angiogenesis

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Abstract—Homeodomain-containing transcription factors are critical in the regulation of cell proliferation, differentiation, and migration, and they play an important role in organogenesis and pattern formation during embryogenesis. There is evidence that some of them are oncogenes or tumor suppressors. The cardiovascular system undergoes extensive remodeling during embryogenesis and disease states such as atherosclerosis and tumor-induced angiogenesis, and homeobox genes may play an important role in regulating these processes. Recently, homeobox genes have been detected in both vascular smooth muscle and endothelial cells, and they are implicated in pathological processes such as arterial restenosis after balloon angioplasty and tumor-induced angiogenesis. The cellular function of some of these genes is beginning to be elucidated. Therefore, we briefly review what is currently known about the involvement of homeobox transcription factors in both physiological and pathological vascular remodeling and angiogenesis. (Circ Res. 2000;87:865-872.)

Key Words: genes, homeobox • endothelium • muscle, smooth, vascular

Remodeling in the vascular system, both at the cellular and organ levels, occurs during normal development and in pathological states. Examples of remodeling during normal development include the formation of the vascular system from the aortic arches and the formation of the capillary networks that provide oxygen and nutrients to organs and limbs during embryogenesis. Vascular remodeling also occurs during wound healing and the female reproductive cycle, where both angiogenesis and the regression of blood vessels occur. Pathological remodeling occurs during the development of atherosclerosis and restenosis after balloon angioplasty, where the migration of proliferating phenotypically-modified vascular smooth muscle cells (VSMCs) from the medial to the luminal side of the vessel is an important mechanism in the intimal thickening that narrows the vessel lumen and leads to ischemia, or even anoxia, in the downstream tissues.1

In addition, vascular remodeling plays a critical role in the biology of tumors, whose growth without a blood supply is limited to <1 mm by the diffusion of oxygen and nutrients through the interstitial fluids. To overcome this limitation, tumors secrete proangiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) to stimulate the ingrowth of new blood vessels.2 These newly formed tumor vessels are heterogeneous and contain endothelial cells (ECs) with an immature phenotype.3 Thus, an improved understanding of the mechanisms behind vascular remodeling could produce insights into such diverse processes as wound healing, vascular diseases such as atherosclerosis and restenosis, and cancer.

Although the receptors and signaling pathways activated by growth factors and cytokines have been extensively studied in both ECs and VSMCs, much less is known about the detailed molecular biology of the downstream transcription factors that are activated by these signaling pathways to regulate the tissue-specific gene expression and the growth and differentiation of these cell types. These transcription factors represent a common mechanism that can be regulated by the interaction of multiple signaling pathways. Homeobox transcription factors regulate cell differentiation, proliferation, and migration in vertebrates and invertebrates.4–9 As such, they are excellent candidates to be involved in the final transcriptional control of the genes responsible for vascular remodeling and angiogenesis.

Homeobox genes were first characterized through the study of mutations that give rise to homeotic mutations in Drosophila.10 Homeotic transformations occur when one body part or segment is replaced with another, normally formed, segment.11 The prototypical example is the Drosophila gene antennapedia, mutations of which can result in the replacement of the fly’s antennae with normally formed legs. Isolation and characterization of many of the genes responsible for homeotic mutations revealed that they encode transcription factors with a common 60 amino acid DNA-binding motif, the homeodomain, containing a helix-turn-helix motif similar to that found in prokaryotic regulatory
proteins such as Cro, CAP, and the A-repressor in *Escherichia coli*. In both *Drosophila* and vertebrates, many, but not all, homeobox genes are arranged in clusters. *Drosophila* has one major homeotic complex (HOM-C), and mice and humans have four unlinked complexes (HOX A through HOX D). In addition, many homeobox genes are located outside of these clusters.

Homeobox proteins are transcription factors that regulate the expression of lineage-specific genes and can control cell and organ differentiation. Homeobox genes are also involved in cell cycle control, and some are oncogenes. For instance, the homeobox genes HSIX1 and HOX 11 have been implicated in disrupting the normal G2 checkpoint. Other homeobox genes appear to act as tumor suppressors. For example, *cdx1* and *cdx2* are expressed primarily in the gut epithelium, and their downregulation is associated with colorectal tumorigenesis, whereas the overexpression of *cdx1* and *cdx2* in colon carcinoma cells decreases in vitro measures of tumorigenicity, such as proliferation and migration rates and resistance to apoptosis. Even more evidence suggesting the role of these genes as tumor suppressors in colon epithelia comes from the observation that homozygous *cdx2* knockout mice die at birth, but the heterozygotes rapidly develop intestinal tumors. It has also been shown recently that the loss of *HoxA5* activity in breast cancer may be a critical feature of tumorigenesis because it positively regulates p53 expression.

Because homeobox genes control body plan formation during development, it is not surprising that they also influence cell migration. For example, injecting *goosecoid* homeobox mRNA into *Xenopus* embryonic cells leads to the induction of region-specific cell migration during gastrulation. Homeobox transcription factors may control cell movement during pattern formation with their ability to regulate the expression of the extracellular membrane proteins that mediate cell-matrix interactions.

Thus, homeobox genes truly have pleiotropic roles in many cell types and can promote either cell proliferation or growth arrest, as well as differentiation or cell de-differentiation. Given the plasticity of the ECs and VSMCs that make up the vascular system, the features of homeobox proteins make them promising candidates as transcription factors that regulate cellular differentiation and remodeling in the vascular system during normal development and in pathological states. Several of these factors have recently been isolated from or detected in the tissues of the vascular system and, in the embryo, several have been implicated in controlling the remodeling that occurs as the vascular system is formed. Given these recent observations, which were made since our last review of homeobox gene involvement in the cardiovascular system, now is an opportunity to review what is known about homeobox gene expression and activity in the vascular system and how these genes might be involved in human diseases in which vascular remodeling plays a role. Because we are primarily interested in processes in which remodeling of the vasculature plays a prominent role, we restricted our discussion mainly to the homeobox genes expressed in VSMCs, ECs, or both.

**Homeobox Genes in Vascular Development**

**HOX Cluster Genes in Vascular Development**

Several members of the HOX clusters are expressed in the cardiovascular system during embryogenesis, and their expression persists after birth. Investigators have screened human aortic smooth muscle cDNA libraries from adult and fetal tissue and isolated cDNAs encoding HOX A2, HOX A4, HOX A5, HOX A11, HOX B1, HOX B7, and HOX C9. Functional evidence for direct HOX cluster gene involvement in vascular patterning has been obtained by targeting various genes by knockout in mouse embryos. For example, transgenic mice with null mutations of the HOX A3 gene die shortly after birth due to multiple defects in the cardiovascular system, which include heart wall malformations, persistent patent ductus arteriosus, and stenosis of the aortic valve. In the vascular system, lack of a right carotid artery was observed in some mutant mice, and in all mice the aorta had a thin wall and was poorly developed. The homozygotes were also athymic, lacked parathyroids, had reduced thyroid and submaxillary tissue, and demonstrated a number of craniofacial defects, with the overall constellation of defects resembling the pathology of the human congenital disorder Di-George’s syndrome.

As a result of cluster-duplication events during evolution, each vertebrate HOX gene can have as many as 3 paralogues (ie, Hox genes in corresponding positions), each within an independent cluster. Because members of each paralogous group have similar DNA-binding domains and expression patterns, they may have synergistic or complementary functions. Thus, targeting groups of paralogous genes, rather than individual genes, can produce defects in embryonic vascular development. For example, antisense targeting of the messages for genes in the paralogous HOX 3 group (HOX A3 and B3) results in the regression of aortic arch 3 in a manner similar to that of arch 2. Similarly, targeting paralogous group 5 genes (HOX A5, B5, and C5) causes the appearance of an additional pharyngeal arch containing a novel and completely independent aortic arch artery. These observations suggest that paralogues may have overlapping functions in the vasculature, such that the organism can compensate for the loss of expression of one but not for the loss of all.

**Prx1 and Prx2 in Vascular Development**

Several non-HOX cluster homeobox genes are expressed in the cardiovascular system during embryogenesis. Prominent among these are *Prx1* (also known as *MHox or Phox*) and *Prx2* (also known as *S8*), which are members of a homeobox subfamily known as the paired-related genes. They are so named for their homology with the *Drosophila* segmentation gene *paired*. *Prx1* was isolated using two independent approaches by two different groups. These methods included (1) complementation screening in yeast for factors able to cooperate with *MCM1*, a yeast mating-type gene containing a MADS box and sharing homology with the serum response factor (SRF), to activate cell type–specific gene expression and (2) detecting the ability of *Prx1* to bind to an A/T-rich site essential for muscle-specific transcription and transactivation by myogenic helix-loop-helix proteins in
the muscle creatine kinase enhancer. Prx1 is upregulated by angiotatin II, and it may play a role in angiotensin II-mediated smooth muscle α-actin expression in VSMCs.

The expression of Prx1 and Prx2 during embryogenesis suggests a role for them in vasculogenesis. These genes are expressed throughout embryogenesis, predominately in mesenchyme-specific patterns, including the mandibular arch, the hyoid and branchial arches, limb buds, somatopleure, cranial mesenchyme, and dermis, with extensively overlapping patterns of expression. In the vascular system, Bergwerff et al. reported that Prx1 and Prx2 expression was associated with the primary vessel wall from early stages and that it became increasingly restricted to the adventitial and outer medial cell layers. Prx1 expression colocalizes with procollagen I and fibrillin-2 but not with smooth muscle α-actin, suggesting that it may be involved in matrix modulation and formation of the adventitial layer in the embryo. Prx2 expression is highly associated with the developing ductus arteriosus and is one of the earliest markers of its differentiation.

Transgenic mice with null mutations of these genes suggest their relative importance in vascular patterning in the embryo, with Prx1 appearing to be more important to vascular formation than Prx2. Prx2−/− mutants are viable and do not show cardiovascular malformations. In addition, the intracardiac morphologies of Prx1+/− and Prx1+/Prx2−/− null mutants also appear normal throughout development. However, the Prx1+/− and Prx1+/Prx2−/− double-null mutants demonstrate a vascular abnormality, described as an abnormal positioning and awkward curvature of the aortic arch, in addition to a misdirected and elongated ductus arteriosus.

Also, in two of seven Prx1+/Prx2−/− mutants analyzed, an anomalous retroesophageal right subclavian artery was described, as was an excessive tortuosity of all great vessels as they run through the mesenchyme. However, the vascular histology and vessel wall thicknesses were normal in all mutants. Although both Prx1+/− and Prx2−/− double-gene-targeted mice revealed similar spectra of vascular anomalies, double mutants appeared to be more seriously affected, suggesting that the function of these two genes overlap.

### Homeobox Gene Expression in Vascular Smooth Muscle

#### Phenotypic Modulation and HOX Cluster Genes in VSMCs

On the basis of cell culture models and in vivo observations, it has been postulated that VSMCs exist within a spectrum of phenotypes ranging from the “contractile” to the “synthetic” state. Cells in the contractile state are quiescent, do not migrate, are relatively insensitive to mitogens, express contractile proteins at a high level (including smooth muscle–specific isoforms of actin and myosin), and are associated with a normal vessel wall. Synthetic state cells, however, can migrate; express lower levels of contractile proteins, with higher levels of nonmuscle isoforms of myosin and actin; secrete extracellular matrix components; and generally resemble their less differentiated precursors in fetal blood vessels. They are also responsive to many growth factors, especially platelet-derived growth factor and bFGF. Because homeobox genes control proliferation, differentiation, and migration, they are ideal candidate regulators of VSMC phenotype plasticity.

Homeobox sequences isolated from an adult rat vascular smooth muscle cDNA library include HOX A2, HOX A4, HOX A5, and HOX B7, whereas the HOX genes HOX A5, HOX A11, HOX B1, HOX B7, and HOX C9 have been detected in human adult and fetal aortic smooth muscle tissue. Of these, HOX B7 and HOX C9 are expressed at markedly higher levels in embryonic VSMCs compared with adult VSMCs, suggesting they play a role in the proliferation and remodeling that occur during embryogenesis. Given their differential expression in fetal tissue compared with adult vascular tissue, HOX B7 and HOX C9 appear to be good candidate genes for regulating the change from the synthetic to contractile phenotype.

Recently, it was shown that overexpression of HOX B7 in C3H10T1/2 cells, a multipotent cell line able to differentiate into VSMCs and osteogenic and chondrogenic lineages, results in increased proliferation, the induction of a VSMC-like morphology, and the expression of early VSMC marker calponin and SM22α, but not of the intermediate VSMC marker smooth muscle myosin heavy chain. These observations imply a role for this gene in vascular remodeling, either in the expansion of immature VSMCs or the change of vascular myocytes to a more immature phenotype, both of which occur in human vascular diseases. Moreover, HOX B7 mRNA was also detected in human atherosclerotic plaques at a higher level than in the normal human artery wall, implying further that it might be involved in the pathogenesis of atherosclerosis or other proliferative arterial diseases in which VSMCs revert to a more immature phenotype. Finally, HOX B7 expression increases bFGF secretion by breast and melanoma tumor cell lines and reduces growth factor independence. These observations, when coupled with its ability to induce early VSMC differentiation and proliferation in multipotent cells, are consistent with the notion that it is a potential regulator of phenotypic modulation.

#### Gax Regulation of VSMC Proliferation and Migration

Gax cDNA was isolated by screening a rat aorta cDNA library with inosine-containing degenerate oligonucleotides directed at the most conserved peptide sequence of antennapedia homeodomain. This gene maps to mouse chromosome 12 and human chromosome 7; therefore, it is not a member of a homeobox gene cluster. Gax expression has been detected in adult cardiovascular tissues including the heart, lungs, and the medial smooth muscle cells of arteries, and embryonic expression has been detected in all 3 muscle lineages (cardiac, smooth, and skeletal muscle), as well as in the brain. Gax overlaps in its expression pattern with myocyte-specific enhancer factor 2 (MEF2), a homologue of the SRF transcription factor, which is a regulator of gene transcription in muscle and neuronal cell types, suggesting that MEF2 may participate in the regulation of Gax expression during embryogenesis. In VSMCs, Gax...
mRNA is rapidly downregulated by mitogenic signals such as serum, platelet-derived growth factor, and angiotensin II and more slowly upregulated by growth arrest signals such as serum deprivation and C-type natriuretic peptide. Gax downregulation in VSMCs has also been observed in vivo after the balloon injury of rat carotid arteries, which gives rise to a proliferative lesion.

The altered expression of Gax has profound effects on VSMC biology. When microinjected into VSMCs, the Gax protein blocks VSMC proliferation and causes G1 cell-cycle arrest. Similarly, VSMC and fibroblast proliferation are strongly inhibited when Gax is overexpressed by placing its cDNA downstream of the cytomegalovirus promoter in an adenoviral vector and infections cultured cells with this construct. Overexpression of Gax also inhibits intimal hyperplasia in vivo in injured rat vessels and rabbit vessels and inhibits the proliferation of cardiac myocytes in the embryonic chick heart. The sustained overexpression of Gax will induce apoptosis in proliferating but not quiescent cells, suggesting that Gax expression and cell cycle activity are incompatible. Gax activates the expression of the cell cycle inhibitor protein p21 through a p53-independent pathway. Because Gax overexpression does not have a growth inhibitory effect in cells derived from p21 knockout mice, the upregulation of this cdk inhibitor can largely account for its antiproliferative actions.

Gax also controls the migration of VSMCs toward chemotactic growth factors, presumably through its ability to alter integrin expression. Transduction of the Gax gene with an adenoviral vector leads to a marked decrease in directed VSMC motility on extracellular matrix. This decrease is dose-dependent and independent of chemotactic growth factors. Cell-cycle arrest is essential for the antimitogenic activity of Gax, because Gax overexpression has no effect on the migration of cells lacking the cdk inhibitor p21 (p21- null cells). Although the inhibition of the cell cycle by itself is not sufficient to suppress migration, the antimigratory effect of Gax can be restored in p21- null cells when cell-cycle arrest is induced by coexpression with exogenous p21 or p16. Furthermore, the effects of Gax on cellular migration can be correlated with its ability to modulate integrin expression. Overexpression of Gax leads to the downregulation of the integrins α5β1 and α6β1 through the specific suppression of β1 and β6 subunits, both in vitro and in vivo. In parallel with its effects on migration, the Gax-induced downregulation of integrin expression does not occur in p21- null cells unless cell-cycle arrest is induced by the coexpression of p21 with p16. Collectively, these data suggest that Gax may function to coordinate vascular cell growth and motility through its ability to regulate integrin expression in a cell cycle–dependent manner. Furthermore, because integrin signaling is an important regulator of cell viability, these data also suggest that the apoptotic activity of Gax overexpression may be mediated by its ability to interfere with integrin-regulated signaling pathways within proliferating vascular cells.

Mice transgenic for a null mutation in Gax (whose mouse homologue is referred to as Mox-2) demonstrate an overall reduction in the skeletal muscle mass of the limbs, consistent with a decrease in the migration of skeletal muscle precursors in the developing limb. This produces a phenotype in which newborn mice manifest an abnormal extended position of the forelimbs and hindlimbs, with markedly diminished motility and an abnormal gait. These animals die shortly after birth from unknown causes, and it is not known whether they have any significant cardiovascular anomalies. If they do not, this would suggest that redundant homeobox factors, such as Mox-1, compensate for a lack of Gax expression in the developing cardiovascular system.

Oct-1

Oct-1 is a member of the POU subfamily of homeodomain proteins. It has been reported that nitric oxide inhibits the binding of Oct-1 to its consensus binding site in VSMCs. Given that nitric oxide is a potent inhibitor of cell cycle activity in VSMCs, such an observation suggests a possible role for Oct-1 in regulating the antiproliferative effects of this agent. Furthermore, Oct-1 binds to a repressor element in the von Willebrand factor gene in human umbilical vein endothelial cells (HUVECs), implying it may also have a role in regulating the expression of EC-specific genes.

Homeobox Gene Expression in Adult Vascular Endothelium

HOX Cluster Genes in Vascular Endothelium

The expression of several members of the HOX A, HOX B, and HOX D clusters has been detected in vascular ECs. Moreover, the expression of these genes is controlled by factors that regulate the growth of these cells. For example, Patel et al detected an alternatively-spliced variant of HOX A9, which was dubbed HOX A9EC. HOX A9EC is of interest because it is thus far the only known homeobox gene whose expression is restricted to ECs. More intriguingly, its expression is rapidly downregulated when ECs are treated with tumor necrosis factor-α (TNF-α), which, in addition to its numerous other activities, is proangiogenic. In another study, Belotti et al reported that tissue plasminogen activator, which induces in vitro morphological modifications mimicking angiogenesis activation, including elongation and formation of tube-like structures, causes an initial downregulation of all HOX B cluster genes expressed in HUVECs, followed by their overall upregulation to varying degrees over 48 hours. VEGF, an EC cytokine that induces differentiation and formation of tube-like structures, causes an initial downregulation of all HOX B cluster genes expressed in HUVECs, followed by their overall upregulation to varying degrees over 48 hours. In contrast, bFGF, a potent mitogen for ECs, and TNP-470, a fumagillin derivative with EC antiproliferative properties, had little or no effect on the expression of most HOX B cluster genes. Because HOX B cluster gene expression does not correlate with the mitogenic state of the cell but was altered with the state of cellular differentiation, Belotti et al suggested that these genes are involved in the morphogenic changes associated with the angiogenic phenotype.

The strongest evidence for the involvement of HOX gene regulation of the angiogenic phenotype in ECs comes from the study of the paralogous HOX genes HOX B3 and HOX D3, each of which seem to have distinctive and complementary roles in this process, much as the paralogous HOX group
3 and 5 genes do in vascular patterning during embryogenesis. Boudreau et al. studied HOX D3 expression in HUVECs. They based their study on previous observations that HOX D3 modulated the expression of the integrin $\beta_3$ subunit in erythroblasts.109 Integrin $\alpha_v\beta_3$ is highly expressed in angiogenic ECs.109,110,111 and antibody inhibitors against this receptor inhibit angiogenesis.72–75,109,112–114 HOX D3 is expressed at high levels in proliferating ECs that are induced to form tubes on reconstituted basement membrane, but not in quiescent ECs, and its expression is induced by bFGF. Moreover, blocking HOX D3 expression by antisense significantly blocks the ability of bFGF to induce the expression of integrin $\alpha_v\beta_3$, and urokinase plasminogen activator, whereas HOX D3 overexpression leads to increased expression of these two proteins and a morphological change of the ECs to an angiogenic phenotype. In a chick chorioallantoic membrane model, HOX D3 overexpression led to the development of endothe-

liomas.109 These observations suggest that HOX D3 is involved in the early, invasive phase of angiogenesis.

More recently, Myers et al. reported that the paralogous gene HOX B3 influences angiogenic behavior in a manner distinct from that of HOX D3. Antisense against HOX B3 impairs the capillary morphogenesis of dermal microvascular ECs cultured on basement membrane extracellular matrices; this effect is associated with the decreased phosphorylation of the Eph A2 receptor and is reversed by the addition of the ephrin A1 ligand. Consistent with this result, constitutive expression of HOX B3 in the chick chorioallantoic membrane results in an increase in capillary vascular density and angiogenesis and does not result in the formation of endothelio-

mas, as overexpressed HOX D3 does. Taken together, the results of these two studies suggest complementary roles for HOX B3 and D3 in angiogenesis, with HOX D3 promoting the invasive or migratory behavior of ECs in response to angiogenic signals, and HOX B3 promoting the subsequent capillary morphogenesis of these new vascular sprouts.

Although disruption of the paralogous gene HOX A3 results in cardiovascular defects,28 histological analyses of HOX D3-deficient mice do not reveal any defects in the heart or blood vessels.29 Likewise, HOX B3-deficient mice show no outward abnormalities in the cardiovascular system.76 HOX D3 and HOX B3 double mutants die either at birth or before weaning;66 however, no studies on the cardiovascular system of these mice have been reported to date.

Hex, an Early Indicator of EC Precursors and Regulator of EC Number During Embryogenesis

The expression of nonclustered homeobox gene expression in ECs has been less well studied, but at least one such gene, $X$-Expression, has been postulated to have a role in EC differentiation. $X$-Expression is a proline-rich divergent homeobox gene that is expressed in a range of multipotent hematopoietic progenitor cells and cell lines, and is generally downregulated during terminal cell differentiation.39 In the mouse embryo, $X$-Expression is an early marker of EC precursors, and it is transiently expressed in the endoderm, the ventral foregut, the nascent blood islands of the visceral yolk sac, and later in embryonic angioblasts and endocardium.50 The Xenopus $X$-Expression homologue of $X$-Expression, $X$-Expression, is expressed in vascular ECs throughout the developing vascular network, with its expression being transient and commencing slightly after the expression of the VEGF receptor gene $flk-1$, which is essential for vascular development.11 Moreover, overexpression of $X$-Expression during embryogenesis leads to a disruption of vascular structures and an increase in the overall number of ECs.81 These observations demonstrate that the endothelial layer is important to vascular patterning during embryogenesis and suggest a role for $X$-Expression in regulating this patterning, as well as a potential role in the EC angiogenic phenotype. However, disruption of the $X$-Expression gene in mouse embryos does not produce detectable abnormalities in the cardiovascular sys-

tem, suggesting that other factors may compensate for this loss of $X$-Expression function.82

Other HOX Genes and Tumor Angiogenesis Versus Physiological Angiogenesis

Angiogenesis is critical to tumor growth, and great effort has been made to understand how tumors induce the endothelium of the surrounding tissue to form new blood vessels. Recently, St. Croix et al. reported the use of serial analysis of gene expression to look for expressed sequence tags (ESTs) whose expression is increased $\geq$10-fold in tumor endothelium relative to normal endothelium. Not surprisingly, many of the ESTs they reported appear to derive from extracellular matrix proteins. One sequence that they found, however, was an EST whose sequence was similar to the homeobox gene $Dlx-3$, a member of the Distal-less family of homeobox genes. Of particular significance, the expression of this EST was not detectable in the developing corpus luteum, implying a molecular distinction between tumor angiogenesis and physiological angiogenesis. Although further studies are required to elucidate the identity and function of the gene encoding this EST, it should be noted that $Dlx-3$ has been previously reported to be required for placental function.84 Other homeobox genes expressed in the placenta include $Dlx-4$, $Gax$, $HB24$, and $msx2$.85 Given the critical role of angiogenesis and blood vessel regression in the placenta, it is reasonable to predict that some of these homeobox genes are involved in these processes.

Conclusions

Since we first reviewed what was known about the involve-

ment of homeobox genes in the vasculature,24 considerably more has been learned, but our knowledge is still not as detailed as it is for the cytokines and growth factors that act on ECs and VSMCs and the signaling pathways activated by these factors. However, it is clear from the Table, in which we summarize the homeobox genes thus far implicated in angiogenesis and vascular remodeling, that multiple homeobox genes participate in vascular remodeling by influencing the behavior of VSMCs, ECs, or both. From the observations summarized in the Table, it is possible to make some general statements regarding the involvement of homeobox genes in vascular remodeling:

1. Homeobox pathways are redundant and overlapping, especially during embryogenesis. Therefore, it is likely that the pattern of homeobox expression, rather than any one individual homeobox gene, regulates the ultimate
behavior of VSMCs and ECs during embryogenesis and vascular remodeling. The roles of *HOX B3* and *HOX D3* in regulating EC phenotype during angiogenesis are a good example. Each of these two genes appears to be involved in a different step of the process, with *HOX B3* influencing the final formation of capillaries and *HOX D3* controlling earlier steps in the process, in a manner consistent with previous work demonstrating complementary, overlapping, or synergistic roles of paralogous *HOX* genes.

2. Little is known about how the homeobox genes implicated in angiogenesis and vascular remodeling exert their effects at the cellular level. However, it is clear that at least a subset of them appear to function by controlling the differentiation, proliferation, and/or migration of VSMCs or ECs (examples include *HOX B3*, *HOX D3*, and *Gax*).

3. Consistent with previous observations of homeobox activity during embryogenesis and in normal and cancerous cells, a subset of homeobox genes might influence angiogenesis and vascular remodeling by activating or repressing the expression of genes encoding cell surface molecules, such as integrins, that control cellular migration (examples include *Gax* and *HOX D3*).

4. Little is known about the precise mechanisms by which homeodomain proteins regulate downstream target genes. Furthermore, where putative downstream genes are known, little is known about the specific sequences by which individual homeobox genes influence gene expression. One exception is *Prx1*, which can activate gene expression either by binding to the ubiquitous transcription factor SRF or by directly binding to a specific gene regulatory sequence. Clearly, one of the most fertile areas for homeobox gene research is the elucidation of their downstream targets and how activation or repression of the expression of these target genes results in a change in cell phenotype or behavior. Given their importance in cell cycle control, cell migration, and cell adhesion, it is likely that many more homeobox genes will be implicated in the regulation of vascular remodeling and angiogenesis. The elucidation of which homeobox genes are involved in these processes, their downstream target genes, and which cell signaling pathways activate and repress homeobox gene expression in vascular ECs and VSMCs will result in a better understanding of the basic cellular mechanisms by which the vascular system is remodeled in response to physiological signals, tumors, or other stimuli and thus may help to identify targets for therapeutic intervention in vascular diseases.
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