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Editorial

Perspectives Series: Cell Adhesion in Vascular Biology

Endothelial Adherens Junctions: Implications in the Control of Vascular Permeability and Angiogenesis

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Introduction

The endothelium is a continuous monolayer formed by cells linked to each other by different types of adhesive structures. These are involved in the control of vascular permeability to plasma proteins and circulating cells, and contribute to the establishment of cell polarity by limiting the diffusion of lipid and transmembrane proteins in the outer leaflet of the plasma membrane bilayer (1).

A major ubiquitous type of endothelial cell–cell junctions are adherens junctions. The general organization of adherens junctions presents many structural and functional similarities in different cell types but its molecular components and biological meaning may vary in the different tissues. In recent years excellent reviews (3–9) have extensively considered the molecular organization and signaling properties of adherens junctions. The present review is devoted to the special properties of these structures in the endothelium and offers some insights on their role in the control of endothelial cell permeability and vascular morphogenesis.

Adherens junctions are formed by transmembrane adhesive proteins, belonging to the cadherin family, that are organized in clusters at cell–cell contacts and connect through their cytoplasmic domain with a complex network of cytoskeletal proteins (3–9).

Cadherins are single chain transmembrane polypeptides that promote, at least in the majority of cases, homophilic type of binding. The extracellular domain of cadherins usually consists of five homologous repeats of 110 residues which contain putative Ca^{2+} binding sequences. The cadherin short cytoplasmic region interacts with three related proteins which belong to the "armadillo" family (β-catenin, plakoglobin, and p120). β-catenin and plakoglobin bind α-catenin, which is homologous to vinculin and mediates the linkage of the cadherin–catenin complex to the actin cytoskeleton (Fig. 1).

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This complex organization of cadherin-catenins and the cytoskeleton is necessary not only to strengthen cell–cell adhesion but also to transfer signals between neighboring cells (3–9).

Adherens junctions can do so in different ways. First, they can act by localizing signaling molecules and thereby facilitating their reciprocal interaction through an increased proximity effect. Kinases (such as src, lyn, and yes), tyrosine phosphatases, components of the Ras signaling pathways, and small GTP binding proteins concentrate at cell–cell contacts in correspondence of adherens junctions (3–9).

Second, adherens junctions may play a more direct role in signaling by controlling the cytoplasmic levels of catenins. There is evidence that β-catenin and plakoglobin can act as signaling molecules when released into the cytosol from adherens junctions. As other members of the "armadillo" family, both proteins can translocate to the nucleus, bind transcription factor(s) and control gene expression (10, 11). In addition, cytosolic β-catenin and plakoglobin can associate with "adenomatous polyposis coli" (APC)[1] an oncogene which inhibits cell cycle progression (6–9) (Fig. 1).

Finally, adherens junctions, by attaching cells to one another, allow justacrine signaling through the local release and binding of growth factors and cytokines.

Molecular and functional specificity of adherens junctions in the endothelium

In endothelial cells, adherens junctions possess several specific features. The major cadherin is VE (vascular endothelial)-cadherin or cadherin-5. This molecule is strictly specific of endothelial cells and its sequence presents homologies but also a few differences with respect to the other members of the family (12, 13).

Like other cadherins, VE-cadherin is linked to catenins and to the actin cytoskeleton. However, in endothelial cells, the cadherin–catenin complex is remarkably dynamic and its composition rapidly changes in relation to the functional state of the cells (14). When the cells have weak junctions such as at early stages of confluency or when they are detaching and migrating from a monolayer, VE-cadherin is heavily phosphorylated in tyrosine and mostly linked to p120 and β-catenin. In these conditions, only a very small amount of the complex is associated with plakoglobin and the actin cytoskeleton. When adherens junctions are stabilized, as in tightly confluent cells, the majority of VE-cadherin looses tyrosine phosphorylation

1. *Abbreviations used in this paper:* APC, adenomatous polyposis coli; VE, vascular endothelial.

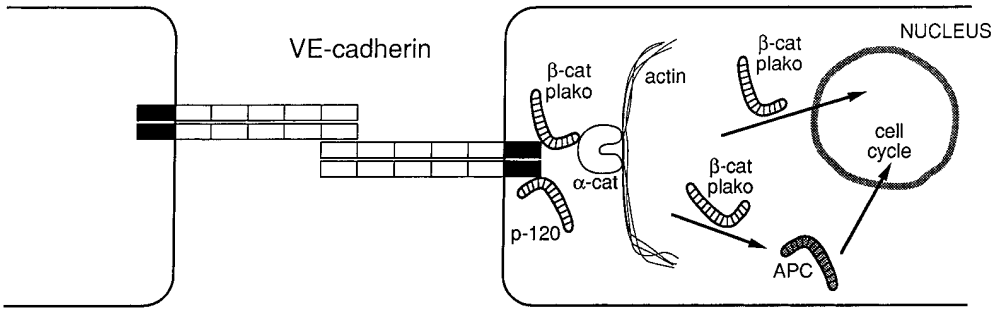

Figure 1. Schematic representation of adherens junction organization in the endothelium. VE-cadherin mediates a calcium-dependent, homophilic binding between cells. The crystal structure of cadherins suggests that they form dimers and interact with dimers on the opposite cell. Inside the cell VE-cadherin binds armadillo proteins (*β-cat,* β-catenin; *plako,* plakoglobin and p-120). β-catenin and plakoglobin associate α-catenin (*α-cat*) which in turn mediates the anchorage to actin microfilaments. Armadillo proteins have signaling properties: β-catenin and plakoglobin can translocate to the nucleus and interact with transcription factors. In addition, they bind the oncogene APC which inhibits cell cycle progression. See text for details.

and combines with plakoglobin and actin while both p120 and β-catenin are strongly reduced in the VE-cadherin complex (14 and M.G. Lampugnani, manuscript in preparation).

An important question is whether the changes in adherens junctions composition that mark a switch from a strong to a weak state and vice versa, may be reproduced by agents which affect endothelial permeability. Findings to date, though not conclusive, appear to be consistent with this assumption and support the general idea that VE-cadherin association with catenins is an important determinant of endothelial paracellular permeability.

When the association of VE-cadherin and catenins is abolished by truncating the cytoplasmic tail of the molecule, control of paracellular permeability is lost even if the mutant molecule can still cluster at junctions and promote cell–cell attachment (15). Thus, the complete organization of adherens junctions organelles and their linkage to actin microfilaments is required for the cells to "compact" one another and to prevent the passage of high molecular weight proteins through the junctions.

Thrombin was found to reduce plakoglobin association to VE-cadherin in parallel with endothelial cell retraction and gap formation in the monolayer (16). Adhesion of polymorphonuclear cells to endothelial cells induces catenin detachment from VE-cadherin and a general disassembly of adherens junctions. This was accompanied by increased endothelial permeability as observed in vivo in correspondence with sustained polymorphonuclear cell emigration (17).

Besides the relationship between adherens junctions organization and permeability, another possibility is that the dynamic interchange of catenins at adherens junctions may induce different intracellular signaling. The dynamic attachment and release of β-catenin, p120 or plakoglobin from adherens junctions may lead to different cytoplasmic levels of these proteins and possibly to different cellular responses depending on endothelial cell confluency or activation by agents such as thrombin or adherent leukocytes.

Adherens junctions and angiogenesis

In general, adherens junctions are crucial for tissue morphogenesis during embryonic development. Cadherins, through their homophilic interactions, are responsible for cell–cell recognition and tissue segregation. In the embryo, β-catenin and plakoglobin exert a general control over cell differentiation and developmental patterning processes (reviewed in references 6 and 11).

As regards the endothelium, adherens junctions may be important in directing the morphogenesis of the vasculature during embryonic development and when angiogenesis occurs in the adult. These structures not only would be able to assemble cells together but through their signaling properties might contribute to the determination of the overall architecture of the vasculature. Indeed, VE-cadherin is synthesized at very early stages in the embryo, at the time of endothelial cell differentiation (18). Its early and ubiquitous distribution along the vascular tree suggests it may be necessary for the correct organization of the vessels.

In human endothelial tumors like angiosarcoma or in hemangiomas where the vascular structures are profoundly altered, VE-cadherin is poorly expressed or is frequently misplaced from cell–cell contacts (1).

In addition, endothelial cells differentiating in vitro from embryonic stem cells in which VE-cadherin gene has been inactivated form cell aggregates but are unable to organize vascular structures (D. Vittet, T. Bouchou, E. Dejana, and P. Huber manuscript in preparation). These observations indirectly suggest that VE-cadherin and adherens junctions in general contribute to a correct vascular morphogenesis, but the question of what mechanisms are responsible for the process remains unanswered.

To form new vessels endothelial cells need to detach from the vascular wall, invade the underlying tissues, and then form tubes which branch and organize into extensive anastomotic networks. This process is complex and involves not only endothelial cell proliferation and migration but also cell–cell and cell–matrix adhesion, proteolytic remodeling of the matrix and changes in integrin expression. These activities need to be strictly controlled for a normal development of the vascular network. For instance, if endothelial cell proliferation or matrix proteolysis is not balanced, the vasculature becomes abnormal with the formation of vascular malformations.

Our hypothesis is that VE-cadherin and adherens junctions in general, may play a role in vascular remodeling by controlling and limiting endothelial cell migration and growth and possibly other specific endothelial cell functions.

During the first steps of angiogenesis, junctions need to become looser to let cells migrate and invade the underlying tissues. Since VE-cadherin inhibits cell migration from a mono-

Table I. Membrane Proteins Clustered at Endothelial Cell–cell Contacts

Proteins	Structure	Activity	Cell expression	References
VE-cadherin	Cadherin	Adherens junction component	Endothelial cells	1
Occludin	Four transmembrane domains	Tight junction component	Cells expressing tight junctions	23
CX43, CX40, CX37	Connexins	Gap junction components	Endothelial and other cells	24
PECAM	Ig	Cell–cell adhesion, leukocyte transmigration	Endothelial cells, platelets and leukocytes	25
S-Endo1 or MUC18	Ig	Cell–cell adhesion	Endothelial, smooth muscle, melanoma and other cells	26
Endoglin	Unique	TGFβ receptor	Endothelial cells	27
CD34	Sialomucin	L-selectin ligand	Endothelial, hemopoietic precursors and other cells	31
BD31	GPI-anchored glycoprotein	Cell–cell adhesion	Epithelial and endothelial cells	32

layer (13), endothelial cell detachment from neighboring cells should be preceded by inactivation of VE-cadherin and adherens junctions in general. Indeed, in other types of cells, growth factors such as epithelial growth factor (EGF) and hepatocyte growth factor (HGF) induce tyrosine phosphorylation of adherens junctions components(19) and this effect is related to their cell scattering activity. Angiogenic factors as vascular endothelial growth factor (VEGF) act in a similar way inducing tyrosine phosphorylation of adherens junctions to allow endothelial cell motility (S. Esser, E. Dejana and W. Risau, manuscript in preparation). Interestingly, VEGF is known to increase endothelial cell permeability, and this effect may reflect, at least in part, a weakening of intercellular junctions.

When the vascular network is formed and the intercellular contacts reestablish, endothelial cell migration and proliferation is again inhibited. The mechanisms that regulate this phenomenon are still largely unknown, but adherens junctions components seem to be involved.

We found that VE-cadherin transfection induced contact inhibition of cell growth in tumor cell lines (20). In addition, the engagement of VE-cadherin in endothelial cells inhibits their proliferation. For this activity, the extracellular adhesive properties of VE-cadherin are not sufficient and the intracellular interaction with catenins is required. As discussed above since β-catenin and plakoglobin have direct signaling properties, it is tempting to speculate that in confluent cells they interchange at junctions, translocate to the nucleus and downregulate the expression and/or activity of cell cycle regulatory molecules (Fig. 1).

Besides contact inhibition of growth, adherens junctions may affect other functions of endothelial cells which are important for the formation of a normal vascular network. Integrins, and αvβ3 in particular, play a key role during the first stages of angiogenesis in cell growth, migration and localization of proteolytic enzymes (21). In keratinocytes, cadherin engagement and expression inhibit integrin synthesis (22) and a similar process may occur also in endothelial cells.

A series of cell activities such as matrix proteolytic enzymes, prostacyclin release, cytoskeletal protein synthesis or inhibition of molecules which regulate cell cycle are strongly influenced by endothelial cell density. Future studies will tell whether molecules at adherens junctions might take part in modulating these activities.

Other molecules at endothelial cell–cell junctions

As reported in Table I many adhesive proteins localize at endothelial cell–cell contacts. It is difficult to believe that this redundancy is simply due to the need for keeping cell–cell attachment. It is more attractive to consider that these structures have different and specific biological activities and possibly distinct intracellular signaling pathways.

Besides adherens junctions, endothelial cells also express tight junctions (1). These organelles have a molecular organization different from adherens junctions suggesting a different biological role. However, adherens junctions are required for tight junction organization and maintenance, indicating that these structures are related (for review see reference 1). Occludin is the only transmembrane protein found so far at tight junctions. It is well expressed in endothelial cells and in particular in vessels which require a strict control of permeability such as in the brain microvasculature. Occludin is directly or indirectly associated with cytoskeletal proteins (such as ZO proteins and cingulin) and the actin cytoskeleton (23).

Co37, Co40, and Co43 are gap junction components (1, 24).

For all the other molecules listed in the Table no direct association with known cytoskeletal/signaling proteins or with defined junctional organelles has been found so far.

Some of them, such as PECAM (25) or S-endo1(26), promote homotypic endothelial cell adhesion and can therefore contribute to the assembly of vascular structures. Some molecules could act as docking structures, promoting cell–cell adhesion to allow the other proteins to cluster and to further stabilize the junctions. Finally, they might have other functional roles unrelated to endothelial cell–cell adhesion. For instance, PECAM promotes leukocyte extravasation, possibly by directing leukocyte movement through the intercellular junctions (25). Endoglin, is a receptor for TGFβ (27) and may localize this growth factor at intercellular contacts and eventually help reciprocal signaling among the cells.

Finally, it is worth noting that VE-cadherin is not the only cadherin expressed in the endothelium; N-cadherin is found in comparable amounts but, surprisingly, it does not concentrate

at cell–cell contacts when VE-cadherin is present (28). This observation poses the question of the biological significance of N-cadherin in these cells. Despite its diffuse distribution, N-cadherin retains robust homotypic adhesive properties and may therefore promote endothelial cell adhesion with other N-cadherin expressing cells such as smooth muscle cells of the vascular tunica media. There is also indirect evidence that N-cadherin can bind the FGF receptor (29) and in this way transfer intracellular signals. Finally cadherins are able to bind bacteria and mediate their entry into epithelial cells (30). A similar mechanism might exist also for N-cadherin in endothelial cells.

Conclusions

Adherens junctions are complex structures formed by the association between adhesive transmembrane proteins and the cytoskeleton. They play a central role in morphogenesis and in determining tissue integrity by promoting cell–cell mechanical linkages and signaling. Information about the molecular organization and functioning of adherens junctions and their interaction with the other adhesive structures at junctions might open new directions for the development of agents to control endothelial cell permeability and angiogenesis.

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