

## CHAPTER 4

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# Embryonic Vasculogenesis and Hematopoietic Specification

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### Abstract

Vasculogenesis is the process by which blood vessels are formed *de novo*. In mammals, vasculogenesis occurs in parallel with hematopoiesis, the formation of blood cells. Thus, it is debated whether vascular endothelial cells and blood cells are derived from a common progenitor. Whether or not this is the case, there certainly is commonality among regulatory factors that control the differentiation and differentiated function of both cell lineages. VEGF is a major regulator of both cell types and plays a critical role, in coordination with other signaling pathways and transcriptional regulators, in controlling the differentiation and behavior of endothelial and blood cells during early embryonic development, as further discussed herein.

### Key Messages

- Blood cells and blood vessel endothelium develop in parallel, but it is still debated whether they develop from a shared precursor.
- VEGF signaling is critical for vascular plexus formation and hematopoiesis.
- VEGF signaling cooperates with other signaling pathways to promote endothelial cell specification, vascular plexus remodeling and hematopoietic specification.
- VEGF signaling is fine tuned via a collection of receptors and co-receptors to modulate endothelial cell behavior.
- The precise contribution of different VEGF signaling pathways to the regulation of vascular development and hematopoiesis is not yet fully understood and needs to be researched further.

### Introduction

Vasculogenesis is the process by which blood vessels are formed *de novo*. This process first occurs in the embryonic yolk sac of mammalian embryos, and then later during development in the embryo proper. During gastrulation, embryonic ectodermal (epiblast) cells are recruited to the primitive streak where they undergo an epithelial to mesenchymal transition. These cells then migrate between the visceral endoderm and epiblast to form either mesoderm or definitive endoderm.<sup>1</sup> In the yolk sac, the visceral endoderm is thought to elicit soluble signals which target the underlying mesoderm to induce the formation of primitive endothelial and hematopoietic cells, the first differentiated cell types to be produced in the mammalian embryo (Fig. 1). Primitive endothelial and hematopoietic cells coalesce to form blood islands that then fuse

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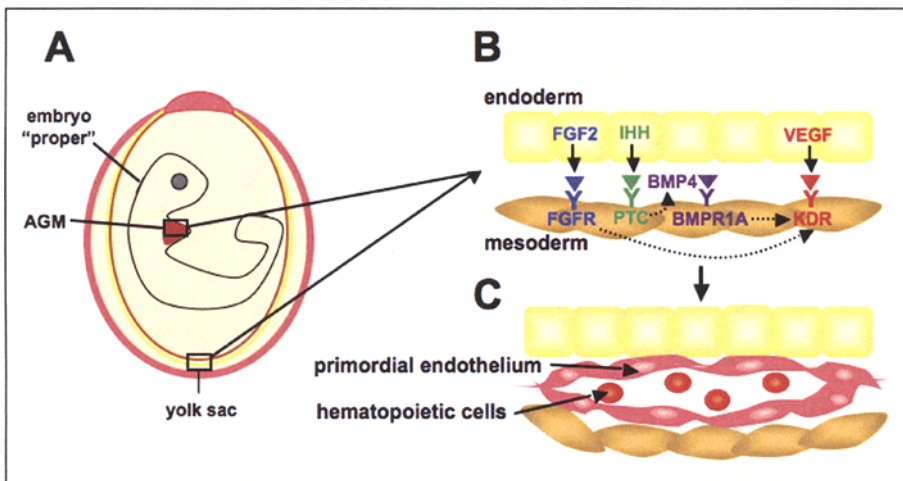


Figure 1. Molecular regulation of primitive hematopoietic specification and vascular remodeling during murine development. A) Schematic representation of the developing murine embryo, highlighting the primary sites of embryonic hematopoiesis. B) Schematic representation of endoderm-derived molecular signals with known roles in the specification of mesodermal precursors. C) Schematic representation of primitive hematopoietic development within the yolk sac vascular plexus.

to form a primitive network of tubules known as a capillary or vascular plexus. Remodeling and maturation of the capillary plexus into a circulatory network requires the subsequent recruitment of mural cells (smooth muscle cells and pericytes) to form the outer blood vessel wall. Concurrent with vascular remodeling is the induction of definitive hematopoiesis, the formation of mature circulating blood cells. This chapter focuses on understanding the sequential processes of vasculogenesis and hematopoiesis, and the major signaling pathways that regulate these processes; a key regulatory factor is vascular endothelial growth factor (VEGF), the main focus of this text.

## Endothelial Specification and Vasculogenesis

During blood vessel development, endothelial cells are predominantly derived from mesoderm, as are blood cells. Mural cells are also largely derived from mesoderm, although neural crest and proepicardial organ cells also contribute mural cells to developing vessels. Vascular development has been well studied in several embryonic model systems including mouse, chick, quail, frog, and zebrafish, but perhaps best genetically manipulated in the murine system. Thus, our discussion of the molecular regulation of vasculogenesis and hematopoiesis will be based largely on insights gained from the study of the developing mouse; relevant insights from other model systems will also be discussed.

### Formation of Mesodermal Precursors

Murine blood vessel formation initiates during gastrulation, wherein posterior epiblast cells migrate through the primitive streak to form mesoderm,<sup>1</sup> from which vascular and blood cells are differentiated. Several signals cooperate to promote mesoderm specification for blood vessel formation (Fig. 1B). One signal needed for specification of mesoderm is the soluble factor bone morphogenic protein 4 (BMP4), whose expression prior to gastrulation is localized to the primitive streak. In the absence of BMP4, mesoderm fails to develop and thus mutant embryos arrest at the egg chamber stage.<sup>2</sup> Another factor required for mesoderm formation is fibroblast growth factor 2 (FGF2, previously known as basic FGF or bFGF). Knockout inactivation of

FGF receptor 1 (FGFR1) results in an accumulation of epiblast cells, as they fail to migrate through the primitive streak and form mesoderm, further emphasizing the importance of FGF signaling in mesoderm formation.<sup>3</sup> VEGF has critical roles in the subsequent stages of mesodermal commitment and differentiation, but unlike BMP4 and FGF2, VEGF is not known to play a role in mesoderm formation.

### ***Commitment of Mesodermal Precursors to the Endothelial Lineage***

During embryogenesis, commitment of multipotent mesodermal cells to an endothelial cell lineage is thought to be regulated by soluble signals derived from adjacent endodermal cells. In the murine yolk sac, visceral endoderm elicits signals needed for vascular induction, although the hierarchy of such signals is not yet clear. One such soluble effector is indian hedgehog (IHH) (Fig. 1B). Using mouse embryo explant cultures lacking endoderm, it was discovered that IHH can respecify anterior epiblast cells, which normally form neuroectoderm, to form endothelial and hematopoietic cells instead,<sup>4</sup> thus demonstrating sufficiency of IHH for vascular induction. IHH's downstream effector in this pathway may be BMP4, as it is upregulated in response to IHH signaling (Fig. 1B).<sup>4</sup> In support of this theory, aberrant vasculogenesis and hematopoiesis has been demonstrated in BMP4-null mutant mouse embryos that survive past the egg cylinder chamber stage.<sup>2</sup>

### ***Role of VEGF/KDR Signaling in Mesodermal Precursors of the Endothelial Lineage***

Synergistic signaling between IHH and endoderm-derived FGF2 is thought to promote the expression of the VEGF receptor KDR (also known as FLK1 or VEGFR2) in adjacent mesodermal cells (Fig. 1B). A role for FGF2 in the formation of endothelial precursors, angioblasts, from mesoderm has been demonstrated in the quail embryo, where explant cultures consisting of only mesoderm lack the ability to undergo angioblast formation unless treated with FGF2.<sup>5</sup> Furthermore, isolated FGF2-treated epiblast cells are capable of forming endothelial cells in vitro.<sup>6</sup> Perhaps the critical role of FGF2 in vivo during vascular induction is the upregulation of KDR. Once KDR is upregulated in mesodermal progenitors (not all of which will become endothelial cells<sup>7</sup>), they become capable of responding to VEGF, which is initially produced only in the visceral endoderm of the mouse yolk sac.<sup>8</sup> KDR-expressing mesodermal progenitors are exquisitely sensitive to the bioactive levels of VEGF, which therefore exerts a dose-dependent effect on vasculogenesis. This is evidenced by the fact that mice heterozygous for a null mutation in the *Vegfa* gene die in utero due to failed vascular development: In these mutants, endothelial cells differentiate, but in a delayed fashion, and this leads to disorganized blood vessels and disrupts hematopoietic cell formation.<sup>9</sup> Similarly, embryos lacking KDR exhibit arrested vascular development and embryonic lethality. These mutants form endothelial precursors, but they fail to differentiate into mature endothelial cells, thereby halting blood island development and vascular plexus formation.<sup>10</sup> The data obtained from the KDR null mice have been corroborated in both the avian and *Xenopus* systems. In quail embryos, KDR expression was knocked down and revealed that primitive endothelial cells developed in the absence of KDR, but no mature endothelial networks formed.<sup>11</sup> In addition, it was demonstrated that quail mesoderm isolated from embryos can form hemangioblasts upon treatment with VEGF, even in the absence of contact with endoderm.<sup>12</sup> When VEGF was ectopically expressed in *Xenopus* embryos, large disorganized vascular structures containing mature endothelial cells formed.<sup>13</sup> Taken together, these data indicate that VEGF is critically important for the differentiation and/or survival of mature endothelial cells.

### ***Role of VEGF/Neuropilin Signaling in the Endothelial Lineage***

Recently, two novel VEGF receptors were discovered and termed neuropilin 1 and 2 (NRP1 and NRP2). Originally, the neuropilins were identified in the nervous system as transmembrane receptors for class III semaphorins, which provide repulsive cues during the axon guidance of

several embryonic nerves.<sup>14,15</sup> Both NRP1 and NRP2 are also expressed on endothelial cells, where they function as isoform-specific VEGF receptors.<sup>16,17</sup> NRP1 null mice are embryonic lethal between E12.5 and E13.5 due to cardiovascular defects, including defective aortic arch remodeling and formation of abnormal capillary networks in the brain and spinal cord.<sup>18</sup> In contrast, NRP2 null mice have been reported to be viable and fertile.<sup>19</sup> However, ablation of both NRP1 and NRP2 results in embryonic lethality by E8.5 in the mouse, as no organized blood vessels are formed, similar to the KDR and VEGF null phenotypes.<sup>20</sup> This demonstrates, once again, the necessity of precise VEGF signaling for early vascular development.

### **Capillary Plexus Formation**

As endothelial cells are committed and differentiated during vasculogenesis, their proliferation, migration and coalescence into a primitive capillary plexus must be well coordinated. VEGF signaling is critically important in this process, as it promotes endothelial cell proliferation<sup>21-23</sup> and modulates migration.<sup>24-26</sup> FGF2 is also involved in the stimulation of endothelial cell proliferation,<sup>27</sup> and has been shown to function synergistically with VEGF.<sup>24,28,29</sup> The mitogenic effects of VEGF are thought to be mediated largely by the KDR receptor.<sup>26,30</sup> In contrast, an alternative VEGF receptor termed FLT1 (also known as VEGFR1) is thought to modulate VEGF's proliferative effects by sequestering locally available soluble VEGF. Consistent with this idea is the phenotype of embryos lacking FLT1: These mutants exhibit excessive endothelial cell formation, which leads to the formation of a disorganized vascular plexus incapable of remodeling into a functional circulatory network.<sup>31,32</sup> In addition, a soluble form of FLT1 termed sFLT1 is thought to be critical to shape VEGF gradients in the environment of growing blood vessels to promote directional vessel growth.<sup>33</sup> These studies emphasize the fact that although VEGF's mitogenic effects are essential for capillary plexus formation, they must be balanced to prevent excessive endothelial cell proliferation and promote sprouting.

### **Vascular Remodeling**

Once a primitive endothelium has been formed and patterned, the next aspect of blood vessel development is remodeling of the plexus to promote the establishment of a mature circulatory network.

### **Endothelial Cell Proliferation, Migration and Survival**

Vascular remodeling is a complex process, in which a balance between signals to induce and inhibit endothelial cell proliferation must be reached. This process involves multiple signaling cascades as well as cell-cell and cell-matrix communications. Factors involved in maintaining the appropriate rate of endothelial cell proliferation include VEGF, FGF2, retinoic acid (RA), and transforming growth factor beta (TGFB1). VEGF and FGF2 are needed for the induction of endothelial cell proliferation. However counteractive anti-proliferative signals such as RA<sup>34</sup> and TGFB1<sup>35</sup> are an equally essential requirement for appropriate blood vessel formation.

Retinaldehyde dehydrogenase 2 (RALDH2) converts retinol (vitamin A) into its biologically active metabolite retinoic acid. Like other mesoderm modulating factors in the developing murine yolk sac, RALDH2 is expressed by the visceral endoderm, where active production of retinoic acid occurs. Retinoic acid is actively secreted from the visceral endoderm and interacts with retinoic acid receptors (RAR1/2) expressed by the vascular endothelium.<sup>35</sup> RARA1/2 signaling directly inhibits endothelial cell cycle progression via upregulation of two cell cycle inhibitors, the cyclin-dependent kinase inhibitors p21 (CDKN1A) and p27 (CDKN1B),<sup>34</sup> and indirectly suppresses growth via upregulation of the anti-proliferative cytokine TGFB1. TGFB1 likely acts through the SMAD5 pathway to upregulate the production of extracellular matrix protein fibronectin, which then functions to promote visceral endoderm survival and signals via integrins present on vascular endothelium. Of particular importance are integrins alpha 5 beta 1 and alpha V beta 3, which both bind fibronectin, but elicit opposite responses. Signalling of integrin alpha 5 beta 1 via a currently undefined intracellular signaling pathway

serves to block endothelial cell proliferation; in contrast, integrin alpha v beta 3 signals via the KDR/MAPK pathway to promote endothelial cell proliferation.<sup>35</sup> The maintenance of a delicate equilibrium between these signaling events is necessary to regulate the proper branching and remodeling required for development of a mature vascular network.

### ***Role of Mechanotransduction and Flow in the Maintenance of Blood Vessels***

Once blood vessels have formed, and likely concurrent with the remodeling process, fortification of the vessel wall is initiated in response to hemodynamic forces, which are exerted upon endothelial cells by the newly established circulatory flow. Genomic studies *in vitro* have identified a multitude of genes that are differentially regulated by various types of hemodynamic forces, including laminar shear stress, turbulent shear stress, and disturbed flow.<sup>36-40</sup> These studies provide strong evidence that endothelial cells not only have the ability to "sense" hemodynamic forces, but that they are also capable of discriminating between different types of biomechanical stimuli. In particular, shear stress has been shown to promote endothelial cell survival via two main events, cell growth and inhibition of cell death (apoptosis). While the role of VEGF in endothelial response to shear stress is currently unknown, its receptor KDR appears of great importance: KDR is a mechanosensor that converts mechanical stimuli into chemical signals, as both its expression and activation level increase in response to laminar shear stress.<sup>41</sup> Upon exposure to fluid shear stress, endothelial cells also upregulate KDR expression to activate both ERK and JNK kinases, which are downstream targets of the MAPK pathway.<sup>42</sup> Once these pathways are initiated, the transcription of immediate early response genes such as monocyte chemoattractant protein 1 and FOS is upregulated to promote endothelial cell growth.<sup>43</sup> Anti-apoptotic signaling pathways are also regulated by shear stress. Activation of the receptor tyrosine kinases KDR and TIE2 initiates a signaling cascade, in which activation of PI3K promotes the phosphorylation of AKT, which in turn triggers the upregulation of nitric oxide production and thereby elicits an antiapoptotic signal to endothelial cells in the blood vessel wall.<sup>44-47</sup> Endothelial expression of p21 drastically increases in response to increased nitric oxide production, resulting in the inhibition of endothelial cell apoptosis.<sup>48</sup> Vice versa, loss of p21 significantly increases endothelial cell death in response to shear stress, demonstrating that p21 is one of the major factors mediating the anti-apoptotic effect of shear stress.<sup>48</sup> In addition to its anti-apoptotic role, p21 creates a G1 to S phase block via cyclin-dependent kinase phosphorylation of the retinoblastoma protein,<sup>49</sup> thus decreasing the rate of DNA synthesis during exposure to laminar shear stress.

## **Hemogenic Specification and Hematopoiesis**

### ***Developmental Origin of Hematopoietic Progenitors***

In the mouse, primitive hematopoiesis is initiated in the yolk sac between embryonic day 7.0 and 7.5, producing predominantly nucleated erythroid cells expressing embryonic globin.<sup>50</sup> A second wave of hematopoiesis is initiated in the yolk sac<sup>51</sup> and embryo proper<sup>52</sup> between E10.0 and E11.0,<sup>53</sup> when definitive hematopoietic stem cells (HSCs), capable of repopulating the neonate or adult blood system, arise in the aorta-gonad-mesonephros (AGM) region. Within the AGM, hematopoietic precursors are seen as clumps of cells that appear to bud from endothelial cells of the ventral wall of the dorsal aorta, and the umbilical and vitelline arteries.<sup>54</sup> Thus, during definitive as well as primitive yolk sac hematopoiesis, there is a close spatial and temporal association between endothelial and hematopoietic cell development. Studies in both chick and mouse have shown that AGM-derived yolk sac cells, which give rise to endothelial cells of the yolk sac vasculature, also have the capacity to generate definitive hematopoietic cells *in vitro*.<sup>55</sup> Together, these findings point to endothelium as the most likely source of definitive hematopoietic precursors in the developing embryo. However, the differing interpretation of the current body of experimental evidence has yielded three major theories for the origin and specification of the hematopoietic lineage (Fig. 2).

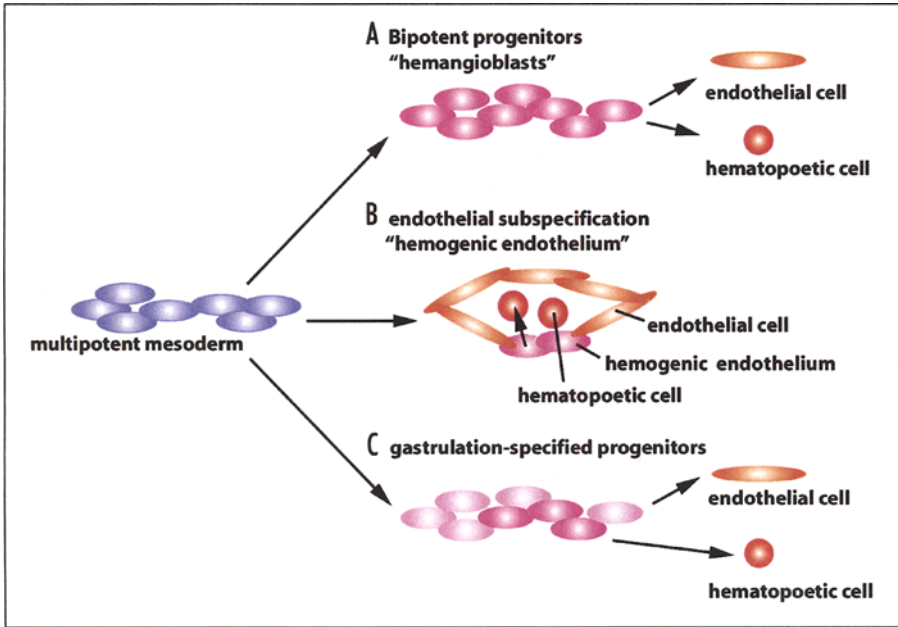


Figure 2. Theories of hematopoietic specification during embryogenesis. Schematic representation of the three major alternative theories for the developmental emergence of primitive endothelial and hematopoietic lineages: (A) the bipotent "hemangioblast", (B) "hemogenic" endothelium, and (C) gastrulation-specified endothelial and hematopoietic progenitors.

### Endothelial and Hematopoietic Cells May Share a Common Progenitor

In blood islands of the yolk sac, where the earliest hematopoietic cells appear, cells in the hematopoietic and endothelial lineage arise almost simultaneously from the extraembryonic paraxial mesoderm to form a primitive capillary plexus, in which primitive nucleated erythroblasts are intimately associated with maturing endothelial cells.<sup>56</sup> This observation has led to the hypothesis that both lineages arise from a common precursor (Fig. 2A). This concept is supported by experimental evidence suggesting the shared expression of hematopoietic and endothelial-specific genes in both lineages. Even though the physical proximity of endothelial and hematopoietic cells in the developing embryo does not prove the existence of a common progenitor, supportive evidence for this concept is provided by *in vitro* studies, which demonstrate the formation of clonal blast colony-forming cells capable of generating both primitive hematopoietic and endothelial cell types under appropriate culture conditions.<sup>57-59</sup> These cells express markers associated with both hematopoietic and vascular development, including KDR,<sup>60-62</sup> TAL1,<sup>50,63</sup> VE-cadherin<sup>64</sup> and GATA1.<sup>65</sup> Cell tracking studies of transgenic murine embryos with GFP targeted to the *brachyury* locus have also identified clonal progenitors in the gastrulating mouse embryo that are capable of forming blast cell colonies with *ex vivo* hematopoietic and vascular potential.<sup>66</sup>

### Hematopoietic Cells May Be Derived from Specialized Hemogenic Endothelium

An alternative theory, equally consistent with existing evidence, postulates that blood-forming cells are specified from mesodermally-derived primitive endothelium known as *hemogenic endothelium* (Fig. 2B). Clusters of hematopoietic precursors that appear to derive directly from the vascular endothelium *in vivo* have been observed in developing blood vessels of numerous vertebrate species, and their appearance correlates with the developmental timing of definitive

hematopoietic cells.<sup>54</sup> In the murine yolk sac and embryo proper, cells with blood-forming capacity have also been found to reside within the vascular endothelium.<sup>67</sup> A population of cells termed side population cells has been isolated from murine yolk sac and embryo prior to the onset of circulation; this cell population resides within the vascular endothelium, expresses high levels of endothelial markers, and fails to generate blood cells *in vitro*; however, as development progresses, this cell population acquires hematopoietic potential and phenotypic characteristics similar to those of bone marrow side population cells, suggesting a developmental transition from an endothelial to a hematopoietic progenitor cell type.<sup>67</sup> Culture studies also provided support for the model of hematopoietic specification from hemogenic endothelium. In particular, a population of primitive endothelial-like cells was isolated from human embryonic stem cell cultures, which expresses the endothelial markers PECAM1, KDR and VE-cadherin (PFV+), but not the hematopoietic lineage marker CD45; when treated with VEGF, these cells were shown to generate mature endothelial cells, whilst treatment with hematopoietic growth factors facilitated hematopoietic development.<sup>68</sup> These studies therefore identified PFV+/CD45- cells as a distinct bipotent progenitor population capable of supporting the progressive development of the endothelial and hematopoietic lineages. Most significantly, these studies demonstrated the stepwise generation of hematopoietic and endothelial cell precursors from multipotent embryonic cells, revealing a hierarchical organization of cell fate commitment, which supports the idea that hematopoietic and endothelial cells derive from a subset of embryonic endothelium with hemangioblastic properties.

### **Endothelial and Hematopoietic Progenitors May Be Independently Specified during Gastrulation**

Other studies suggest that there is no common lineage between blood and endothelial cells, and that both are instead independently specified during gastrulation (Fig. 2C). Studies of the early primitive streak-stage embryo confirmed that cells derived from the primitive streak preferentially contribute to the erythrocyte precursor population of the developing vascular blood islands.<sup>1</sup> In contrast, cells derived from the primitive streak of mid-primitive streak-stage embryos appear to contribute primarily to the endothelium, with little or no erythroid component.<sup>1</sup> Perhaps most significantly, simultaneous contribution of primitive streak progenitors to blood islands and vitelline endothelium is rarely seen to occur at the same location within yolk sac mesoderm.<sup>1</sup> These observations suggest that the allocation and recruitment of early progenitor cells to the endothelial and hematopoietic lineages likely occurs at different stages of gastrulation, and is therefore both spatially and temporally segregated during morphogenesis of the vitelline vessels.

### **Summary**

The relationship between hematopoietic precursors derived from hemogenic endothelium and putative yolk sac hemangioblasts remains unclear. Progenitors capable of reconstituting neonatal hematopoiesis arise in the yolk sac before circulation,<sup>51</sup> and may be related to, or even derived from, the earlier born yolk sac hemangioblast. However, definitive hematopoietic stem cells that first arise in the AGM region are thought to be unrelated by lineage to yolk sac-derived precursors, because their development occurs independently of any circulation of cells from the yolk sac.<sup>53</sup> Isolation of single cells that can give rise to both endothelial and hematopoietic cells *in vitro* provides clear evidence of a common origin for the two lineages. However, it does not distinguish whether this common precursor makes a simple choice between the two lineages, or whether endothelium is formed first and blood forming cells are specified subsequently, or whether there is a more primitive multipotent precursor, whose potency remains undefined.

### **Developmental Regulation of Hematopoietic Progenitors**

Although the cellular origin of hematopoietic cells is still debated, a number of signaling molecules have been shown to play critical roles in the specification and differentiation of blood cells.

## VEGF/KDR

As previously discussed, KDR plays a key role in the developmental regulation of hematopoiesis as well as vasculogenesis. Generally recognized as the earliest antigenic marker of developing endothelial cells, *Kdr* expression can be detected in mesodermally derived blood island progenitors as early as E7.0.<sup>62,69</sup> Mice deficient in KDR fail to develop definitive hematopoietic cells capable of long-term engraftment in an irradiated recipient, nor do they make functional blood vessels, and they therefore die in midgestation, between E8.5 and 9.5.<sup>10,70</sup> In chimeric aggregation studies using wild-type mouse embryos, KDR null ES cells fail to contribute to primitive or definitive hematopoiesis *in vivo*, suggesting a cell autonomous requirement for KDR signaling in hematopoietic development.<sup>70</sup> Furthermore, VEGF signaling from the yolk sac endoderm via KDR is required for the hematopoietic differentiation of mesoderm.<sup>9,32,70</sup> Moreover, both vasculogenesis and hematopoiesis are impaired in mice homozygous for a hypomorphic VEGF allele, and decreased KDR activity.<sup>8</sup> Although the findings described above demonstrate that VEGF/KDR signalling plays a crucial role in promoting hematopoiesis, it may not be essential for the initial specification of hematopoietic precursors. KDR-deficient ES cells retain some hematopoietic potential *in vitro*<sup>70</sup> and KDR null mouse embryos contain normal numbers of hematopoietic progenitors at E7.5, even though they are profoundly deficient at E8.5.<sup>71</sup> Rather than being required for specification, VEGF/KDR signalling may provide an important signal for the subsequent survival, migration and clonal expansion of hematopoietic progenitors. Consistent with this idea, KDR+ cells of mouse embryos carrying a hypomorphic *Vegfa* allele reach the yolk sac on time by E8.5, but are severely compromised in their ability to generate primitive erythroid precursors, perhaps because VEGF normally prolongs the life span of primitive erythroid progenitors by inhibiting apoptosis.<sup>72</sup>

## TAL1

The transcription factor TAL1 (also known as SCL) is first coexpressed with KDR around E7.0 in the visceral mesoderm and *in vitro* promotes the early stages of differentiation of mesoderm into cells of the hematopoietic lineage.<sup>73,74</sup> Gene targeting and chimera analyses demonstrated a requirement of TAL1 for the generation of both primitive and definitive hematopoietic lineages and for the appropriate remodeling of the yolk sac vasculature *in vivo*.<sup>75-77</sup> Specifically, TAL1 null mutant embryos are embryonic lethal, as they fail to initiate yolk sac hematopoiesis. KDR+ cells isolated from differentiating TAL1 null ES cells also fail to generate either blood or endothelial cells *in vitro*,<sup>78</sup> suggesting that KDR signaling and TAL1 expression synergise during early hematopoietic development.

## GATA1, GATA2, LMO2 and RUNX1

The transcription factors GATA1, GATA2, LMO2 and RUNX1 also play important roles in the fate determination of the hematopoietic lineage. GATA1 and GATA2 are essential for both embryonic and adult erythropoiesis: Loss of GATA1 in knockout mouse embryos halts erythroid differentiation at the proerythroblast stage,<sup>79</sup> whilst loss of GATA2 function causes early embryonic lethality due to a primary defect in primitive hematopoiesis.<sup>80</sup> LMO2 is required for both vascular development and yolk sac hematopoiesis, and LMO2 null ES cells fail to contribute to the vascular endothelium of chimeric animals.<sup>81,82</sup> Loss of function of any one of these three transcription factors yields a phenotype similar to that of TAL1 null mutant mice, consistent with the idea that LMO2 may function cooperatively with GATA1 and TAL1 to promote the specification of erythroid cells.<sup>81</sup> Definitive, but not primitive, hematopoiesis is also dependent on the transcription factor RUNX1. RUNX1 null mutant embryos undergo primitive yolk sac hematopoiesis normally, but die between E11.0 and E12.0 due to a failure of definitive hematopoiesis.<sup>83</sup> *LacZ* knock-in mice revealed expression of *Runx1* in a subpopulation of cells in the yolk sac endothelium and in the floor of the dorsal aorta, suggesting that RUNX1 supports the differentiation of hemogenic endothelium *in vivo*, which contributes to definitive hematopoiesis.<sup>84</sup> Consistent with this idea, aortic clusters of hematopoietic cells are absent in RUNX1 null mutants,<sup>85</sup> and *in vitro* RUNX1 null embryoid bodies produce clonal blast colony-forming cells at reduced numbers.<sup>86</sup>



## Conclusions and Future Directions

Experimentation in several embryonic model systems by many different laboratories has revealed that VEGF signaling is critical for the formation of an initial vascular plexus from multipotent mesodermal progenitors. Continued VEGF signaling, fine-tuned via its receptors and coreceptors, then modulates endothelial cell behavior to establish a functional circulatory network. Other signaling pathways function coordinately with VEGF to mediate vascular plexus remodeling, endothelial cell specification and mural cell recruitment. The formation of blood vessels and blood cells occurs concomitantly. The VEGF receptor KDR is widely recognized as a common marker of all cells possessing putative hemangioblast properties.<sup>87</sup> Cells that retain KDR activity have endothelial potential, whereas cells that activate hematopoietic transcription factors such as TAL1 and RUNX1 gain hematopoietic activity. A subset of KDR-expressing cells that are TAL1+ may represent primitive hemangioblasts capable of initiating primitive yolk sac erythropoiesis, while KDR-expressing cells that are both TAL+ and RUNX1+ represent definitive precursors for definitive hematopoiesis in the AGM and yolk sac.<sup>78</sup> This model of successive diversion of subsets of VEGF-responsive, KDR-expressing cells perhaps best explains our current understanding of the formation of endothelial, primitive hematopoietic and definitive hematopoietic precursors in the early embryo. However, continued research is needed to fully understand the complexity of VEGF signaling. Moreover, we need to increase our efforts to elucidate how VEGF signaling cooperates with other signaling pathways during vascular development and hematopoiesis. Insights gained from ongoing work in developmental model systems will likely benefit the optimization of clinical therapies for those prevalent diseases that are associated with disrupted blood cell and blood vessel formation.

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