

Angiogenesis: the VE-cadherin switch

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Because angiogenesis is a key step in a number of pathological processes, including tumor growth and atherosclerosis, many research studies have investigated the regulatory signals active at various stages of vascular invasion. The differential activities of the endothelial junction protein vascular endothelial (VE)-cadherin reflects the versatile behavior of endothelial cells between vascular quiescence and angiogenesis. VE-cadherin function and signaling is deeply modified in proliferating cells and this conversion is accompanied by phosphorylation of the protein on tyrosine residues and enhanced transcription of its gene. Recent advances in the complex interplay between protein tyrosine kinases and phosphatases regulating VE-cadherin phosphorylation and function are discussed in this review.

Introduction

During the past decade, the prominent role of the neovascularization process in tumor growth and metastasis has been clearly established. The vascular endothelium forms a selective barrier between the blood stream and the underlying tissues. In addition, the endothelium is at the origin of neovessel formation by extension of the existing vasculature in a process called angiogenesis. From the biological point of view, the endothelial cells may be considered as epithelial cells, connected by cell-cell junctions and attached to a basal lamina, when embedded in stable vessels, whereas it behaves like mesenchymal cells during the angiogenic process. In established vessels, transmembrane adhesive proteins located at the cell-cell junction maintain the integrity of the endothelial cell lining. The vascular endothelial (VE)-cadherin, originally called cadherin-5 (Suzuki et al. 1991), is the transmembrane component of the endothelial adherens junction and plays a pivotal role in endothelium integrity and in the control of vascular permeability (Breviario et al. 1995). In this review, we will focus on the differential functions of this endothelial-specific molecule in quiescent and angiogenic endothelial cells and we will describe recent advances in our knowledge of its regulation

between these two states. The organization of the endothelial intercellular contacts and the molecular description of the different junctional complexes are described in excellent reviews (Aurrand-Lions et al. 2002, Vestweber 2002, Bazzoni and Dejana 2004) and will not be reported here.

VE-cadherin in quiescent vessels

Adherens junction organization

VE-cadherin belongs to the cadherin family [for review, see Wheelock and Johnson (2003)] whose members develop homophilic adhesive activity through their extracellular domains. Classical cadherins, such as VE-cadherin, are associated with actin via interaction of their C-terminal domain with β -catenin/ α -catenin or plakoglobin/ α -catenin complexes. In addition, association of VE-cadherin with vimentin through plakoglobin/desmoplakin has been reported (Wheelock and Johnson 2003). The linkage of VE-cadherin with the cytoskeleton, especially when plakoglobin is involved, leads to strong cell-cell interaction. Two other catenins, p120 and the p120-related protein p0071, bind to an identical juxtamembrane intracellular domain of VE-cadherin. P120 exerts various functions, including the regulation of cadherin levels by controlling cadherin internalization and degradation as well as cadherin clustering at the junction. P0071 interacts with desmoplakin, which may provide an additional link between VE-cadherin and vimentin. For a review on p120/p0071 in endothelial cells, see Vincent et al. (2004).

Vascular permeability

Besides its preponderant role in endothelial homotypic adhesion, the presence of VE-cadherin at cell junctions decreases paracellular permeability. Endothelial junctions are highly dynamic and vascular permeability is increased by inflammatory cytokines, including histamine, tumor

necrosis factor α (TNF α) [reviewed in Bazzoni and Dejana (2004)] and platelet activating factor (PAF) (Hudry-Clergeon et al. 2005). In addition, vascular endothelial growth factor (VEGF) (Esser et al. 1998), a cytokine promoting vascular proliferation in angiogenic situations, also induces increase in vascular permeability and endothelial cell survival in quiescent vessels. The cellular responses to these effectors involve disruption of the VE-cadherin-based adherens junction.

Cell cycle arrest

It is commonly accepted that contact inhibition of cell proliferation is at least partially mediated by the establishment of cadherin-based junctions. Endothelial cell division is inhibited when cells are plated onto a substrate containing the VE-cadherin extracellular domain (Caveda et al. 1996), indicating that VE-cadherin engagement limits endothelial cell proliferation. Several signaling pathways have been identified to explain the VE-cadherin-dependent cell cycle arrest. First, β -Catenin may translocate in some conditions to the nucleus where it activates genes, including *cyclin D1* and *myc*, which induce cell cycle entry [reviewed in Conacci-Sorrell et al. (2002)]. β -catenin localization at the junction may thus prevent target gene transcription and cell division. Second, VE-cadherin associates with VEGF receptor 2 (R2) upon VEGF induction or angiogenic stimulation (Carmeliet et al. 1999, Lambeng et al. 2005). This association inhibits VEGF R2 phosphorylation, by the action of junctional phosphatases such as DEP-1, and its MAP kinase-dependent proliferative signal (Lampugnani et al. 2003). Third, VEGF induces VE-cadherin association with the adaptor molecule Shc, and this association increases Shc dephosphorylation, probably through the action of junctional phosphatases (Zanetti et al. 2002). As Shc is known to activate the MAP kinase pathway, its capture at the junction by VE-cadherin and its subsequent dephosphorylation may interfere with the VEGF proliferative signal. Fourth, Csk (C-terminal

Src kinase) binds to VE-cadherin when phosphorylated on Tyr 685 (see below), and this binding might also be involved in inhibition of cell growth (Baumeister et al. 2005).

Endothelial cell survival

VEGF transduces a survival signal to endothelial cells through a VE-cadherin-dependent mechanism. This signal is mediated by the PI3-kinase/Akt pathway and needs VEGF R2-VE-cadherin association (Carmeliet et al. 1999). Among the possible target of PI3-kinase signaling, the upregulation of *Gas1*, a growth arrest-specific gene, correlates with inhibition of endothelial cell apoptosis (Spagnuolo et al. 2004).

VE-cadherin requirement and function in angiogenesis

The first evidences of VE-cadherin implication in angiogenesis derives from experiments using function-blocking antibodies in *in vitro* angiogenesis (Matsumura et al. 1997, Bach et al. 1998). In VE-cadherin-deficient embryos and yolk sacs, the vascularization process was arrested at a very primitive stage, with no sign of angiogenic sprouting (Gory-Fauré et al. 1999). For example, the yolk sac blood islands formed normally but did not undergo vascular branching until fetal death at midgestation, indicating that VE-cadherin is required for developmental angiogenesis. More recently, tumor angiogenesis could be blocked by antibodies against VE-cadherin, suggesting that VE-cadherin activity is necessary for vascular proliferation in adults (Corada et al. 2002, Liao et al. 2002). Although cadherins are known to limit cell detachment and migration, downregulation of VE-cadherin activity does not seem to favor a mesenchymal phenotype and VE-cadherin-deficient endothelial cells remained round and weakly mobile (Feraud et al. 2001). The fact that VE-cadherin may be directly involved in endothelial invasive activity is supported by data showing that the VE-cadherin cytoplasmic tail induces cell membrane protrusions (Kouklis et al. 2003). Alternatively, the

vascular defects observed in null mice may be caused by increased endothelial cell apoptosis as, in the absence of VE-cadherin, VEGF-induced survival is inoperative. However, two features argue against that: (i) yolk sac blood islands do not show any sign of leakiness at any time, (ii) the percentage of endothelial cells is normal in VE-cadherin-deficient yolk sacs (Rampon and Huber 2003). One possible reason is that basic fibroblast growth factor (bFGF) anti-apoptotic signaling is not altered by VE-cadherin deficiency and may be sufficient to sustain endothelial survival.

During the angiogenesis process, VE-cadherin disappears from the adherens junction and epitopes are unmasked (Corada et al. 2002, Liao et al. 2002). This is in agreement with the increase in vascular permeability constantly observed in angiogenesis. Other VE-cadherin activities have been reported in the context of vascular proliferation. In the course of migration, VE-cadherin may develop heterophilic adhesive reactions with fibrin, a mechanism potentially relevant in healing angiogenesis [for a review, see Martinez et al. (2001)]. VE-cadherin seems also implicated in lumen formation (Yang et al. 1999) and in cell proliferation through Rho-mediated tension in the actin cytoskeleton (Nelson and Chen 2003). VE-cadherin modulation of VEGF R2 signaling might be modified in angiogenic cells compared to resting cells, as VEGF R2 activation (phosphorylation) by VEGF is stronger in sparse than in confluent cells (Rahimi and Kazlauskas 1999).

Tyrosine phosphorylation of VE-cadherin

Incubation of endothelial cells with VEGF dramatically increases VE-cadherin phosphorylation levels on tyrosine residues (Esser et al. 1998). We recently showed that VE-cadherin phosphorylation levels were significantly increased in ovaries and uterus subjected to hormonal induction, in correlation with the angiogenic switch observed in these tissues,

while they were weak or undetectable in resting tissues (Lambeng et al. 2005). We found that another tyrosine kinase, Src, was constantly associated with VE-cadherin, independent of angiogenic stimulation *in vivo* or VEGF induction *in vitro*. VE-cadherin-associated Src phosphorylation was increased in angiogenic tissues or VEGF-induced endothelial cells. VE-cadherin phosphorylation through the VEGF/VEGF R2 pathway needs Src activity (Weis et al. 2004, Lambeng et al. 2005). Src is required for VEGF-induced angiogenesis and endothelial survival in the angiogenic process (Eliceiri et al. 1999). In addition, Src was shown to associate with VEGF R2 after VEGF induction (Chou et al. 2002). It can be deduced from these data that VEGF induction triggers VEGF R2 association with the VE-cadherin-Src complex. This association leads to Src phosphorylation, which in turn induces VE-cadherin phosphorylation. Furthermore, these results suggest that VE-cadherin phosphorylation is a necessary step for the endothelial switch from the quiescent to the angiogenic phenotype.

It is widely accepted that VE-cadherin phosphorylation triggers cell-cell disruption, possibly through modification of adherens junction composition. But activation of Src in the VE-cadherin complex may also serve other purposes. Src may transduce VEGF survival signal by activation of the PI3-kinase-AKT pathway. In addition, Src is known to regulate membrane ruffles and lamellopodia. Therefore, VE-cadherin-associated Src may also be directly involved in cell migration and vascular invasion.

Other Src family kinases, such as Fyn and Yes, are preferentially associated with VEGF R1 over VEGF R2 (Chou et al. 2002). There is no evidence that VEGF R1 is involved in VE-cadherin phosphorylation. However, activities of Src family members are partially redundant (Eliceiri et al. 1999), and one cannot exclude at present a role for Fyn and Yes in VE-cadherin tyrosine phosphorylation as well as VEGF R2 signal transduction in particular situations.

VE-cadherin contains nine tyrosines in its cytoplasmic domain. The Vestweber lab reported that Tyr685 could be phosphorylated, thereby promoting VE-cadherin-Csk association, and eventually leading to contact inhibition of growth (Baumeister et al. 2005). Consistent with this, we identified Tyr685 as the unique phosphorylation target site in VEGF-induced endothelial cells (Wallez et al, unpublished results). Other tyrosines may be phosphorylated. The Cheresh lab showed that VE-cadherin phosphorylation on Tyr658 or Tyr731, but not other tyrosines, was sufficient to maintain cells in a mesenchymal state (Potter et al. 2005). More work is necessary to understand which tyrosine is targeted in which condition.

VE-cadherin phosphorylation in quiescent vessels is repressed by phosphatase activity (Lambeng et al. 2005). Several phosphatases are localized at endothelial junctions. Among them, protein tyrosine phosphatases VE-PTP and PTP-mu directly interact with VE-cadherin, and there is convincing evidence showing that the activities of either enzymes alter VE-cadherin phosphorylation state (Nawroth et al. 2002, Sui et al. 2005). In addition, Csk binding to phospho-VE-cadherin may inactivate Src by phosphorylation of the Src inhibitory site (Tyr527). This mechanism may represent a negative feedback regulation of VE-cadherin activation.

Angiogenic stimulation of VE-cadherin expression

VE-cadherin was initially considered as a constitutive protein with unregulated expression. However, this issue has to be reconsidered in view of several recent studies. The first description challenging the previous assumption was published by (Parker et al. 2004). The authors showed that endothelial cells from human breast carcinoma contained elevated amounts of VE-cadherin mRNA compared to normal mammary vasculature. We recently reported that VE-cadherin levels in adrenal endothelial cells were dependent upon experimental trophic variation of the gland (Huber et al. 2005). In this model, *VE-cadherin*

promoter activity was downregulated during gland regression and restored after its regeneration. Increased *VE-cadherin* promoter activity was also observed in mouse tumors and in Matrigel plugs impregnated with bFGF (Prandini et al. 2005), suggesting that this cytokine might be the angiogenic factor responsible of *VE-cadherin* transcription enhancement. *VE-cadherin* expression is under control of Ets transcription factors (Gory et al. 1998). One of these factors, Ets 1, is specifically expressed in angiogenic endothelial cells and is capable to transactivate the *VE-cadherin* promoter (Lelievre et al. 2000). Ets1 is thus a good candidate for *VE-cadherin* transcriptional activation in the angiogenesis process. Given that VE-cadherin is involved in vascular morphogenesis and endothelial survival, the fact that its expression is upregulated during vascular proliferation further suggests that VE-cadherin may be a key player in this process.

Conclusion and future directions

Whereas the pleiotropic functions of VE-cadherin in quiescent endothelium have been extensively studied, VE-cadherin role in angiogenesis remains poorly investigated. More specifically, its function in endothelial migration, possibly through membrane protrusion and actin remodeling, is elusive. The transition in VE-cadherin function from the quiescent to the angiogenic states is promoted by VE-cadherin tyrosine phosphorylation and is accompanied by promoter activation leading to increased VE-cadherin content. We propose herein a model (Figure 1) in which the quiescent-to-angiogenic transition of endothelial cells requires a switch in VE-cadherin functions. The interplay between tyrosine kinases and phosphatases interacting with and regulating the VE-cadherin complex must be examined in details to clarify individual activity of each enzymatic partner.

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Figure 1. Model describing VE-cadherin differential activities in quiescent and angiogenic endothelial cells, in connection with VEGF signaling.

In quiescent cells and in absence of VEGF, VE-cadherin molecules are assembled in the adherens junction. Although Src is associated with the complex, VE-cadherin is maintained unphosphorylated by associated phosphatases, such as VE-PTP and PTP- μ . The identity of the targeted phosphotyrosines by either phosphatases is unknown. In presence of VEGF, VEGF R2 associates with VE-cadherin and Src, and the adherens junction is disrupted. VEGF R2 and Src kinase activities lead to VE-cadherin phosphorylation on tyrosines, allowing Csk binding to phospho-VE-cadherin. Both Csk and DEP1 may inhibit VEGF-induced cell proliferation by independent pathways. In addition, VEGF R2/VE-cadherin association induces cell survival through PI3kinase/AKT signaling.

In angiogenic cells, VEGF R2 also associates with the VE-cadherin/Src complex leading to VE-cadherin phosphorylation. However, in absence of protein tyrosine phosphatase activity, signaling is different: cell proliferation is stimulated by ERK/MAPK pathway activation and membrane protrusions are induced through Cdc42 activation. β -catenin may potentially translocate to the nucleus and subsequently activate cell cycle gene transcription. Independently, bFGF signaling presumably leads to enhanced VE-cadherin gene expression. In healing angiogenesis, VE-cadherin may develop heterophilic adhesion with fibrin.