

FGFR1 and the Bloodline of the Vasculature

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Since the recognition of basic fibroblast growth factor (bFGF, FGF-2) and acidic FGF (aFGF, FGF-1) as proliferation and maintenance factors for endothelial cells in culture,^{1,2} much interest has arisen regarding their role as potential angiogenic factors in tumors and tissue healing in vivo. The FGFs, of which presently at least 22 members have been recognized,³ interact with FGF receptors (FGFRs). After FGF binding, the FGFRs dimerize and become activated displaying protein tyrosine kinase activity and subsequent signaling in the cell.^{4,5} Four functional FGFRs have been recognized, FGFR1–4, most of which can be encountered in various alternative spliced isoforms. FGFR1 plays an essential role during early stages of development of the mesoderm and at several levels during organogenesis.^{3,6} FGF/FGFR1 is expressed almost exclusively in the mesenchyme, including endothelial cells, smooth muscle cells, and hemopoietic progenitor cells. FGFR1 is involved in the stimulation and proliferation of endothelial cells and vascularization by FGFs, particularly bFGF and aFGF. FGFs also promote the formation of hematopoietic progenitor cells in early development, which requires the FGFR1 as well.^{7,8} In line with this, chromosomal translocation involving FGFR1 has been observed in patients with stem-cell myeloproliferative disease, probably caused by constitutive activation of the FGFR1 gene.⁹

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It has long been anticipated that hematopoietic progenitor cells and endothelial progenitor cells derive from a common cell type, the hemangioblast.¹⁰ Studies on mouse embryos have shown that such hemangioblast cells indeed exist. These cells develop in the primitive streak of the embryo and migrate to the yolk sac, where they form blood islands.¹¹ The outer cells of these blood islands mature into endothelial cells and form a vascular plexus, while the inner cells develop into primitive and definitive hematopoietic progenitor cells and their subsequent lineages of blood cells. In addition, in the para-aortic splanchnopleura, hemangioblasts develop into vascular structures.¹¹ Later in development the primitive and definitive hematopoietic progenitor (“stem”) cells migrate to

the fetal liver and subsequently to the bone marrow, where they remain life-long active as a source for white and red blood cells, mast cells, and megakaryocytes, from which blood platelets are formed.

One of the issues that remained unresolved was the contribution of FGF signaling to the early development of the vascular tree. Embryos deficient in FGFR1 stop growing at an early stage of gestation because of abnormal mesodermal patterning.^{12,13} However, when embryonic stem cells were derived from FGFR1-deficient blastocysts and administered subcutaneously in nude mice, these embryonic stem cells were still able to differentiate into different types of mesodermal cells.¹⁴ Thus FGFR1 causes inappropriate mesodermal patterning rather than impeding mesodermal differentiation. Because FGFs are required for proper mesodermal pattern development, deficiency of FGFR1 interfered with the early development and viability of embryos in the mouse. To overcome this initial blockade of development, Faloon et al⁷ and Magnusson et al^{15,16} used the technique of growing embryoid bodies from embryonic stem cells. Pluripotent embryonic stem cells can be isolated from the inner cell mass of a blastula stage embryo. These cells can be grown in culture and can be induced to aggregate into spheroid bodies, the embryoid bodies, by specific culture conditions, which includes the withdrawal of the factor LIF. The inner mass of these embryoid bodies subsequently develops into mesodermal cells, a part of which further differentiate into hemangioblast cells and subsequently into hematopoietic cells, endothelial cells, and vascular smooth muscle cells (Figure 1). The embryoid bodies appeared helpful in elucidating the contribution of various factors involved in hematopoietic and endothelial development.^{17–19} Their regulation is highly comparable with that in the yolk sac of murine embryos. It has become clear that the differentiation steps from mesodermal progenitor cells into hemangioblasts and subsequently into primitive and definitive lineages of hematopoietic and endothelial progenitor cells required the expression of growth factors including BMP-4 and VEGF, the growth factor receptors VEGFR-2 (Flk1, KDR) and FGFR1, CD41, and transcription factors, such as SCL/Tal1 and Runx1 (required for hematopoietic cells formation).^{20–29} Members of the transforming growth factor (TGF)- β signaling family, activin A, TGF- β , and possibly BMP-2, act as positive (Activin A) and negative regulators (TGF- β).^{30,21} The various steps thought to be involved in the differentiation of mesoderm into hematopoietic and endothelial progenitor cells are summarized in Figure 2.

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Magnusson et al¹⁵ report on the expression of FGFR-1 in embryoid bodies and its requirement for hematopoietic and endothelial cell development and function. They show that FGFR1 is expressed and becomes activated in a

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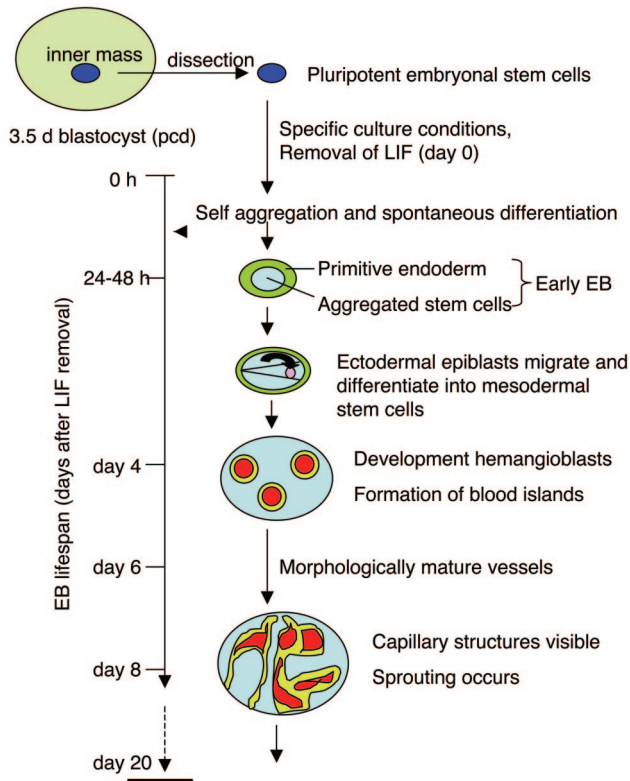


Figure 1. Development of early vasculature in embryoid bodies. EB indicates embryoid body; pcd, postcoital day; LIF, leukemia inhibitory factor.

subpopulation of endothelial cells during the development of embryoid bodies. Its expression was associated with cell division, suggesting that particularly dividing endothelial cells express FGFR1. Whether this is related to the cell cycle state of the cell or reflects a subpopulation of rapidly dividing endothelial cells remains uncertain. In FGFR1-expressing endothelial cells (FGFR1⁺/CD31⁺) bFGF stimulated mitogenesis, both in the absence and presence of VEGF. Addition

of bFGF to embryoid bodies particularly stimulates angiogenesis in embryonic bodies by the length growth of vascular structures, while the stimulatory effect of VEGF regards the formation of a complex capillary plexus.¹⁶ Although embryoid bodies derived from stem cells that lack one or both alleles of FGFR1 are still able to form endothelial cells and vascular structures, the formation of hematopoietic progenitor cells is impaired. Analysis of the mRNA expression in FGFR1^{-/-} embryoid bodies showed a 50% increase of the mesodermal marker *brachyury* at day 4, indicating a delayed progression of differentiation as compared with their FGFR1^{+/+} counterparts. After 8 days, when endothelial cells and vascular structures had become developed, a relatively normal development of the endothelial lineage in these embryonic bodies was observed. However, a number of genes involved in hematopoietic differentiation were highly suppressed in the FGFR1^{-/-} embryoid bodies, including *SCL/Tal1*, *CD41*, and the β -H1 and *GATA-1*, two proteins involved in development of erythrocytes. Indeed, at day 12 virtually no expression of the leukocyte marker *CD45* was seen in FGFR1^{-/-} embryoid bodies, in contrast to the FGFR1^{+/+} ones. This indicates that the differentiation into hematopoietic progenitor cells is severely impaired, and suggests that FGF/FGFR1 signaling is required for this differentiation.

These data are consistent with previous observations of Faloon et al,⁷ who showed a considerable reduction of VEGFR2, *SCL/Tal1*, β -H1, and β -major production in FGFR1^{-/-} embryoid bodies as compared with their FGFR1 wild-type counterparts. *SCL/Tal1* is required for the commitment of hemangioblasts into hematopoietic progenitor cell differentiation.^{21-23,31} Therefore, it is likely that the absence of *SCL/Tal1* in FGFR1^{-/-} embryonic bodies is an important contributor to the lack of hematopoietic differentiation. Overexpression of *SCL/Tal1* in FGFR1^{-/-} embryonic bodies may resolve whether this is the only factor preventing hematopoiesis or whether other factors also contribute to this phenom-

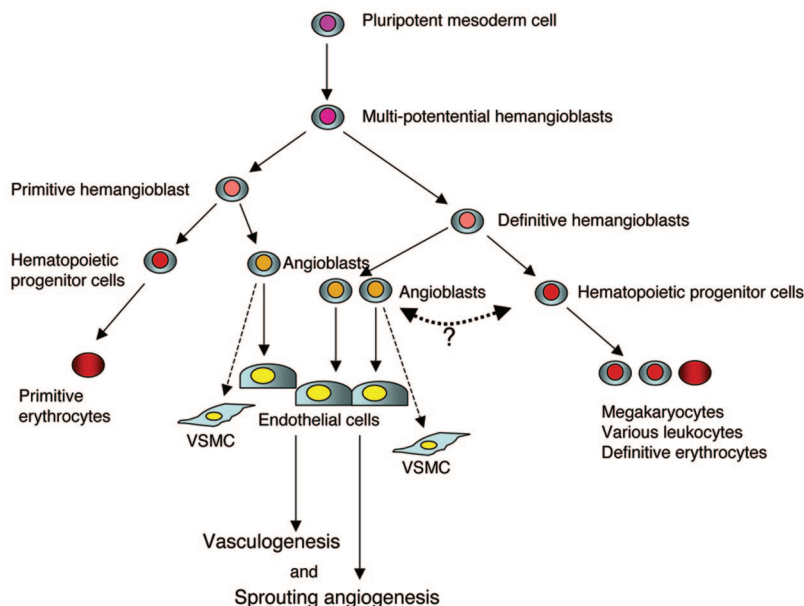


Figure 2. Differentiation of hemangioblasts into hematopoietic cells and endothelial cells.

enon. Faloon et al⁷ indicated that in SCL/Tal1^{-/-} embryoid bodies endothelial markers were elevated 2- to 4-fold, suggesting preferential differentiation into the endothelial lineage. This raises two questions. First, are the endothelial cells differentiating directly from the primitive hemangioblast in FGFR1^{-/-} and SCL/Tal1^{-/-} conditions identical in characteristics as those deriving wild-type conditions from the definitive hemangioblasts? Second, does (over)expression of SCL/Tal1 reduce differentiation into the angioblast and endothelial lineage, or is the increase in endothelial differentiation in FGFR1^{-/-} and SCL/Tal1^{-/-} embryonic bodies merely caused by the lack of transition into hematopoietic progenitor cells? Other studies have shown that SCL/Tal1 deficiency causes defects in endothelial growth and yolk sac vascularization, indicating that this factor also contributes to proper endothelial development.^{31,32} Apparently experimental conditions may influence the degree to which endothelial development occurs in this deficiency. Because replenishment of SCL/Tal1 in 3.5- to 4-day-old SCL/Tal1^{-/-} embryoid cells by Cre-lox-mediated induction or viral transduction showed that the function of hematopoietic and endothelial cells can be normalized,^{31,33} it is likely that SCL/Tal1 deficiency causes deflection of the hemangioblast differentiation into the direction of the angioblast lineage producing endothelial cells and smooth muscle cells. A similar mechanism may occur in the case of FGFR1 deficiency.

Although the studies of Magnusson et al¹⁵ and Faloon et al⁷ indicate that FGFR1 is dispensable for early vascular development in embryoid bodies, this does not exclude that FGFR1 contributes to vascular development and angiogenesis. Several lines of evidence support such a role. First, part of the endothelial cells in the embryoid bodies and in the new vasculature of teratomas does contain FGFR1. A considerable part of these cells is involved in cell division. Whether this expression is paralleled by effects of FGF/FGFR1 on cell division or migration cannot be deduced from these experiments. An alternative explanation might be that FGF/FGFR1 signaling reduces the vulnerability of endothelial cells for apoptosis, as shown in vitro.^{34,35} It may be a general strategy in development to enhance apoptosis vulnerability of cells that make important steps in differentiation or morphogenic patterning, to avoid improper growth. If FGF/FGFR1 signaling would be able to overcome this vulnerability, FGF signaling would act as a "permissive factor" in development. Indeed, such a permissive role of FGF has been well documented in neural stem cell differentiation where FGF has a dual action maintaining the size of the progenitor pool by its proliferative effects as well as stimulating proneural differentiation by modulating signaling.³⁶ Given the involvement of FGF/FGFR1 signaling in the process of differentiation of many different cells, including cardiomyocytes, adipocytes, bone cells, and hematopoietic cells and in patterning of mesoderm and embryo vessels,^{7,15,37-39} this is a concept that warrants further investigation. Such a concept may also explain why the subpopulation of circulating progenitor cells that contain FGFR1 may be particularly successful in differentiating into an endothelial phenotype in adult man.⁴⁰

Interestingly in the FGFR1^{-/-} embryoid bodies the lack of FGFR-1 signaling resulted in an abnormal branching pattern

of the vasculature.^{15,16} Although these in vitro systems do not completely reflect the normal embryonic or organ-specific multicellular environment (embryoid bodies lack the polarity of the embryo) and the precise state of differentiation of endothelial cells, they underpin a potential role of FGF/FGFR1 signaling in patterning of the vascular network. This is in line with studies of Lee et al,²⁹ who used a dominant-negative truncated FGFR1 behind a CMV-promoter, which was injected in the 9-day-old murine embryo, and becomes expressed preferentially in vascular and endocardial endothelial cells. The overexpression resulted in abnormal development including incomplete branching of the vascular network.²⁹

The formation of a vascular network as well as mesodermal development require coordination of cell signaling and stepwise recruitment of cytokines in a temporally and spatially coordinated manner. These processes warrant factors that can change the cellular environment from a nonpermissive one into a permissive milieu for such processes. The current study of Magnusson adds another piece of the puzzle to the concept that FGF/FGFR1 is not a conditional factor but rather one of these permissive factors.

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