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Transcriptional Regulators of Angiogenesis

Anne Hamik, Baiqiu Wang, Mukesh K. Jain

Abstract—Angiogenesis, the process by which new blood vessels develop from a pre-existing vascular network, is essential for normal development and in certain physiological states. Inadequate or excessive angiogenesis has been incriminated in a number of pathologic states. For example, vaso-occlusive disease arising from atherosclerosis can lead to ischemia, a situation in which enhanced angiogenesis would be beneficial. Conversely, overzealous angiogenesis can contribute to tumor development and in this case inhibition of angiogenesis is desirable. Thus, strategies to induce or inhibit angiogenesis are of considerable therapeutic interest. (***Arterioscler Thromb Vasc Biol*****. 2006;26:1936-1947.)**

Key Words: angiogenesis ■ endothelium ■ transcription ■ gene regulation

The angiogenic process is regulated by a wide array of growth factors and signaling pathways. Ultimately, many of these mechanisms converge on nuclear events that regulate cellular gene expression. In this review we focus on a number of transcriptional pathways that have recently been implicated in angiogenesis. In the text and in the Table, we have grouped specific molecules by the transcription factor class to which they belong. In the Figure, these factors are grouped according to cellular function. Because of space limitations we have excluded factors for which direct evidence (in vitro or in vivo) is lacking even if there is correlative evidence available. Furthermore, we will not discuss certain established pathways, such as hypoxia-inducible factor 1 (HIF-1α), that have been the subject of numerous reviews.

Zinc-Finger Proteins

Zinc-finger proteins (ZnF) constitute one of the most abundant classes of DNA binding proteins. The coordination of cysteine (C) and/or histidine (H) residues around a zinc ion forms an independent domain that protrudes as a finger-like projection. The number and spacing of these cysteine and/or histidine residues account for the different subclasses of ZnF proteins. Most DNA-binding ZnF proteins contain $\geq$3 fingers that contact DNA in the major groove.

Kruppel-Like Factors

Kruppel-like factors (KLF) are C2H2 zinc-finger proteins implicated in aspects of cellular growth and differentiation. KLF2 (Lung-Kruppel-like factor) was originally identified by

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Characteristics and Functions of Specific Transcription Factors in Angiogenesis

Factor	Family	Demonstrated Function	In Vivo Model and Phenotype	Angiogenesis-Related Target Genes	Cofactors
Id	bHLH	Required for angiogenesis	KO: embryonic lethal with defects in neural differentiation and angiogenesis	VEGF, α6 integrin, β4 integrin, FGF receptor 1, MMP-2, TSP-1	
SCL/tal-1	bHLH	Promotes angiogenesis by modulating VEGFR2	KO: embryonic lethal with defects in yolk sac angiogenesis	VEGFR2	GATA4, ETS
HAND1	bHLH	Promotes angiogenesis	KO: embryonic lethal with abnormal smooth muscle cell distribution	VEGF, ang-1,ephrin B2, Notch1, Notch4	
HAND2	bHLH	Promotes angiogenesis	KO: embryonic lethal with disrupted EC patterning and smooth muscle cell differentiation	Neuropilin-1	
TFII-I	bHLH	Promotes angiogenesis by activating VEGFR2		VEGFR2	
TFII-IRD1	bHLH	Counter-regulates VEGFR2		VEGFR2	
Hey1/2	bHLH	Promotes angiogenesis	Double KO: embryonic lethal with vascular defects that affect placenta, yolk sac and embryo	CD44, neuropilin1, ephrin B2	
SREBP	bHLH	Pro-angiogenic. Activated by VEGF.	Deficiency in CAM (via treatment with 25-hydroxycholesterol) inhibits angiogenesis	FAS, LDLR, HMGCR	
CREB	bZIP	Promotes angiogenesis by regulating expression of VEGFR1	KO: die immediately after birth from respiratory distress	VEGFR1	
c-Fos	bZIP	Promotes angiogenesis by inducing expression of VEGF, regulating capillary pericyte growth		VEGF, ICAM-1, MCP-1	
Fra-1	bZIP	Promotes angiogenesis	KO: embryonic lethal with inadequate placental vascularization	VEGF, MMP-1, MMP-9, uPA	
Jun-D	bZIP	Inhibits angiogenesis	KO: viable, increased cardiac capillary density in response to chronic pressure overload.	HIF-1α, VEGF	
MEF2C	MADS box	Promotes angiogenesis	KO: embryonic lethal with vascular defects	ang-1, VEGF	
Smad5	Smad	Regulates mesenchymal–endothelial communication during angiogenesis	KO: embryonic lethal with defective angiogenesis		
Smad4	Smad	Inhibits angiogenesis by regulating VEGF and TSP-1		VEGF, TSP-1	
Smad3	Smad	Promotes angiogenesis		VEGF	
Smad2	Smad	Inhibits angiogenesis by regulating TSP-1 and sVEGFR1		TSP-1, sVEGFR1	
Stat3	Stat	Promotes angiogenesis by targeting VEGF	Cardiac-specific KO: viable, reduced myocardial capillary density; cardiac-specific transgenic: viable, increased capillary density	VEGF, HIF-1α	
NFAT	NFAT	Promotes angiogenesis by regulating VEGF-mediated effects and enhancing endothelial cell survival	KO (NFATc3/c4): embryonic lethal with defects in vessel assembly and disorganized vessel growth.	VEGF, TF, COX-2, cFLIP	
KLF2	Znf	Anti-angiogenic	KO shows vascular leak; viral over-expression inhibits VEGF-mediated angiogenesis	VEGFR2, SEMA3F	
KLF5	Znf	Promotes angiogenesis	Heterozygotes show decreased angiogenesis in ischemic tissue and tumor	PDGF-A	RAR
VEZF1	Znf	Down-regulation impairs tissue culture models of angiogenesis	Antisense oligonucleotide impairs network formation	endothelin-1, OP18	p68RacGAP
Egr-1	Znf	Induces angiogenesis	DNAzyme-mediated reduction of Egr-1 blocks angiogenesis	FGF2, multiple pro-angiogenic factors	
COUP-TFII	Nuclear receptors	Pro-angiogenic. Important in mesenchymal-endothelial interactions and invasive phenotype.	KO: embryonic lethal with defects in angiogenesis and heart development	ang-1, MMP-2, uPA	FOG-2
PPARγ	Nuclear receptors	Inhibits VEGF-mediated angiogenesis via several proposed mechanisms	Activation by ligand in CAM and rat cornea inhibits angiogenesis	VEGFR1, Flt-2, CD36, maspin, leptin, TNFα, integrin $\alpha5\beta1$	
HOXD3	Homeobox	Increases invasiveness of EC; regulates uPA and $\alpha v\beta$ integrin expression; enhances wound healing	Viral over-expression in CAM leads to vascular malformations; HOXD3 over-expression in diabetic mice enhanced angiogenesis	uPA, integrin $\alpha v\beta3$, type I collagen	
HOXB3	Homeobox	Enhances capillary morphogenesis	Viral over-expression in CAM increases angiogenesis	ephrin 1A	

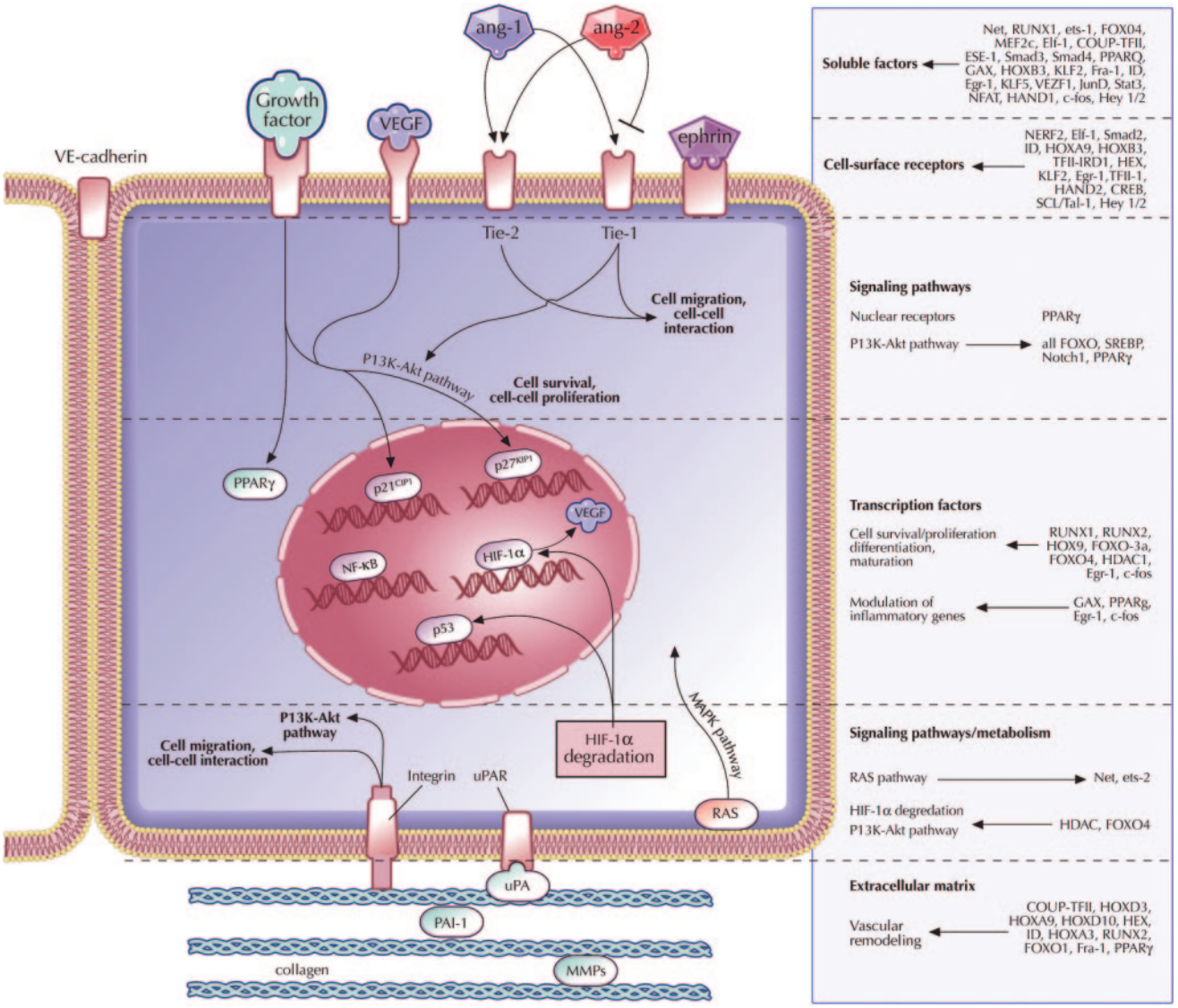
(continues)

Table 1 Continued

Factor	Family	Demonstrated Function	In Vivo Model and Phenotype	Angiogenesis-Related Target Genes	Cofactors
HOXD10	Homeobox	Blocks bFGF-induced angiogenesis. Regulates expression of extracellular matrix proteins	Viral over-expression in CAM blocks angiogenesis	PAI-1, uPAR, β4 integrin, α3 integrin, RhoC, MMP-14	
GAX	Homeobox	Inhibits angiogenic response to growth factors; inhibits NF-κB activity		ID1–4, ang-1, ang-2, ICAM-1, VCAM-1, E-selectin, several CXC chemokines	
HEX	Homeobox	Anti-angiogenic; blocks effects of VEGF		VEGFR1, VEGFR2, Tie-1, Tie-2, uPA, MMP-1, endoglin	
HOXA3	Homeobox	Promotes angiogenesis via upregulation of MMP-14 and uPAR	Over-expression in wounds of diabetic mice enhances angiogenesis	uPAR, MMP-14	
HOXA9	Homeobox	Essential for postnatal neovascularization; upregulates pro-angiogenic factors	Heterozygote and KO mice have decreased No. of endothelial progenitors and impaired post-natal neovascularization	eNOS, VEGFR2, VE-cadherin, EphB4	
ets-1	ETS	Upregulates fli-1	Over-expression via transfection leads to increased ischemic limb perfusion and angiogenesis	ang-2, HGF, VEGF	SP100
NERF2	ETS	Upregulates Tie-2		Tie-2	
Elf-1	ETS	Enhances Tie-2 and ang-2 promoter activity		Tie-1, Tie-2, ang-2	
Ets-2	Helix-turn-helix	Induces CD13/APN expression		CD13/APN	
ESE-1	Helix-turn-helix	Upregulates ang-1 under inflammatory conditions		ang-1	
Net	Helix-turn-helix	Knockdown inhibits angiogenesis and VEGF expression	Homozygous mutant Net mice have decreased angiogenesis in wounds	VEGF	
RUNX1	Runt domain	Pro-angiogenic effects are mediated through repression of IGFBP-3	KO: fetal lethal from massive hemorrhage into the central nervous system, defect in definitive hematopoiesis	ang-1, IGFBP-3	
RUNX2	Runt domain	Regulates VEGF-induced vessel invasion into developing bone. Represses $p21^{CIP1}$ promoter	KO: cartilage angiogenesis does not occur, KO mice have no bones-skeleton is composed entirely of cartilage	uPA	
FOXO1	Forkhead	Essential for embryonic vascular development. Inhibits ang-2, eNOS and $p27^{kip1}$ expression. FOXO1 expression is inhibited by ang-1.	KO: embryonic lethal, impaired vascular development	connexins 37 and 40; ephrin B2, ang-2, eNOS, Elk-3, KLF5, VEGFR1, Id2	
FOXO-3a	forkhead	Inhibits EC proliferation via down-regulation of $p27^{kip1}$; inhibits eNOS expression and postnatal neovascularization	KO: females are infertile, have abnormal ovarian follicular development	ENOS, Elk-3, $p27^{kip1}$	
FOXO4	Forkhead	Promotes degradation of HIF-1α	KO has no obvious phenotype	VEGF, GLUT-1, EPO, $p27^{kip1}$	
HDAC1	Other	Promotes HIF-1α and VEGF expression	Inhibition by trichostatin A in mouse tumor model: decreased angiogenesis in hypoxic tumor regions	p53, VHL, HIF1α, VEGF	
Notch1	Other	Pro-angiogenic; upregulated by VEGF			

ang indicates angiopoietin; CAM, chicken chorioallantoic membrane; cFLIP, cellular Fas-associated death domain-like interleukin 1β -converting enzyme inhibitory protein; COX-2, cyclooxygenase-2; EC, endothelial cell; eNOS, endothelial nitric oxide synthase; EPO, erythropoietin; FAS, fatty acid synthase; FGF, fibroblast growth factor; fli-1, Friend leukemia integration-site 1; FOG-2, friend of GATA-2; GLUT-1, glucose transporter-1; HGF, hepatocyte growth factor; HIF-1α, hypoxia inducible factor 1-α ; HMGCR, HMG COA reductase; ICAM-1, intercellular adhesion molecule-1; IGFBP-3, insulin like growth factor binding protein-3; KO, knock-out; LDLR, low density lipoprotein receptor; MCP-1, monocyte chemoattractant protein-1; MMP, matrix metalloproteinase; OP18, oncoprotein 18; PAI-1 plasminogen activator inhibitor-1; PDGF-A, platelet-derived growth factor-A; RAR, retinoic acid receptor; SEMA3F, semaphorin 3F; TF, tissue factor; TNFα , tumor necrosis factorα ; TSP-1, thrombospondin-1; uPA, urokinase-type plasminogen activator; uPAR, UPA receptor; VCAM-1, vascular cell adhesion molecule-1; VE-cadherin, vascular endothelial-cadherin; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau.

Lingrel et al.[1] KLF2 is induced by laminar flow, an important stimulus for endothelial differentiation and vascular remodeling.[2] As such, it was not surprising that KLF2-null mice exhibited vascular defects. Targeting of this factor in mice has revealed important roles for KLF2 in multiple cell types including endothelial cells. Specifically, KLF2-deficient mice die in mid-gestation because of leaky blood vessels.[3] More recently, studies from our laboratory show that adenoviral overexpression of KLF2 potently abrogates vascular endothelial growth factor (VEGF)-mediated angiogenesis as well as tissue edema.[4] This potent inhibitory effect appears to be caused, at least in part, by a reduction in expression of the key VEGF receptor VEGFR2.[4] In addition, Dekker et al have also shown that KLF2 induces the potent anti-migratory factor SEMA3F that may also contribute to its anti-angiogenic effects.[5] Studies from the laboratory of R. Nagai implicate a



Schematic illustrating the site of cellular functions and pathways regulated by transcription factors recently described to be active in angiogenesis. Transcription factors listed on the right have been shown to regulate or be regulated by the molecules, signaling pathways, or structures included in each titled subsection. Directionality of effect is indicated by the arrows; for example, the RAS pathway regulates Net, and HDACs regulate HIF-1α degradation. Each subsection is illustrated graphically in the portion of the figure directly to its left.

second KLF family member in regulating angiogenesis. KLF5 is expressed in activated endothelial cells and studies using KLF5$^{+/-}$ mice demonstrate impaired angiogenic activity in models of hind-limb ischemia and tumor implantation.[6] Taken together these studies support an important role for KLFs in angiogenesis.

Vascular Endothelial Zinc Finger 1

Using a retroviral entrapment genetic screening strategy, Stuhlman et al identified vascular endothelial zinc finger protein 1 (Vezf1) on the basis of its endothelial restricted expression in the developing embryo.[7] Vezf1 contains 6 C2H2 zinc fingers, is expressed at sites of postnatal angiogenesis, and can regulate endothelial gene products such as endothelin-1.[8] Downregulation of Vezf1 expression in endothelial cells impairs proliferation, migration, and network formation.[9] These data suggest that Vezf1 may normally function to promote aspects of angiogenesis.

Early Growth Response Factor 1

Early growth response factor 1 (Egr-1) is a broadly expressed C2H2 zinc-finger protein first discovered as an immediate early gene induced by serum. Egr-1 can induce a broad spectrum of growth factors, cytokines, receptors, adhesion molecules, and proteases implicated in angiogenesis. Using an RNA-cleaving phosphodiester-linked DNA-based enzyme approach to eliminate Egr-1 expression, Khachigian et al demonstrated a reduction in endothelial cell replication, migration, tubule formation, and fibroblast growth factor (FGF)-dependent angiogenesis.[10] Consistent data were obtained when an inhibitor of Egr-1, Nab2, was shown to inhibit angiogenesis in vitro.[11]

Basic Helix-Loop-Helix

Members of the basic helix-loop-helix (bHLH) family of transcriptional factors share a common sequence motif of a

stretch of basic amino acids responsible for site-specific DNA binding adjacent to a helix-loop-helix domain. bHLH proteins play important roles in regulating gene expression by forming homodimers and heterodimers that bind to a 6-basepair DNA site with the consensus sequence CANNTG.

Inhibition of DNA Binding/Inhibitor of Cell Differentiation

Inhibition of DNA binding/inhibitor of cell differentiation (Id) proteins contain the helix-loop-helix that allows for heterodimerization with other members of this family but lack a DNA binding domain. As a consequence, Id proteins form inactive heterodimers with other bHLH factors and function in a dominant-negative fashion. The Id protein family is comprised of 4 members designated Id1, Id2, Id3, and Id4. They are widely expressed during development and are responsible for regulation of a variety of cellular processes including cell growth, differentiation, and apoptosis.[12] With respect to angiogenesis, Id1 and Id3 appear to be particularly important. Double-knockout mice ($Id1^{-/-}Id3^{-/-}$) are not viable and exhibit defects in neural differentiation and angiogenesis but not vasculogenesis. Furthermore, although mice with targeted disruption of Id1, Id2, or Id3 are viable, they exhibit a marked reduction in tumor angiogenesis and metastasis.[13] The basis for these effects may be multifactorial as Id proteins have been shown to regulate the expression of multiple factors implicated in angiogenesis such as VEGF and its receptor VEGFR2,[13–15] α6 integrin, β4 integrin, FGF receptor 1, matrix metalloproteinase (MMP)-2, and thrombospondin (TSP-1).[16,17]

Stem Cell Leukemia/T-Cell Acute Leukemia-1

Stem cell leukemia (SCL) was originally identified as an oncogene in human T-cell leukemia.[18] Gene-targeting studies showed SCL was essential for both normal yolk sac angiogenesis[19] and adult hematopoiesis.[20,21] In adult animals, SCL expression in nonhematopoietic cells is limited to endothelial cells lining new blood vessels.[22,23] Furthermore, consistent with this observation, SCL expression is robust in endothelial cells of tumor vasculature but not in mature quiescent vasculature.[24] Ectopic expression of SCL augmented endothelial cell chemotactic migration and accelerated capillary formation in vitro and in vivo.[25]

Heart and Neural Crest Derivatives

The heart and neural crest derivative (HAND) subfamily of bHLH proteins comprises 2 members: HAND 1 (eHAND, Hxt, Thing1) and HAND2 (dHAND, Hed, Thing2). Originally identified by protein interaction screens,[26,27] HAND gene expression is restricted to heart, lateral mesoderm and neural crest derivatives. In addition, HAND1 is expressed in extra-embryonic membranes, whereas HAND2 is uniquely expressed within the maternal deciduum.[26–28] Knockout analysis of HAND1 and HAND2 in mouse model resulted in embryonic lethality. Although vasculogenesis was unaltered in $HAND1^{-/-}$ yolk sacs, vascular refinement and smooth muscle cell recruitment was disrupted.[29] In HAND2-null mice, endothelial differentiation was unaltered; however, endothelial cell patterning and smooth muscle cell differentiation were disrupted leading to abnormal vascular development.[30] These studies implicate HAND factors as essential for embryonic vessel development. The role of these factors in adult angiogenesis remains to be elucidated.

Sterol Regulatory Element Binding Proteins

Sterol regulatory element binding proteins (SREBPs) are endoplasmic reticulum-bound transcription factors that are critical regulators of cellular cholesterol synthesis. In sterol-loaded cells SREPBs bind to and are inactivated by SREBP cleavage activating protein (SCAP). When sterol levels drop, SCAP transports SREBPs to the Golgi where they are cleaved, allowing nuclear translocation of the N-terminal end and transactivation of genes such as receptor, hydroxymethylglutaryl coenzyme A (CoA) (3-hydroxy-3-methylglutaryl [HMG] CoA) reductase and fatty acid synthase. Previous studies have shown that VEGF and bFGF modulate the microviscosity of the plasma membrane as part of their pro-angiogenic effect. VEGF activates SREBP1 and SREBP2 in endothelial cell in a SCAP- and Akt-dependent manner.[31] Inhibition of SCAP blocks VEGF-induced pseudopodia extension and migration. Thus, SREBPs may influence angiogenesis via an initial step in the migration of endothelial cells.

Transcription Factor II-I

Transcription factor II-I (TFII-I) is a ubiquitously expressed basal transcription factor activated in response to a number of extracellular signals and functions through a core promoter site termed an initiator element (Inr). Wu and Patterson[32] identified TFII-1 as capable of binding to a site within the basal promoter of the VEGFR2 (KDR/Flk-1) gene and regulating transcriptional activity in vitro and in vivo. More recently, Jackson et al[33] extended these observations to show that TFII-1 can act at both basal and regulatory sites within the VEGFR2 promoter and that small inhibitory RNA (siRNA)-mediated knockdown of TFII-1 reduced VEGFR2 expression. Given the central importance of VEGFR2, these studies support a role for TFII-1 in angiogenesis. However, in vivo studies to date have been lacking.

Hey Factors

The Hey family of factors (Hey 1, Hey2, HeyL) are key effectors of the Notch signaling pathway and critical regulators of cardiovascular development. With respect to vessel biology, these factors have been implicated in regulating vascular development and angiogenesis. In vitro, Hey 1 has been shown to regulate endothelial proliferation, migration, and tube formation.[34] Surprisingly, targeting of Hey1 did not reveal any overt vascular phenotype. However, the combined loss of Hey1 and Hey2 was lethal with global lack of vascular remodeling and impaired arterial fate determination and maturation.[35] Together, these finding suggest an important role of Hey proteins in angiogenesis.

Basic Leucine Zipper Protein

Basic leucine zipper (bZIP) proteins contain 4 or 5 leucine residues spaced at intervals of seven amino acids, resulting in their hydrophobic side chains being exposed at one side of a helical region. Like bHLH, many bZIP transcription factors

exert their functions by forming heterodimers of 2 different polypeptide chains, each containing one bZIP domain. Some members of this family of transcriptional regulators have been implicated to play a role in angiogenesis.

cAMP Response Element-Binding Proteins

The cAMP response element-binding (CREB) proteins are key transcriptional mediators of stimulus-induced nuclear responses that underlie the development and function of diverse cell types. The CREB family consists of CREB and two closely related factors termed CREM and ATF1. Several lines of evidence implicate CREB in angiogenesis. For example, CREB regulates the expression of a number of genes induced by hypoxia such as VEGF.[36] CREB binding has been implicated in the regulation of 2 key VEGF receptors, VEGFR1 (Flt1)[37] and VEGFR2 (Flk). Furthermore, VEGF binding to its cognate receptor VEGFR2 induced CREB phosphorylation at serine 133, and increased DNA-binding and transcriptional activity. Furthermore, overexpression of a constitutively active form of CREB enhanced tumor angiogenesis.

Activator Protein-1

Activator protein-1 (AP-1) transcription factors are dimers of proteins encoded by Jun (c-Jun, JunB, JunD), Fos (c-Fos, Fra-1, Fra-2, and FosB), and ATFs. Dimers formed between ATF and Jun preferentially bind to cAMP-responsive elements. Jun homodimers or the more stable Jun-Fos heterodimers regulate a large variety of biological processes including cell differentiation, proliferation, apoptosis, and oncogenic transformation. Targeting of c-Jun, JunB, Fra-1, and Fra-2 results in embryonic or early postnatal death. With respect to angiogenesis, several family members have been shown to regulate gene products implicated in angiogenesis. For example, c-Fos induces VEGF-D;[38] Fra-1 induces urokinase-type plasminogen activator (uPA), uPA receptor (uPAR) and various matrix metalloproteinases (MMPs),[39,40] and c-Jun/JunB induces proliferin.[41] In vivo evidence supporting a role has been more challenging given that, as noted, knockout of several family members results in nonviable animals. However, examination of the Fra-1-null embryos revealed evidence for inadequate placental vascularization suggesting that this factor promotes vessel development.[42] In the case of JunD, animals are viable and thus amenable to further study. In the context of cancer biology recent studies show that JunD reduces tumor angiogenesis by limiting Ras-mediated production of reactive oxygen species.[43] Furthermore, JunD-null mice exhibit higher cardiac capillary density and increased VEGF levels along with enhanced cardiomyocyte apoptosis in response to pressure overload.[44] Taken together, these data suggest that members of the AP-1 family can differentially regulate angiogenesis.

Nuclear Receptors

Chicken Ovalbumin Upstream Promoter-Transcription Factor II

Chicken ovalbumin upstream promoter-transcription factors (COUPTF) are orphan members of the steroid/thyroid hormone superfamily. COUP-TFII-null mice die as embryos from defects in angiogenesis and heart development.[45] These mice show decreased expression of angiopoietin-1 (ang-1) and defective remodeling of the primitive capillary plexus into large and small microcapillaries, a pattern suggesting disrupted mesenchymal-endothelial interactions. COUP-TFII and ets-1 are colocalized in mesenchymal cells during embryogenesis, and ets-1 can transactivate a COUP-TFII construct in transient transfections.[46] COUP-TFII can confer an invasive phenotype to human lung carcinoma cell lines by inducing MMP-2 and uPA/uPAR,[47] reminiscent of similar modulation of MMP-2 and uPA seen during angiogenesis.

Peroxisome Proliferator Activated Receptors

Peroxisome proliferator activated receptors (PPARs) are ligand-activated nuclear receptors that have well-established pleiotropic roles in cell metabolism and tend to confer anti-proliferative and pro-differentiation properties. The 3 isoforms, PPARα, PPARγ, and PPARβ/δ, are all receptors for the class of synthetic ligands called thiazolidinediones well as for natural ligands, the most widely studied of which is the prostaglandin 15 deoxy-PGJ_2. The effects of PPARs on angiogenesis have been subject to an excellent recent review[48] and readers are referred thereto for comprehensive references. Briefly, PPARγ is expressed in endothelial cells and inhibits angiogenesis at multiple steps. Activation by ligand decreases tube formation, the proliferative response to VEGF, bFGF, and phorbol myristate acetate, and angiogenesis in the rat cornea.[49,50] PPARγ ligands also inhibit leptin-induced Akt-mediated endothelial cell migration.[51] Importantly, PPAR activation can inhibit inflammation-independent angiogenesis such as in the chicken chorioallantoic membrane as well as angiogenesis induced by the inflammatory mediators ELR+ and CXC chemokines IL-8, ENA-78 and Gro-a in tumors.[52]

Helix-Turn-Helix

Homeobox Factors

The homeobox (HOX) family members contain a helix-turn-helix DNA-binding homeodomain and have critical roles in pattern formation and organogenesis, where they regulate cell differentiation, proliferation, and migration. More recently, they have been shown to regulate these same processes during adult neovascularization.[53] Ex vivo models of adult neovascularization indicate that HOX gene products have both complementary and antagonistic functions. For example, HOXD3 increases the invasive and migratory behavior of the transformed cell line HMEC-1, whereas its paralog HOXB3 enhances subsequent capillary morphogenesis.[54] However, HOXD10, GAX and HEX have effects that are nonangiogenic.[55–57] Interestingly, much of the data available thus far indicate that the mechanism of function for these transcription factors is via regulation of genes that contribute to remodeling of the extracellular matrix, rather than a direct effect on endothelial cell proliferation. Thus, HOXD3 up-regulates $\alpha v\beta 3$ integrin and collagen 1,[58,59] and HOXA3 increases, whereas HOXD10 decreases, MMP-14 and uPAR expression.[55,60] A growth factor-independent function of HOXD3 is suggested by a study demonstrating up-regulation of HOXD3 as a result of the binding of the extracellular

protein Del-1 to integrin $\alpha v\beta 5$.[61] More traditional angiogenic targets have also been highlighted in studies of HOXA9, whose pro-angiogenic effects are mediated at least in part via direct effects on the up-regulation of endothelial nitric oxide synthase (eNOS) and VEGFR2[62] and downregulation of ephrin B4.[63]

E26 Transformation Specific

The E26 transformation specific (ETS) family of transcription factors is characterized by a conserved winged helix-turn-helix structural motif that serves as the DNA-binding domain. In the early 1990s, ets-1 was the first member of this family to be recognized as a critical factor in vascular development and angiogenesis. The characterization of ets-1 and early information about other members of this family has been summarized in a review by Sato.[64] Since that time, ets-1 has been shown to be a potent stimulator of angiogenesis in vivo[65] and to be negatively regulated by SP100.[66] Additional members of the ETS family have since been identified as regulators of angiogenesis. For example, novel ets transcriptional factor 2 (NERF2) is highly expressed in endothelial cells and upregulates the expression of Tie2 (the ang-1 receptor), an effect that may be most important under hypoxic conditions.[67] Elf-1 is expressed in a subset of endothelial cells and can transactivate both the Tie1 and Tie2 promoters[68] as well as the angiopoietin-2 (ang-2) promoter.[69] Ras/MAPK-dependent phosphorylation of Ets-2 was demonstrated to be required for tubule formation by transformed EC lines and able to transactivate the CD13/APN promoter, a metallopeptidase with angiogenic properties.[70] ESE-1 expression is induced in endothelial cells in response to inflammatory mediators where it then mediates transcription of ang-1.[71] This is particularly interesting as ang-1, although known to be induced under inflammatory conditions, does not have obvious binding sites for the traditional mediators of inflammation including NF-kB, STAT, NFAT, or C/EPB. Finally, downregulation of Net (Elk-3/Sap-2/ERP) has been demonstrated both in vitro and in vivo to inhibit angiogenesis and VEGF expression. Net is converted from a transcriptional repressor to a transcriptional activator via the Ras/MAPK pathway.[72]

MADS-Box Factors

Myocyte enhancer factor-2 (MEF2) proteins comprise a subfamily of the MADS (MCM1, agamous, deficiens, serum response factor)-box factors and are best known as critical regulators of muscle development and differentiation. Four MEF2 factors—MEF2A, MEF2B, MEF2C, and MEF2D—have been identified to date. MEF2C is expressed in developing endothelial cells, smooth muscle cells, and surrounding mesenchyme during embryogenesis.[73] Mice carrying a mutant MEF2C gene die early during embryogenesis and exhibit multiple vascular defects including the failure of endothelial cells to organize into complex vascular structures and inadequate smooth muscle cell differentiation.[73,74] Information regarding the role of this family of factors in adult angiogenesis is lacking.

Smad Family

The founding member of the Smad family was first identified as the product of *drosophila* gene *mothers against decapentaplegic* (MAD). This discovery led to the identification of Mad-related gene products, referred to as Smads in nematodes and vertebrates. Smad proteins are a component of the transforming growth factor β (TGFβ) signaling pathway and function downstream of the TGFβ receptor to directly transduce signals from the cell membrane into the nucleus. There are 9 vertebrate Smads: pathway-specific Smads1, 2, 3, 5, and 8 and MADH6; the common mediator Smad4; and the inhibitory Smads 6 and 7. Smad2 and 3 act as the downstream mediators of TGFβ and activin receptors, whereas Smad1 and 5 respond to bone morphogenetic protein (BMP) signals. Because TGFβ has been shown to have both pro-angiogenic and anti-angiogenic effects depending on the status of the cell, it is conceivable that Smad proteins are important transcriptional factors that mediate those effects. Studies of Smad5-null embryos revealed obvious defects in angiogenesis.[75] At E9.0, the yolk sacs of Smad5-null mice lacked a well-organized yolk sac vasculature, a defect that may be caused by a failure of communication between endothelium and mesenchyme during angiogenesis. Smad4, the only Smad protein involved in signaling pathways of all members of the TGFβ superfamily, has a central role in mediating TGFβ effects. It was originally identified as a tumor suppressor gene DPC4 (deleted in pancreatic carcinoma). In fact, the most potent mechanism underlying Smad4-mediated tumor suppression is via inhibition of angiogenesis,[76] which involves both downregulation of VEGF and upregulation of TSP-1. Smad3 is becoming recognized as a pro-angiogenic factor due to the fact that it mediates TGFβ1-stimulated VEGF-A expression.[77,78] In contrast, Smad2 has been implicated in promoting the production of anti-angiogenic factors such as TSP-1 and the soluble VEGF-A receptor Flt-1(sVEGFR1).[77] Together, these results demonstrate that Smad proteins play distinct and opposing roles in regulating expression of angiogenic factors.

Signal Transducer and Activator of Transcription

The signal transducer and activator of transcription (STAT) factor are a family of transcription factors that are activated by cytokines, growth factors, and hormones. Seven family members have been identified and shown to play crucial roles in different physiological processes such as cellular differentiation, proliferation, apoptosis, and angiogenesis.[79] Among this family, STAT3 has emerged as particularly important in the context of angiogenesis. STAT3 is a direct transcriptional activator of HIF-1 and VEGF in a broad range of human cancers.[80,81] Transgenic overexpression of STAT3 in the heart resulted in increased VEGF expression and capillary density.[82] Conversely, cardiomyocyte-restricted deletion of STAT3 resulted in reduced myocardial capillary density.[83] Consistent with these observations, targeting of STAT3 with a small-molecule inhibitor blocked HIF-1/VEGF expression in vitro and tumor angiogenesis in vivo.[84] These studies established an essential role of Stat3 in controlling cardiac capillary vasculature.

The Nuclear Factor of Activated T Cells

Members of the nuclear factor of activated T cells (NFAT) family of transcriptional factors normally reside in the cytoplasm and on stimulation translocate to the nucleus to affect cellular gene expression. Four main family members have been identified to date termed NFATc (NFAT2/NFATc1), NFATp (NFAT1/NFATc2), NFAT3 (NFATc4), and NFAT4 (NFATc3). Studies to date implicate several members in embryonic and adult angiogenesis. While targeting of NFATc3 and c4 revealed viable animals, the double-knockout results in embryonic death.[85] Examination of the NFATc3/4-null mice demonstrated disorganized major vessels including the intersomitic vessels, branchial arch arteries, and cranial vessels. No defect was observed in the differentiation and proliferation of mutant endothelial cells. However, null embryos exhibited reduced association of smooth muscle cells and pericytes indicating that NFATc3/4 are required for recruitment of these cells to the vessel wall. In the adult vasculature, NFAT1 is the dominant form expressed. NFAT1 is activated by pro-angiogenic factors such as VEGF/bFGF and inhibited by anti-angiogenic molecules such as pigment epithelial-derived factor (PEDF). NFAT1 activation has been shown to increase pro-angiogenic/pro-inflammatory factors such as tissue factor and cyclooxygenase-2 (COX-2).[86,87] Furthermore, VEGF-induced NFAT directly upregulates expression of the caspase-8 inhibitor cellular Fas-associated death domain-like IL1β-converting enzyme inhibitory protein (c-FLIP), which mediates resistance to apoptotic signaling.[88] Finally, a mechanistic rationale for the potential importance of the NFAT pathway in the angiogenesis seen in diseases such as rheumatoid arthritis is supported by the observation that treatment of endothelial cells with the immunosuppressive drug cyclosporine prevents VEGF-mediated angiogenesis at least in part by inhibiting the NFAT-dependent upregulation of COX-2.[86] Taken together these studies implicate the NFAT family as critical regulators of embryonic and adult angiogenesis.

Runt Domain Factors

The RUNX transcription factors are members of the Ig-loop DNA-binding family of proteins that contain a conserved Runt-homology domain. The group consists of at least 3 phosphorylated α subunits which have several alternative names abbreviated from the core "runt-related gene/core binding factor/acute myeloid leukemia/polyoma enhancer-binding protein 2," ie, RUNX1/Cbfα2/AML1/PEBP2αB, RUNX2/Cbfα1/AML3/PEBP2αA, and RUNX3/Cbfα3/AML2/PEBP2αC. Each of these heterodimerizes with a single β subunit, Cbfβ. Mice deficient in either RUNX1 or RUNX2 die as embryos or soon after birth with a defect in definitive hematopoiesis (RUNX1)[89] or ossification (RUNX2).[90,91] RUNX1 is expressed in endothelial cell lines and at sites of angiogenesis in vivo and is induced by bFGF and VEGF. A dominant-negative construct impairs endothelial cell proliferation, migration, and tube formation,[92] whereas overexpression of RUNX1 in endothelial progenitors leads to enhanced expression of vascular endothelial cadherin (VE-cadherin) and the formation of vascular networks, demonstrating a role in endothelial cell differentiation and maturation.[93] Interestingly, in RUNX2-deficient mice, although there is normal vascularity of the perichondrium and surrounding tissue, there is a lack of blood vessel invasion into areas of hypertrophied chondrocytes, a process required for the transformation of cartilage to bone.[94] In human bone marrow endothelial cells, a dominant-negative RUNX2 causes decreased endothelial cell differentiation and impairs growth arrest.[95]

Forkhead Factors

The FOXO subclass of forkhead transcription factors (FKHR/FOXO1, FKHRL1/FOXO3a, AFX/FOXO4) has been shown to have roles in stress responses, control of cell cycle, and apoptosis. Recent studies also implicate this family of factors in angiogenesis. FOXO1-null mice die on embryonic day 10 to 11 with defects in vasculogenesis and angiogenesis.[96,97] Histological analyses revealed defects in the dorsal aorta, hypoplastic branchial arches, and absence of distinct yolk sac blood vessels. FOXO3a-null mice demonstrated only abnormalities of ovarian follicular development, and FOXO4-null mice had no obvious phenotype.[96] However, both FOXO1 and FOXO3a have been shown to regulate postnatal neovascularization.[98] Overexpression of either of these transcription factors inhibited endothelial cell migration and tube formation in vitro, and knockdown significantly increased migration and sprout formation. Both FOXO1 and FOXO3a bind to and transrepress the eNOS promoter. eNOS is essential for postnatal neovascularization, and FOXO3a-null mice reflect this by demonstrating increased eNOS expression and postnatal neovascularization.

Regulation of the cell cycle by FOXO1 and FOXO4 is mediated by FOXO-dependent induction of expression of $p27^{KIP1}$, which causes growth suppression and increased endothelial cell apoptosis.[99,100] Akt-dependent phosphorylation of FOXO proteins leads to sequestration in the cytoplasm and, thus, inactivation. This mechanism has been demonstrated after cytokine induction of the PI3K/Akt pathway,[99] with treatment of endothelial cells with epoxyeicosatrienoic acid[101] and after addition of ang-1.[102] By activating Akt, ang-1 blocks FOXO1-induced apoptosis and FOXO1-mediated gene expression. Furthermore, microarray analysis of endothelial cells over-expressing FOXO1 shows enhanced expression of genes associated with vascular destabilization and remodeling. Finally, in Tet-on HeLa cells, FOXO4 inhibits hypoxia-induced HIF-1α by mediating proteosomal degradation of HIF-1α by a mechanism different from the previously described von Hippel Lindau-dependent ubiquitin-mediated process.[103] This may represent a novel mechanism for hypoxic regulation. However, endogenous FOXO4 has not been demonstrated in endothelial cells,[101] nor does exogenous expression have an effect on neovascularization.[98]

Histone Deacetylases

Histone deacetylases (HDACs) modulate chromatin structure and associate with transcription factors involved in repressing gene expression. A function in angiogenesis is suggested by the fact that HDAC1 is induced by hypoxia in multiple cell types. Overexpression of HDAC1 downregulated p53 and von Hippel-Lindau tumor suppressor genes and stimulated

angiogenesis in human endothelial cells.[104] Inhibition of HDAC1 upregulated the aforementioned genes and also inhibited HIF-1α and VEGF expression. Thus HDAC1 may regulate hypoxia-mediated angiogenesis.

Notch

Notch family receptors are associated with the plasma membrane and are cleaved on activation by ligand binding. The intracellular domain of the receptor then translocates into the nucleus and acts as a transcriptional coactivator. Members of the Notch family are expressed in the endothelium and targeted mutations of many, including Notch1,[105] result in vascular defects. VEGF, but not bFGF, induces Notch1 and its ligand Dll4 in human arterial endothelial cells (but not in human umbilical vein endothelial cells) via activation of Akt.[106] Constitutive activation of Notch1 enhances formation of arterial endothelial cell tubule formation. Thus Notch1/Dll4 may have a particularly important role in arteriogenesis.

Summary

As reviewed, the past few years have witnessed the identification of many transcriptional regulators of angiogenesis. Although these studies have provided novel scientific information, much remains to be done. Several key questions are: (1) precisely which step(s) in the angiogenic process does each factor affect?; (2) can this effect be verified in more than one model of angiogenesis in vivo?; (3) are the observed effects of a factor critical during embryonic angiogenesis, adult angiogenesis, or both?; (4) does the specific transcription factor cooperate with others to exact its angiogenic effects?; and (5) can the expression/function of a specific transcription factor be altered in a therapeutically beneficial manner?

Angiogenesis is a complex multistep process that involves degradation of the extracellular matrix, endothelial cell migration and proliferation, tube formation, and vessel maturation with investment of pericytes and/or smooth muscle cells. Studies on a specific factor have, to date, often focused on a single or very restricted number of steps in this process. Furthermore, many studies have been limited to in vitro assays. Moving forward, it will be critical that more detailed studies for each individual factor be undertaken in order to gain a more comprehensive understanding of its role in the angiogenic process. Additionally, studies should be extended to at least one (and preferably more than one) in vivo model of angiogenesis. This is an important point as the milieu of tumor angiogenesis may be quite different than that seen, for example, during embryogenesis or in response to a specific stimulus (eg, specific growth factor or cytokine).

Transcription factors clearly do not function in a vacuum. Interaction and cooperation with other transcriptional regulators, components of the general transcriptional machinery, and chromatin-modifying factors can have profound cellular effects. An understanding of the transcriptional hierarchy and network is critical to gaining a more complete understanding of how specific factors regulate the complex angiogenic process.

Finally, identification of novel transcriptional pathways that regulate angiogenesis offers, in principal, the foundation for novel therapeutic strategies. Traditionally, transcription factors have not served as attractive targets. However, the nuclear receptor family (eg, PPARs) is a notable exception. These factors are activated in the cytoplasm by ligand binding, with subsequent translocation to the nucleus to affect gene expression. Of particular interest is that potential PPAR ligands include currently prescribed oral medications. In the same vein, recent studies demonstrate that KLF2 expression can be induced by statins. Given that both high doses of statins and KLF2 can inhibit angiogenesis, this raises the interesting possibility that some of the anti-angiogenic properties of statins may be, in part, KLF2 dependent. If so, a rationale would be provided to explore the Kruppel-like factors as targets for pharmaceutical agents aimed to inhibit angiogenesis.

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