Endothelial Cell-to-Cell Junctions: Adhesion and Signaling in Physiology and Pathology

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Besides intercellular recognition and adhesion, which are primarily performed by the transmembrane components, many of the molecules associated in endothelial cell-to-cell junctions initiate or regulate signal transmission. Clustering of molecules at junctions has the consequence of allowing new local interactions to direct specific cellular responses with crucial effects on the physiology and pathology of the endothelium and, more generally, of the vascular system. The implication is that cell-to-cell junctions could be envisaged as molecular targets for different types of therapeutic intervention. These could be directed to “cure” the defects of endothelial junctions that accompany several pathologies or to reversibly open them in a controlled way for the efficient delivery of drugs to the tissues. These aims can become more and more approachable as the knowledge of the molecular organization and function of endothelial junctions increases and their organ and tissue specificities become understood.

Specialization of the plasmatic membrane at endothelial cell-to-cell junctions is indeed present in the vessels of both adult and developing organisms. Electron microscopy observations supporting the structural distinction of endothelial junctions date from the 1970s (Simionescu et al. 1975). Molecular specialization can be clearly observed using specific antibodies for the immunostaining of tissue sections and, even more convincingly, for whole-mount immunostaining of murine and zebrafish embryos and of transparent organs such as the trachea, diaphragm, and urinary bladder in the adult mouse and the retina in newborn mice (Fig. 1A). This type of analysis is very effective and begins to allow the

definition of junctional specialization in different vascular districts (Baluk et al. 2007) as well as of their modification in pathological conditions (Baluk et al. 2009). This represents a most relevant advance in the field of vascular biology as it offers the basic knowledge of how to design therapeutic interventions targeting the junctional compartment.

MOLECULAR STRUCTURE AND SIGNALING ACTIVITY

Although the basic structure of endothelial cell-to-cell junctions is relatively simple, several aspects contribute to complicating the picture. In the generic model they consist of

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Figure 1. Vascular phenotype after mutation of junctional components in mouse and zebrafish. (A) Endothelial junctions are present in vessels in vivo. *Left* panel shows large veins with branching venules and arteries (blue and red asterisks, respectively) in a mouse trachea stained in whole mount with antibodies to VE-cadherin (arrows), the endothelial-specific transmembrane component of adherens junctions. Endothelial cells in arteries are narrow and elongated in the direction of blood flow whereas in veins they appear wider and less stretched. Bars, 150 and 50 μm, respectively, in *left* and *right* panels. (A courtesy of Fabrizio Orsenigo, Firc Institute of Molecular Oncology, Milan.) (See facing page for legend.)

transmembrane proteins that interact with transmembrane molecules, often identical, on adjoining cells and on the same cell, in *trans*- and *cis*-interactions, respectively, thus developing adhesive links (Dejana et al. 2009). In addition, the transmembrane molecules associate with cytoplasmic, and sometimes transmembrane, components to form multiprotein complexes which have scaffold and signaling properties. In many cases the detailed description of the molecular relationships within these complexes is still incomplete, and knowledge of the mechanisms that regulate such interactions in response to environmental conditions and stimuli is only partial. In addition, the list of molecules that can present junctional localization is constantly increasing (Fig. 1B). This suggests the existence of distinct junctional complexes localized in discrete microdomains. Although this idea still awaits additional experimental evidence, the existence of distinct complexes within the major junctional domains, adherens and tight junctions, respectively (see below), would introduce further local specialization for more subtle regulation of signaling from junctions (Lampugnani 2010; Millan et al. 2010). Lastly, the endothelium of different sides of the vascular network and of different organs presents junctional specialization, as discussed below. Although much work is needed to fully define the molecular properties of cell-to-cell junctions in the different endothelia of the organism, it is clear that these structures can convey signals to the cell. The intercellular recognition and adhesive interaction represent only the most obvious functions. Indeed, the signals transmitted by the molecular complexes in cell-to-cell junctions are multiple and regulate several fundamental aspects of endothelial physiology. This has been observed in vivo in experimental models of genetic

ablation in mice and zebrafish (Table 1) and in some human pathologies, as discussed in the following paragraphs. In addition, junctions might exert an indirect control of transcriptional activity. The most studied example is represented by molecules associated to adherens junctions, the catenins, β -, γ -, or plakoglobin and p120, where they are directly bound to the cytoplasmic domain of VE-cadherin (Table 1 and see below). Catenins can also be found in the nucleus in which they can regulate the activity of transcription factors (Daniel 2007; Shimizu et al. 2008; Dejana 2010; see below for discussion of the case of β -catenin/FoxO1 complex). Although potentially relevant to the coordination of the transcriptional program with the state of junctions, the relationship between the junctional and nuclear pools of catenins has just recently started to be delineated (Maher et al. 2009).

ADHERENS JUNCTIONS AND TIGHT JUNCTIONS

The best and longest recognized distinct junctional domains are adherens junctions and tight junctions. They host molecular complexes with a specific composition and function and occupy selective positions in the junctional cleft. This organization, described in detail for epithelial junctions, is also generally maintained in the endothelium (Fig. 1B; Dejana et al. 2009). The tight junctions occupy the most apical position, sealing the cleft edges toward the luminal surface. Their main function in the endothelium is to restrict paracellular permeability (Table 1; Dejana et al. 2009). Adherens junctions are localized more basally and as well as limiting paracellular permeability they control vessel morphogenesis and stability (Table 1; Dejana et al. 2009). A detailed description of the

Figure 1. (Continued) (B) A schematic representation of the reciprocal relationships between adherens and tight junctions is reported. Tight junctions are apical to adherens junctions and seal the paracellular cleft, introducing a strict control of paracellular permeability. Adherens junctions contribute to the control of paracellular permeability, also positively regulating the transcription of the main transmembrane component of tight junctions, claudin5. They control several aspects of endothelial physiology, allowing the local association and interaction of scaffold and signaling molecules. The main components of adherens and tight junctions are listed. For details and further bibliography see text and Dejana et al. (2009).

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Table 1. Vascular phenotype after mutation of junctional components in mouse and zebrafish

Components	Mutation in the mouse homozygous	Phenotype
Adherens junction		
VE-cadherin	Null, global	Extensive angiogenic defects; embryonic lethality 9 dpc (Carmeliet et al. 1999)
	Truncation (80 aa carboxy-terminal)	Phenotype similar to null one (Carmeliet et al. 1999)
	<i>Zebrafish morpholino</i>	<i>Vascular abnormalities</i> (Montero-Balaguer et al. 2009)
N-cadherin	Null, endothelial specific	Extensive angiogenic defects; embryonic lethality 9.5 dpc (Luo and Radice 2005)
β-catenin	Null, endothelial specific	Abnormal vessel organization and vascular fragility; embryonic lethality 11.5 dpc (Catellino et al. 2003)
	Exon3 deletion, stabilization and gain of function	Defects of vessel sprouting and branching; embryonic lethality 11.5–12.5 dpc (Corada et al. 2010)
p120	Null, endothelial specific	Vascular defects; embryonic lethality starting from 12.5 dpc in 40% of mutants (Oas et al. 2010)
Afadin	Null, endothelial specific	Embryonic lethality in 85% of mutants; impaired angiogenesis in survivors (Tawa et al. 2010)
CCM1	Null, global	Vascular defects, lack of vessel remodeling; embryonic lethality 11 dpc (Whitehead et al. 2004)
CCM2	Null global and endothelial specific	Embryonic lethality 9.5 dpc (Boulday et al. 2009)
CCM3	Null global and endothelial specific	Embryonic lethality 9–9.5 dpc (He et al. 2010)
	<i>Zebrafish</i>	
	<i>CCM1 (Santa) mutant</i>	<i>No concentric growth of myocardium</i> (Mably et al. 2006), <i>big heart, nonpatent branchial arch artery</i> (Mably et al. 2006; Kleaveland et al. 2009)
	<i>CCM2 (Valentine) mutant and morpholino</i>	<i>Vascular dilation and morphogenetic defects</i> (Hogan et al. 2008)
	<i>CCM1 and CCM2, morpholinos and mutants</i>	<i>Nonpatent branchial arch arteries</i> (Voss et al. 2009; Zheng et al. 2010)
	<i>CCM3a and b, 18 aa deletion: STK25/MST4 binding site</i>	<i>Decreased vascular integrity, brain hemorrhages</i> (Gore et al. 2008)
	<i>CCM1 and Rap1, combinatorial morpholinos at low doses</i>	
Rap1b	Null, global	Defective angiogenesis, vascular fragility, hemorrhages; embryonic lethality 13.5 dpc 40%; perinatal lethality 55%; survival 5% (Chrzanowska-Wodnicka et al. 2008)
VE-PTP	Null, global	Embryonic lethality 9.5 dpc (Baumer et al. 2006; Dominguez et al. 2007)
DEP1	Truncation, loss of phosphatase domain (117 aa carboxy-terminal)	Vascular defects; embryonic lethality 11.5 dpc (Takahashi et al. 2003)
Csk	Null, global	Defective vascular remodeling, vitellin vessels absent (Duan et al. 2004)
HEG1	Null, global	Loss of pulmonary and lymphatic vessel integrity. Pulmonary hemorrhages; 55% lethality before weaning (Kleaveland et al. 2009)
	<i>Zebrafish mutant and morpholino</i>	<i>Big heart, block of concentric growth of myocardium block of circulation, nonpatent branchial arch arteries</i> (Mably et al. 2003; Kleaveland et al. 2009)

Continued

Table 1. *Continued*

Components	Mutation in the mouse homozygous	Phenotype
Tight junction		
Claudin5	Null, global	Abnormal blood–brain barrier function; neonatal lethality (Nitta et al. 2003)
JAM A	Null, global	Defective permeability and angiogenesis in the adult (Cera et al. 2004; Cooke et al. 2006)
JAM C	Null, global	Defective permeability in the adult (Orlova et al. 2006)
ESAM	Null, global	Defective angiogenesis and permeability in the adult (Ishida et al. 2003; Wegman et al. 2006)
ZO-1	Null, global	Defective vascular remodeling in the yolk sac; embryonic lethality 11.5 dpc (Katsuno et al. 2008)
Other junctions		
PECAM	Null, global	Increased permeability in response to LPS in adult lung, liver, kidney (Carrithers et al. 2005)

The components of adherens junctions and tight junctions, as well of PECAM, localized in endothelial junctions but outside these domains, either the genetic ablation or mutation or translational down-regulation of which have been reported to produce vascular disorganization and/or dysfunction in mouse and zebrafish models.

The mutation in zebrafish and the respective phenotype are in *italic* type to distinguish them from the murine phenotype.

molecular composition of these two domains can be found in Dejana et al. (2009), and is summarized in Figure 1B. Only a few junctional molecules are endothelial specific; these are vascular endothelial (VE)-cadherin (Lampugnani et al. 1992), the transmembrane organizer of adherens junctions and VE-PTP (Fachinger et al. 1999) associated with VE-cadherin in adherens junctions, and claudin5 in tight junctions (Nitta et al. 2003). Although most components of endothelial junctions are not endothelial specific, some of them appear to play a selective role in endothelial physiology, as indicated by the specific vascular phenotype of KO mice (Table 1; Nyqvist 2008) and in some cases of zebrafish morphants and mutants as discussed below (Table 1).

Although physically restricted to distinct regions of the intercellular cleft, adherens junctions and tight junctions can crosstalk (Dejana et al. 2009). In particular, adherens junctions direct the organization and function of tight junctions. This became evident when studying VE-cadherin null endothelial cells which present defective tight junctions. This is the consequence of transcriptional inhibition of claudin5,

one main transmembrane component of tight junctions, in the absence of VE-cadherin. The effect is mediated through the regulation of claudin5 promoter by a transcriptional repressor complex formed by FoxO1 in association with Tcf4 and β -catenin (Taddei et al. 2008). FoxO1 phosphorylation by Akt and decreased nuclear localization of β -catenin impair the inhibitory complex in wild-type endothelial cells, allowing transcription of claudin5. In the absence of VE-cadherin, Akt activity is reduced. As a consequence, unphosphorylated FoxO1 can accumulate in the nucleus. As the nuclear levels of β -catenin are also increased in VE-cadherin null cells, more transcriptional repressor complex can be formed to inhibit claudin5 expression (Taddei et al. 2008).

Pecam/CD31, a widely used marker of vascular endothelium, as well as being expressed in leukocytes and platelets, is also associated with junctions, although for the most part it does not colocalize either with adherens or tight junctions (Ayalon et al. 1994). However, in association with VE-cadherin and VEGFR2, it can form complexes that have the important role of flow sensors (Tzima et al. 2005). This is a

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particularly relevant function because the endothelium of the vessels in vivo continuously experiences variation of fluid forces. However, these complexes might not be localized in cell-to-cell junctions (Tzima et al. 2005).

Organ and Vascular Side/District Specificity

The heterogeneity and specialization of the endothelium in the different vascular districts and organs are receiving increasing attention and the molecular mechanisms controlling such specification have begun to be delineated (DeVal and Black 2009; Swift and Weinstein 2009; Dejana 2010). Specialization of endothelial junctions appears to be part of such adaptation programs. This concept is well illustrated in lymphatic vessels and in vessels of the central nervous system.

The organization of junctional complexes in lymphatic vessels has been defined in detail (Baluk et al. 2007). In particular, initial lymphatic vessels present a peculiar arrangement in distinct microdomains, “flaps and buttons,” which alternate in the junctions. PECAM and LYVE1 are concentrated along the rim of the overlapping flaps which are anchored in place by buttons. Junctional buttons contain VE-cadherin, and the tight junctions molecules, Claudin5, occludin, JAMA, and ZO1. This organization is functional to the rapid exchange of fluids and cells between vessels and tissues, as it allows for the opening of the paracellular route at specific sites without altering the general organization of the vessels (Baluk et al. 2007). The endothelium in collecting lymphatic vessels, which have a duct role, presents junctions organized as in blood vessels. The stimuli and mechanisms that direct this complex patterning of lymphatic junctions are still to be defined.

Endothelial junctions of the vessels in the central nervous system present a structural specialization which is required for the maintenance of the blood–brain barrier (Engelhardt 2003). That is, the endothelium of cerebral vessels is particularly enriched in tight junctions (Kniesel et al. 1996). The functional consequence is that paracellular permeability is abolished. Central to the maintenance of the

blood–brain barrier is claudin5 (Nitta et al. 2003; see also below), with the cooperation of other claudins, -1, -12, -11, and in particular -3 (Wolburg et al. 2003). The differentiation of specialized tight junctions in brain vessels is under the control of Wnt signaling (Dejana 2010). Brain vascularization is regulated by both Wnt7a and b (Stenman et al. 2008), whereas Wnt3a up-regulates the expression of claudin 3 (Liebner et al. 2008), the formation of tight junctions, and the barrier properties of the endothelium through a β -catenin-dependent canonical Wnt signaling both in vivo and in vitro models. Wnt3a/ β -catenin signaling did not modify Claudin1 and -12, found in the endothelium of the blood–brain barrier (Liebner et al. 2008). In addition, indications start to appear that the molecular composition of the junctions can be influenced by the arterial or venous location of the vessels. The phosphatase VE-PTP is preferentially concentrated in junctions of arteries and arterioles (Fachinger et al. 1999; Baumer et al. 2006; Dominguez et al. 2007). VE-cadherin phosphorylation in tyrosine 658 and 685 is high in venous vessels and low or undetectable in arterial ones (Orsenigo F, Giampietro C, Lampugnani MG, et al., in prep.).

The functional consequences of these and other yet unidentified specializations of junctions in distinct endothelial districts could open up new opportunities for targeted therapies.

Complexes between Adhesion Molecules and Growth Factor Receptors Regulate Angiogenesis and Vessel Stabilization

As discussed in the previous section, molecules in endothelial cell-to-cell junctions can form multi-protein complexes. In particular, components of adherens junctions can be recovered by immunoprecipitation associated with receptors of growth and differentiation factors.

VE-cadherin, the transmembrane organizer of endothelial adherens junctions, has been shown to form complexes with VEGFR2 and TGF β RII/TGF β RI (Lampugnani et al. 2003; Rudini et al. 2008). The ligands of these receptors, in particular VEGFA and TGF- β , are critical

regulators of endothelial differentiation, growth, and stabilization. The complexes between VE-cadherin and growth factor receptors require clustering of VE-cadherin in stable contacts for the regulation of signaling from receptors (Lampugnani et al. 2003; Rudini et al. 2008). This implies that the junctional microenvironment promoting the local association between molecules directs the appropriate transmission of the signals according to the state of the junction.

When associated to VE-cadherin, VEGFR2 promotes antiapoptosis and endothelial stability through the activation of Akt, while its mitogenic potential, mediated by p42/44 MAPKs, is switched off. The proliferative response is preferentially activated by VEGFR2 dissociated from VE-cadherin, and this is also so after internalization of the receptor in endosomal compartments (Lampugnani et al. 2003, 2006). The interaction between VE-cadherin and VEGFR2 requires the cytoplasmic region of VE-cadherin that binds β -catenin as well as β -catenin itself. The cross-talk between VE-cadherin and VEGFR2 could be particularly important to maintain homeostasis of the endothelium in the adult organism. In this condition, low constitutive level of VEGF signals endothelial survival and stabilization (Lee et al. 2007).

When associated to VE-cadherin, TGF- β RII/TGF- β R1 inhibits endothelial proliferation and migration, thus promoting vessel stabilization in response to TGF- β . This effect is mediated by phosphorylation of Smad1/5 and 2/3 and activation of transcriptional responses (Rudini et al. 2008).

Another molecular complex associated to endothelial cell-to-cell junctions in stable vessels is that between Tie2 and VE-PTP (Saharinen et al. 2008). This complex has been shown to contribute to the maintenance of the quiescent phenotype and to the control of paracellular permeability (Nottebaum et al. 2008; Saharinen et al. 2008). In stable vessels Ang1-bound Tie2, (with Ang1 ligand produced by pericytes) is recruited to endothelial cell-to-cell contacts to form, through a bridge of multimeric Ang1, trans-homotypic complexes with Tie2 molecules present on the adjoining cells. The interaction between Tie2 receptors on opposing

cells further activates the receptors. The signaling outcome is endothelial stabilization and decreased permeability through the activation of the Akt pathway and of eNOS, which are also localized in cell-to-cell contacts. VE-PTP also associates to the Tie2 complexes, further contributing to the decrease of paracellular permeability (Saharinen et al. 2008). Association of activated Tie2 to endothelial junctions is particularly relevant as it represents the constitutive situation of adult vessels in vivo (Saharinen et al. 2010).

VE-PTP can also associate to VE-cadherin, further stabilizing the junctions (Nottebaum et al. 2008). It is not known at present whether its interactions with Tie2 and VE-cadherin are mutually exclusive.

ENDOTHELIAL JUNCTIONS IN PHYSIOLOGY AND PATHOLOGY

Various pathological conditions in humans are characterized by alteration of cell-to-cell junctions in the vascular endothelium and often also of the junctions between endothelial and mural cells such as pericytes and astrocytes (Gaengel et al. 2009). Although it remains to be established whether alterations of junctions are a primary effect and play a causal role in the pathology, experimental models in the mouse and in zebrafish clearly indicate that several components of endothelial cell-to-cell junctions are required for correct organization of vessels during embryogenesis and for efficient barrier function in the adult organism.

Morphogenetic Process: Angiogenesis during Embryonic Development

Phenotype of Murine Mutants

The phenotypes of murine mutants indicate that components of adherens junctions regulate the correct organization of new vessels. Most murine models of genetic modification of junctional components consist of constitutive null mutations either ubiquitous or endothelial specific (Table 1). The phenotypes observed are in most cases lethal at early stages of embryonic development (9–11 dpc). The phenotypic

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alterations present common and specific features, which indicates involvement of adherens junction components in common morphogenetic pathways. An example of expression of mutated molecule instead of null mutation is that of the truncated form of VE-cadherin, lacking the carboxy-terminal domain, which also contains the binding site for β -catenin (Carmeliet et al. 1999). Although localized at cell junctions, the truncated VE-cadherin induces a lethal phenotype with features very similar to the null mutation. Besides β -catenin, the truncated VE-cadherin does not associate with either VEGFR2 or TGF β RII. This indicates the importance in vivo of cytoplasmic interactions of VE-cadherin for correct vessel organization.

Whereas vascular abnormalities can be unsurprising after global suppression of endothelial-specific molecules, such as VE-cadherin or VE-PTP, they can be unexpected and indicate a specific vascular role when observed after ablation of ubiquitously expressed genes. This is the case for global mutation of CCM1, -2, and -3 (Whitehead et al. 2004; Bouliday et al. 2009; He et al. 2010), Rap1b (Chrzanowska-Wodnicka et al. 2008), and DEP-1 (Takahashi et al. 2003). In addition, desmoplakin KO produced endothelial alterations in the mouse embryo, besides multi-tissue dysfunctions (Gallicano et al. 2001). For other genes ubiquitously expressed, the constitutive/global ablation of which induces lethality before the organization of the vascular network, a vascular phenotype becomes evident when they are suppressed specifically in the endothelium. This has been the case for the catenins, β -catenin (Cattellino et al. 2003) and p120 (Oas et al. 2010). A deletion of β -catenin, which results in stabilization of the molecule with gain of function and sustained transcriptional signaling, has also been observed to induce a severe vascular phenotype when the expression is endothelial specific (Corada et al. 2010). Also for N-cadherin, a molecule that in normal quiescent endothelium is poorly expressed and which is excluded from junctions by VE-cadherin, the role during embryonic angiogenesis could be recognized after endothelial-specific ablation of the gene (Luo and Radice 2005).

Endothelial-specific and inducible models of null mutations have been described more recently (Pitulescu et al. 2010) for CCM2 and CCM3 (Bouliday et al. 2009; He et al. 2010) and Afadin (Tawa et al. 2010). The constitutive and endothelial-specific mutation of each CCM gene induces a phenotype superimposable to the constitutive global one, further pointing to a crucial role of CCM molecules in vessels morphogenesis and functions (see below for further discussion). The models of inducible mutation will allow the definition of the role of the junctional components at different stages of embryonic and neonatal development and in the adult animal in a resting situation, and after stimulation with different inflammatory or angiogenic agents.

Components of tight junctions mostly impact on the function of the endothelium as a permeability barrier. Null mutation of Claudin5 (Nitta et al. 2003) results in early postnatal lethality owing to impaired barrier functions of the endothelium in brain vessels. Claudin5 is ubiquitously expressed in the endothelium and this distinct phenotype points to a specific role for this molecule in endothelial cells of cerebral vessels. In other cases, as for JAM-A, JAM-C, and ESAM KO, the defective permeability and inflammatory phenotype became evident only after challenging the animals with inflammatory agonists (Ishida et al. 2003; Cera et al. 2004; Cooke et al. 2006; Orlova et al. 2006; Wegman et al. 2006). Similarly, in PECAM KO, LPS induces a delayed increase in vascular permeability (Carrithers et al. 2005).

Zebrafish Embryo and Molecules of Endothelial Junctions

Zebrafish embryos, for their transparency and autonomous development, represent an increasingly popular and informative model in vascular biology (Jin et al. 2005; Isogai et al. 2009). They allow direct observation in vivo of the role of junctional molecules of the endothelium in the organization and function of vessels. This has been reported for VE-cadherin (Montero-Balaguer et al. 2009) and ZO-1 (Katsuno et al. 2008). Combinatorial knockdown,

treating simultaneously with doses of morpholinos minimally or not effective when used individually, can be applied to study synergistic interactions along common pathways. This has been reported for CCM1 and Rap1b (Gore et al. 2008). Morpholino-induced in-frame deletions have also been produced to express a form of CCM3 protein which lacks the MST4/STK24/25 binding region and mimics a mutation observed in patients (Table 1; Voss et al. 2009; Zheng et al. 2010). The vascular phenotype of some morphants and mutants for junctional components in zebrafish larvae is reported in Table 1.

Stabilization of Endothelial Junctions for Organ Homeostasis in the Adult Organism: Experimental Models and Pathologies

Endothelial Junctions and the Control of the Endothelial Barrier: Experimental Model

A direct experimental indication that endothelial junctions, and in particular adherens junctions, are required for the maintenance of the structure and the barrier function of the endothelium in the stabilized vessels of the adult organism has been obtained using antibodies to VE-cadherin. A monoclonal antibody to the extracellular domain of VE-cadherin increased vascular permeability dramatically and acutely (Corada et al. 1999). Extravasation of Evans blue in the heart and lung tissues was observed within 1 hour and with a dose-dependent effect after intravenous injection of the antibody (Corada et al. 1999). The decrease in barrier function was accompanied by disorganization of the endothelial layer, with opening of gaps between cells and exposure of basement membrane in areas at the latest stages.

Endothelial Junctions in Vascular Malformations in Humans

A relevant example of the role of endothelial junctions in the organization and stabilization of vessels is offered by the human pathology cerebral cavernous malformation (CCM). A genetic cause of this pathology is linked to

loss-of-function mutations in any of three independent genes, CCM1 (*Krit1*), CCM2 (*Osm2/Malcavernin*), and CCM3 (*Pcdc10*) (Labauge et al. 2007). The expression of the corresponding proteins is not endothelial specific. However, strong evidence has accumulated indicating that the cause of the pathology is indeed the mutation of the endothelial protein (Boulday et al. 2009; He et al. 2010). The three CCM genes codify for unrelated proteins that can form a complex (Labauge et al. 2007) in which CCM2 bridges CCM1 and CCM3 together. The convergent phenotypic defect of the mutants in any of these genes indicates that a common pathway is affected in the pathology. The vascular “mulberry” structures that characterize this disease are frequently localized in the central nervous system; they can be observed since childhood, and often new lesions appear in monitored patients. The lesions are not only structurally abnormal, but they are also hyperpermeable and unstable. Mild leakiness with deposits of hemosiderine and local edema cause the weaker symptoms that characterize the pathology: headaches and neurological symptoms, while the rupture of the malformation causes hemorrhagic stroke. At electron microscopy analysis, the endothelium of the lesion has been described as lacking tight junctions (Clatterbuck et al. 2001). Molecules of endothelial adherens junctions such as VE-cadherin, β -catenin, and the junctionally associated aPKC ζ are mislocalized in the vessels of the human lesion (Lampugnani et al. 2010) and in the experimental model of inducible mutation in the mouse (Lampugnani et al., in prep.). This also affects apical-basal polarity of the endothelium as indicated by delocalization of podocalyxin from the apical surface (Lampugnani et al. 2010). Adherens and tight junctions control apical-basal polarity in epithelia (Qin et al. 2005; Martin-Belmonte and Mostov 2008), but this is also a fundamental property of mature endothelium.

Delocalization of Rap1 from junctions contributes to their disorganization in the absence of CCM1 (Glading et al. 2007). Rap1 small GTPase (Kawata et al. 1988) is an important mediator of junctional stabilization; Rap1

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associates with CCM1 and requires it for junctional localization. Rap1 is central to diverse signaling networks, some of which are activated by cAMP (Pannekoek et al. 2009). It stabilizes VE-cadherin clustering at junctions, thus promoting the control of paracellular permeability (Kooistra et al. 2005), although its action on VE-cadherin still remains to be defined in molecular terms (Pannekoek et al. 2009).

Hyperactivation of another small GTPase, Rho, also plays an important role in the increased vascular permeability observed in CCM1 and CCM2 mutants (Whitehead 2009; Stockton et al. 2010). Pharmacological treatments with inhibitors of the Rho pathway, simvastatin, to inhibit Rho prenylation (Whitehead et al. 2009) and fasudil to inhibit Rho-activated kinase (Stockton et al. 2010), have been shown to reduce or prevent the increased permeability of vessels in the lung and in the brain of heterozygous CCM1 and CCM2 mice under basal conditions or after LPS or VEGF stimulation. However, the state of endothelial junctions and the effects of these drugs on them have not been reported in these murine models.

The experimental data available up to now do not definitely indicate whether endothelial junctions are the primary target of CCM mutations or whether the junctional effects are secondary responses; however, disorganization of junctions induces undoubtedly very serious functional consequences in this pathology.

Endothelial Junctions in Tumors

Vessels in tumors characteristically show severe structural abnormality (Baluk et al. 2005). These defects are the consequence of the disequibrated microenvironment created by the neoplastic cells which is enriched with growth factors and inflammatory mediators (Fukumura and Jain 2007). The result is hyperproliferating endothelium and lack of stabilizing pericytes. In particular, endothelial cell-to-cell junctions appear profoundly irregular and discontinuous (Baluk et al. 2005) with gaps and irregular distribution/expression of junctional molecules. This condition accompanies and very likely contributes to the defective

control of the barrier functions of the endothelium that result in tissue edema, increased interstitial pressure, irregular distribution of chemotherapeutics, and hypoxia, which is unfavorable for radiotherapy (Jain 2005). In addition, the hypoxic and hyperacidic environment exerts a selective pressure that favors the more resistant and malignant tumor clones (Loges et al. 2009). Antiangiogenic therapies intended to starve the tumor to death, in particular using inhibitors of the VEGF pathway, have been remodulated into approaches aimed at stabilizing and normalizing the vascular network in the tumor (Fukumura and Jain 2007). The experimental and clinical observations strongly indicate that a successful therapy targeting vessels will require the definition of a precise equilibrium between antiangiogenesis and vessel stabilization (Loges et al. 2009).

A detailed analysis of the effect of anticancer therapeutic regimens on the molecular organization of endothelial junctions is still very incomplete. However, functional studies indicate a positive correlation between therapeutic effect and increased tumor perfusion and reduction of edema, which is likely the result of vessel normalization (Fukumura et al. 2010). In this frame, the vessels of tumors implanted in mice haploinsufficient for the oxygen sensor PHD2, show increased expression of junctional VE-cadherin through a HIF-dependent mechanism (Mazzone et al. 2009). Also ZO-1 and Claudin5 occupy longer distances in junctions of tumor vessels in PHD2 heterozygous mice. This goes in parallel with vessel normalization, improved tumor perfusion, and oxygenation and decreased invasive and metastatic behavior (Mazzone et al. 2009).

Observations in experimental tumor models indicate that junctional molecules in the endothelium of neoplastic tissue are indeed in a molecular situation different from that in normal tissues. A monoclonal antibody, clone E4G10, to an epitope in the first 10 amino acids of the extracellular domain of VE-cadherin (May et al. 2005), could recognize VE-cadherin only in tumor vessels, indicating that the epitope was present/accessible only in the junctions of the vessels in the neoplasia (Liao et al. 2002).

In addition, in vivo treatment with this antibody reduced the growth of different types of experimental tumors in the mouse, without affecting vascular permeability (Liao et al. 2002). A detailed analysis of the distribution of the antibody EG10 in the junctions of tumor vessels and of the state of endothelial junctions in the tumors receiving the antibody has not been reported. This type of observation strengthens the concept that endothelial junctions may represent a direct target in an anti-cancer therapeutic approach directed to/including the vascular compartment.

CONCLUSIONS

Endothelial cell-to-cell junctions have been extensively characterized in terms of molecular composition, organization, and function. Much knowledge of the role of specific junctional components has been collected, studying in-vitro models of cultured endothelial cells and angiogenic processes in the murine embryo and, more recently, in zebrafish larvae.

However, the detailed analysis of the organization of junctions in adult stable vessels and of their molecular modifications in pathological situations is still very fragmentary. The murine transgenic models of endothelial-specific and conditional mutation will allow the understanding of the role of specific components of endothelial junctions in adult vessels both under physiological condition and after a pathological challenge.

In addition, the conditional models should allow us to distinguish the specific features of the organization and function of endothelial junctions in distinct vascular districts. A detailed map of the organization and function of endothelial junctions along the body should allow us to precisely monitor the effects of pharmacological treatments and to design more selective drugs.

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