Development and differentiation of endothelium

WERNER RISAU

Max-Planck-Institut für physiologische und klinische Forschung, W.G. Kerckhoff Institut, Abteilung Molekulare Zellbiologie, Bad Nauheim, Germany

Development and differentiation of endothelium. Vascular endothelial cells play an important role in tissue homeostasis, fibrinolysis, and coagulation; blood-tissue exchange, vasotonus regulation, blood cell activation, and migration; and the vascularization of tissues. The formation of new blood vessels comprises two distinct steps: vasculogenesis, the in situ assembly of capillaries, and angiogenesis, the sprouting of capillaries from preexisting ones. Vascular endothelial growth factor (VEGF) is essential for vasculogenesis and angiogenesis. Its expression is high in the embryonic brain and kidney when angiogenesis occurs and low in the adult brain when angiogenesis is absent. In the kidney, VEGF expression remains high in glomerular podocytes even in the adult. VEGF receptors 1 and 2 (flt-1 and flk-1) are endothelial-specific receptor tyrosine kinases. Similar to the ligand, expression of these receptors is high during brain and kidney angiogenesis, low in adult brain endothelium, but high in adult glomerular endothelium. Because VEGF is also a vascular permeability factor, the expression in the adult correlates with the low permeability of blood-brain barrier endothelium and the high permeability of fenestrated glomerular endothelium. Although fenestrae formation can be induced in vitro by VEGF and a basal lamina-type extracellular matrix, blood-brain barrier characteristics seem to require the presence of still unknown brain-derived factors.

DEVELOPMENT OF ENDOTHELIAL CELLS

In the embryo, the cardiovascular system is the first organ system to develop. Angioblasts differentiate from the mesoderm and subsequently form a primitive vascular plexus. This process is called vasculogenesis, as reviewed in [1]. The subsequent process of sprouting of new capillaries from the preexisting network is called angiogenesis. Angioblasts also differentiate from the mesodermal precursors within organ rudiments of the lung, pancreas, and spleen [2]. Notably, all of these organs are of endodermal origin, whereas organs of ectodermal or mesenchymal origin seem to be vascularized, at least partly, by an angiogenic mechanism, such as kidney and brain [3–5]. The molecular mechanisms underlying these processes are still only poorly understood, but progress has been made recently. In an avian model system, endothelial cell differentiation can be induced in vitro by incubation of pluripotent embryonic cells (epiblast cells) with basic fibroblast growth factor (bFGF) [6]. The first molecule so far known to be expressed in a population of mesodermal cells giving rise to angioblasts is the VEGF receptor-2 [VEGFR-2; also known as flk-1 in the mouse and kinase insert domain receptor (KDR) in the human]. The crucial importance of this receptor is underlined by the early lethality resulting from the absence of hematopoietic and endothelial cells in mice deficient for this molecule. Later, during embryonic development, VEGFR-2 becomes restricted to endothelial cells consistent with the function of its ligand VEGF as a specific endothelial cell growth and vascular permeability factor. Mice deficient for a single VEGF allele show embryonic lethality due to defective large vessel development and capillary sprouting. This severe haploid-insufficient phenotype suggests that critical thresholds of VEGF protein may be required for activating the VEGF receptors in early development, as reviewed in [7].

Hypoxia is a rapid and potent inducer of VEGF mRNA expression. Hypoxia-induced transcriptional activation is mediated by direct binding of hypoxia-inducible factor-1 (HIF-1), a basic helix-loop-helix (bHLH) Period, Arylhydrocarbon-receptor, single minded (PAS)-domain transcription factor. VEGF up-regulation is also achieved by increased RNA stability [8, 9]. Hypoxia may play a role in the regulation of VEGF-dependent developmental angiogenesis, for example, in the brain and retina. We have cloned a novel bHLH-PAS-domain protein showing a high degree of homology to HIF-1. This was termed HIF-related factor (HRF). HRF mRNA is highly expressed by brain capillary endothelial cells in the embryo and the adult but also in other vascular beds such as the kidney glomeruli and choroid plexus. Expression of HRF is, however, not restricted to the endothelium: smooth muscle cells, epithelia, and neurons also have detectable amounts of HRF mRNA [10]. HRF is distinguished from its close relative HIF-1 by its distinct expression pattern that implies a role for the factor during tubulogenesis. Tubular networks, which are propagated by branching and sprouting of terminal cells, are also known in invertebrates, such as the tracheal system in insects, the morphogenesis of which closely resembles that of the vertebrate vascular system [11].

Key words: vascular endothelial growth factor, hypoxia-inducible factor, blood-brain barrier, fenestrated endothelial cells.

© 1998 by the International Society of Nephrology
the bHLH-PAS-domain protein tracheless has been identified as a master regulator of tracheogenesis [12]. Interestingly, the amino acid sequence of the DNA binding basic domain is identical to that of HIF-1, and HRF is a more potent inducer of VEGF than HIF-1. Furthermore, tracheae and blood vessels grow in response to hypoxia. Apart from the endothelium, HRF expression correlates with VEGF expression in the kidney glomeruli and the choroid plexus. There, in contrast to VEGF, HRF is highly expressed not only in the epithelial, but also in the endothelial cells where VEGF receptors are present at high levels at this stage of development [13].

**ORGAN-SPECIFIC ENDOTHELIUM**

On the basis of morphology, microvascular endothelium in the normal adult organism is divided into different phenotypes: continuous, fenestrated, and discontinuous. The morphological differences correlate with vascular permeability. In continuous capillaries, the endothelial cytoplasm is continuous, and there is no fusion of luminal and abluminal plasma membranes, except at the cell junctions. Discontinuous endothelial cells usually have clustered holes of 80 to 200 nm in diameter at the tapering edges of the cell. Fenestrated endothelium has similar pores with diaphragms. All of these different characteristics reflect potential differentiation pathways of endothelial cells, which have formed capillaries in a given organ or tissue by vasculogenesis or angiogenesis, as reviewed in [14]. Interaction of both types of endothelium with the organ and tissue environment, either by soluble factors or via cell-cell interaction, leads to the particular phenotype of the endothelium. It is unclear whether the differentiation of organ-specific characteristics in organ-derived endothelium is already predetermined. In any case, a single organ such as the kidney contains many different types of endothelial cells, such as, fenestrated, diaphragmed endothelium in peritubular capillaries, fenestrated, nondiaphragmed (or “porous”) endothelium in glomerular capillaries, and continuous endothelium in other parts. In lymph nodes, “high” endothelial cells are found specifically in postcapillary venules. In the brain, circumventricular organs have fenestrated endothelium, whereas other brain capillaries form the blood-brain barrier. Organ-specific differentiation of endothelial cells is reversible. Fenestrations and blood-brain barrier properties, for example, are lost from endothelial cells invading different organs and tumors [15]. Also, endothelial cells lose organ-specific properties when removed from the organ environment and cultured *in vitro*. This loss of organ-specific characteristics, such as, fenestrations [16] and the high electrical resistance of the blood-brain barrier [17], has been observed in many cultured endothelial cells.

**BRAIN ANGIOGENESIS AND BLOOD-BRAIN BARRIER ENDOTHELIUM**

The development of the brain vascular system begins when angioblasts enter the head region and form the perineural vascular plexus that covers the entire surface of the neural tube. The vascular system within the central nervous system (CNS) does not develop by vasculogenesis but originates from the perineural plexus when vascular sprouts invade the proliferating neuroectoderm at day 10 of embryonic development in rodents. It is maximal in the early postnatal period and down-regulated in the adult brain [18]. Experiments using embryonic brain tissue of the chicken transplanted into ectopic sites of quail embryos have confirmed that the transplanted brain tissue of the chicken can induce ingrowth of vascular sprouts and, therefore, angiogenesis in the quail. Moreover, the grafted neural tissue induces blood-brain barrier characteristics in the host-derived endothelial cells [19, 20].

Brain angiogenesis seems to be regulated by angiogenic factors secreted by the brain. The spatial and temporal expression pattern of VEGF mRNA corresponds closely with angiogenesis during embryonic development in the mouse brain. By *in situ* hybridization, we have shown that in day 17 mouse embryos VEGF mRNA is expressed in the ventricular layer of the developing neuroectoderm [13]. In adult brain, where vascularization is complete and the rate of endothelial cell proliferation is very low, VEGF mRNA is no longer detected in the ependymal cells. It is, therefore, conceivable that during brain development VEGF is released by cells of the ventricular layer, thus promoting angiogenesis by initiating migratory and mitogenic responses by endothelial cells from the perineural vascular plexus. These endothelial cells express VEGFR-2 mRNA during the period of brain vascularization. In the adult brain, when angiogenesis has ceased, expression is very low. Taken together, these findings support the hypothesis that VEGFR-2-expressing capillary sprouts, originating from the perineural vascular plexus, migrate toward an angiogenic stimulus provided by VEGF.

Expression of the tie-1 and tie-2 receptor mRNAs is very similar to that of the high-affinity VEGF receptors, suggesting that they also might be involved in brain angiogenesis. However, the phenotype of the tie-1 and tie-2 receptor-deficient mice differs from that of VEGF receptor-deficient mice [21]. Most strikingly, no sprouting of capillaries into the brain is seen in tie-2 mutant mice, although a perineural plexus is formed normally. Ligands for the tie-2 receptor, called angiopoietins, have been identified recently and are expressed at sites of vascular sprouting and remodeling [22, 23]. Thus, brain angiogenesis requires at least two different ligand-receptor systems that are involved in different aspects of the complex process of vascular sprouting, migration, invasion, and proliferation.
During late embryonic and early postnatal development in rodents, brain capillaries become impermeable to proteins and differentiate to blood-brain barrier endothelium. Tight junctions between brain endothelial cells are the structural basis for the paracellular impermeability and high electrical resistance of these endothelial cells. During brain angiogenesis, the decrease in vessel permeability to protein correlates with a conformational change of the tight junctions. We have analyzed quantitatively the structure and function of tight junctions in primary cultures of bovine brain endothelial cells using quantitative freeze-fracture electron microscopy and ion and inulin permeability [24]. We found a complex network of P-face-associated tight junctions only in the capillaries and postcapillary venules of the brain cortex. Tight junctions in peripheral, nonbarrier endothelial cells are E-face-associated in vivo. Consistent with the development of physiological barrier properties, that is, electrical resistance and low permeability, P-face-associated junctional particles form during the development of blood-brain barrier capillaries from embryonic day 13 to postnatal day 1 [25]. The molecular mechanisms involved in the gain and loss of P-face association of tight junctions are unknown. There is, however, evidence that tight junctions are associated with the cytoskeleton [26], which seems to be important for the regulation of paracellular permeability, suggesting that the cytoplasmic anchoring of the tight junctions plays an important role.

**FENESTRATED ENDOTHELIA**

Fenestrated capillaries are more permeable to low-molecular weight, hydrophilic molecules than continuous capillaries, which is consistent with the presence of fenestrated endothelium at sites of filtration, secretion, and absorption [27]. The only molecular difference from all other endothelial plasma membranes known so far is the clustering of anionic cell-surface molecules at fenestrations [28]. Because fenestrated endothelium occurs in close proximity to epithelium, interaction between the two cell types has been thought to be important for the differentiation and maintenance of fenestrae. As mentioned earlier here, epithelial cells adjacent to fenestrated endothelium, in the glomerulus and choroid plexus, show a high constitutive expression of VEGF. VEGF, by virtue of its permeability-inducing activities, might therefore induce and stabilize the fenestrated capillary phenotype. Recently, direct in vivo evidence has been provided by the demonstration of increased microvascular permeability and endothelial fenestration after topical application of VEGF/VPF in muscle or by intradermal injection. This effect was seen in venous and capillary endothelium that is not normally fenestrated in these organs [29]. In an in vitro model system, we have indeed observed fenestrae induction in endothelial cells cultured on a basal lamina-type extracellular matrix in the presence of VEGF [30]. This unique activity of VEGF therefore provides an opportunity to unravel the molecular mechanisms involved in the differentiation of fenestrated endothelium.

**CONCLUSIONS AND PERSPECTIVES**

Blood-brain barrier and glomerular fenestrated endothelial cells are two functionally important examples of organ-specific endothelial cells. From the data summarized in this brief review, a concept emerges that suggests that continuous expression of VEGF in epithelial cells, such as, podocytes, adjacent to endothelial cells, such as, glomerular endothelium, that express VEGF receptors induces and maintains a fenestrated phenotype in the endothelium (Fig. 1). HRF is a candidate factor expressed in podocytes and
endothelial cells that may be responsible for the high level of continuous VEGF and VEGF receptor expression. In the brain, VEGF and its receptors are down-regulated after brain angiogenesis has ceased (Fig. 1). This may be because of the absence of hypoxia in a fully differentiated, highly vascularized CNS. Although not expressed in brain parenchymal cells, HRF mRNA is still expressed by brain capillary endothelial cells in the adult. The functional consequence of this expression and the target genes are unknown. In any case, in addition to the down-regulation of VEGF in the brain, signals from the brain are needed to induce the particular blood-brain barrier properties in endothelial cells, such as P-face-associated tight junctions.

Reprint requests to Dr. W. Risau, Max-Planck-Institut fu¨r physiologische und klinische Forschung, W.G. Kerckhoff Institut, Abteilung Molekulare Zellbiologie, Parkstrasse 1, D-61231 Bad Nauheim, Germany. Email: WRisau@kerckhoff.mpg.de

REFERENCES