VE-Cadherin
The Major Endothelial Adhesion Molecule Controlling
Cellular Junctions and Blood Vessel Formation

Dietmar Vestweber

Abstract—Vascular endothelial (VE)-cadherin is a strictly endothelial specific adhesion molecule located at junctions between endothelial cells. In analogy of the role of E-cadherin as major determinant for epithelial cell contact integrity, VE-cadherin is of vital importance for the maintenance and control of endothelial cell contacts. Mechanisms that regulate VE-cadherin–mediated adhesion are important for the control of vascular permeability and leukocyte extravasation. In addition to its adhesive functions, VE-cadherin regulates various cellular processes such as cell proliferation and apoptosis and modulates vascular endothelial growth factor receptor functions. Consequently, VE-cadherin is essential during embryonic angiogenesis. This review will focus on recent new developments in understanding the role of VE-cadherin in controlling endothelial cell contacts and influencing endothelial cell behavior by various outside-in signaling processes. (Arterioscler Thromb Vasc Biol 2008;28:000-000.)

Key Words: vascular permeability ■ VE-cadherin ■ leukocyte extravasation ■ cell adhesion

Besides providing a complex system of passively transporting tubes, the endothelium of blood vessels controls actively the entry of leukocytes and substances into tissue. The control of endothelial cell contacts is of vital importance for this process. VE-cadherin is the major determinant of endothelial cell contact integrity and regulation of its activity or its presence at cell contacts is an essential step that controls the permeability of the blood vessel wall for cells and substances.

Cadherins represent one of the major families of cell adhesion molecules. They are defined by the typical extra-cellular cadherin domains (EC-domain) and mediate adhesion via homophilic, Ca²⁺-dependent interactions. Most cadherins typically possess 5 extracellular cadherin domains. Based on sequence comparison they can be divided into different subfamilies of which 2 are the classical type I cadherins such as E- N- P- and C-cadherin, and the type II cadherins, which lack the HAV motif, a classical cadherin binding motif in the EC1 domains of type I cadherins. VE-cadherin belongs to the type II cadherins.

Optimal adhesive function of cadherins requires association of their C terminus with cytoplasmic proteins: the catenins. Cadherins bind directly to β-catenin (alternatively to plakoglobin) and to p120. Plakoglobin and β-catenin can bind to α-catenin, an actin binding protein. For many years it has been the generally accepted view that linkage of the cadherins via the catenins to the actin cytoskeleton is the mechanism by which catenins strengthen cadherin-mediated adhesion. This view has recently been challenged in studies that were unable to verify actin binding to a preformed E-cadherin–β-catenin–α-catenin complex.1 Nevertheless, even if binding of the catenins to a cadherin would not provide linkage to the actin cytoskeleton, it is still commonly accepted that the lack of catenin association destabilizes cadherin-mediated cell adhesion. Consequently the cadherin-catenin association is still the major target of many studies aiming at understanding the mechanism by which cadherin function is regulated. Various intracellular signaling molecules as well as phosphorylation of tyrosine and serine residues of catenins or cadherins have been reported to play a role in cadherin regulation.

Similar to integrins, cadherins are also known for outside-in signaling, a term that describes receptor functions of cell adhesion molecules. VE-cadherin has been described to mediate contact inhibition of cell growth, thereby negatively interfering with VEGFR-2–stimulated cell proliferation. In contrast to this effect, VE-cadherin seems to support VEGFR-2–stimulated antiapoptotic effects. This review will focus on recent progress in understanding the regulation of VE-cadherin mediated adhesion and current concepts for the function of VE-cadherin as a signaling receptor.

Identification of VE-Cadherin and Its Requirement for Endothelial Junction Integrity

VE-cadherin was originally identified and cloned as one of 8 new cadherins using an RT-PCR approach.2 These cadherins were originally named cadherin-4 to 11, and cadherin-5, later renamed as VE-cadherin, was the only endothelial cadherin...
among them. Its complete human cDNA sequence was cloned revealing clear homology to the other known cadherins with clear differences within the cytoplasmic tail. The same cadherin was identified by a monoclonal antibody (mAb) approach as an endothelial cell contact protein. Its cell adhesion function was demonstrated by the ability of transfected CHO cells to aggregate in a Ca\(^{2+}\)-dependent way and the molecule was renamed VE-cadherin. Cloning of mouse VE-cadherin allowed to show its endothelial specificity at the very earliest stages of vascular development in embryonic tissue. This has been confirmed for VE-cadherin in zebrafish embryos. The genomic structure and chromosomal mapping of mouse VE-cadherin were published. A roughly 2.5-kb region of the promotor was characterized to direct endothelial expression of reporter genes in vivo, although expression was not found in all endothelial cells. Stronger endothelial expression of reporter genes in vivo, although expression was not found in all endothelial cells. When intraperitoneally injected, these studies were confirmed in vitro with polyclonal anti–VE-cadherin antibodies and in vivo with yet another mAb against mouse VE-cadherin that induced increased vascular permeability. Adhesion of human VE-cadherin could be blocked with antibodies against EC1 as well as with antibodies against EC3.

**Structural Basis of Homophilic Interactions of VE-Cadherin**

The structural basis of homophilic interactions of cadherins has been extensively analyzed in recent years. Type I and type II cadherins have 5 EC domains and a conserved tryptophan (W2) within their N-terminal EC-1 domain, whereas type II cadherins have an additional tryptophan (W4) within this domain. It was shown for type I cadherins that binding of the W2-residues into a hydrophobic pocket of the adjacent EC1 domain in trans position supports homophilic adhesive contacts between cadherins. In addition, lateral (cis) dimerization of cadherins is believed to provide the interface that supports homophilic interactions of cadherins. It has been suggested that this cis interaction is based on the binding between EC1 and EC2 domains.

For VE-cadherin 2 different modes of interactions have been suggested. One model was proposed based on work with a recombinant form of VE-cadherin expressed in bacteria and containing domains EC-1 to EC-4. This protein was found to form hexameric complexes that were analyzed biochemically and by cryoelectron microscopy. 3D reconstructions of the electron micrographs were used to suggest a homology model (24 Å resolution) based on the known crystal structure of C-cadherin. The arrangement of the 6 VE-cadherin molecules resembled 2 VE-cadherin “triple helices” with each helix containing 3 parallel VE-cadherin monomers and the 2 trimers being connected in trans via “adhesive” interactions between 3 pairs of EC-1 domains. The proposed “triple helix” was supported by trimeric interactions between the EC4 domains.

The second model was based on a recombinant soluble form of mouse VE-cadherin, expressed in mammalian cells, and containing all 5 EC domains connected to the N terminus of the coiled-coil domain of cartilage matrix protein (CMP). This protein formed Ca\(^{2+}\)-dependent ring-like and double ring–like arrangements that suggested interactions between domains 1 and 2 of the ectodomains. This model perfectly agrees with the predicted interactions between adjacent EC1 domains and EC1 and EC2 domains that were suggested based on the crystal structure for C-cadherin. Importantly, only dimers but no hexamers were found in these studies.

Both models reflect on the elongated but substantially curved structure of the crystal data for C-cadherin, although only the studies by the Engel laboratory directly showed this curved shapes in the electron microscope for E-, N-, and VE-cadherin. The second model is quite convincing, reflecting the prominent involvement of the EC1 and EC2 domains reported for C-cadherin. The molecular shapes observed in the electron microscope for analogous multimeric E- and N-cadherin fusion proteins was principally identical, suggesting similar interaction modes for VE-cadherin and “classical” type I cadherins.

The relevance of the hexameric structure has been questioned, because the bacterial recombinant form used in these studies lacked glycosylation and the EC5 domain. In fact, the EC2 to EC5 domains of C-cadherin do contain N- and O-glycosylation sites (almost all on EC3 and EC4) that may well prevent association between these domains, and VE-cadherin contains potential glycosylation sites at similar locations. N-linked glycosylation of VE-cadherin has been demonstrated.

Some biophysical parameters of the homophilic VE-cadherin interaction have been determined by atomic force microscopy using a VE-cadherin-Fc fusion protein. Trans association of VE-cadherin dimers was shown to be a low affinity reaction with a K\(_d\) of 10\(^{-3}\) to 10\(^{-5}\) mol/L. Association required low affinity Ca\(^{2+}\)-binding sites (K\(_d\) = 1.15 mmol/L).

**Regulation of VE-Cadherin Adhesiveness: A Role for Tyrosine Phosphorylation?**

Leukocytes can transmigrate through the endothelial barrier via a junctional as well as a transcellular pathway, with the latter one being much less frequently used. It is generally accepted that endothelial cell contacts need to be opened to let leukocytes pass through the junctions of the endothelial barrier. With VE-cadherin being the major adhesive mechanism for the integrity of endothelial cell contacts, it is reasonable to assume that it needs to be locally downregulated to allow a leukocyte to pass the barrier. Initially, docking of neutrophils to human endothelial cell monolayers was reported to lead to degradation of components of the VE-cadherin/catenin complex by mechanisms within endothelial cells. However, extending these studies revealed that the observed effects had been attributable to proteases released during specimen preparations.

Nevertheless, when the experimental set up was changed and monocyte interactions with endothelial cell monolayers...
were analyzed under flow, it was shown that staining for the VE-cadherin–catenin complex was focally lost at endothelial contacts where monocytes were transmigrating. This finding was confirmed by video microscopy and VE-cadherin–GFP transfected endothelial cells or antibody-labeled VE-cadherin and PECAM-1. It was concluded that VE-cadherin would “move aside like a curtain” to let the leukocyte pass through the junction. An alternative explanation would be endocytosis. Either way, it is likely that VE-cadherin adhesive interactions would open before VE-cadherin molecules move away from sites of transmigration. How this is triggered is not entirely understood.

Several publications report on correlations between changes in the stability of VE-cadherin adhesion and changes in the tyrosine phosphorylation of the VE-cadherin catenin complex. Increasing cell confluence of endothelial cells correlated with increasing tyrosine phosphorylation of VE-cadherin and β-catenin, but not of α-catenin. Neutrophils stimulated by C5a were reported to enhance endothelial cell permeability for albumin, and this correlated with increased tyrosine phosphorylation of VE-cadherin and β-catenin.

It has been suggested that tyrosine phosphorylation of VE-cadherin itself might affect VE-cadherin functions. Based on permeability studies of transfected CHO cells, expressing point mutated forms of VE-cadherin with tyrosine residues replaced by either glutamate or phenylalanine, tyrosine residues 731 and 658 were suggested to participate in the regulation of the adhesive function of VE-cadherin. However, CHO cells expressing these mutants bound as well to immobilized VE-cadherin–Fc as CHO cells expressing non-mutated VE-cadherin. Intriguingly, overexpression of tyrosine/phenylalanine replacement mutants of VE-cadherin for either tyrosine 731 or 658 in primary human umbilical vein endothelial cells (HUVECs) inhibited transendothelial migration of leukocytes, suggesting for the first time that phosphorylation of specific residues is involved in leukocyte extravasation. In addition, crosslinking of intercellular adhesion molecule-1 (ICAM-1) triggered tyrosine phosphorylation of VE-cadherin, a process that required Src and the prolinc-rich tyrosine kinase 2 (Pyk-2). Essentially similar results were shown by Turowsky et al for mouse VE-cadherin, although in this report several more tyrosine mutants of VE-cadherin were analyzed and only the Y731F point mutated form of VE-cadherin inhibited leukocyte diapedesis.

Various factors are known to enhance vascular permeability and to affect phosphorylation of the VE-cadherin–catenin complex. The receptor type protein tyrosine phosphatase (RPTP)-μ is not restricted to endothelial cells and has been described to affect the function of various cadherins. It associates directly with the cytoplasmic tail of VE-cadherin (Table 1) and silencing its expression in human endothelial cells enhanced their permeability for albumin.

Recently, the endothelial specific RPTP, called VE-PTP, was found to associate specifically and selectively with VE-cadherin. Expression of VE-PTP in triple-transfected CHO cells reversed the tyrosine phosphorylation of VE-cadherin elicited by vascular endothelial growth factor-receptor 2 (VEGFR-2). Expression of VE-PTP under an inducible promoter in CHO cells, transfected with VE-cadherin and VEGFR-2, increased VE-cadherin–mediated barrier integrity of a cellular monolayer. The highly specific interaction of VE-PTP with VE-cadherin further supports the hypothesis that tyrosine phosphorylation of the VE-cadherin/catenin complex or of factors in its vicinity is involved in the regulation of VE-cadherin–mediated cell contacts. Indeed, knocking down VE-PTP expression in endothelial cells leads to increased permeability across the endothelial cell layer as well as to increased transendothelial migration of neutrophils (Nottebaum et al, unpublished data, 2004). In addition, the docking of neutrophils to the apical surface of endothelial cells triggers the dissociation of VE-cadherin from VE-PTP (Nottebaum et al, unpublished data, 2005). Thus, VE-PTP is required for the maintenance of VE-cadherin mediated endothelial cell contacts and is likely to participate in neutrophil transmigration.

Vascular endothelial growth factor (VEGF) was found to enhance the permeability of HUVEC monolayers and to increase tyrosine phosphorylation of VE-cadherin, β-catenin, and plakoglobin, although the time courses of both events were not similar. In mice, intravenous injection of VEGF was reported to lead within 2 to 5 minutes to the dissociation of a preexisting complex of the VEGF-receptor 2 with VE-cadherin and β-catenin as well as tyrosine phosphorylation of VE-cadherin and β-catenin. These effects required the kinase Src and were already reversed after 15 minutes. Repeated VEGF injections lead to vascular damages including edema formation.

Association between the VE-cadherin/catenin complex and VEGFR-2 seems to be important for outside in (Table 2) as well as for inside out signaling events (Table 3) concerning VE-cadherin. In cultured cells, and in contrast to the above cited report by Weis et al, VEGF usually leads to transient complex formation (not dissociation) between VE-cadherin and VEGFR-2, an interaction that requires expression of β-catenin and of the scaffold protein IQGAP1. On the one hand this complex is probably important for the counter-regulation of VEGF stimulated proliferation and the regulation of apoptosis as described in more detail below. On the other hand, this complex is most likely important for the regulation of VE-cadherin mediated adhesion. VEGF-stimulated tyrosine phosphorylation of VE-cadherin was reported to require Src, and this kinase was found constitutively associated with VE-cadherin. Furthermore, Src was described to exclusively phosphorylate tyrosine 685 on VE-cadherin. It is yet unknown whether phosphorylation of this tyrosine would affect the adhesive function of VE-cadherin, although it has been reported that inhibition of Src inhibits VEGF-stimulated vascular permeability. A correlation between an increase of VE-cadherin/catenin phosphorylation and a decrease in cell contact integrity has not always been verified. In a thorough kinetic analysis, Seebach et al found that 2 different factors, VEGF and the phosphatase inhibitor pervanadate, each led to a rapid increase (minutes after stimulation) of the tyrosine phosphorylation of VE-cadherin, β-catenin, and plakoglobin, whereas at the same time the transendothelial electrical resistance increased. A decrease in electrical resistance occurred only 40 to 60 minutes later.
An alternative mechanism for the downregulation of VE-cadherin function during VEGF-induced permeability could be based on the phosphorylation of serine 665 in the cytoplasmic tail of VE-cadherin, leading to endocytosis. According to this report VEGF-stimulation leads via Src-dependent phosphorylation of the guanine exchange factor Vav2, to the activation of the GTPase Rac and subsequent phosphorylation of serine 665 of VE-cadherin. This phosphorylation results in the recruitment of β-arrestin2, thereby promoting internalization of VE-cadherin. Intriguingly, serine 665 is located on VE-cadherin directly adjacent to the p120-catenin binding site. Because it has been shown that p120 regulates clathrin-dependent endocytosis, it would be interesting to know whether p120 binding could affect serine 665 phosphorylation.

Partly contradictory results were published for the effects of thrombin on the VE-cadherin catenin complex. Thrombin-induced increase of endothelial permeability was found to go along with the transient dissociation of all catenins from VE-cadherin. Others found no overall dissociation of β-catenin from VE-cadherin on thrombin stimulation. Instead, they observed that the phosphorylation of p120 was upregulated and that of β-catenin was downregulated. A third study demonstrated that thrombin dissociated the phosphatase SHP2 from β-catenin and at the same time increased transiently the tyrosine phosphorylation of β-catenin, plakoglobin, and p120. For histamine it was reported that tyrosine phosphorylation of VE-cadherin, β-catenin, and plakoglobin was rapidly stimulated going along with a better extractability of the complex with detergent. Histidine had no effect on the N-cadherin/catenin complex. These data were confirmed by others.

Small GTPases and Other Molecules Affecting VE-Cadherin Adhesiveness

Small GTPases have been reported to influence the function of various cadherins. In epithelial cells, Rac, Rho, and Cdc42 have all been implicated in the formation and control of E-cadherin-mediated cell–cell adhesion. The functional link between VE-cadherin and Rho-like GTPases in endothelial cells is less clear. Dominant negative Rac (RacN17) has been described as blocking thrombin-induced permeability in HUVECs. In agreement with the latter report, flow-induced increase of endothelial barrier function and VE-cadherin clustering were both inhibited by dominant-negative Rac. Interestingly and in contrast with the generally reported correlations, increase of barrier function correlated with an increase of tyrosine phosphorylation of the VE-cadherin/catenin complex. Cdc42 was suggested to stimulate the interaction between α-catenin and β-catenin thereby preventing the increase of permeability in lung endothelium caused by a dominant negative form of VE-cadherin.

On the other hand it was found that VE-cadherin localization in primary endothelial cells was independent of Rac and Rho activity. However, VE-cadherin expression in VE-cadherin null endothelial cells induced actin rearrangement, augmented the level of active Rac, decreased active Rho, and stimulated expression of the Rac-specific guanosine exchange factor Tiam-1.

An increasing effect of Rac on endothelial permeability was described using a cell-penetrating constitutively active form of Rac (Tat-RacV12) in HUVECs. Tat-RacV12 also induced reactive oxygen species in endothelial cells and induced tyrosine phosphorylation of α-catenin. This is one of the very rare cases that shows α-catenin as target of tyrosine phosphorylation. In a detailed analysis the dissociation of endothelial cell contacts with anti–VE-cadherin antibodies was investigated. It was found that loss of cell contacts was preceded by and dependent on the rapid activation of Rac1 and increased production of reactive oxygen species. In addition, VE-cadherin–associated β-catenin became tyrosine phosphorylated. Interestingly, a dominant negative form of redox-sensitive proline-rich tyrosine kinase 2 (Pyk2) inhibited tyrosine phosphorylation of β-catenin as well as the loss of endothelial cell contacts.

Other mechanisms that could control VE-cadherin-mediated endothelial cell contacts rely on the cAMP-triggered activation of the guanine nucleotide exchange factor Epac1 that in turn activates the small GTPase Rap1, which supports the function of VE-cadherin. How Rap1 improves the adhesive function of the cadherin is not known, but it has been proposed for E-cadherin, that Rap-1 is necessary for proper targeting of E-cadherin to maturing cell contacts. Sphingosin 1-phosphate (SIP), a phospholipid that binds to a class of G protein–coupled receptors (GPCR) was shown to stabilize endothelial cell contacts and enhance the expression of VE-cadherin at cell contacts of recently confluent endothelial cells, a process that required Rho and Rac. Knocking down the expression of the SIP receptor 1 resulted in a loss of VE-cadherin and PECAM-1 expression at cell contacts. Interestingly, early effects of SIP on endothelial barrier enhancement were found to be independent of the presence of VE-cadherin. In general, vascular permeability is affected by a complex set of physiological and pathophysiological factors of which many act via different targets including VE-cadherin. An excellent overview on this topic is given in a recent comprehensive review.

Two adhesion molecules at endothelial tight junctions that are involved in leukocyte extravasation or the regulation of endothelial cell contacts could potentially affect VE-cadherin function. The junctional adhesion molecule JAM-C, one of a small subgroup of tight junction-located Immunoglobulin superfamily proteins involved in leukocyte extravasation, was recently found to affect junction stability in endothelial cells. Downregulation of its expression by siRNA in cultured endothelial cells led to a decrease of endothelial cell permeability and enhanced VE-cadherin adhesive function. Both effects were dependent on Rap1. A structurally related protein to JAM-C, the endothelial cell-selective adhesion molecule (ESAM), was recently found to play a role in vivo in neutrophil extravasation and in the regulation of endothelial cell contacts. Gene-deficient mice displayed a delay in leukocyte extravasation and a delay in the increase of vascular permeability induced by VEGF. In the context of these effects, it is intriguing that MAGI-1, a PDZ protein that
binds to ESAM, is required for Rap-1 activation and for the enhancement of VE-cadherin–mediated cell contacts. VE-Cadherin Is an Essential Adhesion Molecule in Angiogenesis Disrupting the VE-cadherin gene in mouse embryonic stem (ES) cells allowed to investigate its role in the development of vascular structures. Analyzing embryoid bodies developed from such ES cells revealed that the differentiation of endothelial cells was not impaired, yet they remained dispersed and failed to organize a vessel-like pattern. Mice deficient for VE-cadherin died at midgestation of vascular malformations. Defects were more severe in the extraembryonic vasculature. No capillary plexus was formed in the allantois, although interendothelial junctions did form as shown by electron microscopy. Thus VE-cadherin was dispensable for initial vasculogenesis but was required for subsequent remodeling and morphogenesis. Another study showed similar results analyzing VE-cadherin null mice as well as mice lacking the C-terminal 82 amino acids of VE-cadherin, containing the β-catenin binding site. Mice deficient for VE-cadherin died at midgestation of vascular malformations. Defects were more severe in the extraembryonic vasculature. No capillary plexus was formed in the allantois, although interendothelial junctions did form as shown by electron microscopy. Thus VE-cadherin was dispensable for initial vasculogenesis but was required for subsequent remodeling and morphogenesis. Another study showed similar results analyzing VE-cadherin null mice as well as mice lacking the C-terminal 82 amino acids of VE-cadherin, containing the β-catenin binding site. Mice deficient for VE-cadherin died at midgestation of vascular malformations. Defects were more severe in the extraembryonic vasculature. No capillary plexus was formed in the allantois, although interendothelial junctions did form as shown by electron microscopy. Thus VE-cadherin was dispensable for initial vasculogenesis but was required for subsequent remodeling and morphogenesis. Another study showed similar results analyzing VE-cadherin null mice as well as mice lacking the C-terminal 82 amino acids of VE-cadherin, containing the β-catenin binding site. Mice deficient for VE-cadherin died at midgestation of vascular malformations. Defects were more severe in the extraembryonic vasculature. No capillary plexus was formed in the allantois, although interendothelial junctions did form as shown by electron microscopy. Thus VE-cadherin was dispensable for initial vasculogenesis but was required for subsequent remodeling and morphogenesis.

### Table 1. Proteins That Are Directly or Indirectly Associated With VE-Cadherin

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Details About the Interaction</th>
<th>References</th>
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<tbody>
<tr>
<td><strong>Intracellular binding partners</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGFR-2</td>
<td>Stimulation with VEGF triggers</td>
<td>44,45</td>
</tr>
<tr>
<td>SHP-2</td>
<td>Binds to β-catenin, thrombin triggers dissociation</td>
<td>58</td>
</tr>
<tr>
<td>RPTP-μ</td>
<td>Binds directly to the cytoplasmic tail of VE-cadherin</td>
<td>40</td>
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<tr>
<td>DEP-1</td>
<td>Phosphatase trapping mutants bind β-catenin, plakoglobin and p120 from epithelial cell lysates, however, co-precipitation with VE-cadherin has not yet been analysed</td>
<td>102</td>
</tr>
<tr>
<td>Csk</td>
<td>Binding to phosphorylated tyrosine 685 of VE-cadherin</td>
<td>100</td>
</tr>
<tr>
<td>PAR-3</td>
<td>This polarity protein binds via its third PDZ domain to the C-terminal 5 amino acids of VE-cadherin, no binding to N- or E-cadherin</td>
<td>103</td>
</tr>
<tr>
<td>PAR-6</td>
<td>This polarity protein binds to a region between aa 621–689 and does not compete with p120 binding</td>
<td>103</td>
</tr>
<tr>
<td><strong>Extracellular binding partners</strong></td>
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<tr>
<td>VE-PTP</td>
<td>This receptor type tyrosine phosphatase associates via extracellular domains with VE-cadherin</td>
<td>41</td>
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<tr>
<td>Fibrin</td>
<td>NDSK-II, a natural fragment of fibrin, and the fibrin derived peptide (β 15–42) bind to VE-cadherin, binding requires domains EC3 and EC4 and affects leukocyte extravasation</td>
<td>104,105,106</td>
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<tr>
<td>Fragment of t-RNA-synthetase</td>
<td>A natural fragment of tryptophanyl RNA synthetase with antiangiogenic activity binds to VE-cadherin</td>
<td>107</td>
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Binding of β-catenin, α-catenin, and p120 is similar to other cadherins and has not been listed.

Cardiac vessels—deteriorated first. In allantois explants, an adhesion-blocking antibody against VE-cadherin did not block the formation of nascent blood vessels but disassembled nascent vessels, supporting the idea that VE-cadherin is required for the maintenance of nascent vessels. It is unknown which other adhesive mechanisms mediate endothelial cell contact formation in the absence of VE-cadherin. N-cadherin would be an obvious candidate, because it is also expressed in endothelial cells. In transfected CHO cells it was found that VE-cadherin competes with N-cadherin for junctional localization and drives it out of junctions. It has not been analyzed whether N-cadherin is located in interendothelial cell contacts of nascent vessels from VE-cadherin null mice. The only defect observed was a lack of pericyte covering of endothelial outgrowths. It is intriguing, that gene disruption of the VE-cadherin–associated receptor type tyrosine phosphatase, VE-PTP, also leads to embryonic lethality before E10 because of a defect in

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cells were found next to vessel structures growing as cell cavities. In explant cultures of mutant allantoides, endothelial vessels in the yolk sac developed into dramatically enlarged the intraembryonic vascular system soon deteriorated. Blood angiogenesis. Although blood vessels were initially formed, the intraembryonic vascular system soon deteriorated. Blood vessels in the yolk sac developed into dramatically enlarged cavities. In explant cultures of mutant allantoides, endothelial cells were found next to vessel structures growing as cell layers. No obvious signs for enhanced endothelial apoptosis or proliferation were observed. A subsequent study essentially confirmed these results. Although VE-cadherin could have a role in mechanisms that causes the observed vascular defects, VE-PTP also associates with Tie-2 and may have yet other substrates that could be involved. Based on the idea that VE-cadherin transmits antiapoptotic signals and thereby supports the survival signals of VEGFR-2, antibodies against VE-cadherin were screened for their ability to block this function to obtain a reagent that could possibly drive tumor endothelium into apoptosis. Indeed an anti-mouse VE-cadherin adhesion blocking antibody reduced tumor growth in a Lewis lung metastasis model. However, because of its potent antiadhesive effects, slightly higher doses than those that efficiently inhibited tumor growth were lethal because they caused increased vascular permeability and edema in the lung. To circumvent this problem antibodies were screened that would block VE-cadherin functions during tumor angiogenesis without affecting vascular permeability. An antibody was described (E4G10) that specifically only bound to endothelial cells in a subset of tumor vasculature but not to normal vasculature. It was speculated that the epitope would only be accessible in recently formed adherens junctions of tumor endothelium while masked in resting vasculature. In a similar study, another mAb was described, which had a similar function and bound to domain EC4.

### VE-Cadherin Mediated Signals Inhibit Endothelial Cell Proliferation

Similar to other cadherins, VE-cadherin has antiproliferative effects that mediate cell contact–driven inhibition of cell

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<th>Table 2. Outside-In Signaling, Receptor-Like Function of VE-Cadherin</th>
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<td>Factors and Proposed Mechanisms</td>
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<tr>
<td>Prosurvival signals (antiapoptotic effects)</td>
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<tr>
<td>VE-cadherin/β-catenin forms a complex with VEGFR-2 and PI3-kinase that is required for VEGF-triggered phosphorylation of protein kinase Akt and induction of Bcl2, both mediators of the antiapoptotic machinery.</td>
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<td>Sensing of shear forces</td>
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<td>PECAM-1 acts as a sensor for shear and transmits signals via VE-cadherin (acting as an adaptor) and VEGFR-2, which activate PI3-kinase.</td>
</tr>
<tr>
<td>Antiproliferative effects, contact inhibition of cell growth</td>
</tr>
<tr>
<td>VE-cadherin engagement reduces VEGF-triggered tyrosine phosphorylation of VEGFR-2 and expression of VE-cadherin extends the half life of VEGFR-2.</td>
</tr>
<tr>
<td>Lack of VE-cadherin enhances VEGF-triggered VEGFR-2 phosphorylation, accelerates uptake and extends internalization times of VEGFR-2. Silencing of DEP-1 had the same effects.</td>
</tr>
<tr>
<td>Increased cell density triggers binding of C-terminal src kinase (Csk) to phosphorylated tyrosine 685 of VE-cadherin, Csk slows down proliferation and a VE-cadherin-Y685F mutant partially abolishes contact inhibition of cell growth.</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th>Table 3. Inside-Out Signaling, Stimuli and Mechanisms That Affect the Adhesive Function of VE-Cadherin</th>
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</thead>
<tbody>
<tr>
<td>Stimulus</td>
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<tr>
<td>Thrombin</td>
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<td>VEGF</td>
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<tr>
<td>Shear force</td>
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<td>Unknown</td>
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It should be noted that none of these studies (except for) directly analyzed the adhesive function of VE-cadherin, but rather endothelial cell contact integrity. The detected effects on tyrosine phosphorylation and protein–protein interactions correlated with changes in cell contact stability, however causal relationships have not yet been strictly established. P-Y indicates phospho-tyrosine.
growth in cultured endothelial cells. This effect requires the presence of the last 82 amino acids at the C terminus of VE-cadherin, the same part of the protein that is required to support survival of endothelial cells. Thus, surprisingly, the same part of VE-cadherin mediates effects that in one case support VEGFR-2 signaling (antiapoptosis, prosurvival effects) and in the other case inhibit VEGFR-2 signaling (proliferative effects). Indeed VE-cadherin–mediated inhibition of VEGFR-2 signaling had already been published around the same time. This report demonstrated that VEGF-triggered phosphorylation of VEGFR-2 was reduced at high cell density and was enhanced if confluent endothelial cell layers were pretreated with an adhesion blocking antibody against VE-cadherin. The same group found that overexpression of VE-cadherin in endothelial cells increases the half-life of VEGFR-2. In agreement with these studies, it was described that the lack of VE-cadherin enhanced VEGF-triggered phosphorylation of VEGFR-2. It was suggested that VEGF stimulation leads to the association of the receptor with VE-cadherin, a process that requires the presence of β-catenin. This association would trigger dephosphorylation of the receptor, possibly mediated by DEP-1 because downregulation of this phosphatase enhanced VEGF-2 phosphorylation and cell proliferation. The same group showed in a later report that VEGF triggered clathrin-dependent internalization of VEGF-2, and this internalization was more rapid and VEGFR-2 remained longer in endosomal compartments if VE-cadherin was absent or cells were sparsely confluent. Endocytosed VEGF-2 was still phosphorylated and inhibited of internalization reestablished contact inhibition of growth. Silencing DEP-1 enhanced internalization and signaling of VEGF-2. The same group reported that VEGF triggered the association of the adaptor protein Shc with VE-cadherin, an association that required the C-terminal 82 amino acids of VE-cadherin. Phosphorylation of Shc lasted longer in VE-cadherin null endothelial cells compared with VE-cadherin expressing cells.

An alternative mechanism for VE-cadherin–mediated contact inhibition of growth was recently suggested by the finding that the C-terminal Src kinase (Csk), a kinase that phosphorylates and thereby inhibits Src, binds specifically to phosphorylated tyrosine 685 of VE-cadherin. This is the same tyrosine that was later described as exclusively phosphorylated by Src (see above). Binding of Csk increased with higher cell density and silencing Csk expression enhanced endothelial cell proliferation, suggesting that Csk binding to VE-cadherin may be involved in VE-cadherin–mediated contact inhibition of cell growth. Although Csk binding to tyrosine 685 was not found to affect VE-cadherin adhesive activities in transfected cells, a function of tyrosine 685 in the regulation of VE-cadherin adhesion in endothelium should not be excluded.

Another molecule suggested to be involved in VE-cadherin–mediated contact inhibition of growth is surviving because it was found to be slightly upregulated in angiogenically active endothelial cells and downregulated in culture by cell confluence and VE-cadherin expression.

**Concluding Remarks**

Investigating VE-cadherin over the last 15 years has established it as one of the 2 to 3 most intensely studied cadherins known today. Its accessibility from within the vasculature, its prominent role as the dominant adhesion molecule responsible for the maintenance and control of endothelial cell contacts, and its essential role during morphogenesis of the blood vessel system has made VE-cadherin a prime research target among adhesion molecules in the vasculature. Much progress has been made in identifying signaling partners (Table 2) and factors that influence VE-cadherin function (Table 3). Based on these findings several major goals and questions have emerged that will be important in the future. As for other cadherins, it will be important to understand in detail, how phosphorylation of the cadherin/catenin complex and of other substrates in its close vicinity translate into changes in adhesive strength. Furthermore, how do other adhesion mechanisms at endothelial junctions (ESAM, JAM-C, or maybe PECAM-1) modify the function of VE-cadherin? How do small GTP binding proteins such as Rap1 affect VE-cadherin? To what extent is the regulation of the VE-cadherin–mediated barrier key to the process of leukocyte extravasation in inflammation and lymphocyte homing? Which of the many different factors that influence vascular permeability do indeed affect VE-cadherin function and which ones act indirectly via other molecular mechanisms? Which endothelial adhesion mechanisms mediate the formation of nascent blood vessels in VE-cadherin–deficient mice? How do mechanisms that control endothelial cell contacts and VE-cadherin function affect or even control endothelial cell sprouting, proliferation and branching? These are only some of the most fascinating questions connected to the central role of VE-cadherin in vascular biology. Studying them will reveal important insights into basic mechanisms of angiogenesis, inflammation, and vascular biology as a whole.

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**Disclosures**

None.

**References**


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Dietmar Vestweber

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