

Transcriptional regulation of endothelial cell and vascular development

Changwon Park, Tae Min Kim and Asrar B. Malik

Department of Pharmacology and Center for Lung and Vascular Biology,
The University of Illinois College of Medicine, Chicago, IL USA

Correspondence: Asrar B. Malik (abmalik@uic.edu)

Key words: endothelial cells, vasculogenesis, angiogenesis, transcription factors,
reprogramming

Abstract

The establishment and maintenance of the vascular system is critical for embryonic development and postnatal life. Defects in endothelial cell development and vessel formation and function lead to embryonic lethality and are important in the etiology of vascular diseases. Here we review the underlying molecular mechanisms of endothelial cell differentiation, plasticity, and the development of the vasculature. This review focuses on the interplay among transcription factors and signaling molecules that specify the differentiation of vascular endothelial cells. We also discuss recent progress on reprogramming of somatic cells towards distinct endothelial cell lineages and its promise in regenerative vascular medicine.

A. Introduction

The vasculature consisting of arterial, venous and lymphatic vessels forms through two distinct processes during embryogenesis: *vasculogenesis*, defined as *de novo* vessel formation induced by differentiation of angioblasts and *angiogenesis* defined as new vessel formation secondary to proliferation of endothelial cells from pre-existing vessels.¹⁻³ While vasculogenesis is the major mechanism of formation of blood island vessels, dorsal aorta, endocardium and vitelline vessels in the embryo, angiogenesis is the predominant means of vascularization of all organs. Vasculogenesis was thought to occur only in developing embryos, recent studies show that vasculogenesis persists during vascular repair in the adult through differentiation of endothelial progenitor cells (EPCs).⁴

Although there is no established distinction between angioblasts and EPCs based on specific markers, we will use the term angioblast to represent the precursor endothelial cell responsible for vasculogenesis in the developing embryo, whereas EPC denotes the progenitor cell that differentiates to endothelial cells during vessel formation in adult. We will not deal with the debate and controversy about bone marrow derived cells that have been referred to as “EPCs”. For the definition of these controversial cells, their origins, and presumed functions, the reader is referred to the review.⁵

The first identifiable structures of developing mammalian embryos are blood vessels and the heart which provide perfusion and nutrient delivery necessary for organogenesis. Early embryonic lethality is invariably the consequence of impaired cardiovascular development. The first sign of blood vessel formation occurs at the gastrulation stage as early as mouse embryonic day (E) 7.5 in the extra-embryonic yolk sac blood island (Figure 1).⁶⁻⁸ Blood vessels in the blood island are lined by endothelial cells and are perfused by primitive erythrocytes. The blood island subsequently fuses to form the primary plexus, the immature vascular network,

which is followed by the phase of vascular remodeling in the yolk sac leading to formation of the complex yolk sac vasculature (Figure 1).

Vessel formation in the embryo proper is preceded by the appearance of angioblasts at E7.5,² crucial cells which establish the vasculature of intra-embryonic regions including the dorsal aorta and vitelline vessels, and primary plexuses of lungs, spleen, and heart.³ The more complex phase of formation of the embryonic vascular networks occurs by angiogenesis during which newly formed vessels are stabilized through interactions of endothelial cells with each other via endothelial junction proteins and with recruited mural cells, the pericytes, and an ordered extracellular matrix.^{2,3,9}

The newly formed vessels of the developing embryo thereafter further specialize into arteries, veins and capillaries, which have distinct functions based on the presence and amount of smooth muscle cells and specific extra-cellular matrix characteristics of the vessel wall.¹⁰ While capillaries are not invested with smooth muscle cells, arteries develop a thick tunica medium consisting of elastic fibers and smooth muscle cells required for their vasomotor tone and conduit function. Veins by contrast contain fewer elastic fibers and smooth muscle cells (and hence are compliant) and have valves to prevent blood back-flow.¹⁰ At E10.5-11.5, lymphatic endothelial cells are generated from a sub-population of cardinal vein endothelial cells as well as the intersomitic vessels, and they migrate dorso-laterally to form lymphatic sacs and the lymphatic vasculature (the so called “third circulation”), which functions to regulate tissue fluid balance and provide immune surveillance through lymphocyte trafficking (Figure 1).^{11,12} In this review, we focus on transcriptional regulation and essential signaling components of vascular development and cell reprogramming by transcription factors required for differentiation of endothelial cells and for vascular development. Abbreviations are listed in Table 1.

B. Development of vascular structures

Hemangioblasts and the establishment of distinct vascular structures

The close relationship between hematopoiesis and vessel formation has led to the canonical view that both hematopoietic cells and endothelial cells develop from a common progenitor cell, termed the hemangioblast.⁶⁻⁸ However, the hemangioblast as a cell remains poorly defined and elusive. During *Drosophila* embryogenesis, the lymph gland, the major site for hematopoiesis, develops in close proximity of the aorta.¹³ Analysis of expression markers and lineage tracing studies using Flp - FRT (flippase - flippase recognition target) recombination indicated that the cardioblast, a type of vascular progenitor cell, and lymph gland (comprising hematopoietic cells) developed from a common progenitor population in the cardiogenic mesoderm.¹⁴ Studies in zebrafish described a *cloche* (*clo*) mutant displaying defects in both hematopoietic and endothelial differentiation.¹⁵⁻¹⁷ *clo* was shown to act upstream of the hematopoietic and endothelial genes *flk1* (*vascular endothelial growth factor receptor 2*), *scl* (*stem cell leukemia*), and *etsrp* (*ets related protein*). Another report using zebrafish showed that *lysocardiolipin acyltransferase* (*lycat*) was critical for both hematopoietic and endothelial cell development.¹⁸ Fate mapping in zebrafish showed the existence of bipotential cells (hemangioblast-like cells) in embryos capable of generating both hematopoietic and endothelial cells, but not other cell types.¹⁹

Initial evidence of the existence of hemangioblasts in mammalian cells comes from studies using the *in vitro* embryonic stem (ES) cell differentiation system.²⁰⁻²² Vascular endothelial growth factor (VEGF) stimulation of ES cells induced the formation of a transient cell population (termed BL-CFCs, blast colony-forming cells) expressing hematopoietic and endothelial lineage markers. These cells upon re-plating differentiated clonally into distinct hematopoietic and endothelial cell populations. Studies in mice showed that Brachyury/GFP⁺FLK1⁺ cells appearing at E7.5 embryos formed blast colonies similar to the ES cell-derived blast colonies and gave rise to both hematopoietic and endothelial cells.²³

While the above studies suggested that endothelial cells share their early developmental program with hematopoietic cells, others have challenged this fundamental concept. In

zebrafish, lineage tracing experiments showed that only a certain fraction of endothelial cells originated from hemangioblasts¹⁹ raising the possibility of another population giving rise to endothelial cells. Studies have also reported a differential time course of emergence of endothelial and hematopoietic cells in the developing mouse embryo.²⁴ Angioblasts were found as early as E5.5 whereas hematopoietic progenitors were observed at E6.5,²⁴ suggesting non-parallel events. While these studies showed that generation of hematopoietic and endothelial cells can follow a different time course, they do not rule out the presence of a common precursor cell. A plausible explanation for these findings is that generation of hematopoietic and endothelial cells is a stochastic process, thus, while the cells may have a same precursor, their generation follows different routes.

The hemangioblast concept has been further challenged by the recognition of a specialized endothelial cell population capable of generating hematopoietic cells, known as the hemogenic endothelium.^{25,26} Zovein et al²⁵ showed by lineage tracing endothelial cells with VE-cadherin CreERT (Cre recombinase - estrogen receptor T2; tamoxifen inducible Cre) and RosaR26LacZ reporter that VE-cadherin lineage cells located within the aorta-gonad-mesonephros (AGM) generated hematopoietic stem cells. In subsequent studies, the genesis of hematopoietic stem cells from endothelial cells within the AGM was seen in dramatic images of these cells being “shed” from the endothelium of mice and zebrafish.²⁷⁻²⁹ Additionally, it was shown that ES cell-derived BL-CFCs generated hematopoietic cells through a presumptive hemogenic endothelium defined by TIE2^{hi}c-KIT⁺CD41⁻ cells.³⁰

First emerging FLK1/VEGFR2⁺ cells are multipotent progenitors of the cardiovascular system

Despite the incompletely controversy surrounding the hemangioblast origin of endothelial cells and blood cells, it is clear that VEGF-FLK1 signaling is essential for endothelial cell generation and vessel development.^{31,32} We will discuss below the developmental kinetics

of endothelial cell lineage with a focus on the key role of FLK1⁺ cells in giving rise to endothelial cells.

Endothelial cells are derived developmentally from the mesoderm, which can be marked definitively by the expression of *Brachyury/T box* gene (Figure 2).³³ *Brachyury* is expressed throughout the primitive streak during gastrulation. Mice deficient in *Brachyury* were embryonically lethal with defects in primitive streak, notochord, and allantois.^{34,35} A chimeric aggregation study between *Brachyury*-null ES cells and wild type embryos demonstrated that *Brachyury* was essential for the proper movement of mesoderm from the primitive streak.³⁶ Using ES cells in which the expression of GFP is controlled by the *Brachyury* promoter,³⁷ it was shown that *Brachyury*⁺ mesodermal cells were the first to appear and subsequently expressed FLK1, thus becoming *Brachyury*⁺FLK1⁺ cells during differentiation *in vitro*. In developing mouse embryos, the expression of *Flk1* was first detected in the posterior portion of the primitive streak, followed by preferential expression in vascular endothelial cells of the yolk sac and embryonic vasculature including the endocardial tube.³⁸ Deficiency of *Flk1* induced embryonic lethality due to lack of yolk sac blood island and blood vessel and endocardium formation.³¹ A Chimeric aggregation study using wild type embryos showed that *Flk1*-null ES cells failed to induce vessel development and hematopoiesis.³⁹ Therefore, the first emerging FLK1⁺ cells represent hemangioblasts, and FLK1 is indispensable for the development of both blood and endothelial cell lineages.

Cell lineage tracing experiments have provided further insights into blood and endothelial cell potential of FLK1⁺ cells. FLK1⁺ cells present in differentiating ES cells were fractionated by the expression of SCL (stem cell leukemia), a basic helix-loop-helix transcription factor, which plays an important role of hematopoiesis and vessel remodeling (see below),⁴⁰ and thereby tracked for their developmental potential. To track and isolate the cells expressing SCL, a truncated human *CD4* gene, a co-receptor for T cell receptor signaling, consisting of only extracellular and transmembrane domain⁴¹ was knocked into the endogenous *Scf* locus

and thus SCL⁺ cells could be identified with hCD4 expression without affecting cell viability.²² A subset of FLK1⁺ cells was shown to progress to either FLK1⁺SCL⁺/hCD4⁺ cells or FLK1⁺SCL⁻/hCD4⁻ cells. However, the hemangioblast activity was enriched only in the FLK1⁺SCL⁺/hCD4⁺ cells.²² Interestingly, endothelial cells could also develop independently of SCL expression in that both FLK1⁺hCD4⁺ and FLK1⁺hCD4⁻ cells generated endothelial cells on further culture.²² These findings suggest that angioblasts and hemangioblasts represent two distinct origins of endothelial cells. The first arising FLK1⁺SCL⁺ cells represented the *in vitro* equivalent of hemangioblasts, and that later endothelial cells can also develop independently of hematopoiesis directly through angioblasts.

Other studies utilizing the genetic cell tracing method have pointed to a broader potential of FLK1⁺ cells as the FLK1⁺ cells obtained from differentiating ES cells were also shown to generate smooth muscle actin (SMA) cells.^{42,43} In addition, FLK1⁺ cells marked by *Flk1Cre;Rosa26* reporter (*R26R*) were detected in skeletal muscles and cardiomyocyte of E10.5 embryos,⁴⁴ besides the expected endothelial and blood cells.⁴⁴ Another study⁴⁵ showed the presence of FLK1⁺/LacZ⁺ cells in cardiac and skeletal muscles; however, upon re-analysis of FLK1⁺/LacZ mice after deleting the Neo cassette (*Flk1/LacZ-neo-out*), a much stronger and broader LacZ stained cell population was found compared to a previous study with FLK1⁺/LacZ-neo-in mice.³¹ Thus, the FLK1⁺ mesoderm likely represents a multipotent progenitor cell population in addition to blood and endothelial cell progenitors of the cardiovascular system.

C. Transcriptional regulation of vessel development

The details of transcriptional control of vessel development are summarized in Figure 3 as well as Table 2 and discussed under the headings below that describe the function of individual transcription factors.

ER71 (ETS-related 71)/ETV2

ETS (E-twenty-six specific) transcription factors are highly homologous in a short stretch of 85 amino acids (ETS DNA binding domain) located at the carboxyl terminus.⁴⁶⁻⁴⁸ A winged

helix-turn-helix motif formed by the ETS domain binds the consensus sequence (5'-GGAA/T-3') to regulate expression of target genes.⁴⁷ In addition to the ETS domain, all ETS factors contain a transactivation domain, and some members of the family, ETS1, FLI1 (Friend leukemia integration 1), and ERG (Ets related gene), contain a Pointed (PNT) domain that mediates protein-protein interactions and oligomerization.^{46,49} Up to now 27 ETS factors have been reported in humans and mice.⁴⁸ The presence of ETS binding consensus sequence is seen in more than 200 genes involved in tumorigenesis, apoptosis, angiogenesis, and hematopoiesis,⁵⁰ suggesting that ETS factors are critical in many biological and pathological processes.

Among the ETS factors regulating vascular development, recent attention has focused on ER71 (also known as ETV2).⁵¹ ER71 is a 38kDa protein comprising a N-terminus transactivation domain and C-terminus ETS DNA binding domain (Figure 4A).^{52,53} ER71 was initially thought to be testis-specific;^{53,54} however, more thorough analysis showed it to be present in the posterior mesoderm, extra-embryonic mesoderm, lateral plate mesoderm, and cardiac crescent in embryos between E7.0-E7.75.⁵⁵⁻⁵⁸ Most ER71⁺ cells were also positive for FLK1⁵⁷ consistent with its importance in vascular development. At later embryonic stages (E8.25–E9.5), *Er71* expression was mainly seen in major vessels, specifically in the dorsal aorta, cardinal vein, branchial arch, intersomitic vessels as well as endocardium, followed by a sharp decrease beyond E11.5.⁵⁵⁻⁵⁸ This “on-off” expression pattern was also conserved in differentiating ES cells *in vitro*.^{55,57} *Er71* expression preceded the *Flk1* message and showed a transient pattern with a peak occurring at D3 of ES cell differentiation and was undetectable at D4 and thereafter. When sorted at D2.75, the *Er71* message was highly enriched only in FLK1⁺ cells.^{55,57}

With regard to *Er71* expression relative to *Flk1*, a study showed an unusual result that FLK1⁺ cells appeared prior to ER71⁺ cells.⁵⁷ This observation cannot be reconciled with what appears to be an important function in ER71 in giving rise FLK1⁺ cells. The discrepancy might be explained by different culture conditions [i.e., embryoid body (EB) formation under serum vs.

ES differentiation on OP9 under cytokines] and the genetic manipulations carried out (wild type vs. *Er71-VENUS*). In addition, another general concern is that both EB formation and OP9 co-culture methods have been used in different studies for differentiating mouse ES cells *in vitro*, which could account for discrepancies in this and other findings. In the EB formation method, ES cells differentiate in suspension and form cell aggregates, the EBs which contain three embryonic germ layer lineage cells. In this protocol, EB thus mimics a three-dimensional structure of an embryo that could be critical for cell-cell interactions needed for embryogenesis.^{59,60} In contrast, the ES cells differentiating in a feeder cell line OP9 do not form EBs.^{61,62} In this co-culture system, one can efficiently evaluate the effects of added cytokines or growth factors, compared to the EB protocol. ES cells in the latter system may be directed to differentiate into certain cell lineages by factors including soluble cytokines and growth factors as well as signals from cell surface molecules on OP9 cells. Thus, in analyzing the results of differentiation, it pays heed to note and be cognizant of the differences such as these in differentiation protocols.

Gene knockout studies showed an indispensable function of ER71 in both vessel and blood development.^{55,56} Mice deficient in *Er71* died at E10.5 and displayed complete lack of vessel structures and hematopoietic cells.^{55,56} Overexpression of *Er71* in ES cells induced the generation of FLK1⁺ cells as well as endothelial and hematopoietic cells.⁵⁵ ER71 was shown to function by interacting with FoxC2, which induced the expression of key endothelial genes that are critical for endothelial and hematopoietic lineages, *Flk1*, *VEcadherin*, *Tie2*, *Scl*, *Notch4*, and *Nfatc1*.^{55,63,64} These findings have implicated a fundamental role of ER71 in vasculogenesis and hematopoiesis (Figure 4B).

A vasculogenic role of ER71 has also been reported in the zebrafish and *Xenopus*, indicating that the gene is evolutionarily well conserved. *etsrp*, an *Ets* transcription factor in zebrafish, was expressed preferentially in the vasculature, similar to mouse ER71, and identified to be downregulated in the *clo* mutant.^{17,65} Knockdown of *etsrp* in zebrafish embryos led to

complete absence of vascular structures, whereas overexpression of *etsrp* induced genes required for differentiation of endothelial and hematopoietic lineages expressing *flk1* and *scl*.^{17,65,66} *etsrp* also rescued vascular defects seen in the *c/o* mutant described above.¹⁷ The *Xenopus* ortholog *er71* similarly exhibited a conserved function to the mouse and zebrafish homologs.⁶⁷

While the above studies describe the important role of ER71 in differentiation of endothelial cells and vessel development, the upstream mechanisms regulating ER71 expression itself are less well understood. Using mouse ES cell differentiation system, Yamamizu et al⁶⁸ reported that protein kinase A (PKA) signaling activated the expression of *Er71* through the cAMP response element binding protein (CREB) (Figure 4B). In another study,⁵⁶ *Er71* was found as one of the significantly downregulated transcripts in the cardiac progenitors deficient in *Nkx2-5*, a homeobox transcription factor required for heart morphogenesis including endocardial cushion formation.⁶⁹⁻⁷¹ NKX2-5 was expressed in both myocardium and endocardium,⁶⁹⁻⁷¹ but *Er71* message was only detected in the endothelium/endocardium in E8.5 hearts,⁵⁶ suggesting a genetic interaction of NKX2-5 and ER71. Indeed, NKX2-5 directly bound to the *Er71* promoter through NKX2-5 responsive element, and activated *Er71* expression (Figure 4B).⁵⁶ In zebrafish, *foxc1a/b* also bound the promoter to induce expression of *etsrp* (Figure 4B),⁷² indicating that ER71 functions requires entrainment of multiple transcription factors for its full effect.

Early activation of ER71/ETV2 is critical for FLK1⁺/VEGFR2⁺ mesoderm specification and vessel development

Attempts have been made to identify the transcription factors responsible for differentiation of FLK1⁺ mesoderm into endothelial and hematopoietic cell vs. those required for cardiac lineage specification. Studies showed that FLK1⁺PDGFR α ⁺ (platelet-derived growth factor receptor α) cells had a distinct cardiogenic potential whereas FLK1⁺PDGFR α ⁻ cells were

enriched in endothelial and hematopoietic progenitors.^{73,74} Since ER71 is an important cell fate transcriptional determinant of the FLK1⁺ mesoderm,⁵⁵ studies were made in which ER71 was deleted in ES cells or mouse embryos.^{57,75} Deletion of ER71 markedly reduced the generation of FLK1⁺ cells.⁷⁵ Interestingly, most of the FLK1⁺ cells derived from *Er71*^{-/-} ES cells also expressed PDGFR α and differentiated into cardiac lineages.^{57,75} Also overexpression of ER71 in wild type ES cells significantly enhanced the generation of FLK1⁺PDGFR α ⁻ cells over that seen in FLK1⁺PDGFR α ⁺ cells,⁷⁵ suggesting a determinant role of ER71 for FLK1⁺ mesoderm specification towards endothelial and blood lineages. In addition, morpholino knockdown of *etsrp* in zebrafish expanded the myocardium with reduced generation of vascular endothelial cells.⁶⁴ *etsrp*-GFP⁺ cells in the absence of *etsrp* also expressed *hand2*,⁶⁴ a cardiomyocyte marker.^{76,77} Ectopic expression of *etsrp* inhibited expression of cardiac markers but augmented endothelial markers⁶⁴ further supporting the role of ER71 in mediating the differentiation of FLK1⁺ cells specifically towards the endothelial lineage.

The WNT signaling pathway might play an important role in inducing the ability of ER71 to differentiate FLK1⁺ cells towards endothelial cells.⁷⁵ Overexpression of *Er71* impaired cardiac mesoderm formation (as evident by decreased generation of FLK1⁺PDGFR α ⁺ cells) and repressed expression of the WNT- β -catenin downstream genes, but this was rescued by WNT agonists. Inversely, deficiency in *Er71* exhibited enhanced WNT- β -catenin activity.⁷⁵ Elevated level of hemangiogenic mesoderm formation upon *Er71* overexpression was rescued by overexpressing β -catenin. Although these findings suggest a critical role of β -catenin in ER71-mediated FLK1⁺ mesoderm specification towards endothelial and hematopoietic cells, gene knockout studies in mice have not been consistent.⁷⁸ Inactivation of β -catenin in FLK1⁺ cells (i.e., *Flk1Cre*;floxed β -catenin mice) induced defective angiogenesis in the central nervous system with phenotypically normal development of the cardiac and hemo-vascular systems.⁷⁸

The findings that NKX2-5 is expressed in both myocardium and endocardium and regulates ER71 expression⁵⁶ and that ER71 specifies the endothelial and hematopoietic lineages by suppressing the cardiac lineage are seemingly contradictory.^{57,64,75} This apparent contradiction might be due to the definition of “cardiac lineage”. Endothelial lineages including the endocardium, cardiac lineages (i.e. myocardium/cardiomyocyte) and hematopoietic cells originate from FLK1⁺ cells.^{44,45,79,80} Thus, it is plausible that ER71 can specify FLK1⁺ cells capable of generating hematopoietic cells and endothelium/endocardium at the expense of myocardium generation. Endocardium-specific activation of ER71 by NKX2-5 remains unknown.

A time course study of ER71 illustrates the importance of the transient nature of its expression in the developing embryos in mediating the formation of blood vessels. The expression of *Er71* became undetectable beyond E11.5 of mouse gestation and was very low in mature endothelial cells,^{55-58,81} suggesting that ER71 function is tightly regulated to ensure proper development of the vascular system. In accord with this observation, a study⁸² has reported that persistent expression of *Er71* in developing embryos induced developmental abnormalities in vessels and hematopoietic cells. The sustained expression in TIE2⁺ cells (*Tie2Cre;RosaR26R-Er71*) caused abnormal vascular development evidenced by dilated yolk sac vessels accompanied with hemorrhaging.⁸² This might be a function of continuous expression of pro-angiogenic genes including *Flk1* with reduced expression of endothelial cell maturation or stability genes *Klf2* (*Kruppel-like factor 2*), *Klf4* (*Kruppel-like factor 4*), *Timp1* (*tissue inhibitor of metalloproteinase 1*), *Timp2* (*tissue inhibitor of metalloproteinase 2*) and *Cyr61* (*cysteine rich protein 61*). Transient expression of *ER71* with continuous expression of *ERG1* and *FLI1* can directly convert amniotic cells to mature endothelial cells,⁸³ consistent with the fundamental role of the transient and early-onset of expression of ER71 in mediating endothelial cell specification in the developing embryo. In the same context, the time-dependent downregulation of *ER71* expression may be needed to ensure endothelial cell maturation and formation of inter-endothelial junction integrity required for functionally normal vessels.

SCL/TAL 1 (stem cell leukemia/T-cell acute lymphoblastic leukemia)

SCL/TAL transcription factor belongs to the basic helix loop helix (bHLH) transcription factor family.^{40,84,85} SCL expression is seen in yolk sac blood progenitors and endothelial cells.⁸⁶⁻
⁸⁸ Ectopic expression of *scf* in zebrafish induced both hematopoietic and endothelial genes.^{16,89} Defects in blood and vessel formation in both *clo* and *etsrp* knock-down zebrafish were partially rescued by overexpressing *scf*.^{16,89,90} Although *Scf*^{-/-} mice embryos died due to complete lack of hematopoiesis,⁸⁶⁻⁸⁸ chimeric analysis of wild type and *Scf*^{-/-} ES cells showed that SCL was also required for vascular remodeling but apparently not for vasculogenesis.⁹¹ This was evident by the finding that the mutants with high degree of chimerism exhibited disorganized yolk sac vessels lacking vitelline vessels.⁹¹ Further, *Scf*^{-/-} ES cells failed to contribute to the formation of large vessels.⁹¹

SCL has been postulated to be critical for the specification of the hemangioblast rather than angioblasts.^{21,22,89,92} Hemangioblast activity was enriched in FLK1⁺SCL⁺ cells, whereas ES cells deficient in *Scf* failed to generate blast colonies.^{21,22,92} Ectopic expression of *Scf* in the locus of *Flk1* increased blast colony formation compared to *Flk1*^{+/+} cells.⁴³ Thus, SCL appears to be dispensable for vasculogenesis but it is required for hemangioblast/vascular remodeling in the developing embryo.

Although SCL acted downstream of FLK1,^{22,43} the presence of a putative SCL binding site in the *Flk1* promoter⁹³ suggests additional regulatory roles of SCL in *Flk1* expression. Studies showed that SCL working with ID1 (Inhibitor of Differentiation and DNA binding 1) can modulate the promoter activity of *Flk1* by binding E2-2, a suppressor of *Flk1* promoter activation and angiogenesis.⁹⁴ The functional significance of SCL interaction with ID1 in regulating angiogenesis *in vivo* needs further investigation.

Overexpression of *Er71* in ES cells and zebrafish enhanced the expression of *Scf*, but knockdown of *Er71* induced a reduction of *Scf*.^{17,55} Genome wide sequence analysis showed that *Scf* contains the binding site for ETS.⁶³ Luciferase and ChIP experiments positioned *Scf*

downstream of ER71.⁶³ The mechanism of ER71 regulation of SCL and how it regulates endothelial cell and vessel development remains unexplored, and it is potentially a fruitful area since it will help to connect the function of these two key transcription factors.

GATA2 (GATA binding protein 2)

The GATA transcription factors (GATA1 through GATA6) belong to C2H2 zinc-finger transcription factors and bind (T/A)GATA(G/A) in the genomic DNA.^{95,96} Among the known six GATA members, initial analysis has described GATA1, GATA2 and GATA3 as the “hematopoietic GATAs” due to their preferential expression in hematopoietic cells.⁹⁷ *Gata1*^{-/-} mouse embryos displayed defects in erythropoiesis.^{98,99} Mouse embryos deficient in *Gata2* died by E11.5 and exhibited anemia.¹⁰⁰ *Gata2*^{-/-} ES cells and yolk sac cells generated significantly reduced number of multipotent progenitors.^{100,101} However, evidence of endothelial expression of GATA2 in developing embryos¹⁰²⁻¹⁰⁴ suggests that GATA2 might also play an important role in vessel development. Indeed, Lugus et al¹⁰⁵ reported that expression of *Gata2* was enriched in BL-CFCs and that overexpression of *Gata2* in ES cells enhanced the generation of FLK1⁺ cells as well as endothelial cells *in vitro*. The findings that several key endothelial genes contain GATA binding sites¹⁰⁶⁻¹⁰⁸ further suggest that GATA2 regulates vessel development through transcriptional activation of endothelial genes including *Flk1* and *VEcadherin*. In this regard, a recent report showed that GATA2 regulates endothelial specific gene expression and thus endothelial specificity through epigenetic modification.¹⁰⁹ In this study, the authors found that *ENDOMUCIN*, an endothelial specific gene, contained preferential GATA2 binding sites in transcription start site (TSS) as well as -139 kb region of human dermal microvascular endothelial cells (HMVECs). An epigenetic experimental approach also demonstrated that both regions are in active chromatin state in HMVECs. In contrast, the erythroid cells, K562, which also highly express GATA2, contained preferential GATA2 binding sites in SCL/TAL1.¹⁰⁹ *ENDOMUCIN* TSS and -139 kb region in K562 showed enrichment of H3K9me3.¹⁰⁹ Interestingly, knockdown of GATA2 in HMVECs significantly reduced the expression of endothelial genes

including *ENDOMUCIN* and *KDR/VEGFR2*, but also increased expression of non-endothelial cell genes such as *SM-ACTIN* and *SNAIL*.¹⁰⁹ Together, these results suggest that GATA2 plays an important role in mediating endothelial gene expression and the maintenance of endothelial cell fate. The mechanisms of GATA2-mediated endothelial cell specification in developing embryos remain to be elucidated. A fundamental question is whether ER71 can form a transcriptional complex containing GATA2 to regulate endothelial gene expression.

A recent study has also described the previously unknown function of GATA2 in lymphatic vessel development.¹⁰⁴ The authors generated mice in which *Gata2* is conditionally inactivated by Cre-ERT under *Gata2* vascular endothelial (VE) enhancer, and found that the CKO embryos died at ~E16.5, and importantly showed abnormal lymphatic vessel formation manifested by subcutaneous edema and a presence of mixed lymphatic-blood vessel structures. Since mice with germ line knockout¹⁰⁰ and conditional deletion of *Gata2*¹⁰⁴ develop normal vascular structure during the early embryo stage (E10-E11), it appears that GATA2 plays an important role in lymphangiogenesis, but it is not required for endothelial cell specification and the initial establishment of primary vascular structure. But it is still not possible to exclude functional redundancy in studies in which *Gata2* is deleted; that is, loss of GATA2 in developing mouse embryos (prior to E16.5) could be compensated by other GATA members, hence resulting in normal embryonic vasculature development.^{100,104} The finding that GATA3 directly binds the TIE2 promoter in adult endothelial cells¹¹⁰ suggests such a redundancy concept if GATA3 is upregulated following deletion of GATA2.

ID (Inhibitor of Differentiation and DNA binding)

ID proteins have been implicated in embryonic and postnatal angiogenesis.¹¹¹ IDs, consisting of 4 members (ID1, ID2, ID3, ID4), belong to the bHLH transcription factors, but lack DNA binding domains;¹¹¹ thus, they function by “sequestering” transcription factors that target genes.¹¹¹ Expression of *Id1* and *Id3* was detected in embryonic vessels and also mice deficient in *Id1* and *Id3* showed abnormal vascular development.^{112,113} In cultured endothelial cells,

overexpression of *Id1* enhanced endothelial cell proliferation and migration, whereas *Id1* knockdown inhibited these responses,^{114,115} suggesting a pro-angiogenic role of ID1. IDs together with SCL de-repressed *Flk1* promoter activity by interacting with E2-2, a basic helix-loop-helix transcription factor, functioning as a negative regulator of *Flk1* expression.^{94,116} In addition, studies showed that ID1 and ID3 are direct downstream targets of the BMP-SMAD pathway^{114,117} suggesting that BMP signaling may be important in activating IDs and hence in generating FLK1⁺ endothelial cells.

Role of Other ETS Transcription Factors

The ETS transcription factors display a broad range of expression patterns and activities in developing mouse embryos as well as in adults. However, some of these factors show a preferential expression in endothelial cells, and it is important to consider their role in generation of endothelial cells and vessels during development. Expression of the ETS transcription factors, *Ets1* and *Fli1*, can be detected in the yolk sac blood islands in the early stage embryos and their expression is maintained in developing vessels.¹¹⁸⁻¹²⁰ Whereas the ETS transcription factor, *Erg1*, is highly expressed in mesodermal lineages including endothelial cells,¹²¹ another ETS transcription factor *Etv6* shows ubiquitous expression pattern throughout embryogenesis and in adults.^{122,123} The high degree of variability in the expression patterns of the ETS transcription factors makes it difficult to ascribe specific functions in mediating differentiation of endothelial cells and vascular development.

Gene knockout mouse studies however have demonstrated in a general sense the importance of the ETS transcription factors in mediating vessel remodeling and structural integrity.¹²⁴ Germ line deletion of *Ets1* did not cause vascular defects.¹²⁵ However, *Ets1*^{-/-};*Ets2*^{-/-} mouse embryos died *in utero* and showed vessel branching defects,¹²⁶ indicating the redundant role of ETS1/2 in embryonic vessel formation. In addition, a recent study suggests a novel function of ETS1/2 in lymphatic vessel formation (see PROX1 below).¹²⁷

The role of FLI1 in embryonic vessel development is intriguing. Studies from *Xenopus* showed that morpholino knockdown of *fli1* led to a significant reduction of expression of genes critical for early hematopoietic and endothelial cell development *scl*, *lmo2* and *flk1*.¹²⁸ The expression of *fli1* was not affected in *c/o* mutant or in zebrafish treated with *scl* or *lmo2* morphants. These results suggest that *fli1* is one of the earliest transcription factors involved in endothelial and hematopoietic cell specification. However, knockdown of zebrafish *fli1* did not recapitulate the defects seen in *Xenopus*.¹²⁸ Similarly, *Fli1*^{-/-} mouse embryos did not display severe vascular defects; *Fli1*^{-/-} embryos died around E11.5 with extensive hemorrhaging,¹²⁹ indicating its dispensable role in endothelial cell specification unlike the findings in *Xenopus* and *c/o* mutant.¹²⁸ This discrepancy might be explained by redundant role of other ETS transcription factors such as ERG1 in zebrafish and mice knockout studies.

Homozygous mouse embryos for ERG^{Mld2/Mld2} where serine at residue 329 on ERG1 is substituted with proline, resulting in non-functional ERG due to missense mutation, died due to hematopoietic defects and exhibited dilated vessels in the brain.¹³⁰ In a subsequent study, it was demonstrated that ERG1 directly induced the expression of *CDH5* (*vascular endothelial cadherin*; *cadherin 5, type 2*) in HUVECs,¹³¹ suggesting that ERG1 regulates endothelial cell-cell interaction possibly through formation of adherens junctions.

Forkhead Transcription Factors

The forkhead transcription factor family (also known as forkhead/winged helix domain) is characterized by the conserved the DNA-binding domain, which recognizes the consensus sequence 5'-TTGTTTAC-3'.^{132,133} Among the forkhead transcription factors, FOXO and FOXC are implicated in vascular development and endothelial cell generation.¹³³ There are four members of FOXO family, FOXO;FOXO1/FKHR, FOXO3/FKHRL1, AFX/FOXO4 and FOXO6. FOXOs functioning mainly through PTEN (phosphatase and tensin homologue deleted on chromosome 10) are involved in cell survival/apoptosis, cell cycle, DNA repair and reactive oxygen species regulation.^{133,134} In mouse embryos, *FoxO1* is highly expressed in the

developing vessels and *FoxO1*^{-/-} mice embryos failed to survive beyond E10.5 because of defective vascular development in the yolk sac and embryo.^{135,136} A detailed gene expression analysis uncovered decreased expression of *Gja4* and *Gja5*, both of which are components of gap junction.¹³⁵ As gap junction genes are preferentially expressed in developing embryos,^{137,138} the functional interaction between FOXO1 and GJA4/GJA5 may be important in the development of the embryonic vasculature. Mice deficient in *FoxO3* or *FoxO4* in contrast to *FoxO1* did not show vascular defects.¹³⁶ However, overexpression of *FoxO1* and *FoxO3* inhibited endothelial tube formation and migration partly through direct inhibition of eNOS,¹³⁹ believed to be a proangiogenic factor.¹⁴⁰ Overexpression of FOXO3 induced angiogenesis as evidenced by enhanced recovery of blood flow from ischemic injury with a concomitant increase in capillarity.¹³⁹ Conditional deletion of *FoxO1*, *FoxO3* and *FoxO4* using *Mx1Cre* mice led to the development of hemangiomas,¹⁴¹ indicating suppressive function of FOXOs in endothelial cell proliferation.

FOXC1 and FOXC2, members of the C subgroup of forkhead transcription factor family, have also been found to regulate arterio-venous specification and lymphatic vessel differentiation.¹³³ Promoters of multiple endothelial genes *Flk1*, *VEcadherin*, *Pecam1*, *Tie2* and *Scl*, contain evolutionarily conserved FOX:ETS binding motifs.⁶³ As discussed above, ER71 and FOXC2 were shown to bind the FOX:ETS motifs present in promoters and enhancer elements of these genes and to function cooperatively to activate transcription (Figure 4B). *foxc1a/foxc1b* in zebrafish can also function as an upstream regulator of *etsrp*,⁷² indicating a critical role of cooperation of FOXC members with ER71 in mediating endothelial cell development. *FoxC1* deficient mice died pre- and post-natally with multiple vascular defects including coarctation of the aortic arch.¹⁴² *FoxC2* deficient mice exhibited similar defects but with abnormal development of lymphatic vessels manifested by lack of valves and increased vascular smooth muscle cells.¹⁴³ Studies on double *FoxC1/FoxC2* deleted mice demonstrated a role of FOXC in determining artery and venous endothelial cell fate as demonstrated by the presence of arterio-

venous malformations in the mutant embryos and failure to express the arterial markers *Dll4* and *Notch* with unaltered expression of the venous markers, *Coup-TFII* and *EphB4*.^{144,145} FOXC1 and FOXC2 may function by activating the *Dll4* promoter through a direct binding on the FOX binding sites of this promoter.¹⁴⁵ FOXC2 together with [Su(H)](Suppressor of Hairless)-NICD(Notch intracellular domain) may also function by directly activating the *Hey2* (Hairy/enhancer-of-split related with YRPW motif protein 2) promoter to mediate arterial endothelial cell specification.¹⁴⁶

In addition, FOXC2 functions in proper lymphatic vessel development. In the absence of *FoxC2*, the mutant embryos developed lymphatic vessels with defective lymphatic valves and abnormally enhanced pericytes recruitment.¹⁴³ FOXC2 was shown to bind and activate the *Nfatc1* (nuclear factor of activated T cells, cytoplasmic 1) promoter.¹⁴⁷ Cyclosporine A-mediated inactivation of NFAT signaling mimicked the lymphatic phenotypes seen in *Fox2^{-/-}* mouse embryos.¹⁴⁷ Taken together with the finding that inactivating mutations in *FOXC2* are responsible for lymphedema-distichiasis in human,¹⁴⁸ it appears that FOXC2 is a versatile transcription factor that has a role in FLK1⁺ cell generation, arterial specification, and lymphatic vessel development (Figure 4B).

Other subclasses of forkhead transcription factors FOXF1, FOXH1 and FOXM1 have also been implicated in vessel and endothelial cell development. *FoxF1* is expressed in the posterior primitive streak, allantois, amnion and yolk sac vasculature.^{149,150} Germ line deletion of *FoxF1* led to avascular yolk sacs and allantois.¹⁵⁰ FOXF1 may act downstream of hedgehog signaling to induce BMP expression which is an important inducer for vasculogenesis.¹⁵¹ FOXF1 was also shown to function as an upstream activator of *Flk1* gene expression.¹⁵² A potential binding site for FOXF1 was identified in the specific locus (Distal-Multipotent-Mesodermal-Enhancer, DMME) of the *Flk1* gene.¹⁵² In the zebrafish, *foxh1* bound to the *flk1* promoter and repressed gene expression and induced defective vessel formation.¹⁵³ In contrast, FOXM1 is dispensable for vessel development in developing embryos since mice lacking *FoxM1* in endothelial cells

(*Tie2Cre;floxed FoxM1*) were born alive.^{154,155} However FOXM1 was demonstrated to have an entirely novel function in mediating EC proliferation and vascular repair.^{154,155} In response to vascular injury elicited by lipopolysaccharide (LPS), vessels of *Tie2Cre;floxed FoxM1* mice exhibited severely impaired ability to repair compared to controls. This defective vessel repair program was ascribed to reduced expression of β -catenin in adherens junctions of ECs deficient in *FoxM1* and reduced EC proliferation.^{154,155} Thus, FOXM1 functioned by transcriptionally activating the expression of β -catenin through FOXM1 binding sites on the promoter.¹⁵⁵

HEY1/HEY2 (Hairy/enhancer-of-split related with YRPW motif protein1/2)

The NOTCH pathway controls endothelial cell specification (arterial vs. venous endothelial cells) and plays an important role in endothelial cell sprouting.^{156,157} HEY1 and HEY2, members of hairy and enhancer of split-related family of bHLH transcription factors, are direct transcriptional targets of the NOTCH pathway.¹⁵⁸ *Hey1*^{-/-} and *Hey2*^{-/-} mouse embryos developed defective vessels and died at E9.5.^{159,160} In these mice, vasculogenesis occurred normally, but vessel remodeling in the yolk sac and placenta was impaired and some mice showed poorly developed dorsal aorta and cardinal veins. Expression of arterial markers was also significantly reduced in these mice. A similar finding was seen in *gridlock (grl)* zebrafish (*grl* is the zebrafish ortholog of mammalian *Hey2*) where dorsal aorta development was defective.¹⁶¹ These studies collectively suggest the potentially important role of HEY1/HEY2 in arterio-venous specification.

In line with the above findings, deficiency of *Rbpj* (*recombination signal binding protein for immunoglobulin kappa J region*) in mice, an obligatory transcriptional factor of the NOTCH pathway,¹⁶² also induced defective arterial vessel formation. These studies showed that the NOTCH pathway in endothelial cells controls the development of arteries through RBPJ-HEY1/2 signaling.

SOX7, SOX17 and SOX18, members of the SOX (Sry-related HMG box) transcription factor family

The SOX (SRY-like HMG-box) transcription factor family shares a conserved DNA binding domain, known as HMG (high mobility group), that recognizes the consensus sequence 5'-(A/T)(A/T)CCA(A/T)G-3' present in various genes.¹⁶³ Among 20 different members of the SOX factors, the SOX F group (Sox7, 17 and 18) has been found to play an important role in vascular development (Figure 4C).¹⁶⁴ Expression of *Sox18* and *Sox7*, but not *Sox17*, was detected between E7.5 and E8.5 in endothelial cells of the yolk sac vasculature and the dorsal aorta, and *Sox17* was expressed in endothelial cells of the dorsal aorta at E9.5.¹⁶⁵⁻¹⁶⁷ *Sox18* was also evident in endothelial cells of the developing lymphatic vessels.¹⁶⁸

Linkage analysis combined with DNA sequencing showed that SOX18 was responsible for the phenotypic defects observed in four spontaneous mouse mutants, *Ragged (Ra)*, *Ragged-Opposum (RaOp)*, *Ragged-Jackson*, and *Ragged-like*, all of which carried a mutation in *Sox18* gene, making a dominant negative form of SOX18.^{169,170} *RaOp* homozygous mice initially developed a normal vasculature but died at E14.5 with defective lymphatic vessel development accompanied by hemorrhage and edema.¹⁷⁰ Also *Sox18*^{-/-} mouse embryos in C57BL/6 background phenocopied the defects seen in *RaOp* homozygous mice;¹⁶⁸ the mutant embryos showed lethality at E14.5 and developed no PROX1 (Prospero homeobox transcription factor 1)⁺ lymphatic endothelial cells, indicating a critical role of *Sox18/RaOp* in lymphatic endothelial cell specification. SOX18 binding to sites present in *Prox1* promoter may be responsible for *Prox1* expression.¹⁶⁸ Overexpression of *Sox18* in differentiating ES cells induced expression of genes critical for lymphatic endothelial cells *Prox1* and *Podoplanin* (Figure 4D), but *Sox18/RaOp* suppressed these genes.¹⁶⁸ Deficiency of *Sox18* in 129-CD1 mixed background, however, did not show these defects, and the mice were born alive and apparently normal.¹⁷¹ This discrepancy in these mixed background mice may due to the compensatory expression of SOX7 and SOX17 in lymphatic endothelial cells since these genetic changes did not occur in

pure C57/B6 background.^{168,171,172} These findings collectively suggest that SOX7 and SOX17 function as genetic modifiers of lymphatic vessel development (Figure 4C).

SOX18 can also function redundantly with SOX7 or SOX17 in arterial-venous specification of endothelial cells. Zebrafish injected with double morphants of *sox7* and *sox18* showed augmented expression of the venous markers *dab2* and *flt4* in arteries coupled with decreased expression of the arterial markers *notch3*, *ephrinB2* and *dll4*.¹⁷³ In a similar study, double knockdown of *sox7* and *sox18* in zebrafish resulted in ectopic expression of *flt4* with concomitant decrease of *ephrinB2* in the dorsal aorta.¹⁷⁴ When both *Sox17* and *Sox18* were deleted in mixed background mice, the mutants displayed defects in anterior dorsal aorta and endocardial tube development.¹⁶⁷ In contrast, Matsui et al¹⁶⁶ reported that mixed background *Sox17^{+/-};Sox18^{-/-}* mice were perinatally lethal and developed abnormal vascular structures. Endothelial cells of *Sox17^{+/-};Sox18^{-/-}* mice also showed significantly impaired tube forming ability *in vitro*.¹⁶⁶ Taken together, these studies suggest that the SOX F group transcription factors function as an important regulator for endothelial cell specification; arterio-venous specification, and venous-lymphatic specification.

PROX1 (Prospero homeobox transcription factor 1)

Prox1 expression at E9.5 was detected in a subpopulation of cells dorsolateral to the cardinal vein.¹⁷⁵ Thereafter, *Prox1* expression became negative in the cardinal vein and it was confined to the cells migrating out of the cardinal vein which formed the lymphatic sac and eventually the lymphatic vasculature itself.^{176,177} *Prox1* continued to be expressed in the adult lymphatic endothelial cells. A number of studies have established a critical role of PROX1 as a supreme regulator of lymphatic endothelial cell specification and maintenance. *Prox1^{+/-}* mice died shortly after birth with defects of enteric lymphatic vessels.¹⁷⁵ *Prox1^{-/-}* (*Prox1^{LacZ/LacZ}*) mouse embryos examined at E14.5 showed no sign of lymphatic vessel formation with normal development of blood vessels.^{175,177} In these mice, *Prox1^{LacZ/LacZ}* cells began to bud from the cardinal vein but failed to migrate, resulting in the lack of lymphatic sac and lymphatic vessel

development. Also the cells deficient in *Prox1* did not express lymphatic markers VEGFR3 and LYVE1 (lymphatic vessel endothelial hyaluronan receptor 1) while they continued to maintain their vascular endothelial identity.¹⁷⁷ These results together show the requisite role of PROX1 activity in mediating lymphatic endothelial cell specification but they also show that PROX1 is dispensable for lymphatic endothelial cell budding from the cardinal vein.

Conditional deletion of *Prox1* in embryos as well as adult led to reprogramming of lymphatic endothelial cells into blood endothelial cells.¹⁷⁸ *Prox1* knockdown in adult lymphatic endothelial cells showed the similar results seen in *in vivo* experiments.¹⁷⁸ Overexpression of *Prox1* in blood endothelial cells induced the expression of lymphatic makers concomitant with decreased expression of vascular endothelial genes.^{179,180} In addition, it has been reported that PROX1 can bind with ETS1/2 and that its interaction in a synergistic manner promote the expression of VEGFR3.¹²⁷ These findings together show the essential role of PROX1 as a cell fate determinant of lymphatic endothelial cells (Figure 4D).

A recent study on revisiting the issue of lymph-angiogenic function of PROX1 identified another source and mechanism of lymphatic endothelial progenitors.¹² Using serial thick vibratome sections, Yang et al¹² found at E10.5 distinct PROX1⁺ cell populations in the intersomitic vessels (ISV) as well as the cardinal vein. These PROX1⁺ cells at both sites acquired expression of PODOPLANIN, which was identified as another accessory protein involved in the specification of lymphatic endothelial cells.¹⁸¹

COUPTFII (Chicken ovalbumin upstream transcription factor II)

COUP-TFII, a member of the NR (nuclear receptor) 2F subfamily, is a nuclear orphan receptor that functions in a wide range of biological processes including angiogenesis and neural development.^{182,183} Expression of *Coup-TFII* starting from E8.5 is limited to endothelial cells of veins and lymphatics, not the endothelium of arteries.^{182,184} Targeted deletion of *Coup-TFII* caused abnormal development of the heart and impaired angiogenesis with missing or collapsed cardinal veins, leading to embryonic lethality.¹⁸² Inactivation of *Coup-TFII* in

endothelial cells of *Tie2Cre* mice resulted in death of mutant embryos and loss venous identity as evident by expression of arterial markers *Jag1*, *Notch1*, *ephrinB2* and *Np1* in the veins.¹⁸⁴ Endothelial expression of *Coup-TFII* induced aberrant vessel development and fusion of artery-vein structures, the same defects seen in *Np1* and *Notch1* knockout mice.^{185,186} These findings suggest a regulatory loop between COUP-TFII and the NOTCH pathway needed for artery-vein specification (Figure 4D). In support of this concept, double knockout of *Coup-TFII* and NOTCH signaling component, *Rbpj*, in endothelial cells partially rescued the loss of venous identity seen with endothelial deletion of COUP-TFII.¹⁸⁷

Despite the strong evidence of the critical role of COUP-TFII in venous endothelial cell specification, the mechanisms regulating the expression of COUP-TFII in this process are poorly understood. A recent study showed that *Coup-TFII* expression was regulated by BRG1 (brahma-related gene1) in veins.¹⁸⁸ BRG1 is a chromatin-remodeling enzyme and a component of SWITCH/sucrose non-fermentable (SWI/SNF)-like complex.¹⁸⁹ Deletion of *Brg1* in TIE2⁺ cells led to embryonic lethality and defective primitive erythropoiesis and yolk sac vessel formation.¹⁹⁰ Venous endothelial cells deficient in *Brg1* showed decreased level of *Coup-TFII* expression with aberrantly elevated expression of arterial markers *ephrinB2*, *Nrp1*, and *Dll4*.¹⁸⁸ BRG1 binding to the -1.2 kb promoter region of *Coup-TFII* was also identified, and knockdown of *Brg1* caused chromosome condensation of the promoter regions. These results together suggest that BRG1 lying upstream can *Coup-TFII* expression through chromosome remodeling and thus induce venous endothelial cell specification.

COUP-TFII is also an important regulator of lymphatic endothelial cell identity. Co-expression of COUP-TFII and PROX1 and their interaction is involved in mediating lymphatic endothelial cell specification throughout embryogenesis and in postnatal life.^{191,192} The interaction of COUP-TFII and PROX1 regulated the expression of lymphatic markers *Fgfr3* and *Vegfr3*.^{191,192} Further, it has been shown that COUP-TFII binds the promoter of *Prox1* through

COUP-TFII binding sequences. Taken together, these observations support the concept that COUP-TFII regulates lymphatic cell development through an interaction with PROX1.

D. Role of Crucial Signaling Pathways in Regulating Endothelial Transcription Factors and Vascular Development

VEGF Signaling

VEGF-activated signaling is the major pathway regulating multiple aspects of endothelial cell function including survival, proliferation and vessel permeability.^{193,194} Upon binding of VEGF, its receptor, FLK1/KDR, transmit signals through a number of downstream molecules MAPK-ERK, p38-MAPK, phospholipase C, and phosphatidyl inositol 3-kinase (PI3K)/Akt/protein kinase B to regulate endothelial function.¹⁹⁵⁻¹⁹⁸ Also (importantly for this review), VEGF signaling plays a critical role in vessel development during embryogenesis. *Vegf*^{+/−} mouse embryos died due to defects in endothelial and hematopoietic cell development.^{32,199} Further, VEGF signaling has a role in arteriovenous specification. Morpholino knockdown of *veg*f in zebrafish prevented the expression of the arterial marker *ephb2* accompanied by sustained expression of venous marker in dorsal aorta.²⁰⁰ Ectopic expression of *veg*f rescued defective arterial differentiation mediated by cyclopamine, a sonic hedgehog inhibitor.^{200,201} VEGF also induced the expression of arterial markers *EphrinB2*, *Np1*, and *Gja5* in primary EphrinB2[−] endothelial cells isolated from E10.5 mouse embryos.²⁰² In addition, overexpression of VEGF in cardiomyocytes (α MHC-VEGF mice) led to significant increase in number of cells expressing the arterial marker *EphrinB2* with decreased number of *EphB4*⁺ venous endothelial cells.²⁰³ The molecular mechanisms of VEGF-mediated arterial specification are still unclear. Recent studies suggest that transcription factors FOXC1/FOXC2 interact with the VEGF pathway components to promote arterial specification of endothelial cells through the NOTCH signaling pathway (see below).

NOTCH Signaling

The NOTCH pathway is critical for arterial specification of embryonic vasculature.^{204,205} Four NOTCH receptors (1 through 4) and five ligands (jagged1, 2, Delta like ligand (Dll)1, 3, 4)

have been identified in mammals.²⁰⁴ Binding between the ligand and the receptor induces proteolytic cleavage of NOTCH receptor, resulting in generation of intracellular form of the receptor (NICD, NOTCH intracellular domain) that translocates into the nucleus to induce its downstream targets HEY1 and HEY2.^{158,206} Mice lacking a single copy of *Dll4* exhibited severe remodeling defects in yolk sac vessels and smaller dorsal aorta¹⁶² consistent with the expression of components of NOTCH signaling, NOTCH1, NOTCH4 and Jagged1, Jagged2 and Dll4 selectively in arterial endothelial cells. Also, these embryos developed abnormal arteriovenous vessels due to fusion between the dorsal aorta and common cardinal vein. In a related study, *Dll4*^{-/-} mouse embryos completely lost arterial identity.²⁰⁷ Overexpression of *Dll4* caused abnormal vessel development with enhanced arterialization.²⁰⁸ Mice deficient in both *Notch1* and *Notch4* died *in utero* with a severe vessel remodeling defects in both yolk sac and embryo.²⁰⁹ Thus, NOTCH, which can be activated by VEGF (please see below),²⁰⁰ has an essential role in mediating arterial specification.

The role of VEGF and NOTCH pathways in promoting arterial endothelial cell specification points to a crosstalk of both pathways. The evidence for this comes from the zebrafish in which activation of *notch* was able to rescue arterial defects caused by morpholino knockdown of *vegfr*.²⁰⁰ Likewise it was shown that VEGF induced the expression of *Notch1* and *Dll4* through PI3K/AKT pathway in cultured endothelial cells.²¹⁰ The VEGF-mediated NOTCH activation was specifically seen in arterial endothelial cells (as opposed to venous endothelial cells) *in vitro*. Another study has shown that the cross-talk between both pathways could be mediated by endothelial transcription factors, FOXC1/FOXC2. As discussed above, deletion of *FoxC1/FoxC2* led to arterial defects in developing mouse embryo.¹⁴⁵ *In vitro* analysis revealed that FOXC1/FOXC2 upregulated the expression of the arterial endothelial markers *Notch1*, *Notch4*, *Dll4*, *Hey2* and *EphrinB2* through direct transcriptional activation.¹⁴⁵ Interestingly, FOXC1/FOXC2-mediated promoter activation of *Dll4* and *Hey2* was augmented by VEGF treatment.¹⁴⁶ Such augmentation was impaired by inhibiting PI3K. These intriguing results

suggest that the VEGF and NOTCH pathway promote arterial endothelial cell specification through FOX transcription factors. The expression of FOXC1 and FOXC2 is not activated by VEGF treatment alone,^{211,212} indicating a complex mechanism requiring further study. The reciprocal interaction between VEGF and NOTCH signaling has also been extensively studied during sprouting angiogenesis, where tip cells direct the growth of sprouts towards a gradient of tissue-derived VEGF. Although the review's intent is not to cover tip cell function, information in this area can be found in other literatures.^{213,214}

Signaling via Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs) belong to transforming growth factor (TGF) β superfamily that regulates a multitude of biological processes including embryonic vessel development.^{215,216} Approximately 20 mammalian BMPs have been identified and they function through serine/threonine kinase receptors composed of type I and type II forms.²¹⁵ Upon binding to BMPs, the type II receptor, which is a constitutively active kinase, activates the type I receptor by phosphorylating specific serine and threonine residues. The activated type I receptor in turn phosphorylates SMADs (SMAD1, 5, and 8) to transmit BMP signaling. Subsequently, the phosphorylated SMADs interact with SMAD4, a common SMAD, and translocate into the nucleus to induce the expression of genes such as ID.¹¹⁴

Mouse gene knockout studies showed a key role of the BMP pathway in vascular development.^{216,217} Mice deficient in both *Id1* and *Id3*, the downstream BMP/SMAD targets, showed vascular defects in the developing brain (see also "ID" above).¹¹³ While *Bmp4* deficient mice died without posterior mesodermal differentiation,²¹⁸ germ line deletion of *Alk-3*, a type IA BMP receptor, failed to survive up to E9.5 stage with defects in mesoderm formation.²¹⁹

Park et al²²⁰ demonstrated that BMP4 via SMAD1/5 signaling induced the generation of FLK1⁺ cells from mouse ES cells in serum-free differentiation condition. Blockade of BMP4 by its antagonist, Noggin,²²¹ reduced FLK1⁺ cell generation.²²⁰ GATA2 together with BMP4 was shown to also promote mouse ES cell differentiation to FLK1⁺ cells.¹⁰⁵ BMP4-mediated FLK1⁺

cell development was mediated by the transcription factor, ER71/ETV2.⁵⁵ The expression of ER71 was decreased by inhibiting BMP signaling.⁵⁵ In addition, overexpression of ER71 rescued the impairment of FLK1⁺ cell generation by Noggin and DKK1 (WNT inhibitor).²²²

WNT Signaling

WNT signaling is critical for embryogenesis and disease development.^{223,224} WNT proteins are ligands that bind their receptors to convey signals through three distinct signaling routes; the canonical WNT/ β -catenin pathway; WNT/ Ca^{2+} pathway; and planar cell polarity pathway (PCP).²²⁵⁻²²⁷ The canonical WNT/ β -catenin pathway has been the best studied and has a crucial role in vascular development. In the off-state (i.e., without engagement of WNT proteins and receptors), β -catenin, a downstream molecule of the pathway, is phosphorylated by GSK3 β , and the “marked” β -catenin is ubiquitinated for degradation by proteasomes. Binding of WNT proteins to their receptor, Frizzled/Lrp (Low-density lipoprotein receptor), activates Dishevelled (Dvl) which inhibits GSK3 β and thus releases β -catenin from the degradation pathway, allowing the translocation of β -catenin to the nucleus. Lengerke et al²²⁸ showed that inhibition of WNT led to decreased generation of FLK1⁺ cells. Another report²²⁹ showed that the expression of genes associated with development of primitive streak, endoderm, and mesoderm was downregulated by inhibiting the WNT/ β -catenin pathway during ES cell differentiation. Unexpectedly, however, the forced expression of the stabilized form of β -catenin failed to stimulate these marker genes. These studies suggest that the WNT/ β -catenin signaling is required but it is not essential for formation of FLK1⁺ mesoderm. Thus, WNT/ β -catenin signaling may have a supportive role in vascular development upon BMP signaling. Together these studies point to a key role of WNT signaling in vascular endothelial cell specification through ID1, GATA2 and ER71/ETV2. How these factors interact with each other to activate Flk1 gene expression remains an open question.

Other studies in developing mouse embryos showed an important role of β -catenin, which can be stabilized downstream of WNT signaling, in vessel development. Inactivation of β -

catenin in TIE2⁺ cells resulted in embryonic lethality with vascular remodeling defects and hemorrhages.²³⁰ The mutant embryos also displayed defects in endocardial cushion and cardiac valve formation.²³¹ Further, the canonical WNTs and β -catenin were shown to be required for development of CNS vessels.^{78,232} Embryos with sustained expression of β -catenin in TIE2⁺ cells lost their arteriovenous identity.²³³ In this study, β -catenin was shown to directly bind *Dll4* promoter²³³ linking the Wnt and NOTCH signaling pathways. Together these findings suggest a role for WNT- β -catenin signaling in vessel development, although the role of this pathway in endothelial cell specification is less clear.

E. Endothelial cell plasticity and cell reprogramming

It is now generally accepted that terminally differentiated somatic cells are in the “ground state” where the fates of the cells remain unchanged throughout life. Waddington’s epigenetic landscape model has been supported this deterministic view.²³⁴ In this model, a hypothetical ball will roll down from the top of a hill and complete its journey at the lowest point; the pluripotent cell (analogous to Waddington’s ball) loses its potential, undergoes differentiation and becomes a terminally differentiated somatic cell. However, emerging evidence has challenged this prevailing concept. Building upon experiments by John Gurdon²³⁵ showing that enucleated eggs receiving nuclei isolated from fully differentiated frog cells generated adult frogs, it is now clear that lineage specific transcription factors can change identity of certain types of cells including fully matured somatic cells. Weintraub et al²³⁶ demonstrated that ectopically expressed MyoD, a transcription factor critical for muscle differentiation, converted fibroblasts as well as differentiated cell lines including melanoma and neuroblastoma into skeletal muscle cells. Overexpression of *C/EBP* (*CCAAT/enhancer-binding protein*) transcription factor led to cell fate change of B lymphocytes to macrophage-like cells.²³⁷ As shown by Yamanaka, the pluripotency transcription factors, OCT4, NANOG, SOX2, KLF4 and MYC, are sufficient to generate ES-like cells (induced pluripotent cells, iPSCs) through a de-differentiation mechanism (Figure 5A).²³⁸⁻²⁴¹

With regard to endothelial cells, recent studies have shown that endothelial cells exhibit a certain degree of plasticity which is controlled by transcription factors. Overexpression of *Prox1* conferred the identity of lymphatic endothelial cell to blood endothelial cells.^{179,180} Lymphatic endothelial cells deficient in *Prox1* lost their lymphatic identity and became blood endothelial cells.¹⁷⁸ Similarly, specification of arterial and venous endothelial cells is determined by a reciprocal function of NOTCH signaling and COUP-TFII as discussed above.¹⁸⁴ These observations suggest that endothelial cell identity is interchangeable through transcription factor-mediated reprogramming.

Given such an important role of endothelial cell transcription factors in regulating cell fate, one can envision that appropriate combinations of endothelial transcription factors can directly reprogram terminally differentiated somatic cells into three different types of endothelial cells. A study reported that a combination of ETS factors with concomitant repression of TGF β signaling converted amniotic cells to endothelial cells (Figure 5B).⁸³ The key finding was that transient overexpression of *ER71* converted the amniotic cells into immature endothelial cells which subsequently became mature endothelial cells with sustained expression of *ERG1* and *FLI1*. The authors chose mid-gestation c-KIT⁻ amniotic cells for reprogramming, but failed to reprogram postnatal fibroblasts with the same approach. Although the generated endothelial cells showed vessel forming capacity *in vivo*, it is not clear whether the amniotic cells were reprogrammed at the level of their epigenetic status in this study. Two groups have recently reported that somatic cells can be converted to cells having features of endothelial cells. In one study, cells partially reprogrammed using the Yamanaka factors (OCT4, SOX2, KLF4 and c-MYC) were isolated (referred to as PiPS: partially induced pluripotent stem) and thereafter further differentiated into endothelial cells under defined culture conditions.²⁴² Although PiPS-derived endothelial cells had potential for neovascularization *in vivo*, the identity of PiPS and also PiPS-derived endothelial cells was not clear. In another study,²⁴³ human fibroblasts were induced to become CD34⁺ angioblast-like cells in two successive phases; “plastic induction

phase” driven by ectopic expression of Yamanka factors, followed by “mesodermal induction phase” driven by a chemically defined medium. The CD34⁺ cells were able to differentiate into endothelial cells as well as smooth muscle cells; however, it was not demonstrated whether endothelial cells could be generated concomitantly with hematopoietic cells as would be expected of CD34⁺ cells. These studies open a new possibility of generating endothelial cells from terminally differentiated somatic cells with certain codes of endothelial/pluripotent stem transcription factors. Considering specific functions of each endothelial transcription factors described above, it is reasonable to speculate that immature endothelial cells derived from somatic cells could be specified into arterial, venous or lymphatic endothelial cells in combination of HEY1/2, COUP-TFII and PROX1 as suggested in Figure 5B. If so, it remains to be seen whether these cells will have the ability to regenerate different portions of the vasculature.

F. Concluding remarks

Cardiovascular diseases such as high blood pressure, diabetes and coronary heart disease and vascular diseases associated with acute and chronic inflammation are the leading causes of morbidity and mortality in the United States and other advanced countries. Approximately 37% of the population suffers from cardiovascular diseases in the United States alone. Development of new cell therapeutics based upon detailed understanding of generation of endothelial cells holds great promise. However, more knowledge and deeper understanding of endothelial cell and vascular development is urgently needed. Questions such as how the vascular system comprising endothelial cells develops prenatally and how it can be repaired by activating specific signaling pathways remain to be fully addressed. Additionally, there is the fundamental unexplored area of the importance of hemodynamic forces in mediating endothelial cell gene expression and functionality in arteriovenous endothelial cell specification during embryonic vessel development. Although hemodynamic forces play a key role in development of vascular diseases such as atherosclerosis,²⁴⁴⁻²⁴⁶ their involvement in vascular development is

unexplored territory. In this regard, an interesting study²⁴⁷ (which presages future work that is needed in this field) reported that both *Gja4* and calcineurin/NFAT played a critical role for the formation of lymphatic valve. In the lymphatic valve formation, flow dependent expression of PROX1 and FOXC2 induced expression of *Gja4* and activation of calcineurin/NFAT signaling.

In the future, defining the mechanisms regulating vascular development would be the fundamental for treating diseases related to aberrant vessel growth and dysfunction of endothelial cells. This knowledge would be a new research platform for generating functional and specific types of endothelial cells as well as organ-specific endothelial cells through directed differentiation from pluripotent stem cells and directed conversion of patient-specific somatic cells including blood cells. This is the hope for the future but as discussed in this review some important foundations have been already laid.

Acknowledgements

This work was supported by the March of Dimes Foundation, 5-FY12-44 (to CP), the American Heart Association, 11SDG7390074 (to CP) and NIH R01 HL45638 (to ABM), NIH R01 HL90152 (to ABM), NIH P01 60678 (to ABM), and NIH P01 77806 (to ABM).

Competing interests statements

The authors declare that they have no competing financial interests.

Table1. Abbreviations

Abbreviations	Full name
<i>Bmp4</i>	bone morphogenetic protein 4
<i>Brg1</i>	brahma-related gene 1
<i>Coup-TFII</i>	chicken ovalbumin upstream promoter transcription factor II
<i>CREB1</i>	cAMP response element-binding protein
<i>dab2</i>	disabled homolog 2
<i>Dll1/4</i>	delta-like 1/4
<i>eNOS</i>	endothelial nitric oxide synthase
<i>EphrinB2</i>	ephrin type-B receptor 2
<i>EphB4</i>	ephrin type-B receptor 4
<i>Erg</i>	ets related gene
<i>Er71</i>	ets-related 71
<i>Ets1/2</i>	e26 avian leukemia oncogene 1/2
<i>etsrp</i>	ets1-related protein
<i>Fgfr3</i>	fibroblast growth factor receptor 3
<i>Flk1</i>	fetal liver kinase 1
<i>Fli1</i>	friend leukaemia integration-site 1
<i>flt4</i>	fms-related tyrosine kinase 4
<i>Fox</i>	forkhead transcription factor
<i>Gata</i>	GATA binding protein
<i>Gja4/5</i>	gap junction alpha 4/5
<i>grl</i>	gridlock
<i>Hand2</i>	heart- and neural crest derivatives-expressed protein 2
<i>hCD4</i>	human cluster of differentiation 4
<i>Hey1/2</i>	hairy/enhancer-of-split related with YRPW motif protein 1/2
<i>Id</i>	Inhibitor of differentiation and DNA binding 1
<i>Klf4</i>	krüppel-like factor 4
<i>lycat</i>	lysocardiolipin acyltransferase
<i>Lyve1</i>	lymphatic vessel endothelial hyaluronan receptor 1
<i>Nfatc1</i>	nuclear factor of activated T-cells, cytoplasmic 1
<i>NICD</i>	notch intracellular domain
<i>Nkx2-5</i>	nk2 homeobox 5
<i>Np1</i>	neuropilin 1
<i>Oct4</i>	octamer-binding transcription factor 4
<i>Pdgfra</i>	platelet-derived growth factor receptor α
<i>Prox1</i>	prospero-related homeobox
<i>Rbpj</i>	recombination signal binding protein for immunoglobulin kappa J region
<i>Scl (Tal1)</i>	stem cell leukemia (T-cell acute lymphocytic leukemia protein 1)
<i>SMAD</i>	sma/mad homology
<i>Smc-α</i>	smooth muscle actin alpha
<i>Sox</i>	sry-related HMG box
<i>Su(H)</i>	suppressor of hairless
<i>Tel</i>	translocated ets leukemia
<i>Tgfβ</i>	transforming growth factor beta
<i>Timp</i>	tissue inhibitor of metalloproteinase 1
<i>Vcam1</i>	vascular cell adhesion molecule 1
<i>VEcadherin</i>	vascular endothelial cadherin
<i>Vegfr2/3</i>	vascular endothelial growth factor receptor 2/3

Table 2. Phenotypes of mice and zebrafish deficient in genes critical for cardiovascular development

Genes	Experiments	Findings	References
<i>Lycat</i>	*MO study in zebrafish	reduction of both hematopoietic and endothelial lineages	18
<i>Brachyury</i>	spontaneous mutants	lethal before E11.0 due to the defects in primitive streak, notochord, and allantois	34-36
<i>Scl</i>	MO study in zebrafish	decrease of hematopoietic and endothelial gene induction	16
	knockout mice	lethal around E9.5 due to lack of hematopoiesis	86-88
<i>Er71</i>	knockout mice	lethal by E10.5 due to complete lack of vessel and hematopoietic cells	55,56
	MO study in zebrafish	deficient in endothelial cell development and definitive hematopoiesis	17,90
	MO study in xenopus	defects in vessel formation, hematopoiesis was not affected	67
<i>Gata2</i>	knockout mice	lethal by E10.5 due to defects in primitive erythropoiesis	100
<i>Id1/Id3</i>	knockout mice	lethal by E13.5, vascular defects in the forebrain, absence of branching of blood vessels into the neuroectoderm	113
<i>FoxO1</i>	knockout mice	lethal around E11.0 due to the defects in the branchial arches and vessels of embryos and yolk sacs, retarded cardiac looping and distended pericardium	135,136
<i>FoxO3</i>	knockout mice	no apparent vascular defect	136,139
<i>FoxO4</i>	knockout mice	no apparent vascular defect	136
<i>FoxO1;O3;O4 compound knockout</i>	Mx1-Cre conditional knockout	endothelial cell over-proliferation and liver hemangiomas	141
<i>foxc1a</i>	MO study in zebrafish	reduction in the number of intersomitic vessel	63
<i>foxc1b</i>	MO study in zebrafish	reduction in the number of intersomitic vessel	63
<i>foxc1a/1b</i>	MO study in zebrafish	no intersomitic vessel was detected at 24hpf, decreased formation of axial vessels	63
<i>FoxC1</i>	knockout mice	perinatal death due to interruption or coarctation of the aortic arch and valve hypoplasia	142
<i>FoxC2</i>	knockout mice	abnormal lymphatic vascular patterning, increased pericyte investment of lymphatic vessels, agenesis of valves and lymphatic dysfunction	143

<i>FoxC1/C2</i>	knockout mice	lethal by E9.5, abnormal vessel remodeling, defective arterio-venous specification, lack of arterial marker expression, impaired cardiac development	144,145
<i>FoxF1</i>	knockout mice	lethal by E10.0, yolk sac and allantois vasculature malformation, expansion of VCAM1 ⁺ cell expansion in extraembryonic mesoderm	150
<i>Dll4</i>	knockout mice	haploinsufficient lethality at E9.0-E10.0, impaired vascular remodeling, pericardial effusions, absence of vasculature in yolk sac	162
<i>Hey1</i>	knockout mice	no apparent vascular defect	159
<i>Hey1/Hey2(grl)</i>	knockout mice	embryo dies after E9.5, vasculogenesis occurred but subsequent remodeling was impaired, defects in arterial specification.	159,160
<i>Hey2</i>	MO study in zebrafish	decrease of arterial gene expression, increased venous gene expression	161
<i>Rbpj</i>	knockout mice	defects in vascular remodeling, impaired vascular penetration of the labyrinthine layer of the placenta, loss of arterial specification	162
<i>Sox17</i>	knockout mice	aberrant heart looping, enlarged cardinal vein, mild defects in anterior dorsal aorta formation	167
<i>Sox18</i>	knockout mice	lethal after E14.5, subcutaneous edema due to the defects in lymphatic vessel patterning	168
<i>Sox7/Sox18</i>	MO study in zebrafish	absence of circulation in trunk and tail, defects in arterio-venous specification	173,174
<i>Sox17/Sox18</i>	knockout mice	malformation of dorsal aorta and head vasculature, defects in endocardial cell development and endocardial tube fusion	167
<i>Sox17^{+/-};Sox18^{-/-}</i>	knockout mice	reduced vessel growth in the liver sinusoids and kidney medulla, impaired endothelial cells sprouting and vessel remodeling	166
<i>Prox1</i>	knockout mice	lethal by E14.5, impaired sprouting and budding of lymphatic endothelial cells, normal blood endothelial cell development	175
<i>Coup-TFII</i>	knockout mice	lethal around E10.0 due to hemorrhage and edema in the brain and heart, abnormal cardinal vein formation	182,183
	Tie2 conditional knockout	loss of vein identity, expression of arterial markers in vein	184
<i>Nfatc1</i>	knockout mice	lethal by E17.5, absence of aortic and pulmonary valves, poor coalescence into lymph sacs, defective lymphatic vessel patterning	248,249
<i>Ets1</i>	knockout mice	no vessel defect, 50% of lethality by 4 weeks.	125
	MO study in zebrafish	reduced number of intersomitic vessel sprouting, defects in trunk circulation	250
<i>Ets2</i>	knockout mice	lethal by E8.5 due to impaired development of the extra-embryonic	251

		tissues	
<i>Ets1/Ets2</i>	knockout mice	lethal during E11.5-15.5 due to dilated vessels, edema and hemorrhage	126
<i>Erg</i>	ENU mutagenesis	embryonic lethal by E13.5, defects in definitive hematopoiesis, development of the embryonic vasculature unaffected	130
<i>Fli1</i>	knockout mice	lethal by E12.5 due to the defects in hematopoietic cell differentiation and hemorrhage	129
	MO study in zebrafish	reduction in the number of intersomitic vessel sprouting	250
<i>Tel</i>	knockout mice	lethal between E10.5 and E11.5, defective yolk sac vessel remodeling	122

* morpholino knock-down

References

1. Poole TJ, Coffin JD. Vasculogenesis and angiogenesis: two distinct morphogenetic mechanisms establish embryonic vascular pattern. *J Exp Zool.* 1989;251:224-231.
2. Flamme I, Frolich T, Risau W. Molecular mechanisms of vasculogenesis and embryonic angiogenesis. *J Cell Physiol.* 1997;173:206-210.
3. Risau W. Mechanisms of angiogenesis. *Nature.* 1997;386:671-674.
4. Asahara T, Kawamoto A, Masuda H. Concise review: Circulating endothelial progenitor cells for vascular medicine. *Stem Cells.* 2011;29:1650-1655.
5. Fadini GP, Losordo D, Dimmeler S. Critical reevaluation of endothelial progenitor cell phenotypes for therapeutic and diagnostic use. *Circ Res.* 2012;110:624-637.
6. Sabin FR. Studies on the origin of blood vessels and of red corpuscles as seen in the living blastoderm of the chick during the second day of incubation. *Contributions to Embryology.* 1920;9:213-262.
7. Murray PDF. The development in vitro of the blood of the early chick embryo. *Proc R Soc Lond B Biol Sci.* 1932;11:497-521.
8. Choi K. The hemangioblast: a common progenitor of hematopoietic and endothelial cells. *J Hematother Stem Cell Res.* 2002;11:91-101.
9. Patan S. Vasculogenesis and angiogenesis. *Cancer Treat Res.* 2004;117:3-32.
10. Rhodin JAG. Architecture of the Vessel Wall. In: Terjung R, ed. *Comprehensive Physiology.* Hoboken, NJ: John Wiley & Sons, Inc.; 2011:1-31.
11. Alitalo K. The lymphatic vasculature in disease. *Nat Med.* 2011;17:1371-1380.
12. Yang Y, Garcia-Verdugo JM, Soriano-Navarro M, et al. Lymphatic endothelial progenitors bud from the cardinal vein and intersomitic vessels in mammalian embryos. *Blood.* 2012;120:2340-2348.
13. Meister M, Laguerreux M. Drosophila blood cells. *Cell Microbiol.* 2003;5:573-580.
14. Mandal L, Banerjee U, Hartenstein V. Evidence for a fruit fly hemangioblast and similarities between lymph-gland hematopoiesis in fruit fly and mammal aorta-gonadal-mesonephros mesoderm. *Nat Genet.* 2004;36:1019-1023.
15. Liao W, Bisgrove BW, Sawyer H, et al. The zebrafish gene cloche acts upstream of a flk-1 homologue to regulate endothelial cell differentiation. *Development.* 1997;124:381-389.
16. Liao EC, Paw BH, Oates AC, Pratt SJ, Postlethwait JH, Zon LI. SCL/Tal-1 transcription factor acts downstream of cloche to specify hematopoietic and vascular progenitors in zebrafish. *Genes Dev.* 1998;12:621-626.
17. Sumanas S, Lin S. Ets1-related protein is a key regulator of vasculogenesis in zebrafish. *PLoS Biol.* 2006;4.
18. Xiong J-W, Yu Q, Zhang J, Mably JD. An acyltransferase controls the generation of hematopoietic and endothelial lineages in zebrafish. *Circ Res.* 2008;102:1057-1064.
19. Vogeli KM, Jin S-W, Martin GR, Stainier DYC. A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula. *Nature.* 2006;443:337-339.
20. Choi K, Kennedy M, Kazarov A, Papadimitriou JC, Keller G. A common precursor for hematopoietic and endothelial cells. *Development.* 1998;125:725-732.
21. Faloon P, Arentson E, Kazarov A, et al. Basic fibroblast growth factor positively regulates hematopoietic development. *Development.* 2000;127:1931-1941.
22. Chung YS, Zhang WJ, Arentson E, Kingsley PD, Palis J, Choi K. Lineage analysis of the hemangioblast as defined by FLK1 and SCL expression. *Development.* 2002;129:5511-5520.

23. Huber TL, Kouskoff V, Fehling HJ, Palis J, Keller G. Haemangioblast commitment is initiated in the primitive streak of the mouse embryo. *Nature*. 2004;432:625-630.
24. Furuta C, Ema H, Takayanagi S-I, et al. Discordant developmental waves of angioblasts and hemangioblasts in the early gastrulating mouse embryo. *Development*. 2006;133:2771-2779.
25. Zovein AC, Hofmann JJ, Lynch M, et al. Fate tracing reveals the endothelial origin of hematopoietic stem cells. *Cell Stem Cell*. 2008;3:625-636.
26. Hirschi KK. Hemogenic endothelium during development and beyond. *Blood*. 2012;119:4823-4827.
27. Bertrand JY, Chi NC, Santoso B, Teng S, Stainier DY, Traver D. Haematopoietic stem cells derive directly from aortic endothelium during development. *Nature*. 2010;464:108-111.
28. Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. *Nature*. 2010;464:116-120.
29. Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel type of cell transition. *Nature*. 2010;464:112-115.
30. Lancrin C, Sroczynska P, Stephenson C, Allen T, Kouskoff V, Lacaud G. The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. *Nature*. 2009;457:892-895.
31. Shalaby F, Rossant J, Yamaguchi TP, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature*. 1995;376:62-66.
32. Carmeliet P, Ferreira V, Breier G, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature*. 1996;380:435-439.
33. Wilkinson DG, Bhatt S, Herrmann BG. Expression pattern of the mouse T gene and its role in mesoderm formation. *Nature*. 1990;343:657-659.
34. Gluecksohn-Schoenheimer S. The development of normal and homozygous Brachy (T/T) mouse embryos in the extraembryonic coelom of the chick. *Proc Natl Acad U S A*. 1944;30:134-140.
35. Fujimoto H, Yanagisawa KO. Defects in the archenteron of mouse embryos homozygous for the T-mutation. *Differentiation*. 1983;25:44-47.
36. Wilson V, Manson L, Skarnes WC, Beddington RS. The T gene is necessary for normal mesodermal morphogenetic cell movements during gastrulation. *Development*. 1995;121:877-886.
37. Fehling HJ, Lacaud G, Kubo A, et al. Tracking mesoderm induction and its specification to the hemangioblast during embryonic stem cell differentiation. *Development*. 2003;130:4217-4227.
38. Yamaguchi TP, Dumont DJ, Conlon RA, Breitman ML, Rossant J. flk-1, an flt-related receptor tyrosine kinase is an early marker for endothelial cell precursors. *Development*. 1993;118:489-498.
39. Shalaby F, Ho J, Stanford WL, et al. A requirement for Flk1 in primitive and definitive hematopoiesis and vasculogenesis. *Cell*. 1997;89:981-990.
40. Bloor AJ, Sánchez MJ, Green AR, Göttgens B. The role of the stem cell leukemia (SCL) gene in hematopoietic and endothelial lineage specification. *J Hematother Stem Cell Res*. 2002;11:195-206.
41. Bedinger P, Moriarty A, von Borstel RCn, Donovan NJ, Steimer KS, Littman DR. Internalization of the human immunodeficiency virus does not require the cytoplasmic domain of CD4. *Nature*. 1988;334:162-165.
42. Yamashita J, Itoh H, Hirashima M, et al. Flk1-positive cells derived from embryonic stem cells serve as vascular progenitors. *Nature*. 2000;408:92-96.

43. Ema M, Faloon P, Zhang WJ, et al. Combinatorial effects of Flk1 and Tal1 on vascular and hematopoietic development in the mouse. *Genes Dev.* 2003;17:380-393.
44. Motoike T, Markham DW, Rossant J, Sato TN. Evidence for novel fate of Flk1+ progenitor: Contribution to muscle lineage. *Genesis.* 2003;35:153-159.
45. Ema M, Takahashi S, Rossant J. Deletion of the selection cassette, but not cis-acting elements, in targeted Flk1-lacZ allele reveals Flk1 expression in multipotent mesodermal progenitors. *Blood.* 2006;107:111-117.
46. Sharrocks AD. The ETS-domain transcription factor family. *Nat Rev Mol Cell Biol.* 2001;2:827-837.
47. Wei G-H, Badis G, Berger MF, et al. Genome-wide analysis of ETS-family DNA-binding in vitro and in vivo. *EMBO J.* 2010;29:2147-2160.
48. Hollenhorst PC, McIntosh LP, Graves BJ. Genomic and biochemical insights into the specificity of ETS transcription factors. *Annu Rev Biochem.* 2011;80:437-471.
49. Verger A, Duterque-Coquillaud M. When Ets transcription factors meet their partners. *Bioessays.* 2002;24:362-370.
50. Hsu T, Trojanowska M, Watson DK. Ets proteins in biological control and cancer. *J Cell Biochem.* 2004;91:896-903.
51. Lammerts van Bueren K, Black BL. Regulation of endothelial and hematopoietic development by the ETS transcription factor Etv2. *Curr Opin Hematol.* 2012;19:199-205.
52. De Haro L, Janknecht R. Functional analysis of the transcription factor ER71 and its activation of the matrix metalloproteinase-1 promoter. *Nucleic Acids Res.* 2002;30:2972-2979.
53. De Haro L, Janknecht R. Cloning of the murine ER71 gene (Etsrp71) and initial characterization of its promoter. *Genomics.* 2005;85:493-502.
54. Brown TA, McKnight SL. Specificities of protein-protein and protein-DNA interaction of GABP alpha and two newly defined ets-related proteins. *Genes Dev.* 1992;6:2502-2512.
55. Lee D, Park C, Lee H, et al. ER71 acts downstream of BMP, Notch, and Wnt signaling in blood and vessel progenitor specification. *Cell Stem Cell.* 2008;2:497-507.
56. Ferdous A, Caprioli A, Iacovino M, et al. Nkx2-5 transactivates the Ets-related protein 71 gene and specifies an endothelial/endocardial fate in the developing embryo. *Proc Natl Acad U S A.* 2009;106:814-819.
57. Kataoka H, Hayashi M, Nakagawa R, et al. Etv2/ER71 induces vascular mesoderm from Flk1+PDGFR α + primitive mesoderm. *Blood.* 2011;118:6975-6986.
58. Rasmussen TL, Kweon J, Diekmann MA, et al. ER71 directs mesodermal fate decisions during embryogenesis. *Development.* 2011;138:4801-4812.
59. Doetschman TC, Eistetter H, Katz M, Schmidt W, Kemler R. The in vitro development of blastocyst-derived embryonic stem cell lines: formation of visceral yolk sac, blood islands and myocardium. *J Embryol Exp Morphol.* 1985;87:27-45.
60. Keller GM. In vitro differentiation of embryonic stem cells. *Curr Opin Cell Biol.* 1995;7:862-869.
61. Yoshida H, Hayashi S, Kunisada T, et al. The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. *Nature.* 1990;345:442-444.
62. Nakano T, Kodama H, Honjo T. Generation of lymphohematopoietic cells from embryonic stem cells in culture. *Science.* 1994;265:1098-1101.
63. De Val S, Chi NC, Meadows SM, et al. Combinatorial regulation of endothelial gene expression by Ets and Forkhead transcription factors. *Cell.* 2008;135:1053-1064.

64. Palencia-Desai S, Kohli V, Kang J, Chi NC, Black BL, Sumanas S. Vascular endothelial and endocardial progenitors differentiate as cardiomyocytes in the absence of *Etsrp/Etv2* function. *Development*. 2011;138:4721-4732.
65. Sumanas S, Joraniak T, Lin S. Identification of novel vascular endothelial-specific genes by the microarray analysis of the zebrafish *cloche* mutants. *Blood*. 2005;106:534-541.
66. Sumanas S, Gomez G, Zhao Y, Park C, Choi K, Lin S. Interplay among *Etsrp/ER71*, *Scl*, and *Alk8* signaling controls endothelial and myeloid cell formation. *Blood*. 2008;111:4500-4510.
67. Neuhaus H, Müller F, Hollemann T. *Xenopus* *er71* is involved in vascular development. *Dev Dyn*. 2010;239:3436-3445.
68. Yamamizu K, Matsunaga T, Katayama S, et al. PKA/CREB signaling triggers initiation of endothelial and hematopoietic cell differentiation via *Etv2* induction. *Stem Cells*. 2012;30:687-696.
69. Lyons I, Parsons LM, Hartley L, et al. Myogenic and morphogenetic defects in the heart tubes of murine embryos lacking the homeo box gene *Nkx2-5*. *Genes Dev*. 1995;9:1654-1666.
70. Tanaka M, Chen Z, Bartunkova S, Yamasaki N, Izumo S. The cardiac homeobox gene *Csx/Nkx2.5* lies genetically upstream of multiple genes essential for heart development. *Development*. 1999;126:1269-1280.
71. Stanley EG, Biben C, Elefanty A, et al. Efficient Cre-mediated deletion in cardiac progenitor cells conferred by a 3'UTR-ires-Cre allele of the homeobox gene *Nkx2-5*. *Int J Dev Biol*. 2002;46:431-439.
72. Veldman MB, Lin S. *Etsrp/Etv2* is directly regulated by *foxc1a/b* in the zebrafish angioblast. *Circ Res*. 2012;110:220-229.
73. Sakurai H, Era T, Jakt LM, Okada M, Nakai S, Nishikawa S. In vitro modeling of paraxial and lateral mesoderm differentiation reveals early reversibility. *Stem Cells*. 2006;24:575-586.
74. Hirata H, Kawamata S, Murakami Y, et al. Coexpression of platelet-derived growth factor receptor alpha and fetal liver kinase 1 enhances cardiogenic potential in embryonic stem cell differentiation in vitro. *J Biosci Bioeng*. 2007;103:412-419.
75. Liu F, Kang I, Park C, et al. *ER71* specifies *Flk-1+* hemangiogenic mesoderm by inhibiting cardiac mesoderm and *Wnt* signaling. *Blood*. 2012;119:3295-3305.
76. Yelon D, Ticho B, Halpern ME, et al. The bHLH transcription factor *hand2* plays parallel roles in zebrafish heart and pectoral fin development. *Development*. 2000;127:2573-2582.
77. Schoenebeck JJ, Keegan BR, Yelon D. Vessel and blood specification override cardiac potential in anterior mesoderm. *Dev Cell*. 2007;13:254-267.
78. Stenman JM, Rajagopal J, Carroll TJ, Ishibashi M, McMahon J, McMahon AP. Canonical *Wnt* signaling regulates organ-specific assembly and differentiation of CNS vasculature. *Science*. 2008;322:1247-1250.
79. Lugus JJ, Park C, Ma YD, Choi K. Both primitive and definitive blood cells are derived from *Flk-1+* mesoderm. *Blood*. 2009;113:563-566.
80. Proulx K, Lu A, Sumanas S. Cranial vasculature in zebrafish forms by angioblast cluster-derived angiogenesis. *Dev Biol*. 2010;348:34-46.
81. Hollenhorst PC, Jones DA, Graves BJ. Expression profiles frame the promoter specificity dilemma of the ETS family of transcription factors. *Nucleic Acids Res*. 2004;32:5693-5702.
82. Hayashi M, Pluchinotta M, Momiyama A, Tanaka Y, Nishikawa S, Kataoka H. Endothelialization and altered hematopoiesis by persistent *Etv2* expression in mice. *Exp Hematol*. 2012;40:738-750 e711.

83. Ginsberg M, James D, Ding B-S, et al. Efficient direct reprogramming of mature amniotic cells into endothelial cells by ETS factors and TGF β suppression. *Cell*. 2012;151:559-575.
84. Orkin SH. Development of the hematopoietic system. *Curr Opin Genet Dev*. 1996;6:597-602.
85. Anantharaman A, Lin IJ, Barrow J, et al. Role of helix-loop-helix proteins during differentiation of erythroid cells. *Mol Cell Biol*. 2011;31:1332-1343.
86. Robb L, Lyons I, Li R, et al. Absence of yolk sac hematopoiesis from mice with a targeted disruption of the scl gene. *Proc Natl Acad U S A*. 1995;92:7075-7079.
87. Shivdasani RA, Mayer EL, Orkin SH. Absence of blood formation in mice lacking the T-cell leukaemia oncoprotein tal-1/SCL. *Nature*. 1995;373:432-434.
88. Porcher C, Swat W, Rockwell K, Fujiwara Y, Alt FW, Orkin SH. The T cell leukemia oncoprotein SCL/tal-1 is essential for development of all hematopoietic lineages. *Cell*. 1996;86:47-57.
89. Gering M, Rodaway ARF, Göttgens B, Patient RK, Green AR. The SCL gene specifies haemangioblast development from early mesoderm. *EMBO J*. 1998;17:4029-4045.
90. Ren X, Gomez GA, Zhang B, Lin S. Scl isoforms act downstream of etsrp to specify angioblasts and definitive hematopoietic stem cells. *Blood*. 2010;115:5338-5346.
91. Visvader JE, Fujiwara Y, Orkin SH. Unsuspected role for the T-cell leukemia protein SCL/tal-1 in vascular development. *Genes Dev*. 1998;12:473-479.
92. Robertson SM, Kennedy M, Shannon JM, Keller G. A transitional stage in the commitment of mesoderm to hematopoiesis requiring the transcription factor SCL/tal-1. *Development*. 2000;127:2447-2459.
93. Kappel A, Schlaeger TM, Flamme I, Orkin SH, Risau W, Breier G. Role of SCL/Tal-1, GATA, and Ets transcription factor binding sites for the regulation of Flk-1 expression during murine vascular development. *Blood*. 2000;96:3078-3085.
94. Tanaka A, Itoh F, Nishiyama K, et al. Inhibition of endothelial cell activation by bHLH protein E2-2 and its impairment of angiogenesis. *Blood*. 2010;115:4138-4147.
95. Weiss MJ, Orkin SH. GATA transcription factors: key regulators of hematopoiesis. *Exp Hematol*. 1995;23:99-107.
96. Molkenstein JD. The zinc finger-containing transcription factors GATA-4, -5, and -6. Ubiquitously expressed regulators of tissue-specific gene expression. *J Biol Chem*. 2000;275:38949-38952.
97. Orkin SH. GATA-binding transcription factors in hematopoietic cells. *Blood*. 1992;80:575-581.
98. Pevny L, Lin CS, D'Agati V, Simon MC, Orkin SH, Costantini F. Development of hematopoietic cells lacking transcription factor GATA-1. *Development*. 1995;121:163-172.
99. Fujiwara Y, Browne CP, Cunniff K, Goff SC, Orkin SH. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. *Proc Natl Acad Sci U S A*. 1996;93:12355-12358.
100. Tsai FY, Keller G, Kuo FC, et al. An early haematopoietic defect in mice lacking the transcription factor GATA-2. *Nature*. 1994;371:221-226.
101. Tsai FY, Orkin SH. Transcription factor GATA-2 is required for proliferation/survival of early hematopoietic cells and mast cell formation, but not for erythroid and myeloid terminal differentiation. *Blood*. 1997;89:3636-3643.
102. Lee ME, Temizer DH, Clifford JA, Quertermous T. Cloning of the GATA-binding protein that regulates endothelin-1 gene expression in endothelial cells. *J Biol Chem*. 1991;266:16188-16192.

103. Minko K, Bollerot K, Drevon C, Hallais MF, Jaffredo T. From mesoderm to blood islands: patterns of key molecules during yolk sac erythropoiesis. *Gene Expr Patterns*. 2003;3:261-272.
104. Lim KC, Hosoya T, Brandt W, et al. Conditional Gata2 inactivation results in HSC loss and lymphatic mispatterning. *J Clin Invest*. 2012;122:3705-3717.
105. Lugus JJ, Chung YS, Mills JC, et al. GATA2 functions at multiple steps in hemangioblast development and differentiation. *Development*. 2007;134:393-405.
106. Kobayashi-Osaki M, Ohneda O, Suzuki N, et al. GATA motifs regulate early hematopoietic lineage-specific expression of the Gata2 gene. *Mol Cell Biol*. 2005;25:7005-7020.
107. Khandekar M, Brandt W, Zhou Y, et al. A Gata2 intronic enhancer confers its pan-endothelia-specific regulation. *Development*. 2007;134:1703-1712.
108. Pimanda JE, Chan WY, Wilson NK, et al. Endoglin expression in blood and endothelium is differentially regulated by modular assembly of the Ets/Gata hemangioblast code. *Blood*. 2008;112:4512-4522.
109. Kanki Y, Kohro T, Jiang S, et al. Epigenetically coordinated GATA2 binding is necessary for endothelium-specific endomucin expression. *EMBO J*. 2011;30:2582-2595.
110. Song H, Suehiro J, Kanki Y, et al. Critical role for GATA3 in mediating Tie2 expression and function in large vessel endothelial cells. *J Biol Chem*. 2009;284:29109-29124.
111. Benezra R, Rafii S, Lyden D. The Id proteins and angiogenesis. *Oncogene*. 2001;20:8334-8341.
112. Jen Y, Manova K, Benezra R. Each member of the Id gene family exhibits a unique expression pattern in mouse gastrulation and neurogenesis. *Dev Dyn*. 1997;208:92-106.
113. Lyden D, Young AZ, Zagzag D, et al. Id1 and Id3 are required for neurogenesis, angiogenesis and vascularization of tumour xenografts. *Nature*. 1999;401:670-677.
114. Valdimarsdottir G, Goumans MJ, Rosendahl A, et al. Stimulation of Id1 expression by bone morphogenetic protein is sufficient and necessary for bone morphogenetic protein-induced activation of endothelial cells. *Circulation*. 2002;106:2263-2270.
115. Sakurai D, Tsuchiya N, Yamaguchi A, et al. Crucial role of inhibitor of DNA binding/differentiation in the vascular endothelial growth factor-induced activation and angiogenic processes of human endothelial cells. *J Immunol*. 2004;173:5801-5809.
116. Tanaka A, Itoh F, Itoh S, Kato M. TAL1/SCL relieves the E2-2-mediated repression of VEGFR2 promoter activity. *J Biochem*. 2009;145:129-135.
117. Korchynskyi O, ten Dijke P. Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the Id1 promoter. *J Biol Chem*. 2002;277:4883-4891.
118. Kola I, Brookes S, Green AR, et al. The Ets1 transcription factor is widely expressed during murine embryo development and is associated with mesodermal cells involved in morphogenetic processes such as organ formation. *Proc Natl Acad Sci U S A*. 1993;90:7588-7592.
119. Maroulakou IG, Papas TS, Green JE. Differential expression of ets-1 and ets-2 proto-oncogenes during murine embryogenesis. *Oncogene*. 1994;9:1551-1565.
120. Melet F, Motro B, Rossi DJ, Zhang L, Bernstein A. Generation of a novel Fli-1 protein by gene targeting leads to a defect in thymus development and a delay in Friend virus-induced erythroleukemia. *Mol Cell Biol*. 1996;16:2708-2718.
121. Vlaeminck-Guillem V, Carrere S, Dewitte F, Stehelin D, Desbiens X, Duterque-Coquillaud M. The Ets family member Erg gene is expressed in mesodermal tissues and neural crests at fundamental steps during mouse embryogenesis. *Mech Dev*. 2000;91:331-335.

122. Wang LC, Kuo F, Fujiwara Y, Gilliland DG, Golub TR, Orkin SH. Yolk sac angiogenic defect and intra-embryonic apoptosis in mice lacking the Ets-related factor TEL. *EMBO J*. 1997;16:4374-4383.
123. Lopez RG, Carron C, Oury C, Gardellin P, Bernard O, Ghysdael J. TEL is a sequence-specific transcriptional repressor. *J Biol Chem*. 1999;274:30132-30138.
124. Bartel FO, Higuchi T, Spyropoulos DD. Mouse models in the study of the Ets family of transcription factors. *Oncogene*. 2000;19:6443-6454.
125. Barton K, Muthusamy N, Fischer C, et al. The Ets-1 transcription factor is required for the development of natural killer cells in mice. *Immunity*. 1998;9:555-563.
126. Wei G, Srinivasan R, Cantemir-Stone CZ, et al. Ets1 and Ets2 are required for endothelial cell survival during embryonic angiogenesis. *Blood*. 2009;114:1123-1130.
127. Yoshimatsu Y, Yamazaki T, Mihira H, et al. Ets family members induce lymphangiogenesis through physical and functional interaction with Prox1. *J Cell Sci*. 2011;124:2753-2762.
128. Liu F, Walmsley M, Rodaway A, Patient R. Fli1 acts at the top of the transcriptional network driving blood and endothelial development. *Curr Biol*. 2008;18:1234-1240.
129. Spyropoulos DD, Pharr PN, Lavenburg KR, et al. Hemorrhage, impaired hematopoiesis, and lethality in mouse embryos carrying a targeted disruption of the Fli1 transcription factor. *Mol Cell Biol*. 2000;20:5643-5652.
130. Loughran SJ, Kruse EA, Hacking DF, et al. The transcription factor Erg is essential for definitive hematopoiesis and the function of adult hematopoietic stem cells. *Nat Immunol*. 2008;9:810-819.
131. Birdsey GM, Dryden NH, Amsellem V, et al. Transcription factor Erg regulates angiogenesis and endothelial apoptosis through VE-cadherin. *Blood*. 2008;111:3498-3506.
132. Furuyama T, Nakazawa T, Nakano I, Mori N. Identification of the differential distribution patterns of mRNAs and consensus binding sequences for mouse DAF-16 homologues. *Biochem J*. 2000;349:629-634.
133. Papanicolaou KN, Izumiya Y, Walsh K. Forkhead transcription factors and cardiovascular biology. *Circ Res*. 2008;102:16-31.
134. Burgering BMT, Medema RH. Decisions on life and death: FOXO Forkhead transcription factors are in command when PKB/Akt is off duty. *J Leukoc Biol*. 2003;73:689-701.
135. Furuyama T, Kitayama K, Shimoda Y, et al. Abnormal Angiogenesis in Foxo1 (Fkhr)-deficient Mice. *J Biol Chem*. 2004;279:34741-34749.
136. Hosaka T, Biggs WH, Tieu D, et al. Disruption of forkhead transcription factor (FOXO) family members in mice reveals their functional diversification. *Proc Natl Acad U S A*. 2004;101:2975-2980.
137. Delorme B, Dahl E, Jarry-Guichard T, et al. Expression pattern of connexin gene products at the early developmental stages of the mouse cardiovascular system. *Circ Res*. 1997;81:423-437.
138. Simon AM, McWhorter AR. Role of connexin37 and connexin40 in vascular development. *Cell Commun Adhes*. 2003;10:379-385.
139. Potente M, Urbich C, Sasaki K-i, et al. Involvement of Foxo transcription factors in angiogenesis and postnatal neovascularization. *J Clin Invest*. 2005;115:2382-2392.
140. Bir SC, Xiong Y, Kevil CG, Luo J. Emerging role of PKA/eNOS pathway in therapeutic angiogenesis for ischaemic tissue diseases. *Cardiovasc Res*. 2012;95:7-18.
141. Paik J-H, Kollipara R, Chu G, et al. FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell*. 2007;128:309-323.

142. Winnier GE, Kume T, Deng K, et al. Roles for the winged helix transcription factors MF1 and MFH1 in cardiovascular development revealed by nonallelic noncomplementation of null alleles. *Dev Biol.* 1999;213:418-431.
143. Petrova TV, Karpanen T, Norrmen C, et al. Defective valves and abnormal mural cell recruitment underlie lymphatic vascular failure in lymphedema distichiasis. *Nat Med.* 2004;10:974-981.
144. Kume T, Jiang H, Topczewska JM, Hogan BL. The murine winged helix transcription factors, Foxc1 and Foxc2, are both required for cardiovascular development and somitogenesis. *Genes Dev.* 2001;15:2470-2482.
145. Seo S, Fujita H, Nakano A, Kang M, Duarte A, Kume T. The forkhead transcription factors, Foxc1 and Foxc2, are required for arterial specification and lymphatic sprouting during vascular development. *Dev Biol.* 2006;294:458-470.
146. Hayashi H, Kume T. Foxc transcription factors directly regulate Dll4 and Hey2 expression by interacting with the VEGF-Notch signaling pathways in endothelial cells. *PLoS ONE.* 2008;3.
147. Norrmen C, Ivanov KI, Cheng J, et al. FOXC2 controls formation and maturation of lymphatic collecting vessels through cooperation with NFATc1. *J Cell Biol.* 2009;185:439-457.
148. Fang J, Dagenais SL, Erickson RP, et al. Mutations in FOXC2 (MFH-1), a forkhead family transcription factor, are responsible for the hereditary lymphedema-distichiasis syndrome. *Am J Hum Genet.* 2000;67:1382-1388.
149. Peterson RS, Lim L, Ye H, Zhou H, Overdier DG, Costa RH. The winged helix transcriptional activator HFH-8 is expressed in the mesoderm of the primitive streak stage of mouse embryos and its cellular derivatives. *Mech Dev.* 1997;69:53-69.
150. Mahlapuu M, Ormestad M, Enerback S, Carlsson P. The forkhead transcription factor Foxf1 is required for differentiation of extra-embryonic and lateral plate mesoderm. *Development.* 2001;128:155-166.
151. Astorga J, Carlsson P. Hedgehog induction of murine vasculogenesis is mediated by Foxf1 and Bmp4. *Development.* 2007;134:3753-3761.
152. Ishitobi H, Wakamatsu A, Liu F, et al. Molecular basis for Flk1 expression in hemato-cardiovascular progenitors in the mouse. *Development.* 2011;138:5357-5368.
153. Choi J, Dong L, Ahn J, Dao D, Hammerschmidt M, Chen J-N. FoxH1 negatively modulates flk1 gene expression and vascular formation in zebrafish. *Dev Biol.* 2007;304:735-744.
154. Zhao YY, Gao XP, Zhao YD, et al. Endothelial cell-restricted disruption of FoxM1 impairs endothelial repair following LPS-induced vascular injury. *J Clin Invest.* 2006;116:2333-2343.
155. Mirza MK, Sun Y, Zhao YD, et al. FoxM1 regulates re-annealing of endothelial adherens junctions through transcriptional control of beta-catenin expression. *J Exp Med.* 2010;207:1675-1685.
156. Harvey NL, Oliver G. Choose your fate: artery, vein or lymphatic vessel? *Curr Opin Genet Dev.* 2004;14:499-505.
157. Swift MR, Weinstein BM. Arterial-venous specification during development. *Circ Res.* 2009;104:576-588.
158. Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol.* 2003;194:237-255.
159. Fischer A, Schumacher N, Maier M, Sendtner M, Gessler M. The Notch target genes Hey1 and Hey2 are required for embryonic vascular development. *Genes Dev.* 2004;18:901-911.

160. Kokubo H, Miyagawa-Tomita S, Nakazawa M, Saga Y, Johnson RL. Mouse hesr1 and hesr2 genes are redundantly required to mediate Notch signaling in the developing cardiovascular system. *Dev Biol.* 2005;278:301-309.
161. Zhong TP, Childs S, Leu JP, Fishman MC. Gridlock signalling pathway fashions the first embryonic artery. *Nature.* 2001;414:216-220.
162. Krebs LT, Shutter JR, Tanigaki K, Honjo T, Stark KL, Gridley T. Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants. *Genes Dev.* 2004;18:2469-2473.
163. Lefebvre V, Dumitriu B, Penzo-Mendez A, Han Y, Pallavi B. Control of cell fate and differentiation by Sry-related high-mobility-group box (Sox) transcription factors. *Int J Biochem Cell Biol.* 2007;39:2195-2214.
164. Francois M, Koopman P, Beltrame M. SoxF genes: Key players in the development of the cardio-vascular system. *Int J Biochem Cell Biol.* 2010;42:445-448.
165. Pennisi D, Gardner J, Chambers D, et al. Mutations in Sox18 underlie cardiovascular and hair follicle defects in ragged mice. *Nat Genet.* 2000;24:434-437.
166. Matsui T, Kanai-Azuma M, Hara K, et al. Redundant roles of Sox17 and Sox18 in postnatal angiogenesis in mice. *J Cell Sci.* 2006;119:3513-3526.
167. Sakamoto Y, Hara K, Kanai-Azuma M, et al. Redundant roles of Sox17 and Sox18 in early cardiovascular development of mouse embryos. *Biochem Biophys Res Commun.* 2007;360:539-544.
168. Francois M, Caprini A, Hosking B, et al. Sox18 induces development of the lymphatic vasculature in mice. *Nature.* 2008;456:643-647.
169. Downes M, Koopman P. SOX18 and the Transcriptional Regulation of Blood Vessel Development. *Trends Cardiovasc Med.* 2001;11:318-324.
170. James K, Hosking B, Gardner J, Muscat GEO, Koopman P. Sox18 mutations in the ragged mouse alleles ragged-like and opossum. *Genesis.* 2003;36:1-6.
171. Pennisi D, Bowles J, Nagy A, Muscat G, Koopman P. Mice null for sox18 are viable and display a mild coat defect. *Mol Cell Biol.* 2000;20:9331-9336.
172. Hosking B, Francois M, Wilhelm D, et al. Sox7 and Sox17 are strain-specific modifiers of the lymphangiogenic defects caused by Sox18 dysfunction in mice. *Development.* 2009;136:2385-2391.
173. Herpers R, van de Kamp E, Duckers HJ, Schulte-Merker S. Redundant roles for sox7 and sox18 in arteriovenous specification in zebrafish. *Circ Res.* 2008;102:12-15.
174. Pendeville H, Winandy M, Manfroid I, et al. Zebrafish Sox7 and Sox18 function together to control arterial-venous identity. *Dev Biol.* 2008;317:405-416.
175. Wigle JT, Oliver G. Prox1 function is required for the development of the murine lymphatic system. *Cell.* 1999;98:769-778.
176. Hong YK, Harvey N, Noh YH, et al. Prox1 is a master control gene in the program specifying lymphatic endothelial cell fate. *Dev Dyn.* 2002;225:351-357.
177. Wigle JT, Harvey N, Detmar M, et al. An essential role for Prox1 in the induction of the lymphatic endothelial cell phenotype. *EMBO J.* 2002;21:1505-1513.
178. Johnson NC, Dillard ME, Baluk P, et al. Lymphatic endothelial cell identity is reversible and its maintenance requires Prox1 activity. *Genes Dev.* 2008;22:3282-3291.
179. Petrova TV, Mäkinen T, Mäkelä TP, et al. Lymphatic endothelial reprogramming of vascular endothelial cells by the Prox-1 homeobox transcription factor. *EMBO J.* 2002;21:4593-4599.
180. Kim H, Nguyen VPKH, Petrova TV, Cruz M, Alitalo K, Dumont DJ. Embryonic vascular endothelial cells are malleable to reprogramming via Prox1 to a lymphatic gene signature. *BMC Dev Biol.* 2010;10.

181. Schacht V, Ramirez MI, Hong YK, et al. T1alpha/podoplanin deficiency disrupts normal lymphatic vasculature formation and causes lymphedema. *EMBO J.* 2003;22:3546-3556.
182. Pereira FA, Qiu Y, Zhou G, Tsai MJ, Tsai SY. The orphan nuclear receptor COUP-TFII is required for angiogenesis and heart development. *Genes Dev.* 1999;13:1037-1049.
183. Naka H, Nakamura S, Shimazaki T, Okano H. Requirement for COUP-TFI and II in the temporal specification of neural stem cells in CNS development. *Nat Neurosci.* 2008;11:1014-1023.
184. You LR, Lin FJ, Lee CT, DeMayo FJ, Tsai MJ, Tsai SY. Suppression of Notch signalling by the COUP-TFII transcription factor regulates vein identity. *Nature.* 2005;435:98-104.
185. Kawasaki T, Kitsukawa T, Bekku Y, et al. A requirement for neuropilin-1 in embryonic vessel formation. *Development.* 1999;126:4895-4902.
186. Huppert SS, Le A, Schroeter EH, et al. Embryonic lethality in mice homozygous for a processing-deficient allele of Notch1. *Nature.* 2000;405:966-970.
187. Srinivasan RS, Geng X, Yang Y, et al. The nuclear hormone receptor Coup-TFII is required for the initiation and early maintenance of Prox1 expression in lymphatic endothelial cells. *Genes Dev.* 2010;24:696-707.
188. Davis RB, Curtis CD, Griffin CT. BRG1 promotes COUP-TFII expression and venous specification during embryonic vascular development. *Development.* 2013;140:1272-1281.
189. de la Serna IL, Ohkawa Y, Imbalzano AN. Chromatin remodelling in mammalian differentiation: lessons from ATP-dependent remodellers. *Nat Rev Genet.* 2006;7:461-473.
190. Griffin CT, Brennan J, Magnuson T. The chromatin-remodeling enzyme BRG1 plays an essential role in primitive erythropoiesis and vascular development. *Development.* 2008;135:493-500.
191. Shin JW, Min M, Larrieu-Lahargue Fd, et al. Prox1 promotes lineage-specific expression of fibroblast growth factor (FGF) receptor-3 in lymphatic endothelium: a role for FGF signaling in lymphangiogenesis. *Mol Biol Cell.* 2006;17:576-584.
192. Lee S, Kang J, Yoo J, et al. Prox1 physically and functionally interacts with COUP-TFII to specify lymphatic endothelial cell fate. *Blood.* 2009;113:1856-1859.
193. Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. *Curr Opin Cell Biol.* 2009;21:154-165.
194. Bates DO. Vascular endothelial growth factors and vascular permeability. *Cardiovasc Res.* 2010;87:262-271.
195. Rousseau S, Houle F, Landry J, Huot J. p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. *Oncogene.* 1997;15:2169-2177.
196. Takahashi T, Yamaguchi S, Chida K, Shibuya M. A single autophosphorylation site on KDR/Flk-1 is essential for VEGF-A-dependent activation of PLC-gamma and DNA synthesis in vascular endothelial cells. *EMBO J.* 2001;20:2768-2778.
197. Abid MR, Guo S, Minami T, et al. Vascular endothelial growth factor activates PI3K/Akt/forkhead signaling in endothelial cells. *Arterioscler Thromb Vasc Biol.* 2004;24:294-300.
198. Olsson AK, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol.* 2006;7:359-371.
199. Ferrara N, Carver-Moore K, Chen H, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature.* 1996;380:439-442.

200. Lawson ND, Vogel AM, Weinstein BM. sonic hedgehog and vascular endothelial growth factor act upstream of the Notch pathway during arterial endothelial differentiation. *Dev Cell*. 2002;3:127-136.
201. Incardona JP, Gaffield W, Kapur RP, Roelink H. The teratogenic Veratrum alkaloid cyclopamine inhibits sonic hedgehog signal transduction. *Development*. 1998;125:3553-3562.
202. Mukouyama YS, Gerber HP, Ferrara N, Gu C, Anderson DJ. Peripheral nerve-derived VEGF promotes arterial differentiation via neuropilin 1-mediated positive feedback. *Development*. 2005;132:941-952.
203. Visconti RP, Richardson CD, Sato TN. Orchestration of angiogenesis and arteriovenous contribution by angiopoietins and vascular endothelial growth factor (VEGF). *Proc Natl Acad Sci U S A*. 2002;99:8219-8224.
204. Gridley T. Notch signaling in vascular development and physiology. *Development*. 2007;134:2709-2718.
205. Gridley T. Notch signaling in the vasculature. *Curr Top Dev Biol*. 2010;92:277-309.
206. Andersson ER, Sandberg R, Lendahl U. Notch signaling: simplicity in design, versatility in function. *Development*. 2011;138:3593-3612.
207. Duarte A, Hirashima M, Benedito R, et al. Dosage-sensitive requirement for mouse Dll4 in artery development. *Genes Dev*. 2004;18:2474-2478.
208. Trindade A, Kumar SR, Scehnet JS, et al. Overexpression of delta-like 4 induces arterialization and attenuates vessel formation in developing mouse embryos. *Blood*. 2008;112:1720-1729.
209. Krebs LT, Xue Y, Norton CR, et al. Notch signaling is essential for vascular morphogenesis in mice. *Genes Dev*. 2000;14:1343-1352.
210. Liu ZJ, Shirakawa T, Li Y, et al. Regulation of Notch1 and Dll4 by vascular endothelial growth factor in arterial endothelial cells: implications for modulating arteriogenesis and angiogenesis. *Mol Cell Biol*. 2003;23:14-25.
211. Minami T, Horiuchi K, Miura M, et al. Vascular endothelial growth factor- and thrombin-induced termination factor, Down syndrome critical region-1, attenuates endothelial cell proliferation and angiogenesis. *J Biol Chem*. 2004;279:50537-50554.
212. Abid MR, Shih SC, Otu HH, et al. A novel class of vascular endothelial growth factor-responsive genes that require forkhead activity for expression. *J Biol Chem*. 2006;281:35544-35553.
213. Eilken HM, Adams RH. Dynamics of endothelial cell behavior in sprouting angiogenesis. *Curr Opin Cell Biol*. 2010;22:617-625.
214. Siekmann AF, Affolter M, Belting HG. The tip cell concept 10 years after: New players tune in for a common theme. *Exp Cell Res*. 2013.
215. Miyazono K, Kamiya Y, Morikawa M. Bone morphogenetic protein receptors and signal transduction. *J Biochem*. 2010;147:35-51.
216. Cai J, Pardali E, Sanchez-Duffhues G, ten Dijke P. BMP signaling in vascular diseases. *FEBS Lett*. 2012;586:1993-2002.
217. Goumans MJ, Mummery C. Functional analysis of the TGFbeta receptor/Smad pathway through gene ablation in mice. *Int J Dev Biol*. 2000;44:253-265.
218. Winnier G, Blessing M, Labosky PA, Hogan BL. Bone morphogenetic protein-4 is required for mesoderm formation and patterning in the mouse. *Genes Dev*. 1995;9:2105-2116.
219. Mishina Y, Suzuki A, Ueno N, Behringer RR. Bmpr encodes a type I bone morphogenetic protein receptor that is essential for gastrulation during mouse embryogenesis. *Genes Dev*. 1995;9:3027-3037.

220. Park C, Afrikanova I, Chung YS, et al. A hierarchical order of factors in the generation of FLK1- and SCL-expressing hematopoietic and endothelial progenitors from embryonic stem cells. *Development*. 2004;131:2749-2762.
221. Zimmerman LB, De Jesus-Escobar JM, Harland RM. The Spemann organizer signal noggin binds and inactivates bone morphogenetic protein 4. *Cell*. 1996;86:599-606.
222. Mao B, Wu W, Davidson G, et al. Kremen proteins are Dickkopf receptors that regulate Wnt/beta-catenin signalling. *Nature*. 2002;417:664-667.
223. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*. 2004;20:781-810.
224. Nusse R. Wnt signaling in disease and in development. *Cell Res*. 2005;15:28-32.
225. Kuhl M. The WNT/calcium pathway: biochemical mediators, tools and future requirements. *Front Biosci*. 2004;9:967-974.
226. Cirone P, Lin S, Griesbach HL, Zhang Y, Slusarski DC, Crews CM. A role for planar cell polarity signaling in angiogenesis. *Angiogenesis*. 2008;11:347-360.
227. MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell*. 2009;17:9-26.
228. Lengerke C, Schmitt S, Bowman TV, et al. BMP and Wnt specify hematopoietic fate by activation of the Cdx-Hox pathway. *Cell Stem Cell*. 2008;2:72-82.
229. Lindsley RC, Gill JG, Kyba M, Murphy TL, Murphy KM. Canonical Wnt signaling is required for development of embryonic stem cell-derived mesoderm. *Development*. 2006;133:3787-3796.
230. Cattelino A, Liebner S, Gallini R, et al. The conditional inactivation of the beta-catenin gene in endothelial cells causes a defective vascular pattern and increased vascular fragility. *J Cell Biol*. 2003;162:1111-1122.
231. Liebner S, Cattelino A, Gallini R, et al. Beta-catenin is required for endothelial-mesenchymal transformation during heart cushion development in the mouse. *J Cell Biol*. 2004;166:359-367.
232. Daneman R, Agalliu D, Zhou L, Kuhnert F, Kuo CJ, Barres BA. Wnt/beta-catenin signaling is required for CNS, but not non-CNS, angiogenesis. *Proc Natl Acad Sci U S A*. 2009;106:641-646.
233. Corada M, Nyqvist D, Orsenigo F, et al. The Wnt/beta-catenin pathway modulates vascular remodeling and specification by upregulating Dll4/Notch signaling. *Dev Cell*. 2010;18:938-949.
234. Waddington CH. *The strategy of the genes*. London: George Allen and Unwin; 1957.
235. Gurdon JB. From nuclear transfer to nuclear reprogramming: the reversal of cell differentiation. *Annu Rev Cell Dev Biol*. 2006;22:1-22.
236. Weintraub H, Tapscott SJ, Davis RL, et al. Activation of muscle-specific genes in pigment, nerve, fat, liver, and fibroblast cell lines by forced expression of MyoD. *Proc Natl Acad Sci U S A*. 1989;86:5434-5438.
237. Xie H, Ye M, Feng R, Graf T. Stepwise reprogramming of B cells into macrophages. *Cell*. 2004;117:663-676.
238. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006;126:663-676.
239. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell*. 2007;131:861-872.
240. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science*. 2007;318:1917-1920.
241. Park IH, Zhao R, West JA, et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*. 2008;451:141-146.

242. Margariti A, Winkler B, Karamariti E, et al. Direct reprogramming of fibroblasts into endothelial cells capable of angiogenesis and reendothelialization in tissue-engineered vessels. *Proc Natl Acad Sci U S A*. 2012;109:13793-13798.
243. Kurian L, Sancho-Martinez I, Nivet E, et al. Conversion of human fibroblasts to angioblast-like progenitor cells. *Nat Methods*. 2013;10:77-83.
244. Hahn C, Schwartz MA. Mechanotransduction in vascular physiology and atherogenesis. *Nat Rev Mol Cell Biol*. 2009;10:53-62.
245. Jones EA. The initiation of blood flow and flow induced events in early vascular development. *Semin Cell Dev Biol*. 2011;22:1028-1035.
246. Mammoto A, Mammoto T, Ingber DE. Mechanosensitive mechanisms in transcriptional regulation. *J Cell Sci*. 2012;125:3061-3073.
247. Sabine A, Agalarov Y, Maby-El Hajjami H, et al. Mechanotransduction, PROX1, and FOXC2 cooperate to control connexin37 and calcineurin during lymphatic-valve formation. *Dev Cell*. 2012;22:430-445.
248. Kulkarni RM, Greenberg JM, Akeson AL. NFATc1 regulates lymphatic endothelial development. *Mech Dev*. 2009;126:350-365.
249. Ranger AM, Grusby MJ, Hodge MR, et al. The transcription factor NF-ATc is essential for cardiac valve formation. *Nature*. 1998;392:186-190.
250. Pham VN, Lawson ND, Mugford JW, et al. Combinatorial function of ETS transcription factors in the developing vasculature. *Dev Biol*. 2007;303:772-783.
251. Yamamoto H, Flannery ML, Kupriyanov S, et al. Defective trophoblast function in mice with a targeted mutation of Ets2. *Genes Dev*. 1998;12:1315-1326.