The molecular organization of endothelial junctions and their functional role in vascular morphogenesis and permeability

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ABSTRACT We review here our work on the molecular and functional organization of endothelial cell-to-cell junctions. The first part of the review is dedicated to VE-cadherin, characterized by our group few years ago. This protein is a member of the large family of transmembrane adhesion proteins called cadherins. It is endothelial cell specific and plays a major role in the organization of adherens junctions. Inactivation of VE-cadherin gene or in vivo truncation of its cytoplasmic tail leads to a lethal phenotype due to the lack of correct organization of the vasculature in the embryo. We found that the defect was due to apoptosis of endothelial cells, which became unresponsive to the survival signal induced by vascular endothelial cell growth factor. Our data indicate that VE-cadherin may act as a scaffolding protein able to associate vascular endothelial cell growth factor receptor and to promote its signaling. In the second part of the review we consider another protein more recently discovered by us and called junctional adhesion molecule (JAM). This protein is a small immunoglobulin which is located at tight junctions in the endothelium and in epithelial cells. Evidence is discussed indicating that JAM takes part in the organization of tight junctions and modulates leukocyte extravasation through endothelial intercellular junctions in vitro and in vivo. The general role of tight junctions in endothelial cells is also discussed.

KEY WORDS: endothelium, adherens junctions, tight junctions, angiogenesis, permeability.

Introduction

Adhesion of endothelial cells to one another is mediated by various surface receptors that belong to families of ubiquitously expressed cell adhesion molecules, such as cadherins, integrins, immunoglobulins, and proteoglycans. Besides providing attachment, most adhesive receptors interact with cytoskeletal and signaling molecules and contribute to the regulation of cell morphology and signaling. We concentrated our work on the adhesive molecules that mediate intercellular binding between adjacent endothelial cells and that are usually organized in adherens and tight junctions. These structures can modulate formation of new vessels by regulating cell migration, proliferation and the organization of a three-dimensional network of patent vascular tubes. Some of the molecules involved in cell-cell interactions have been now characterized. The intracellular signaling pathways activated by these molecules are on the contrary, still rather obscure (Hodivala and Watt 1994; Hermiston and Gordon 1995; Finnemann et al., 1995; Monier-Gavelle and Duband 1997).

Intercellular junctions are specialized regions of the plasma membrane which are organized when two cells come into contact. They sustain recognition and adhesion between adjoining cells (Gumbiner, 1996). In the endothelium two classical complexes, with an adhesive transmembrane component associated to a group of regulatory cytoplasmic proteins, can be distinguished: the adherens junction (Aberle et al., 1996) and the tight junction (Anderson and Van Itallie 1995). The general molecular organization and reciprocal relationships of cell to cell adhesive systems are basically similar in the endothelium in comparison to other cell types (Lampugnani and Dejana 1997). Adherens junctions and tight junctions differ on the basis of their components, localization along the lateral membrane and distribution in the vascular tree. Tight junctions present a more restricted distribution, being particularly enriched in endothelia presenting a strict control of permeability, such as arterial endothelium and brain capillaries.

Abbreviations used in this paper: JAM, junctional adhesion molecule; VE, vascular endothelial; PECAM, platelet endothelial cell adhesion molecule; VCAM, vascular cell adhesion molecule; CAM, cell adhesion molecule; HMG, high mobility group; VEGF, vascular endothelial cell growth factor; FGF, fibroblast growth factor.

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VE-cadherin and adherens junctions

Cadherins are recognized as important regulators of tissue morphogenesis both in the embryo and in the adult (Huber et al., 1996a) and their role in the organization of new vessels has been recently reported (Carmeliet et al., 1999; Gory-Faure et al., 1999; Radice et al., 1997). The endothelial specific VE-cadherin, as all the other members of the family, sustains cell-cell recognition and adhesion, homophilically binding an identical cadherin molecule present on an adjoining cell (Breviario et al., 1995). This cadherin represents the major transmembrane component of adherens junctions (Fig. 1).

In the embryo VE-cadherin is first expressed at very early stages of vascular development in mesodermal cells of the yolk sac mesenchyme. At later embryonic stages VE-cadherin expression is restricted to the peripheral layer of blood islands that gives rise to endothelial cells (Breier et al., 1996).

VE-cadherin gene is composed of 12 exons spanning more than 36 kb and like other cadherin genes it harbors large introns, especially at the S’ side (Huber et al., 1996b). The consistently large size of cadherin gene introns may be important for transcriptional regulation. It was shown that a tissue specific enhancer was present in the second intron of L-CAM (Sorkin et al., 1993) and of the P-cadherin gene (Hatta and Takeichi 1994). The cytoplasmic tail of VE-cadherin is encoded by one exon while in the other classic cadherins it is encoded by three exons. Taken together all the data collected indicate a simple transcriptional and mRNA processing pathway. No alternative spliced forms of VE-cadherin have been found so far.

The gene locus was found to be linked to the loci of other cadherins (E-, P- and M-cadherin) on mouse chromosome 8, indicating the presence of a cadherin gene cluster composed by at least four members of the family (Huber et al., 1996b). Despite the mapping on a similar region, it is unlikely that these cadherins genes share common regulatory elements since they have different and specific cell distribution.

A stretch of 2,500 bp in the VE-cadherin promoter region was found to direct transcription of the gene in vitro and in vivo in the endothelium of adult mice and during vascular development (Gory et al., 1998; Gory et al., 1999). VE-cadherin expression was specific for the endothelium of about all types of vessels. In the promoter region several binding sites for transcriptional factors including Sp1, Sp3 and Ets were found. The tissue specificity of VE-cadherin has few exceptions such as the cytotrophoblast. During establishment of human placenta cytotrophoblast invades the uterine interstitium and vasculature anchoring the fetus to the mother and establishing the blood flow to the placenta (Zhou et al., 1997). Cytotrophoblasts invading spiral arterioles replace the maternal endothelium and express a series of adhesion molecules which are typical of endothelial cells such as platelet endothelial cell adhesion molecule (PECAM), vascular cell adhesion molecule (VCAM), the integrin αvβ3 and VE-cadherin (Zhou et al., 1997). Interestingly, in the pregnancy disorder preeclampsia cytotrophoblasts do not express most of endothelial markers including VE-cadherin and their vascular invasion remains superficial (Zhou et al., 1997).

VE-cadherin positive progenitors cells with hemoangiogenic potential were identified by differentiation of totipotent embryonic stem cells in vitro or were isolated from the yolk sac of 9.5 E embryos. These cells would lose VE-cadherin expression while differentiating to hemopoietic progenitors (Nishikawa et al., 1998).

The VE-cadherin carboxy-terminal domain binds to two intracellular proteins, β-catenin and plakoglobin. These proteins belong to the armadillo family and by linking α-catenin promote the anchorage of cadherins to the actin cytoskeleton (Dejana et al., 1995; Takeichi, 1993; Aberle et al., 1996; Gumbiner, 1996). Deletion of a stretch of 83 COOH-terminal amino acids of VE- and other cadherins prevents the binding of both β-catenin and plakoglobin. E-cadherin lacking this domain is unable to promote homophilic aggregation while VE-cadherin and C-cadherin retain their aggregating properties (Navarro et al., 1995; Ozawa and Kemler 1998; Yap et al., 1998).

The amino acid residues in the membrane proximal domain of VE-cadherin cytoplasmic region presents low homology to other cadherins (Breviario et al., 1995). It may be that the membrane proximal regions of each cadherin exhibit specific functional characteristics including the binding to different intracellular regulatory proteins. This region can bind p120, which is another member of the armadillo family related to β-catenin and plakoglobin (Yap et al., 1998; Lampugnani et al., 1997). p120 is unable to bind α-catenin and therefore to anchor the complex to actin cytoskeleton. Tyrosine phosphorylation of VE-cadherin regulates its association with p120 (Lampugnani et al., 1997).

While truncated VE-cadherin can still promote homotypic recognition and adhesion it is unable to control paracellular permeability (Navarro et al., 1995), suggesting that the anchorage of VE-cadherin complex to the cytoskeleton is required to stabilize the junctions.

VE-cadherin transfection can also confer contact inhibition of cell growth while this property is lost by truncated VE-cadherin (Caveda et al., 1996). This would suggest that besides adhesive properties, VE-cadherin clustering at junctions may transfer intracellular signals, and that its binding to β-catenin and/or plakoglobin is required. E-cadherin expression was also related to inhibition of tumor cell growth (Watabe et al., 1994). In the transfected cells expression of α-catenin was needed for the tumor suppressor activity suggesting that the anchorage to actin is an important determinant for such action.

The pathway through which cadherins and in particular VE-cadherin transfer intracellular signals is still obscure. VE-cadherin-catenin complex is dynamic and its composition may change according to the functional state of the cells (Lampugnani et al., 1995). At early stages of confluency VE-cadherin is heavily tyrosine phosphorylated and mostly linked to p120 and β-catenin. When the junction stabilizes the tyrosine residues in VE-cadherin tend to loose phosphorylation. P120 and β-catenin partially detach from the complex and are substituted by plakoglobin (Lampugnani et al., 1997).

It may be that when dissociated from junctions, p120 and more importantly β-catenin become available for signaling. It has been demonstrated that β-catenin directly participates in Wnt growth
factor signaling cascade. The binding of Wnt to its receptor(s) inactivates glycogen synthase kinase-3 (GSK-3) which, in its active form, is responsible for phosphorylation and the rapid inactivation via ubiquitin-proteasome pathway of β-catenin. This process is regulated by the adenomatous polyposis coli (APC) protein and axin/contactin which can bind to GSK-3 and further facilitate β-catenin phosphorylation and degradation (Nusse, 1997; Ben-Ze’ev and Geiger 1998; Bullions and Levine, 1998). Free β-catenin can translocate to the nucleus and bind transcription factors of the high mobility group (HMG). This process may regulate the expression of a series of homeobox genes which are involved in cell growth and differentiation (Nusse 1997; Ben-Ze’ev and Geiger 1998; Bullions and Levine 1998).

Taking all this into account, however, the specific role of β-catenin in endothelial cells is still largely unknown and most of the information comes by analogy from other cellular systems.

A general and relevant issue is whether and how cadherins may transfer specific intracellular signals despite the fact that they bind to the same intracellular molecules. It is known that cadherins may direct different morphogenic programs in the embryo. Embryonic stem cells lacking E-cadherin differentiate to epithelia when rescued by transfection with E-cadherin whereas the same cells transfected with N-cadherin would form cartilage and neuroepithelium (Larue et al., 1996). In addition N-cadherin clustering promotes the activation of a myogenic differentiation program (Goichberg and Geiger 1998).

Endothelial cells express high amounts of both VE- and N-cadherin. Inactivation of the VE-cadherin gene (Carmeliet et al., 1999; Gory-Faure et al., 1999) causes severe alteration in the development of the vasculature and embryos invariably die within E9.5-10 of development. Endothelial cells in the mutant animals are still able to form a primitive vascular plexus. However, the vasculature does not undergo remodeling in absence of VE-cadherin and the vessels tend to regress and collapse leading to the death of the embryos (Fig. 2). The apparent cause of this severe alteration is that endothelial cells in VE-cadherin−/− animals undergo apoptosis. In addition, in vitro studies on cells cultured from mutant animals showed that in the absence of VE-cadherin, they lose the capacity to respond to vascular endothelial cell growth factor (VEGF) which protects them from apoptosis. This was related to the observation of a myogenic differentiation program (Goichberg and Geiger 1998).

As mentioned before cadherins require the contribution of cytoplasmic partners, the catenins, to transmit signals to the cell. The type of catenins associated to cadherins (Nusse, 1997).

The molecular constituents described at tight and adherens junctions in the endothelium, as well as their functional activities are represented. While characterized by distinct and specific components, both types of junctions comprise transmembrane molecules for cell-to-cell recognition and adhesion and intracellular-associated partners for the transmission of functional and structural information. At adherens junctions catenins comprise β-catenin and plakoglobin, directly connected to the cytoplasmic tail of VE-cadherin, α-catenin indirectly linked to VE-cadherin through the other two catenins and anchoring the complex to the actin cytoskeleton, and p120, directly bound to VE-cadherin, but not interacting with the other catenins. The only endothelial selective component of adherens junctions is VE-cadherin. At tight junctions, among claudins, claudin-5 is specifically expressed by the endothelium, while occludin and JAM as well as the cytoplasmic components are concentrated at junctions also outside the endothelium.

The formation of a vascular plexus in the yolk sac requires fusion of the preexisting blood islands (Risau 1995). This process does not take place in the N-cadherin null mutated embryos, suggesting that N-cadherin may have a role in vasculogenesis. It remains an open question whether the absence of N-cadherin in endothelial cells exclusively or the lack of association of endothelial cells with pericytes and smooth muscle cells expressing N-cadherin, could be responsible for the observed effect (Folkman and D’Amore 1996).

As mentioned before cadherins require the contribution of cytoplasmic partners, the catenins, to transmit signals to the cell. The type of catenins associated to VE-cadherin is different in an endothelial layer in dynamic situations (i.e. during migration or organization of a subconfluent culture) in comparison to a resting and established cell layer (Lampugnani et al., 1995). Null mutations of α-catenin, β-catenin and plakoglobin, respectively, result in embryonic lethality which is at a preimplantation stage for α-catenin (Torres et al., 1997), and post-implantation for both β-catenin (Haegel et al., 1995) and plakoglobin (Ruíz et al., 1996). The absence of β-catenin induces lethality at a stage (6.5-7.5 days of embryogenesis) which precedes blood vessel formation. It is intriguing to note that β-catenin can regulate epithelial tubulogenesis in vitro (Pollack et al., 1997). In this model system an NH2-terminal deleted β-catenin inhibits the formation of tubes in MDCK cells, possibly altering the dynamic interaction between APC and endogenous β-catenin, independently of its association to cadherins (Nusse, 1997).

Lethality of plakoglobin null embryo, which is delayed to 12-16 days, is due to impaired myocardial architecture (Ruíz et al., 1996).
was mostly guessed on the basis of the ultrastructure of these areas. Junctions is the control of paracellular permeability and polarity. This which in turn binds desmopakin (Valiron et al., 1996; Kowalczyk et al., 1998). These structures are formed by VE-cadherin linked to plakoglobin ß−catenin was found to codistribute with desmoplakin. These data suggest that endothelial cells assemble unique cell-to-cell adhesive structures which contain desmosomal constituents such as desmoplakin and intermediate filaments, but have VE-cadherin as transmembrane adhesive protein instead of desmoglin and desmocollins as in epithelial desmosomes. The role of these junctional structures in comparison to adherens junctions is still obscure but some evidence suggests that they appear at later stages of junction maturation and may be important to stabilize and strengthen cell to cell adhesion (Valiron et al., 1996).

Tight junctions

The main function which has been attributed so far to tight junctions is the control of paracellular permeability and polarity. This was mostly guessed on the basis of the ultrastructure of these areas which present an apparent fusion of the outer leaflet of the plasmatic membrane (Anderson and Van Itallie, 1995) and which, being located in a very apical position of the lateral junction, can well represent sealing areas of the intercellular space. Also their expression, specifically in epithelia and endothelia of strictly cohesive cell sheets, was compatible with such interpretation.

Although membrane lipids may participate in the molecular architecture of tight junctions, these structures are primarily composed of proteins. Transmembrane tight junction proteins comprise the tetrahedral molecules occludin (Furuse et al., 1993) and claudins (Furuse et al., 1998a) and the immunoglobulin-like cell adhesion molecule JAM (Martin-Padura et al., 1998). Intracellular tight junction proteins are represented by the Membrane-Associated Guanylate Kinase family members ZO-1 (Stevenson et al., 1986), ZO-2 (Gumbiner et al., 1991), and ZO-3 (Haskins et al., 1998) by the small G-proteins rab3b (Weber et al., 1994) and rab13 (Zahraoui et al., 1994) as well as by cingulin (Citi et al., 1988), 7H6 (Zhong et al., 1993) and symplekin (Keon et al., 1996). Within the cardiovascular system, endothelial tight junctions (Fig. 1) regulate (together with adherens junctions) the diffusion of plasma proteins and the passage of blood cells between neighboring cells.

The restraint exerted by tight junctions to the paracellular diffusion of ions and solutes might be attributable to the ability of transmembrane tight junctions proteins to seal together adjacent endothelial cells, even if experimental proof is only indirect. For instance, brain vessels are enriched in tight junctions and stain intensely for occludin (Furuse et al., 1993). Also, permeability inversely correlates with the number of tight junctions strands, which contain occludin (Furuse et al., 1993) and claudins (Furuse et al., 1998b) while an anti-occludin peptide enhances permeability in vitro (Wong and Gumbiner 1997). Finally, transfection of JAM in cells that do not normally form tight junctions increases their resistance to the diffusion of soluble tracers (Martin-Padura et al., 1998). However, we still do not know whether occludin, claudins, and JAM mediate homophilic (or even heterophilic) adhesion, and whether their effects are either independent or cooperative. Interestingly, genetic ablation of occludin does not affect permeability or tight junction organization, suggesting that its function may be vicariously compensated by other molecules (Saitou et al., 1998). Far from representing a static barrier to the diffusion of solutes, tight junctions are dynamically regulated structures, and control of perijunctional actin may be the final effector mechanism for modulating paracellular permeability (Madara, 1998). Also, several signaling receptors and messengers colocalize with tight junctions components (Mitic and Anderson 1998), and their effects on permeability are often associated with changes in the organization of both actin and tight junctions (Anderson and Van Itallie 1995).

Some of the cytoplasmic components of tight junctions are suggested to have signaling roles, and can even show nuclear localization (Gottardi et al., 1996; Muller et al., 1993). Interestingly a cytoplasmic component, ZO-1, which is also expressed by endothelial cells (Balda and Anderson 1993), presents nuclear staining in subconfluent, but not in confluent cultures and this distribution is stimulated at sites of wounding in epithelial cells in vitro and along the outer tip of the villus in tissue sections (Gottardi et al., 1996). An intriguing extrapolation from these data is the possible involvement of tight junction components in morphogenetic processes, among which the organization of endothelial cells in new vessels. However this aspect remains at the present totally unexplored.

The role of tight junctions in the control of vascular permeability to plasma components and circulating cells, is supported by little
evidence. A mAb directed to JAM inhibits leukocyte extravasation in vitro. (Martin-Padura et al., 1998). In vivo the mAb is effective in a model of skin inflammation (Martin-Padura et al., 1998) and cytokine induced meningitis (Del Maschio et al., 1999). This effect is likely mediated by inhibition of leukocytes adhesion to JAM present at interendothelial junctions and the block of their passage through endothelial junctions.

Transfection of JAM cDNA reduces paracellular permeability and promotes occludin localization at intercellular junctions suggesting that this molecule helps, through its homotypic multimerization the organization of tight junctions (G. Bazzoni, submitted).

Transfection of claudins in cells that do not normally express tight junctions induces the formation of strands at intercellular junctions which closely resemble tight junctions (Furuse et al., 1998b). Claudins form a large family of at least 18 homologous proteins which show some cell specificity of expression. In addition, the same cell may express different types of claudins on its surface and they may interact in an homotypic and heterotypic way (Furuse et al., 1999). Gene inactivation of the claudin family members may clarify their role even if it may well be that they may substitute for each other and compromise interpretation of the results.

Conclusions

It is now acquired that morphogenetic processes resulting in the formation of new vessels are directed by soluble mediators which can either stimulate or inhibit the process acting either directly on endothelial cells or on perivascular cells (Hanahan, 1997). Endothelial adhesive molecules promoting anchorage to matrix or cell-to-cell adhesion are important modulators of the morphogenesis of the vascular system. Indications that endothelial cell growth factors can modify proteins at cell-cell and cell-matrix junctions are also available. Understanding the mechanisms through which messages coming from soluble mediators affect the effectors of the architectural behaviour of endothelial cells, such as adhesive receptors for the matrix and adjoining cells, will help to build up a more integrated model of vascular morphogenesis.

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