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## Transcriptional Regulation of Angiogenesis

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## INTRODUCTION

Until recently, the transcription factors necessary for regulating vascular development were largely unknown. This is in sharp contrast with other developmental processes, such as hematopoiesis and myogenesis, in which several cell- or tissue-specific transcription factors have been identified. Vascular development requires the differentiation of endothelial cells from pluripotent stem cells. Progress in identifying the molecular mechanisms underlying vascular development has lagged considerably, in large part the model systems for studying vascular blood vessel development are more limited. The identification of several vascular-specific genes involved in vasculogenesis and the genomic regulatory regions required for directing their expression over the past decade has facilitated the identification of the transcriptional mechanisms required for vascular-specific gene expression. Targeted disruption of additional transcription factors that have been associated with vascular defects led to the elucidation of a role for these factors in vascular development. Angiogenesis, the development of additional blood vessels from a primary vascular network, may recapitulate many of the molecular events occurring during vascular development.

## ANIMAL MODELS OF VASCULAR DEVELOPMENT

One of the major difficulties in identifying the specific transcription factors involved in regulating vascular-specific gene expression, particularly as it relates to blood vessel development, is the difficulty in isolating either embryonic or extraembryonic blood vessels during mouse embryogenesis. Because the process of blood vessel development is highly conserved over evolution, the use of alternate model systems has permitted easier access to studying blood vessel development. Two animal models that have been particularly useful for these studies are the developing zebrafish and chicken. Both have the advantages of allowing direct visualization of blood vessels. Two genes that have been identified in zebrafish and appear to be critical early regulators for initiating vascular development are *cloche* and *spade tail* (1). Similarly, the stem cell leukemia transcription factor, SCL, was also shown to promote vasculogenesis, hematopoiesis, and endothelial differentiation when expressed ectopically in zebrafish mesoderm (2). The ETS transcription factor Fli-1 has also been shown to be enriched in the developing blood vessels of zebrafish embryos (3). As an alternative model of blood vessel development, several investigators have used the developing chicken because of the easier access to developing blood vessels, particularly in the extraembryonic chorioallantoic membrane.

These blood vessels can be microdissected at different stages of development, facilitating the determination of whether specific genes are upregulated or enriched in developing blood vessels. This approach was used to identify which of the members of the ETS transcription factor family are upregulated during blood vessel development. A novel role for the ETS factor E74-like factor (ELF)-1 in vascular development was identified using this approach (4). *In situ* hybridization and immunohistochemical experiments confirmed the enriched expression of this factor in extraembryonic and embryonic blood vessels of the developing chicken embryo (4). The ETS factor ETS-1 has also been shown to be enriched in the developing blood vessels of the chicken, and antisense oligonucleotides have been shown to inhibit angiogenesis when delivered to the chicken chorioallantoic membrane (5).

## CONSERVATION OF TRANSCRIPTION FACTORS INVOLVED IN VASCULAR DEVELOPMENT

One potential criticism of using nonmammalian models to identify the transcription factors involved in regulating blood vessel development is that the same factors may not be evolutionarily conserved. Arguing against this is the fact that studies in the chicken and zebrafish have demonstrated that the factors not only are conserved with regard to protein sequence, but also show a similar enriched expression pattern during vascular development. For example, the helix-loop-helix transcription factor, SCL, is expressed in developing blood vessels and in the vasculature of both the developing mouse and zebrafish (2,6). The ETS factor ELF-1, which has previously been identified for its role for T-cell specific gene expression, has also been shown to be a strong transactivator of the *Tie1* and *Tie2* genes and is highly enriched in developing blood vessels of the developing chicken embryo. The overall homology between the chicken and human ELF-1 protein is 80% (4). Similarly, the ETS factor Fli-1 has recently been shown to be a critical regulator of blood vessel development, not only in zebrafish, but also in the mouse (3,7). *In situ* hybridization studies of the developing mouse have also demonstrated that ETS-1 is expressed in developing blood vessels associated with tumor angiogenesis (8). Targeted disruption of Fli-1 in mice results in a loss of vascular integrity accompanied by bleeding and embryonic lethality at d 11.5 (7). Expression of the *Tie2* gene is also down regulated in these mice. The expression of two GATA factors, GATA-2 and GATA-3, has recently been examined in human fetal tissues. Both factors are enriched in the developing dorsal aorta at 5 wk of age (9).

## TRANSCRIPTIONALLY MEDIATED HYPOXIA RESPONSES DURING ANGIOGENESIS AND LATER STAGES OF BLOOD VESSEL DEVELOPMENT

After the development of a primary vascular network, the developing embryo requires the formation of additional blood vessels or angiogenesis. This process is largely driven by hypoxia, which serves as a stimulus for the release of angiogenic growth factors. One of the main classes of transcription factors that promote this process is the basic helix–loop–helix (bHLH) PAS domain family. A prototype member of this family is the arylhydrocarbon-receptor nuclear translocator (ARNT) (10). ARNT forms a heterodimeric complex with another PAS transcription factor, hypoxia-induced factor (HIF)-1 $\alpha$  (11). In response to oxygen deprivation, these transcription factors stimulate the expression of such angiogenic factors as vascular endothelial growth factor (VEGF) (12). Targeted disruption of the ARNT gene results in embryonic lethality by d 10.5 (13). Although a primary vascular network forms, the predominant defective angiogenesis occurs in the yolk sac and branchial arches, and overall growth of the embryos is stunted. These defects are similar to those observed in VEGF or tissue factor-deficient mice (14,15). Thus, although the primary vascular network develops, the angiogenic responses to hypoxia are severely impaired. Similar findings are observed in HIF-1 $\alpha$  knockout mice in which embryonic lethality occurs by d 10.5 as a result of cardiac and vascular malformations (16). Although neither of these transcription factors is expressed in a vascular-specific way, their roles in angiogenesis and vascular development are primarily related to their ability to stimulate the production of angiogenic factors such as VEGF in response to hypoxia. A third member of this family of transcription factors, endothelial PAS domain protein 1 (EPAS1), was recently identified (17). EPAS is predominantly expressed in endothelial cells and can also heterodimerize with ARNT. Targeted disruption of the EPAS gene has been evaluated by two different groups, resulting in two different phenotypes (18,19). Tian et al. (18) detected abnormalities in catecholamine homeostasis in EPAS $-/-$  mice and no distinct abnormalities in blood vessel formation, whereas Peng et al. (19) identified vascular defects at later stages of embryogenesis during vascular remodeling in their EPAS $-/-$  mice. The differences in the phenotype cannot be attributed to differences in targeting construct, since both groups disrupted the expression of the bHLH domain, but were more likely attributed to differences in the strain of the mice or subtle differences in the embryonic stem (ES) cells used. Although the formation of a primary vascular network or vasculogenesis occurs, later defects in

vascular remodeling are observed during large vessel formation associated with hemorrhaging and the inability of the vessels to fuse properly. This suggests that all three of these PAS family members play a similar role in facilitating later stages of vascular remodeling and angiogenesis in the developing embryo.

Modulation of the function of HIF-1 $\alpha$  is also achieved by interaction with other proteins. The transcriptional adapter proteins p300 and CREB-binding protein (CBP) form a multiprotein/DNA complex together with HIF-1 $\alpha$  on the promoters of the VEGF and erythropoietin genes to promote expression of these genes in response to hypoxia (20). CBP-deficient mice exhibit abnormalities in both vasculogenesis and angiogenesis (21). In contrast, the von Hippel–Lindau tumor suppressor protein (pVHL) has been shown to promote proteolysis of HIF-1 $\alpha$  through ubiquitylation under normoxic conditions. Defective VHL function is associated with cancers that exhibit dysregulated angiogenesis and upregulation of hypoxia inducible genes (22).

The signaling mechanisms by which hypoxia activates HIF-1 $\alpha$  are beginning to be elucidated. The catalytic subunit of PI3-kinase, p110, plays a pivotal role in the induction of HIF-1 activity in response to hypoxia (23). Both induction of VEGF gene expression and HIF-1 $\alpha$  activity in response to hypoxia could be blocked by the addition of a PI3-kinase inhibitor. Further support of this concept comes from experiments in which VEGF gene expression and HIF-1 activity is induced by cotransfection of p110. Other studies have recently demonstrated that HIF-1 $\alpha$  activity may also be modulated by the mitogen-activated protein kinases p42 and p44 (24).

## INDUCTION OF ANGIOGENESIS IN THE SETTING OF INFLAMMATION

In addition to hypoxia, inflammation is a potent stimulus of angiogenesis. Inflammation is associated with the release of inflammatory cytokines such as interleukin (IL)-1  $\beta$  or tumor necrosis factor (TNF)- $\alpha$ . These inflammatory cytokines have been shown to promote the induction of a number of angiogenic growth factors including VEGF, growth factor (FGF), and, more recently angiopoietin-1 (AP-1). The classic transcription factor involved in mediating several inflammatory responses is nuclear factor (NF)- $\kappa$ B. One of the main sources of VEGF in the setting of inflammation is the macrophage. The induction of VEGF in response to inflammatory cytokines in the macrophage has recently been shown to be largely dependent on the activation of NF- $\kappa$ B (25). The regulatory elements responsible for AP-1 gene induction do not contain

classical NF- $\kappa$ B sites used for personal communication. In contrast, we have identified a role for the Ets factor ESE-1 as a transcriptional mediator of AP-1 induction in the setting of inflammation. We have previously shown that ESE-1 is induced in a number of cell types in response to inflammatory cytokines and interacts with NF- $\kappa$ B to regulate several genes, including nitric oxide synthase (26). This suggests that the molecular mechanisms by which angiogenic growth factors are activated at the transcriptional level may be very different from those in the setting of hypoxia and that each angiogenic factor is independently regulated at the transcriptional level.

### TARGETED DISRUPTION AND OVEREXPRESSION STUDIES OF ADDITIONAL TRANSCRIPTION FACTORS

An alternative approach that has resulted in the identification of other transcription factors required for blood vessel development is through targeted disruption. In many cases this has unexpectedly resulted in determining a novel role for a particular factor in blood vessel development. An example is targeted disruption of the AP-1 transcription factor family member Fra1, which leads to abnormalities in extraembryonic vascularization (27). The zinc finger transcription factor, lung krueppel-like factor (LKLF), is expressed in a variety of vascular and nonvascular cell types. However, targeted disruption of this transcription factor leads to abnormalities in later stages of blood vessel development (28). Although the early events of both angiogenesis and vasculogenesis were normal in LKLF-deficient mice, they develop abnormalities in the smooth muscle architecture of the tunica media, leading to aneurysmal dilatation of the blood vessels with eventual blood vessel rupture. Diminished numbers of endothelial cells, pericytes, and extracellular matrix deposition are also seen. The transcription factor Tfeb, a bHLH transcription factor, was recently shown to be required for vascularization of the placenta (29). The homeobox gene *Hox D3* is induced in endothelial cells in response to basic fibroblast growth factor (bFGF), and antisense oligonucleotides to *Hox D3* block the ability of bFGF to induce urokinase plasminogen activator (uPA). Overexpression of *Hox D3* increases integrin expression in endothelial cells (30). Another homeobox transcription factor that may contribute to both hematopoiesis and endothelial differentiation is hhx. Overexpression of this factor in zebrafish embryos leads to enhanced endothelial and erythroid differentiation (31).

## ENDOTHELIAL DIFFERENTIATION

One of the first steps during vascular development is the differentiation of endothelial cells from pluripotent stem cells. This process initially involves the expression of other endothelial-specific markers such as CD31 (PECAM-1). VE-cadherin is associated with the differentiation of these cells into mature endothelial cells. The specific transcription factors that mediate these events have not yet been identified. However, because there are conserved binding sites for several of the transcription factors involved in hematopoiesis in the regulatory regions of vascular-specific genes, it is suggested that members of the same transcription factor families are also involved in the process of endothelial differentiation.

Several studies have recently suggested the existence of a common precursor for both endothelial cells and cells of hematopoietic origin. The possible existence of a common precursor was originally suggested because of the close association of hematopoietic cells and endothelial cells in the developing embryos in the so-called blood islands. Hematopoietic and endothelial cells coexpress a number of genes. One of the earliest markers expressed on cells of endothelial and hematopoietic origin is the VEGF receptor *flk-1*. Further support for the existence of the hemangioblast comes from differentiation of pluripotent embryonic stem cells along endothelial and hematopoietic lineages (32,33). When individual blast colonies are allowed to differentiate further, they form adherent cells that express more endothelial-specific markers such as PECAM-1 and Tie2, whereas many of the nonadherent cells presumed to be hematopoietic origin express such genes as  $\beta$ -H1 and  $\beta$ major consistent with cells derived from the erythroid lineage. Furthermore, when Flk-1-positive cells were isolated from ES cells and allowed to differentiate in vitro, they could be sorted into cells of both endothelial and hematopoietic origin by flow cytometry using surface markers specific for endothelial or hematopoietic cells (34). Some of the specific transcription factors required for endothelial differentiation have recently been identified. The vascular defects seen in mice with targeted disruption of the immediate-early gene *Fra1* were partially attributed to a marked reduction in the number of endothelial cells. The defects were mainly seen in the placenta with severely impaired vascular development leading to embryonic lethality between E10.0 and E10.5 (27). The zinc finger transcription factor *Vezf1* is expressed solely in vascular endothelial cells and their precursors (35). Endothelial-specific expression of *Vezf1* was also observed in endothelial cells of the developing dorsal aorta, the branchial arch artery, and endocardium and co-localized with Flk-1 expression.

## ENDOTHELIAL TUBE FORMATION

Following their differentiation from pluripotent stem cells, endothelial cells migrate and form primitive tubes. The bHLH transcription factor *HESR1* has recently been shown to be upregulated during endothelial tube formation (36). Overexpression of this gene in endothelial cells results in downregulation of Flk-1, which may result in inhibiting endothelial cell proliferation by diminishing endothelial responsiveness to VEGF. Antisense oligonucleotides directed against *HESR1* were able to block the formation of capillary tubes. The homolog of this factor in zebrafish is called gridlock and is a critical mediator of the development of arteries such as the aorta but not of veins (37). The homeobox gene *HOX B3* has recently been shown to be involved in facilitating capillary morphogenesis (38). Overexpression of this factor in the chicken chorio-allantoic membrane leads to increased capillary vascular density, and antisense oligonucleotides inhibit endothelial tube formation of microvascular endothelial cells cultured on extracellular matrix. Another transcription factor involved in endothelial tube formation is nuclear receptor peroxisome proliferator-activated receptor (PPAR)- $\gamma$ . In contrast to *HESR1* and *HOX B3*, ligand activation of this transcription factor blocks endothelial tube formation and endothelial proliferation (39).

## SMOOTH MUSCLE CELL DIFFERENTIATION

After initial endothelial tube formation, vessel maturation requires the subsequent recruitment of surrounding mesenchymal cells and their differentiation into vascular smooth muscle cells. This process involves the interaction of endothelial cells with mesenchymal cells and the release of specific growth factors such as platelet-derived growth factor (PDGF) (40,41). A number of transcription factors have also been shown to be critical for smooth muscle differentiation (Table 1). One such family is the MADS-box transcription factor family. Two members of this family, SMAD5 and MEF2C, are important in vascular development and in smooth muscle cell differentiation (42,43). Targeted disruption of SMAD5 leads to vascular defects resulting in embryonal lethality at d 10.5–11.5. The defects included enlarged blood vessels with diminished numbers of vascular smooth muscle cells. The absence of SMAD5 results in apoptosis of mesenchymal cells and marked reduction in the differentiation of mesenchymal cells into vascular smooth muscle cells (43). Similarly, the targeted disruption of MEF2C leads to abnormalities in smooth muscle cell differentiation and the inability of endothelial cells to form into vascular structures (42). LKLF is a member of the krueppel-like family of zinc finger transcription factors. Targeted dis-

Table 1  
Transcription Factors, Their Families, and Their Roles

| <i>Transcription factor (ref.)</i> | <i>Family</i>    | <i>Role</i>  |
|------------------------------------|------------------|--|
| AML-1 (53)                         | CBF              | Angiogenesis   |
| ELF-1 (4)                          | ETS (wHTH)       | <i>Tie2</i> gene regulation                              |
| Ets-1 (5)                          | ETS (wHTH)       | Angiogenesis   |
| Fli-1 (7)                          | ETS (wHTH)       | Vascular development, <i>Tie2</i> gene regulation        |
| NERF2 (48)                         | ETS (wHTH)       | <i>Tie2</i> gene regulation                              |
| TEL (49)                           | ETS (wHTH)       | Yolk sac angiogenesis                                    |
| MEF2 (42)                          | MADS box         | Vascular development, smooth muscle cell differentiation |
| SMAD5 (43)                         | MADS box         | Smooth muscle differentiation, angiogenesis              |
| SCL/tal-1 (6)                      | bHLH             | Vascular development                                     |
| dHAND (45)                         | bHLH             | Vascular smooth muscle differentiation                   |
| Tfeb (29)                          | bHLH-Zip         | Placental vascularization                                |
| HESR1, gridlock (36,37)            | bHLH             | Aorta development, endothelial tube formation            |
| EPAS (18,19)                       | PAS-bHLH         | Angiogenesis   |
| HIF-1a (16)                        | PAS-bHLH         | Angiogenesis   |
| ARNT (13)                          | PAS-bHLH         | Angiogenesis   |
| Fra1 (27)                          | bZip             | Endothelial differentiation                              |
| Vezf1 (35)                         | Zinc finger      | Endothelial differentiation                              |
| LKLF (28)                          | Zinc finger      | Vascular smooth muscle differentiation                   |
| HOXD3 (30)                         | Homeobox         | Endothelial response to angiogenic factors               |
| COUP-TFII (54)                     | Nuclear receptor | Yolk sac angiogenesis                                    |

*See text for abbreviations.*

ruption of this gene leads to vascular defects. Most notably, there is a reduction in the number of differentiated smooth muscle cells and pericytes. These defects result in aneurysmal dilatation of the large vessels and eventual rupture with intra-amniotic hemorrhage (28). A similar phenotype was recently reported for mice lacking the cytoplasmic domain of Ephrin B2, suggesting that signaling through ephrin B2 may involve activation of LKLF or similar transcription factors during later stages of blood vessel development (44). The bHLH transcription factor dHAND has recently been shown to be crucial for yolk sac vascular development. In dHAND null mice, endothelial cell differentiation and

recruitment of surrounding mesenchymal cells occurs normally. However, the mesenchymal cells fail to differentiate into vascular smooth muscle cells (45). One of the genes that was shown to be downregulated in these mice was the VEGF<sub>165</sub> receptor neuropilin, suggesting that dHAND may be a critical mediator of the VEGF signaling pathway.

### OVERLAPPING TRANSCRIPTIONAL MECHANISMS BETWEEN THE HEMATOPOIETIC AND ENDOTHELIAL LINEAGES

One of the most recent findings regarding the transcriptional regulation of vascular development was the determination that the transcription factor SCL/tal-1, which was originally thought to play a role strictly in hematopoiesis, also appears to be critical for embryonic blood vessel development. Targeted disruption of this gene leads to embryonic lethality by d 9.5 as a result of an absence of yolk sac erythropoiesis (46). However, it was unclear whether this gene might also contribute to nonhematopoietic pathways at later stages of development. By performing transgenic experiments in which the GATA-1 promoter is used to restore SCL gene expression in hematopoietic lineages in SCL<sup>-/-</sup> mice, the mice develop striking abnormalities in yolk sac angiogenesis (6). This suggests that certain transcription factors may be critical for both the normal development of hematopoietic cells and blood vessels and that there may be a common stem cell precursor for both lineages. The most striking defects were a disorganized array of capillaries and absence of normal vitelline blood vessel formation. Although the larger vitelline blood vessels were not present, a smaller network of interconnecting vessels did exist. The architecture of these vessels revealed normal-appearing endothelial cells as well as the smooth muscle cells or pericytes that constituted the outer lining of the blood vessels. The expression of a number of vascular-specific genes including *Tie-1*, *Tie-2*, *Flk-1*, and *Flt-1* also appeared normal. Members of the ETS transcription factor family that were originally described for their role in lymphoid development have now also been shown to regulate vascular specific genes. The ETS factor *NERF* was originally identified for its role in regulating the expression of B-cell-specific genes such as the tyrosine kinase *blk* (47). The *NERF* gene is expressed as at least three isoforms, *NERF1a*, *NERF1b*, and *NERF2*. Whereas *NERF2* is a potent transactivator, the *NERF1* isoforms have a truncated transactivation domain and act as natural dominant negative forms of *NERF2*. These isoforms are differentially expressed in different cell types. Whereas *NERF1a* and *1b* are expressed in B-cells, *NERF2* is highly expressed in

endothelial cells and is a strong transactivator of the endothelial-specific *Tie1* and *Tie2* genes (48). Similarly, the related ETS factor *ELF-1*, which was originally shown to regulate T-cell-specific genes, was also shown to be enriched in developing blood vessels of the chicken (4). The ETS factor *Tel* was originally identified for its role as a proto-oncogene in the development of human leukemias. Targeted disruption of this factor led to defects not only in hematopoiesis, but also in extraembryonic angiogenesis (49).

Another mechanism for providing cell type specificity, even though the particular factor may be expressed in several cell types, is through differential expression of functionally different isoforms of the transcription factor in different cell types. The ETS transcription factor *NERF*, for example, which was originally identified as being important in B-cell function by regulating the B-cell-specific tyrosine kinase *blk*, has also subsequently been shown to regulate the *Tie2* tyrosine kinase in endothelial cells (47,48). The *NERF* gene has multiple isoforms that are differentially expressed in B-cells compared with endothelial cells (48).

## TEMPORAL AND SPATIAL ASPECTS OF VASCULAR DEVELOPMENT

Differentiating cells migrate to the proper location in the correct spatial and temporal organization to form specific structures such as organs or tissues. Blood vessel development similarly involves the correct spatial organization of differentiating endothelial and vascular smooth muscle cells. Endothelial differentiation is an early event followed by the formation of primitive tubes. The subsequent recruitment of surrounding mesenchymal cells and their differentiation into vascular smooth muscle cells is a later event leading to the formation of stable blood vessels. Growth factors including PDGF, bFGF, VEGF, AP-1, and transforming growth factor (TGF)- $\beta$  are key mediators of these events, promoting the proliferation and migration of cells. Several of the transcription factors described above are key regulators of the expression of either growth factors or their receptors or mediators of the cellular responses to these growth factors. A summary of the temporal role for these transcription factors is shown in Fig. 1. One of the earliest transcription factors required for the differentiation of a pluripotent stem cell into a hemangioblast is *SCL/tal-1* (50). Knockout studies suggest that two transcription factors that may be required for differentiation or survival of endothelial cells early in development are *Fra1* and *Vezf1* (27,35). Another early step in the differentiation of endothelial cells is the expression of VEGF receptors that promote not only the

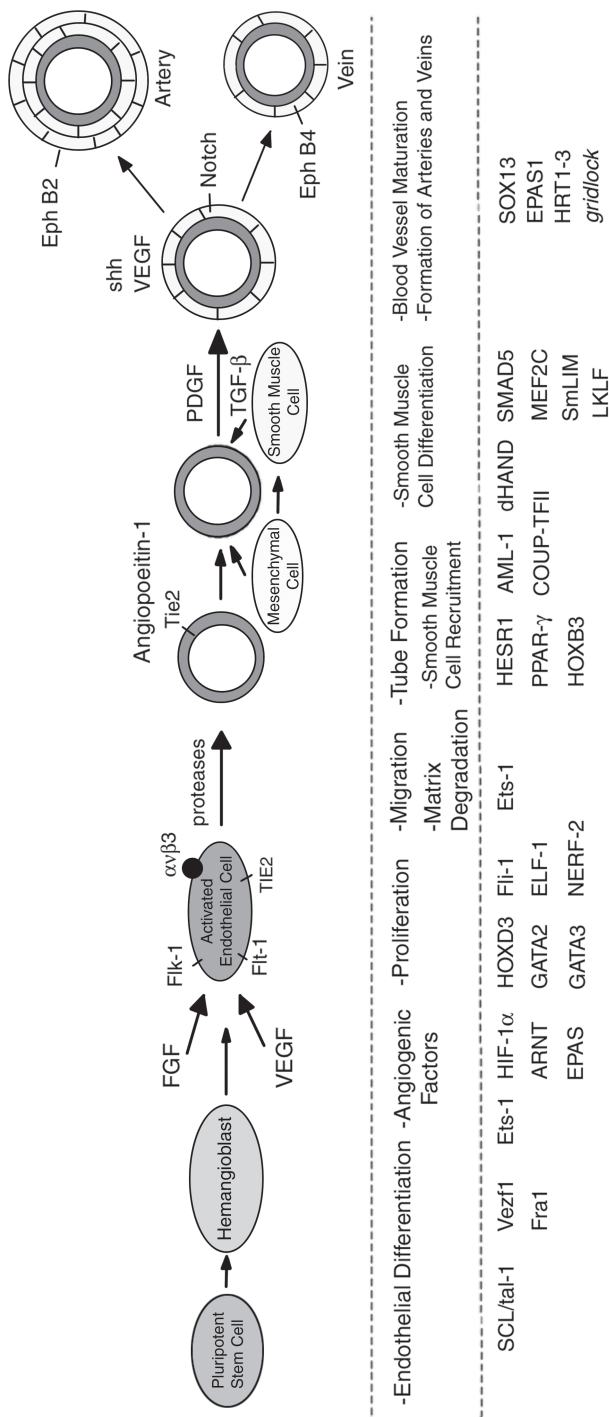


Fig 1. The role of transcription factors during different stages of vascular development.

differentiation but also the proliferation of endothelial cells. Regulation of the VEGF receptor's gene expression is mediated by the Ets transcription factors, GATA factors, and bHLH factor dHAND (45,51,52). The expression of VEGF is largely mediated by the PAS domain family of transcription factors, including HIF-1 $\alpha$ , EPAS, and ARNT, in response to hypoxia. The next stage of blood vessel development involves the proliferation and migration of endothelial cells and their formation into primitive tubes. Endothelial tube formation is regulated at least in part by the transcription factors HESR1 and PPAR- $\gamma$  (36,39). Maturation of primitive endothelial tubes into mature blood vessels requires the recruitment of surrounding mesenchymal cells or pericytes and their differentiation into vascular smooth muscle cells. This process is largely mediated by the angiopoietins and the Tie2 receptor. *Tie2* gene expression has been shown to be regulated by the ETS factors NERF, ELF-1, and Fli-1 (4,7,48). One of the key regulators of AP-1 expression is the transcription factor AML1. Targeted disruption of this factor led to abnormalities in angiogenesis that could be rescued by administration of AP-1 (53). Another transcription factor that also appears to regulate AP-1 levels is the nuclear receptor *COUP-TFII* (54). Targeted disruption of this gene is associated with angiogenic defects and marked reductions in the level of AP-1. The differentiation of mesenchymal cells into vascular smooth muscle cells is also a highly orchestrated process. Members of the MADS-box factors such as SMAD5 and MEF2C mediate the effects of TGF- $\beta$ , thereby promoting endothelial mesenchymal interactions and smooth muscle cell differentiation. Crucial gaps in our understanding of the role of specific transcription factors in this process include the lack of identification of transcriptional mediators that mediate endothelial responses to growth factors such as VEGF and AP-1. The list of factors mentioned above likely represents only a small subset of the factors required for vascular development. Several additional factors likely exist for the different stages of vascular development.

## CLINICAL IMPLICATIONS

The identification of the genomic regulatory regions and the specific transcription factors required for vascular-specific gene expression has several implications regarding the potential treatment of several diseases. First, the identification of vascular-specific fragments allows the possibility of delivering genes and their protein products specifically to blood vessels. The *Tie1* promoter has been used not only to direct the expression of the  $\beta$ -galactosidase gene in an endothelial-specific fashion but has also been used to express growth hormone (55). Although these

experiments were performed in transgenic animals, they could similarly be used in viral vectors to direct endothelial-specific gene expression. Potential therapeutic uses of these vectors include the expression of modulators of inflammation or cell growth in diseases such as the restenosis associated with angioplasty, vasculopathy related to cardiac transplantation, and chronic inflammation associated with atherosclerosis. An example of a protein that has been successfully used to treat restenosis is the Fas ligand, which promotes cell death (56). However, if this gene was expressed in nonvascular cells, it could lead to significant adverse effects. In addition to inflammatory conditions, a vascular-specific promoter might also be used to block vascular growth during tumor growth, since most endothelial cells are not actively proliferating. The identification of transcription factors that may serve as master switches of endothelial differentiation or angiogenesis may also allow the use of these factors to be used in a therapeutic manner or serve as a therapeutic target for blocking angiogenesis. The ability of two transcription factors to direct angiogenesis was recently shown in two studies. In the first study the delivery of the early response gene transcription factor *egr-1* in a wound-healing model enhanced the degree of angiogenesis and promoted normal healing (57). Similarly, the administration of a constitutively active form of the transcription factor HIF-1 $\alpha$  augmented the angiogenic response by expression of this transcription factor *in vivo* in a rabbit model of hindlimb ischemia (58). The fact that several other transcription factors have been shown to be enriched during blood vessel development or that the targeted disruption of these genes is associated with significant vascular defects, as described above, suggests that these factors may also be used therapeutically to promote angiogenesis. Alternatively, several of these newly identified transcription factors could serve as targets for inhibiting blood vessel development or angiogenesis. Drugs used to augment or interfere with the function of these factors could enhance the development of angiogenesis in diseases such as ischemic heart disease, where the development of new blood vessel development may be beneficial. Downregulation or blockade of the function of these factors might also be effective in inhibiting the angiogenesis that promotes such diseases as cancer, rheumatoid arthritis, or diabetic retinopathy.

## SUMMARY

Angiogenesis requires the carefully orchestrated proliferation and migration of endothelial cells, followed by their formation into primitive tube-like structures. Maturation of these primitive tubes into fully devel-

oped blood vessels requires the recruitment of surrounding pericytes and their differentiation into vascular smooth muscle cells. Many of the events that occur during angiogenesis recapitulate events that occur during embryonic blood vessel development. More recently, it has also been shown that endothelial progenitors can be mobilized from the bone marrow to active sites of angiogenesis, thereby providing another source of endothelial cells. Two of the main stimuli that promote angiogenesis are hypoxia and inflammation. Transcription factors have been shown to serve as master switches for regulating a number of developmental processes, such as vascular development, and similarly act to orchestrate angiogenesis. The purpose of this review is to provide an update on the progress that has been made in our understanding of the transcriptional regulation of angiogenesis over the past few years. Ultimately, a better understanding of the molecular mechanisms underlying angiogenesis may provide insights into novel and better therapeutic approaches to promote angiogenesis in the setting of ischemic heart disease.

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