Building blood vessels—stem cell models in vascular biology

Lars Jakobsson, Johan Kreuger, and Lena Claesson-Welsh

Department of Genetics and Pathology, Uppsala University, SE-751 85 Uppsala, Sweden

Spheroids of differentiating embryonic stem cells, denoted embryoid bodies, constitute a high-quality model for vascular development, particularly well suited for loss-of-function analysis of genes required for early embryogenesis. This review examines vasculogenesis and angiogenesis in murine embryoid bodies and discusses the promise of stem cell–based models for the study of human vascular development.

Introduction

In the early 1980s, methodology was established to allow for culture of pluripotent embryonic stem cells (ESCs) isolated from the inner cell mass of mouse blastocysts (for review see Glaser et al., 2005). Gene targeting in ESCs was soon thereafter achieved through homologous recombination, followed by generation of mouse strains from such manipulated ESCs. Occasionally, gene targeting results in early embryonic lethality, which precludes understanding of the contribution of genes to subsequent developmental processes including de novo blood vessel formation (vasculogenesis) and formation of new blood vessels from preexisting vessels (angiogenesis).

Blood vessels are essential for the delivery of nutrients and oxygen to tissues, as well as for removal of waste products. All blood vessels share a number of basic features, although the detailed gene expression pattern, morphology, and function vary between different vascular beds (e.g., arteries, veins, and capillaries). The inside of blood vessels is lined with endothelium, a thin layer of endothelial cells (ECs), which separates the blood from tissues. The outside of the endothelium is covered with a specialized layer of connective tissue (the basement membrane) followed by a layer of mural cells (pericytes and vascular smooth muscle cells). Angiogenesis is a tightly controlled process where EC proliferation and migration is regulated by secreted factors as well as by surrounding cells and matrix. There are currently considerable efforts invested into the development of drugs aimed to control blood vessel growth in conditions such as ischemia and cancer, characterized by deficient or excessive vessel growth, respectively. To this end, it is essential to create easily accessible models by which vessel development can be both manipulated and studied at high resolution.

Vascular development and sprouting angiogenesis in embryoid bodies

The isolation of EC lines and the establishment of conditions required for their maintenance in cell culture represents a milestone in the vascular biology field (Gimbrone et al., 1973). However, such cultures do not provide a proper microenvironment, involving three-dimensional (3D) interactions between ECs and adjacent supporting cells and matrix that are known to be absolutely vital in regulation of vascular processes. In contrast, cultures of human and murine ESCs possess the capacity to differentiate into most if not all major cell lineages (Thomson et al., 1998), creating an environment with parallel development of several cell types. Thus, in differentiating ESCs assembled into embryoid bodies (EBs), vascular development occurs in a context of continuous interactions with adjacent non-ECs. The first indication that EC development and subsequent vascular morphogenesis in differentiating ESC cultures proceed in an in vivo–like fashion was provided by Doetschman et al. (1985).

Formation of EBs can be controlled through aggregation of ESCs in hanging drops (Fig. 1, A and B), after the removal of feeder cells and leukemia inhibitory factor that otherwise are used to keep the ESCs pluripotent. The hanging drop culture proceeds for a few days to allow EB growth and differentiation, followed by seeding into a two-dimensional (2D) culture (Fig. 1, C and E), or into a 3D collagen gel (Fig. 1, D and F). For more information on EB culture procedures, see Jakobsson et al. (2006) (detailed protocols will be made available upon request to the authors). At d 3 of differentiation, the onset of vasculogenesis is demonstrated by the presence of a precursor common for endothelial and hematopoietic cells, the hemangioblast. The hemangioblast, which expresses T cell acute leukemia 1/stem cell leukemia (TAL/SCL), vascular endothelial growth factor receptor (VEGFR)-2, and brachyury, has also been detected in human EBs (Choi et al., 1998; Kennedy et al., 2007). Subsequently, hemangioblasts will be committed to either the hematopoietic or the EC lineage. The EC precursors, the angioblasts, undergo sequential maturation to eventually express a set of markers characteristic for mature ECs such as VEGFR-2, CD31,
vascular endothelial (VE)-cadherin, Tie-1, and Tie-2. Angio-
blast development in EBs thus closely mimics the in vivo matu-
rates (Vittet et al., 1996).

The primary vascular plexus in the EB is remodeled from
d 6 and onwards, by sprouting angiogenesis. This process is regu-
lated by growth factors, which may be produced endogenously or
added as exogenous factors. Also without growth factor treat-
ment but in the presence of 15% serum, vascular development is
evident by the presence of blood islands that differentiate to form
small networks of ECs located in the center of the EB. Addition of
growth factors stimulates further expansion of the endothelium.

There is a distinct morphology of the vascular plexus formed in 2D
EB cultures dependent on the growth factor present in the culture;
VEGF isoforms VEGF-A121 and VEGF-A165, fibroblast growth
factor-2, and platelet-derived growth factor (PDGF)-BB each en-
hance vessel formation in a distinct pattern. Typically, VEGF-A165
stimulates the formation of a peripheral capillary plexus in 2D EB
cultures (Fig. 1, C and E) (Jakobsson et al., 2006).

Invasive angiogenesis in 3D collagen gels is preferen-
tially induced by VEGF-A165 and manifested around d 8 by
the formation of EC sprouts protruding from the central core
of the EB. The stalk cells are guided by tip cells with numer-
ous filopodia, a process with striking similarities to vascular
development in zebra fish and the retina (Fig. 1, D and F; and
Fig. 2 A) (Lawson and Weinstein, 2002; Gerhardt et al., 2003).
Subsequently, the sprouts branch and occasional tip cells fuse
with adjacent vessels to form networks. The EC sprouts are
surrounded by perivascular cells that share features such as
morphology (i.e., close apposition to the endothelial cells) and
protein expression pattern (expressing nerve-glial2 [NG2] and/or
α-smooth muscle actin [αSMA]) with pericytes seen in vivo
(Fig. 2 A). Furthermore, the vessels are enclosed by a vascular
basement membrane whose detailed composition, dynamics, and
Table I. Vascular phenotypes in mouse embryos and EBs, as a consequence of specific gene targeting

<table>
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<tr>
<th>Genotype</th>
<th>Embryo</th>
<th>EB</th>
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<tbody>
<tr>
<td>vegfr2+/−</td>
<td>E8.5–9.5. Defective blood-island formation and vasculogenesis (Shalaby et al., 1995)</td>
<td>Defective EC development and vascular remodeling [Jakobsson et al., 2006]</td>
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<tr>
<td>vegfa+/−</td>
<td>E11–12. Defective vascular development (Carmeliet et al., 1996; Ferrara et al., 1996)</td>
<td>Reduced EC development and vascular remodeling; partial rescue by exogenous VEGF [Bautch et al., 2000]</td>
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<tr>
<td>vegfr2+/−</td>
<td>E9.5–10.5. Generated by aggregation of ESCs with tetraploid embryos. More severely affected than vegfa+/− embryos (Carmeliet et al., 1996)</td>
<td>Attenuated EC development and vascular remodeling; partial rescue by exogenous VEGF [Bautch et al., 2000; Ng et al., 2004]</td>
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<tr>
<td>VE-cadherin+/−</td>
<td>E9.5. Defective vascular development and angiogenesis (Carmeliet et al., 1999)</td>
<td>Defective vascular formation and morphogenesis [Vittet et al., 1997]</td>
</tr>
<tr>
<td>N-cadherin+/−</td>
<td>E10. Impaired angiogenesis, defective yolk sac vasculature (Radice et al., 1997)</td>
<td>Reduced pericyte coating, otherwise intact vascular sprouting and remodeling [Tillet et al., 2005]</td>
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<td>pdgfrβ+/−</td>
<td>Lethal shortly before birth, hemorrhagic (Soriano, 1994). Loss of vSMC recruitment to small arteries in limb, heart and skin (Hellström et al., 1999)</td>
<td>Loss of vSMC/pericyte recruitment to angiogenic sprouts [Rolny et al., 2006]</td>
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<tr>
<td>Ndst1/2+/−</td>
<td>Lethal before gastrulation [Holmborn et al., 2004]</td>
<td>Attenuated vascular development; rescue by HS presented in trans by non-EC [Jakobsson et al., 2006]</td>
</tr>
<tr>
<td>fgfr1−/−</td>
<td>E9.5. Reduced blood-island formation (Deng et al., 1994)</td>
<td>Exaggerated vascularization and angiogenic sprouting in the absence of exogenous growth factors [Magnusson et al., 2005]</td>
</tr>
<tr>
<td>β1integrin+/−</td>
<td>E5.5 (Fässler and Meyer, 1995)</td>
<td>Poor vessel branching, disturbed VEGF-induced morphogenesis [Bloch et al., 1997]</td>
</tr>
</tbody>
</table>

Indicates time point of embryonic lethality.

function in the EBs remain to be described. Lumen formation is detectable at about d 10 of EB differentiation, and occasionally a mature lumen is evident at d 12 (Fig. 2A). Subsequently, large lumenized vascular networks become established. It is an interesting possibility that the EB endothelium has the capacity to undergo arterial/venous specification, as endothelial cells formed from ESCs in vitro specifically express either ephrin B2 or EphB4, which are markers for arterial and venous endothelium, respectively (Muller-Ehmsen et al., 2006).

Embryoid bodies; beyond early lethality of transgenic animals

Inactivation of genes with a vascular function (vegfr2, vegfa, VE-cadherin, or pdgfrβ) results in similar phenotypes in the EB model as in vivo, with regard to temporal effects on development and consequences for EC morphology (Table I) (Olsson et al., 2006). For example, deletion of VEGF-A, one of the main VEGFR-2 ligands, results in an arrest in vascular development and remodeling in vivo as well as in vitro (Ng et al., 2004). The EB model is particularly suitable in this context because it allows rapid and easy testing of the unique contribution of different VEGF-A isoforms to vascular development. Accordingly, treatment of VEGF-A–deficient EBs with purified VEGF-A165 rescued EC morphogenesis (Bautch et al., 2000), in agreement with the fact that mice expressing only VEGF-A165 display normal vascular development. In certain cases, data generated using in vivo models have been extended by studies performed in different ESC-based culture models. For example, VEGFR-2 was shown by differentiation of vegfr2+/− ESCs to be required for vascular morphogenesis but not essential for early endothelial and hematopoietic cell commitment (Schuh et al., 1999). Furthermore, a number of gene deletions have resulted in developmental arrest before the onset of vasculogenesis, for example due to defects in implantation. However, by generation of ESCs from recombinant blastocysts, critical stages incompatible with in vivo growth may be studied in the EB model (see Table I for a comparison of vascular phenotypes in gene-targeted embryos and EBs).

A severe developmental phenotype (lethal before gastrulation) is caused by simultaneous deletion of the enzymes N-deacetylase/N-sulfotransferase 1 and 2 (NDST1/2), central to the synthesis of heparan sulfate (HS) (Holmborn et al., 2004). Proteins modified by attachment of HS, so-called proteoglycans, are essential co-receptors for many tyrosine kinase receptors, including VEGFR-2. Deletion of the NDST1/2 enzymes severely hampers ESC differentiation with a close to complete loss of vascular development (Fig. 2). However, rescue of vascular development was achieved in chimeric EB cultures composed of a mix of Ndst1/2−/− and vegfr2−/− ESCs (Jakobsson et al., 2006). Here, endothelial cells derived from the HS-deficient Ndst1/2−/− stem cells (expressing VEGFR-2) were complemented by normal HS produced by pericytes derived from the vegfr2−/− ESCs (Fig. 2). This exemplifies the versatility of the EB model, which allows combinations of knock-out ESCs to study the requirement for genes in subpopulations of cells during cell specification and development, to unravel new mechanisms in cell communication.

Restrictions of the EB model

Although ESCs have the capacity to produce cells of essentially any type, it is likely that complex processes that require progression through several developmental stages may be underestimated. For example, although blood EC development is faithfully reproduced, the subsequent differentiation of lymphatic ECs from blood vessels is difficult to control (Kreuger et al., 2006). Furthermore, EBs lack blood flow, and dependent
Importantly, both mouse and human ESCs can be used to generate functional ECs contributing to formation of stable vessels that connect to the host circulation (Yurugi-Kobayashi et al., 2003; Wang et al., 2007). The use of human ESCs for therapeutic purposes obviously presents a moral dilemma. Possibly, retrieval of ESCs from other locations than the fetus, such as umbilical cord blood or amniotic sources may present a feasible alternative in the future (Zhang et al., 2006). Furthermore, it is an interesting possibility that pathological conditions characterized by impaired blood and lymph vessel function may be treated by administration of adult stem cells or progenitors isolated from the patient’s own bone marrow. For a recent review on the contribution of circulating stem cells to angiogenesis, see Kopp et al. (2006).

Despite striking similarities between mouse and human development, numerous therapies developed in mice (e.g., to treat diseases such as cancer) have failed when tested in humans. A contributing factor to such failures may be genetic differences between the species. Because most experimentation on humans is prohibited for ethical reasons, preclinical testing has relied solely on animal experimentation. In the future, application of human ESCs may constitute an additional step in the development and testing of drugs, with regard to toxicity and teratogenic effects. Furthermore, experimentation with human ESCs offers means to study human embryonic development. Already, homologous recombination and introduction of RNAi have been demonstrated in human ESCs, paving the way for new insights in human biology (Zwaka and Thomson, 2003).

References


