Cell-cell adhesion in lung endothelium

D. Michael Shasby
Department of Medicine, University of Iowa College of Medicine, Iowa City, Iowa

Shasby DM. Cell-cell adhesion in lung endothelium. Am J Physiol Lung Cell Mol Physiol 292: L593–L607, 2007. First published November 3, 2006; doi:10.1152/ajplung.00386.2006.—Homotypic cell-cell adhesion is essential for tissue and organ development, remodeling, regeneration, and physiological function. Whereas a significant number of homotypic cell-cell adhesion molecules have been identified, much more is known about those concentrated in epithelia than in endothelia. Among the endothelial cell-cell adhesion molecules, very little is known that is specific to endothelium in the pulmonary and bronchial circulations. This review focuses primarily on homotypic cell-cell adhesion molecules that are or are likely to be important in lung endothelium.

What We Learn From Mice Deficient in Endothelial Cell-Cell Adhesion Molecules

Vascular endothelial-cadherin. Cadherins are the single-pass transmembrane molecules that mediate homotypic and hemophilic calcium-dependent cell-cell adhesion. In 1990, Heimark et al. (50) identified a calcium-dependent cell-cell adhesion molecule on bovine aortic endothelium that had properties consistent with a cadherin. In 1991, Suzuki et al. (121) reported the DNA sequences of eight additional cadherin species and reported that one of these, labeled cadherin 5, was present in human umbilical vein endothelial cells (HUVEC). In 1992, Lampugnani et al. (71) reported an antibody that recognized an antigen expressed only on endothelium, at sites of cell-cell contact, and that interrupted the barrier created by cultured HUVEC. The antigen recognized by the antibody reacted with antibody to conserved portions of the cadherin cytoplasmic domain. The antibody did not react with the previously described N-cadherin that was also present in HUVEC (71). More complete descriptions of function and expression followed from Dejana and colleagues (13, 70), and vascular endothelial (VE)-cadherin became recognized as the unique endothelial cadherin that mediated calcium-dependent homotypic endothelial cell-cell adhesion throughout most of the vascular system.

Mice deficient in VE-cadherin do not survive embryogenesis (14, 25, 44). VE-cadherin is not necessary for early endothelial cell association or early vasculogenesis, but it is necessary for maintenance of the endothelial-epithelial sheet, subsequent angiogenesis into complex vessels and tissues, and prevention of endothelial apoptosis and regression of vascular structures. The resistance to apoptosis depends on a linkage between the vascular endothelial growth factor receptor and the VE-cadherin complex. Other cell-cell adhesion molecules such as N-cadherin, junctional adhesion molecule (JAM), zona occludens-1 (ZO-1), occludin, and platelet/endothelial cell adhesion molecule (PECAM) were expressed in normal amounts in VE-cadherin-deficient embryos, and whereas apparently sufficient for nascent vessel formation, these molecules were not sufficient for preservation of the endothelial-epithelial sheet or angiogenesis (14). Mice expressing VE-cadherin deficient in the binding domain for β-catenin suffered embryonic death at...
the same time and in the same fashion as mice deficient in VE-cadherin itself, emphasizing a critical role of the binding of β-catenin to VE-cadherin in preserving VE-cadherin function. Hence, an intact VE-cadherin-β-catenin complex is essential for development and preservation of the endothelial-epithelial sheet and complex vascular patterning and maintenance.

**N-cadherin.** N-cadherin, also one of the classic cadherins, is expressed in all endothelium. Early reports suggested its primary role was endothelial cell adhesion to other cell types, such as smooth muscle cells, and its distribution was described as diffuse around the periphery of the cell (70, 112). When mice were made conditionally deficient in endothelial N-cadherin, it produced a phenotype almost identical to that of VE-cadherin-deficient mice. In fact, mice conditionally deficient in endothelial N-cadherin were also deficient in endothelial VE-cadherin, through a posttranscriptional mechanism (80). N-cadherin-deficient mice also had lower levels of the armadillo protein, p120, that binds to the juxtamembrane domain of cadherins and is important for preventing cadherin degradation (67). As in VE-cadherin-deficient mice, mice conditionally deficient in N-cadherin had normal amounts of other endothelial cell-cell adhesion molecules such as PECAM. Small interfering RNA (siRNA) used to reduce VE-cadherin expression in cultured endothelial cells did not reduce N-cadherin levels, similar to the observations in the VE-cadherin-deficient mice (14). Hence, whereas VE-cadherin expression is dependent on N-cadherin expression, N-cadherin is expressed in the absence of VE-cadherin. However, the expression of N-cadherin alone is not sufficient to support angiogenesis and sustain an endothelial-epithelial sheet.

**E-cadherin.** E-cadherin, another of the classic cadherins, is usually considered the adherens junction cell-cell adhesion molecule in epithelia. However, E-cadherin is expressed in brain microvessel endothelium and more recently was identified in pulmonary capillary endothelium (95, 108). E-cadherin is essential for epithelial development, and the E-cadherin-null mouse does not progress beyond the blastocyst (73). I found no reports of conditionally silencing E-cadherin in endothelium or lung.

**β-Catenin, plakoglobin, and desmoplakin.** β-catenin is an armadillo family protein that binds VE-cadherin in the carboxy-terminal 100 amino acids of the VE-cadherin cytoplasmic domain. As noted above, conditional expression of VE-cadherin, deficient in its β-catenin binding domain, produced a phenotype almost identical to VE-cadherin deficiency (14). Conditional inactivation of β-catenin in endothelium also caused embryonic death in mice but at a little further on in development [embryonic day (E)11.5–13.5 vs. 9.5 for VE-cadherin-deficient mice] (15). At E9.5, the nascent vasculature of mice conditionally deficient in β-catenin was not different from wild-type mice. However, by E10.5, the yolk sac appeared poorly perfused with smaller vessels, and umbilical vessels were smaller with aberrant patterning. Smaller vessels, aberrant patterning, and frequent hemorrhages were present in the embryo itself. Electron microscopic sections demonstrated poorly developed sites of cell-cell adhesion and a marked increase in fenestrations within endothelial cells. Cultured cells deficient in β-catenin were long and thin, were more migratory, and had more plakoglobin and desmoplakin at cell-cell contacts. Cultured monolayers of β-catenin-deficient endothelial cells had increased permeability to dextran compared with wild-type cultured cells. Mice deficient in the VE-cadherin β-catenin binding domain and mice deficient in endothelial β-catenin both emphasize the critical role of β-catenin binding to VE-cadherin for development, remodeling, and stability of the endothelial-epithelial sheet.

Carmeliet et al. (14) proposed that in the absence of β-catenin, VE-cadherin bound plakoglobin (γ-catenin), another armadillo protein. Plakoglobin binds to the same site on cadherin as does β-catenin. However, plakoglobin links the adherens complex to desmoplakin and vimentin, an adherens junction pattern seen in lymphatics and high endothelial venules, called complex adherentes (14). Although able to support nascent vessel formation, this adhesion complex was not sufficient for more complex angiogenesis.

Plakoglobin, also known as γ-catenin, is also an armadillo protein that binds in the same β-catenin binding domain. Mice deficient in plakoglobin also die as embryos, but they die from defects in heart intercalated discs and not vascular defects (107). Hence, it was surprising that mice deficient in desmoplakin, a protein linking plakoglobin to intermediate filaments, in the complex adherentes, died at E10.5 of defects in the microvasculature, with a fivefold reduction in capillaries (41). The basis for failed microvascular development in these mice is unexplained.

**PECAM.** PECAM is a 130-kDa immunoglobulin-like cell-cell adhesion molecule expressed on endothelial and hematopoietic cells including endothelial cells, platelets, and leukocytes. The initial description of mice null for PECAM revealed no significant phenotypic differences from wild-type mice (31). However, when the background mice were changed from C57BL/6 to FVB/n, a defect in leukocyte recruitment from the vascular space was evident (114). No significant vascular structural defects were reported for PECAM deficiency on either background.

Although PECAM-deficient mice did not have abnormal vasculature, a recent report identified abnormal postnatal alveolar development in PECAM-deficient mice (29). The defect in alveolar development was linked to abnormal endothelial cell function, in particular, defective migration, as an antibody to PECAM that inhibited endothelial migration, but not proliferation, reproduced the same effects on alveolar development. The migration defect, and not limited cell survival or proliferation, was confirmed in the PECAM-deficient mice.

**JAM.** JAM-A is a 32-kDa immunoglobulin-like adhesion molecule that is localized to sites of cell-cell adhesion in endothelia and epithelia and is also expressed on leukocytes and platelets (81). In epithelia, tight junctions are consistently apical to and better separated from adherens junctions than they are in endothelia. In epithelia, JAM-A localizes to sites of tight junction cell-cell adhesion. Mice deficient in JAM-A demonstrate alterations in leukocyte trafficking, with increased dendritic cell trafficking to lymph nodes and increased contact hypersensitivity (16). JAM-A-deficient mice also demonstrate impaired neutrophil recruitment to sites of inflammation. The defects in dendritic cell trafficking and neutrophil recruitment are reproduced by JAM-A-deficient marrow in mice expressing JAM-A in endothelium, but not with JAM-A-expressing marrow in mice with JAM-A deficiency in the endothelium (22). Hence, the critical effect on leukocyte trafficking appears to be dependent on leukocyte expression of JAM-A.

L594
deficient mice, doubly deficient for JAM-A and apoprotein E, demonstrated limited monocyte infiltration of vascular wounds. However, the wounds did not demonstrate any defects in endothelial cell recovery and repair (97).

JAM-B is expressed primarily in high endothelial venules, whereas JAM-C is expressed in endothelial cells (especially in lymphatics), platelets, and lymphocytes (8). Both appear to function primarily in leukocyte trafficking, and I could not find data on mice deficient in either.

ESAM. Endothelium-selective adhesion molecule (ESAM) is another immunoglobulin-like cell-cell adhesion molecule expressed only in endothelium (61). Mice deficient in ESAM did not demonstrate developmental vascular defects. However, ESAM-deficient mice did not support the growth of implanted tumors as well as did the wild-type mice. Tumor volumes were less than 50% of those in control mice, the tumors had decreased vascular density, and endothelial cells from ESAM-deficient mice demonstrated impaired tube formation in vitro angiogenesis assays.

CD146 (MUC18, S-endo-1). CD146 (MUC18, S-endo-1) is a transmembrane immunoglobulin-like endothelial cell-cell adhesion molecule. CD146 does not seem to colocalize with either the adherens or the tight junction, and its expression increases with confluency of cultured cells (5). It makes heterophilic bonds with an undetermined partner (64). Antibody to CD146 inhibits angiogenesis in chick allantoic membrane (142). There are no descriptions of its deletion in mice. In Zebra fish, morpholino mutation of CD146 resulted in abnormal angiogenesis with small intersomitic vessels and decreased blood flow (18). In normal Zebra fish embryos, CD146 expression was higher in arteries than in veins early in embryogenesis.

CD148, DEP-1, CD148 or DEP-1 (density-enhanced protein) is a receptor-like tyrosine phosphatase. Mice deficient in DEP-1 die in utero at E10.5 with vascular deformities including larger vessels, endothelial cell proliferation, and failure to form a hierarchy of mature vessels (122). DEP-1 associates with the VE-cadherin complex and dephosphorylates VEGF receptor 2, thereby supporting VE-cadherin-based contact inhibition of cell growth (45). Inhibition of VEGF signaling requires that both DEP-1 and β-catenin bind to VE-cadherin, reemphasizing the importance of an intact VE-cadherin-β-catenin complex in preserving the phenotype of contact growth inhibited, adhering endothelial cells. Hence, whereas DEP-1 is not an adhesion molecule itself, it is an essential component of the endothelial adherens junction complex.

Occludin. Occludin is a four-pass transmembrane protein component of tight junctions. Mice deficient in occludin survive to birth but demonstrate postnatal retarded growth and male infertility. No vascular defects were described. Although occludin was absent from epithelial tight junctions, no other functional or structural defects were detected, and epithelial adherens junctions were intact (110). Occludin-deficient mice did have gastric epithelial inflammation, dystrophic brain calcification, thinner compact bone, and abnormal testes.

Occludin is expressed strongly in brain endothelium and in testis, but its expression in endothelium of other tissues is less certain (52). In a report focusing on larger vessels, occludin was detected at higher levels in arteries than in veins, and higher levels of expression were associated with stronger barrier properties in cultured cells (65). No occludin was detected in dermal microvessels or lung microvessels (83, 84).

Claudin. Tight junctions are remarkably complex structures of 40 or more proteins and have been recently reviewed in detail (130). Claudins are the proteins that create the characteristic strands of tight junctions and are responsible for the tight junction seal and its pore properties. Claudin-5 was identified as the endothelial claudin, in part because it was expressed in almost all tissues, even those without epithelia (84). In the brain and lung, claudin-5 was expressed in all vessels, and its expression pattern overlapped with VE-cadherin expression (84). In contrast, in kidney and intestine, claudin-5 was expressed in arteries but not capillaries or veins. Mice deficient in claudin-5 survive embryogenesis but demonstrate impaired movement at birth and die shortly after birth (90). The vessels of the brain and other organs were normal in developmental appearance, and a precise cause of death could not be determined. In the brain, claudin-12 was expressed in the microvessels, creating tight junctions. However, in the absence of claudin-5, these junctions showed increased permeability to molecules of less than 800 kDa.

Endoglin, TGF-β receptors, and ephrins. Deficiencies of transforming growth factor-β (TGF-β), TGF-β receptors 1 and 2, endoglin that is an ancillary TGF-β receptor, and Eph B4 and ephrin B2 all result in embryonic death with impaired angiogenesis (143). Similar defects in angiogenesis occur with deficiencies of angiopoietin and its receptor Tie-2. These are not cell-cell adhesion molecules, but their regulatory effects in angiogenesis are germane to how cell-cell adhesion molecules function. In this role, the ephrins are especially informative. The receptor Eph B4 is expressed on veins, whereas its obligate ligand, ephrin B2, is expressed only on arteries. Their ligation initiates signaling in both cells. A deficiency in ephrin B2 resulted in aberrant embryonic capillary remodeling in both venous and arterial segments (132). Mutations in endoglin or alk-1, the type 1 TGF-β receptor, are linked to the vascular malformations of hereditary hemorrhagic telangiectasia and reflect failure of patterning between arteries and veins with resultant arterial-venous shunts (75).

Summary of Information from Cell-Cell Adhesion Molecule-Deficient Mice

Tissue development involves the gathering together of cells with similar function, and cell-cell adhesion molecules are important to this process. Mesodermal angioblasts gather together and form a group of uniform-sized tubes composed only of endothelial cells in the process of vasculogenesis. It appears that there is enough redundancy among the known endothelial cell-cell adhesion molecules that elimination of any one of them does not disrupt vasculogenesis.

Tissue development also involves the organization of groups of similar cells with other cell types to form more complex structures. In the lung, the mesodermal angioblasts that have formed tubes of endothelium must interface with the endoderm that is developing into the airways. Similar interactions occur with other portions of the endoderm and the ectoderm in other organs. These events result in the patterning we recognize as a mature vasculature, the process of angiogenesis. This process incorporates dissociation of endothelial cells in tubules, proliferation of and then reassociation of endothelial cells with...
themselves and with new mesodermal and endodermal partners, and the development of a mature vascular pattern. In the process of proliferation, cell-cell adhesion molecules have to let go, reassociate both homotypically and heterotypically, and respond to signals from their new partners and from the molecular complexes that form at sites of cell-cell adhesion. In this process, VE and N-cadherin, β-catenin, CD146, DEP-1, the TGF-β signaling group of the TGF receptors, endoglin, and the ephrins are all essential. The effects of deficiencies in these molecules are manifest so early that I could not find any references to specific defects in the pulmonary vasculature, although hereditary hemorrhagic telangiectasia does manifest as arterio-venous malformations within pulmonary vessels.

More on structure and function. Although many of the endothelial cell-cell adhesion molecules are not essential for mouse embryonic development, they may still have important or essential roles that are required beyond development or that are more subtle and not yet detected in deletion models.

Immunoglobulin-like molecules. Figure 1 provides a general outline of the structure of the immunoglobulin-like cell-cell adhesion molecules.

PECAM. PECAM is a 130-kDa immunoglobulin-like single-pass transmembrane cell adhesion molecule found on all vascular cells, platelets, monocytes, leukocytes, some T cells, and endothelial cells (58). PECAM is expressed on all endothelial cells and is localized to regions of cell-cell contact and to the luminal surface. As alluded to above, deficiency of PECAM was not associated with abnormal vessels but was associated with abnormal trafficking of leukocytes to extravascular tissues and with impaired postnatal alveolar and airway development (29, 114).

More recent studies have emphasized PECAM’s role in signaling in which PECAM acts as a scaffold for signaling and adaptor molecules (58). PECAM’s cytoplasmic domain contains an immunoregulatory tyrosine-based activation motif, and phosphorylation of Tyr663 and Tyr686 within this sequence regulates binding of SH2-containing signaling and adaptor molecules such as SHP-2, PLC-γ, and phosphatidylinositol 3-kinase.

Earlier studies suggested that PECAM was essential for shear stress-initiated signaling in endothelium and that the SH2-SHP-2 interaction was involved (94). More recent studies with cells from PECAM-deficient mice indicate that other molecules can mediate the response to shear in the absence of PECAM, and PECAM’s role is uncertain (120).

Deletion studies in mice, referenced above, emphasized the importance of an intact β-catenin-VE-cadherin complex for the integrity of the endothelial sheet (14, 25, 44). PECAM may help maintain this complex. Tyrosine-phosphorylated β-catenin binds to PECAM near the SHP-2 binding domain. Mutation to phenylalanine of the PECAM tyrosines mediating the SH2-dependent binding of SHP-2, or depletion of PECAM, resulted in increased tyrosine-phosphorylated β-catenin (59).

PECAM deficiency or antibody to PECAM was associated with abnormal postnatal alveolar and airway development (29). The authors hypothesized that alveolar development might be linked to angiogenesis. Prior work from the same authors had demonstrated abnormal tumor angiogenesis in mice treated with antibody to PECAM (147).

JAMs. JAM-A, -B, and -C are 32- to 35-kDa single-pass transmembrane immunoglobulin-like molecules expressing V_{H} and C_{2} type immunoglobulin folds in their extracellular domain, a transmembrane domain and a short (40–50 amino acids) cytoplasmic tail with a type 2 PDZ binding motif (8, 81). JAM-A is expressed on epithelia and endothelia where it localizes to tight junction structures. JAM-A is also expressed on circulating leukocytes and platelets. JAM-B is expressed primarily in high endothelial venules. In mice and humans, JAM-C is expressed on endothelial cells. In humans, JAM-C is also expressed on platelets, T cells, natural killer (NK) cells, and dendritic cells (69). Two very distinct roles have been suggested for JAM proteins. JAM proteins are linked to trafficking of leukocytes from the circulation into tissues. In addition, localization of JAM-A and JAM-C to tight junctions in epithelial and endothelial cells has led to the hypothesis that they participate in formation of tight junctions.

JAM proteins demonstrate homophilic and heterophilic binding. It is not yet clear if JAM-A homophilic binding

---

Fig. 1. Schematic of basic structure of the immunoglobulin-like cell-cell adhesion molecules with variable numbers of V_{H} and C_{2} immunoglobulin folds in the extracellular domains and significant differences in the length and content of their cytoplasmic domains. ESAM, endothelium-selective adhesion molecule; CAR, coxsackie adenovirus receptor; PECAM, platelet/endothelial cell adhesion molecule. JAM, junctional adhesion molecule; TM, transmembrane; AA, amino acids.
mediates binding between homotypic cells (e.g., endothelial to endothelial cells) or if it primarily mediates heterotypic adhesion such as leukocytes to endothelium (8, 32). JAM-A, JAM-B, and JAM-C also bind leukocyte integrins LFA-1, VLA-4, and Mac-1, respectively, and this heterophilic binding has been hypothesized to be important in leukocyte trafficking (113).

Recent work from Corada et al. (22) provided a surprise and has clarified the role of JAM-A in neutrophil trafficking to sites of acute inflammation. Mice globally deficient in JAM-A demonstrated decreased neutrophil diapedesis into the peritoneum or pericardium in response to inflammation. In mice deficient in JAM-A, only in the endothelium, neutrophil migration was normal. In contrast, migration of JAM-A-deficient neutrophils was blunted in mice with normal JAM-A expression in endothelium. Hence, neutrophil expression of JAM-A is essential for normal neutrophil recruitment to sites of acute inflammation, and endothelial JAM-A is not necessary. It is uncertain what the endothelial ligand is for leukocyte JAM-A. The authors speculated it could be JAM-C. This hypothesis is consistent with the observations of Ludwig et al., who found that antibody to JAM-B and JAM-C limited leukocyte trafficking into skin in response to an inflammatory stimulus (79a). The recent observations of Corada et al. (22) clarify the role of JAM-A in neutrophil trafficking. However, the roles of endothelial JAM-B and -C in the trafficking of leukocytes and lymphocytes are still not so well resolved.

Homophilic JAM-C binding has been hypothesized to be relevant to tumor metastasis (113). JAM-C expressing lung carcinoma cells bound to HUVEC, and the binding was inhibited with soluble JAM-C or amino-terminal JAM-C peptides. In contrast, the binding to HUVEC of another lung carcinoma cell line that does not express JAM-C was not inhibited by soluble JAM-C or the amino-terminal peptides.

In epithelia, JAM-A associates with the tight junction protein ZO-1 through PDZ domain-dependent binding to afadin. Antibodies to JAM-A and JAM-A-Fc chimeric protein slow but do not prevent recovery of transepithelial resistance in epithelial cells after calcium depletion (76, 79). The effects of antibodies to JAM-A and JAM-A-Fc chimeras specifically affected reassembly of tight junctions, as reassembly of E-cadherin-based adherens junctions was not affected. In epithelium, the tight junction is distinctly apical to other sites of cell-cell contact, and JAM-A clearly localizes to this apical site. The JAM-A afadin link and the tendency of JAM-A to localize to early sites of cell-cell attachment has led to the hypothesis that JAM-A may participate in the early development of nascent cell-cell adhesion and membrane partitioning, but does not have much of a role in more robust and mature cell-cell adhesion structures (32). In endothelium, with flatter cells, the tight junction is not so clearly separated from other sites of lateral cell-cell adhesion, and JAM-A is similarly not so clearly separated from adherens junctions (28). The same uncertainties about the role of JAM-A in epithelial homotypic cell-cell adhesion apply to endothelial homotypic cell-cell adhesion.

JAM-A may have an important role in angiogenesis. JAM-A associates with the endothelial integrin αvβ3. Beta fibroblast growth factor (bFGF), an important angiogenic growth factor, dissociates JAM-A and αvβ3. Antibodies to JAM-A or function-blocking mutations in the cytoplasmic domain of JAM-A block the ability of bFGF to induce morphological changes in endothelium, migration of endothelial cells, endothelial cell tube formation, and angiogenesis in chick allantoic membrane (88). Since the JAM-A-deficient mouse has normal vascular development, there must be redundancy in this cycle.

JAM-A is expressed on platelets and is phosphorylated when platelets are activated by thrombin or collagen, although the physiological relevance of this phosphorylation remains undetermined (118).

ESAM, CAR. ESAM and coxsackie adenovirus receptor (CAR) are also immunoglobulin superfamily cell-cell adhesion molecules that localize to tight junctions. The extracellular domains of ESAM and CAR contain the same V1 and C2 domains as JAM-A-JAM-C, but the cytoplasmic domains are larger with 105–120 amino acids, and the carboxy terminus contains a type 1 PDZ binding motif rather than the type 2 form found in JAM-A-JAM-C. The longer cytoplasmic domain and the type 1 vs. the type 2 PDZ binding motif suggest different binding partners for ESAM and CAR than for JAM-A-JAM-C. ESAM and CAR bind homophilically and support cell aggregation, a manifestation of cell-cell adhesion that has yet to be demonstrated for JAM-A-JAM-C (32).

ESAM is localized to tight junctions in endothelium but is also expressed on megakaryocytes and platelets (53). ESAM binds the multidomain adaptor protein membrane-associated guanylate kinase with inverted domain structure (MAGI-1) that is capable of bundling multiple proteins to the tight junction complex. As noted above, whereas ESAM-deficient mice developed a normal vasculature, angiogenesis in implanted tumors was defective (61).

CAR localizes to tight junctions in epithelial cells. Earlier reports suggested CAR was expressed on cultured endothelial cells. However, a more recent and very complete survey of mouse tissues did not detect CAR in endothelium in any organs (103). CAR was detected in the heart, in intercelledated discs between cardiomyocytes and in the mesothelial cells of the epicardium.

CD146. CD146, also known as MUC-18 or S-endo-1 Ag, is another immunoglobulin superfamily molecule expressed on all endothelial cells. In contrast to JAM, ESAM, and CAR, the extracellular domain contains two V1 and three C2 regions. The cytoplasmic domain contains 61 amino acids. CD146 localizes at sites distinct from adherens or tight junctions. Ligation of CD146 in cultured cells with antibody stimulates intracellular signaling, including increases in cell calcium and tyrosine phosphorylation of p130cas, Pyk2, FAK, and paxillin (3, 5). CD146 supports cell aggregation, and antibody to CD146 increases diffusion of solute across monolayers of HUVEC. CD146 appears to have a heterophilic physiological ligand that remains unknown (3, 5). As noted, mutations of CD146 caused severe aberrant angiogenesis in Zebra fish with failure of intersomitic vessel development (18). Consistent with an important role in angiogenesis, antibody to CD146 inhibited new vessel development and tumor growth in mice (142).

Nectin. Nectin, another immunoglobulin superfamily molecule, is an important component of developing adherens junctions in epithelia (123, 124). Reviews of endothelial cell-cell adhesion molecules always include nectin, but the primary data for nectin in endothelium is scant. Nectin 2 expression was detected on cultured HUVEC and human placental and skin
Claudin-5 is the claudin expressed in most endothelia (84). Nectin 1 was found in murine corneal microvessels (128). Otherwise, I could find no other primary data for nectin and endothelium.

In epithelia, nectin facilitates establishment of E-cadherin adherens junctions (123, 124). When filopodia from adjacent cells connect, E-cadherin cis dimers bind in trans between the cells. Nectin cis dimers also bind in trans (both homophilic and heterophilic) at the sites of cell-cell contact, and the initial rate of nectin binding is faster than the rate of E-cadherin binding. Nectin binding initiates a signaling cascade of c-Src-Crk-C3G that activates FRG and Rap1 and thereby Cdc42, accelerating E-cadherin binding in trans between the cells. The increased E-cadherin binding transforms the spot puncta of adhesion into linear bands of E-cadherin-based adhesion (36, 37). Cdc42 accelerates the process by stimulating actin-based filopodia with consequent increased numbers of cell-cell contact sites. The Rac small G proteins then stimulate lamellipodia at the sites of contact, creating a zipperlike effect that results in the formation of adherens junctions with E-cadherin binding in trans.

Nectin’s cytoplasmic domain has a PDZ sequence that binds afadin, and afadin binds actin. Mice deficient in afadin develop defects in ectoderm at gastrulation, similar to E-cadherin-deficient mice, emphasizing the important role of nectin and afadin in cadherin cell-cell adhesion. Nectin and afadin may also play a role in tight junction development, although the data for this is more indirect than that for adherens junctions (123, 124). I could find no direct examination of the nectin-afadin system in endothelium.

Claudins and occludin. Tight junctions contain three transmembrane proteins, claudins, occludin, and JAMs. Claudins are the proteins that constitute the tight junction membrane strands and are essential to the barrier of tight junctions that contains 40 other proteins. There are recent comprehensive reviews of claudins (38–40, 130). Claudins are small, 20- to 27-kDa proteins, with four transmembrane domains, a very short cytoplasmic amino terminus of 2–6 residues, two extracellular domains, and a cytoplasmic carboxy terminus of 21–63 residues that links to ZO-1 and other cytoplasmic tight junction proteins through a PDZ binding motif (130). A W-GLW-C-C sequence in the first extracellular loop is characteristic for all claudins.

Claudin-5 is the claudin expressed in most endothelia (84). In the lung, claudin-5 is expressed in arteries, capillaries, and veins. However, in some vascular beds, such as kidney, claudin-5 is expressed in arteries but not capillaries or veins. As noted above, mice deficient in claudin-5 had increased paraacellular permeability to small (<800 Da) solutes in the brain. No specific comments were made about the lung in that report (90). Claudin-5 was reported to be expressed in airway epithelium along with claudin-1 and -3, although Morita et al. (83) in their original description of claudin-5 did not detect it in lung epithelium. In freeze fracture preparations, claudin-5 particles are primarily associated with the extracellular face of the cell membrane, as opposed to claudin-1 and other epithelial claudins that are predominantly associated with the protoplasmic face (84). The other claudins coexpressed in endothelium with claudin-5 may have important effects on the permeability of different vascular beds.

The carboxy PDZ binding motifs of claudins bind to PDZ domains in ZO-1, -2, and -3, MUPPI1, and PATJ that are intracellular scaffolding proteins for the other constituents of the tight junctions. Elimination of the PDZ binding motifs of claudins disrupts their organization but does not preclude their forming strands in the cell membrane (130). In epithelia, different claudins demonstrate differing permeability to differently charged solutes. Selective permeability of claudin barriers to positively or negatively charged solutes is affected by amino acid charges in the first extracellular loop of the claudin, with increased permeability associated with opposite charges on the solute and the claudin loop (21).

The understanding of the regulation of claudin function is not well developed. Phosphorylation of serines and threonines in the carboxy cytoplasmic domain of claudins has been associated with increased permeability and decreases in transcellular resistance. Changes in the dynamic processes of endocytic recycling of claudins into and from the membrane and in claudin expression have also been associated with changes in barrier function (130).

Occludin is a 60-kDa tight junction protein with four transmembrane domains, two extracellular domains, and cytoplasmic amino and carboxy domains. The precise role of occludin in tight junction formation and function remains uncertain. Occludin localized to the tight junction is phosphorylated on serines and threonines, whereas unphosphorylated occludin is more diffusely distributed on the basolateral membrane (115). The tyrosine kinase Yes associates with occludin, and tyrosine phosphorylation of occludin was linked to reassembly of tight junctions after calcium depletion and repletion (19). A more recent report linked occludin with the TGF-β receptor and a role in the epithelial mesenchymal transition that results from ligation of the receptor, suggesting it may have a more prominent role in signaling than structure (6).

Cadherins. Figure 2 is a schematic of a cadherin complex. Discovery and expression. Cadherins are the family of cell-cell adhesion molecules responsible for homotypic calcium-dependent cell-cell adhesion. In most of the systemic circulation and in the conduit pulmonary circulation, the dominant adhesive cadherin is VE-cadherin (28). N-cadherin is expressed in all endothelium, but its role in endothelial cell-to-endothelial cell adhesion is less well defined. In the brain and pulmonary microcirculations, E-cadherin is also expressed on the microvascular endothelial cells (95, 96, 108).

N-cadherin is one of the classic cadherins and was described in chick retina by Thiery et al. (125) in 1977 and in brain by Hatta et al. (48) in 1985. It was detected in endothelium in 1992 (112). Most reports on N-cadherin in endothelium describe a diffuse cortical distribution of the protein and suggest that N-cadherin participates in heterotypic adhesion between endothelial cells and mesenchymal cells such as smooth mus-
involved in cell and fibroblasts that also express N-cadherin (9, 112). A more recent observation in mice deficient in endothelial N-cadherin and referred to above indicates an important role for N-cadherin in the development of homotypic endothelial cell-cell adhesion (80).

E-cadherin is also one of the classic cadherins. It was described by Bertolotti et al. (10) in 1980 as liver cell adhesion molecule (L-CAM). Gallin et al. (42) subsequently described the disruptive effect of antibody to L-CAM (E-cadherin) on tissue patterning in the developing chick. Cereijido et al. (17) described a technique for growing monolayers of Madin-Darby canine kidney (MDCK) cells that developed a resistance to current flow across the epithelium, and the resistance was abolished by calcium depletion. Gumbiner and Simons (46) made antibodies to MDCK cell surface proteins and used the preparation described by Cereijido et al. to find antibodies that interrupted the calcium-dependent transepithelial resistance. They identified a 118-kDa protein that reacted with a monoclonal antibody that disrupted the resistance across the MDCK monolayers. Antibody to uvomorulin, a calcium-dependent cell surface molecule, important for embryonal compaction identified by Hyafil et al. (57), bound the same protein. Yoshida-Noro et al. (145) developed a monoclonal antibody that disrupted calcium-dependent adhesion of epithelial cells, demonstrated that it reacted with the same molecule that Hyafil et al. had identified, and suggested that the cell adhesion molecule be called cadherin. Nagafuchi et al. (86) cloned cDNA for E-cadherin and expressed it in fibroblasts where it transformed the cell phenotype and promoted cell-cell aggregation. Nagafuchi and Takeichi (87) removed some of the coding elements for the cytoplasmic domain from E-cadherin cDNA and demonstrated that cell aggregation was dependent on the cytoplasmic domain. Of note, a construct missing only the most carboxy 37 amino acids, a portion of the cytoplasmic domain that includes what is now known to be the β-catenin binding domain, failed to cause aggregation, emphasizing the importance of β-catenin binding in cadherin-based cell adhesion.

E-cadherin is usually thought of as the cadherin localized to adherens junctions in epithelia. However, Pal et al. (95) reported that E-cadherin was expressed on brain microvessel endothelial cells and that antibody to E-cadherin or a decapentapeptide mimicking the amino terminus of E-cadherin interrupted cell-cell binding of brain microvessel endothelial cells. In a report directly relevant to lung endothelium, Safdar et al. (108) reported that lung alveolar endothelium stained with antibody to E-cadherin. More recently, Parker et al. (96) reported that cultured pulmonary microvascular endothelial cells had a 20-fold lower hydraulic conductance than cultured conduit pulmonary artery endothelial cells. In the same report, they demonstrated that the dominant cadherin in pulmonary microvascular endothelial cells was E-cadherin, not VE-cadherin. It is of interest that both the brain and pulmonary microvessels express E-cadherin. Both sites require a very tight barrier to ensure vital organ function. E-cadherin and epithelia are usually associated with much tighter resistances than endothelium. These resistances are generally attributed to the tight junctions and different claudin expression, and as discussed above, perhaps levels of occluadin expression (130). It will be important to examine the pulmonary microvessel cells for the different tight junction proteins. It will also be important to study their response to inflammatory stimuli in the context of E-cadherin adhesion as discussed below.

The evolution of the discovery and development of the body of knowledge on VE-cadherin was outlined. In addition to its critical role in angiogenesis, persistent VE-cadherin adhesion is essential to normal endothelial function. Systemic venous administration of antibody to VE-cadherin caused edema in the heart and lungs of mice (23).

Structure of classic cadherins and their complexes. N-cadherin, E-cadherin, and VE-cadherin have extracellular domains containing five repeats of ~110 amino acids per repeat, a transmembrane domain, and a highly conserved cytoplasmic domain of ~150 amino acids. By definition, cadherin extracellular repeats contain the conserved sequences DRE, HAV, and DXD (136). N- and E-cadherin have a histidine, alanine, valine sequence in the amino-terminal (EC1) extracellular repeat, characteristic of type I cadherins. Type I cadherin extracellular domains form homodimers in cis, at low calcium concentrations (500 μM). Cis homodimers bind homotypically in trans at physiological extracellular calcium (~1 mM) (98, 102). Peptides containing the amino-terminal repeat HAV sequence and antibodies to the amino-terminal repeat inhibit cadherin adhesion. Although the EC1 repeat initiates trans binding of cadherins, the full strength of trans binding is dependent on binding and the conformation of the other repeat, especially EC3 (102). Specificity of trans binding is determined by the EC1 repeat.

The cytoplasmic domains of N-, E-, and VE-cadherin are essential to cadherin adhesive and signaling function and are highly conserved. The β-catenin binding domains of E- and VE-cadherin are made up of ~30 amino acids within the carboxy-terminal 72 amino acids of each protein. There is more than 65% amino acid identity in the catenin binding domains among the three classic cadherins, with identical positioning of phosphorylatable serines, threonines, and tyrosines. β-catenin binds to cadherin in the endoplasmic reticulum. This β-catenin binding shields PEST sequences in the cadherin cytoplasmic domain that, without β-catenin binding, directs the cadherin to ubiquitination and proteolysis and prevents the cadherin from reaching the cell membrane (55, 56). Phosphorylation of some of the eight serines in the catenin binding domain of E-cadherin increases the affinity of β-catenin binding to cadherin by three logs, with a kDa of 50 pM (20). Hence, β-catenin avidly binds E-cadherin, and this binding is essential for the stability of E-cadherin. Since the conditional deletion of β-catenin in endothelium caused a phenotype very similar to deletion of VE-cadherin, it is likely that a similar principle applies to VE-cadherin and β-catenin (15).

p120 catenin binds to a membrane proximal segment of the cadherin cytoplasmic domain. The critical residues reside in segment 758–773 of human E-cadherin (93, 126). This region is also highly conserved across cadherins and across species. p120 binding to cadherin is necessary for stable and strong cadherin adhesion, and cadherin localization to the cell membrane is necessary for p120 localization to the cell membrane (126). p120 has been reported to enhance and to limit cadherin adhesion (4). These apparent discrepancies in the effects of p120 may be related to regulatory molecules that dock to p120 (60). For example, the tyrosine kinase Fer binds to the amino terminus of p120. The tyrosine phosphatase PTP1B binds to cadherin in the catenin binding domain. Fer keeps Tyr152 in PTP1B in an
active phosphorylated state. The phosphatase activity of PTP1B keeps β-catenin tyrosine residue 654 unphosphorylated. Loss of catalytic function mutations in Fer results in dephosphorylation of PTP1B with consequent phosphorylation of Y-654 in β-catenin and loss of cadherin adhesion (77). On the other hand, the tyrosine kinase Fyn also binds to p120. Fyn phosphorylates Tyr142 on β-catenin, and this phosphorylation interrupts binding of α-catenin to β-catenin (101). As discussed above, loss of β-catenin binding to cadherin results in ubiquitination and proteolysis of cadherin. This pathway is consistent with the observation that p120 limits endocytosis and proteolysis of VE-cadherin (140).

α-Catenin binds to the amino terminus of β-catenin. Historically, α-catenin was thought to link cadherin–β-catenin to the cortical actin cytoskeleton. However, recent reports from Drees et al. (30) and Yamada et al. (141) have convincingly demonstrated that α-catenin cannot bind β-catenin and actin or β-catenin and other actin binding proteins such as α-actinin or vinculin at the same time. Instead, α-catenin appears to function more as a regulator of actin that can be released from the cadherin complex to compete with Arp 2/3 to limit actin bundling.

Effects of cadherin complexes. Since both E- and VE-cadherin seem to be expressed in lung endothelium, it is appropriate to discuss the complexes associated with each. Most of the work on VE-cadherin comes from studies of systemic endothelium. Most of the work on E-cadherin comes from epithelia.

Integrity of cadherin-based adherens junctions is essential to the barrier function of endothelium and epithelium. Although the restrictive properties of the barrier are determined principally by claudins, disruption of E- or VE-cadherin adhesion totally disrupts the barrier. Antibody to VE-cadherin disrupts endothelial barriers in vitro and in vivo (23, 71, 85). Similarly, antibody to E-cadherin interrupts epithelial barriers (46, 139). Cadherin is the calcium-dependent homotypic cell–cell adhesion molecule, and chelation of extracellular calcium increases endothelial permeability to solutes, just as it eliminates the transepithelial resistance of an epithelium, by interrupting cadherin adhesion (17, 46, 117, 139). More recent work from our lab and the laboratory of Dr. Asrar Malik (66, 116, 137–139) has identified VE and E-cadherin adhesion as the targets for signaling pathways activated by histamine and the protease-activated receptor (PAR) receptors, agonists that increase endothelial and epithelial permeability in the setting of inflammation.

Loss of barriers may have effects other than increasing the transfer of solutes and water. In the airways, receptors for some infectious agents are restricted to the basolateral surface of the cells. Loss of E-cadherin adhesion by calcium chelation or stimulation of the cells with histamine increased viral infection of the airway cells (146). Similarly, epithelial growth factor receptors are restricted to the basolateral surface of airway epithelia, and interruption of E-cadherin adhesion by chelating calcium stimulated receptor phosphorylation by mitogens usually restricted to the apical surface liquid (131). The separation of the apical and basolateral surfaces of airway epithelium is greater in distance than those of conduit or alveolar endothelium, but the tight junctions of endothelium do sustain separate apical and basolateral membranes. Similar effects of disrupting the barrier and exposing receptors to ligands normally restricted to the other membrane is a concept that should be tested in endothelium.

Dejana (28) has highlighted signaling and regulatory roles of the VE-cadherin complex. In endothelium, the intact VE-cadherin complex (including binding of β-catenin), similar to E-cadherin in epithelium, plays a major role in the contact inhibition of growth and prevention of endothelial apoptosis. Some of the inhibition of cell growth is due to maintaining β-catenin at the cell membrane, thereby limiting its movement to the nucleus and activation of the Tcf/LEF pathways leading to cell proliferation and responsiveness to mitogens. Additional inhibition of cell growth is due to association of the VE-cadherin complex with VEGF receptor-2 and the phosphatase DEP-1 (CD148). DEP-1, associated with the VE-cadherin complex, dephosphorylates VEGF receptor-2, thereby limiting the proliferative signal from VEGF and preserving VE-cadherin adhesion that is otherwise interrupted by VEGF (45). At the same time that the VE-cadherin complex limits the proliferative signals from VEGF receptor-2, the association of VEGF receptor-2 with the VE-cadherin–β-catenin complex facilitates VEGF-A activation of Akt and survival signals, inhibiting endothelial apoptosis, a role highlighted in the targeted deficiency of VE-cadherin or truncation of the β-catenin binding site from VE-cadherin (14). Hence, maintenance of an intact VE-cadherin complex, including β-catenin, is necessary to prevent an epithelial-mesenchymal transition of endothelium and to prevent apoptosis of endothelium.

In addition to VEGF signaling, an intact VE-cadherin complex may also influence G protein regulation of the actin cytoskeleton. Endothelial cells made deficient in VE-cadherin assumed a mesenchymal phenotype. Expression of VE-cadherin reversed the phenotype back to epithelial, increased the membrane localization of Rac and its GEF, Tiam, and was associated with reorganization of cell actin (72). The recent work from Drees et al. (30) and Yamada et al. (141) also links the cadherin complex to the actin cytoskeleton. α-Catenin, when bound directly to β-catenin in the cadherin–β-catenin complex, cannot bind actin, vinculin, or α-actinin. Hence, the old concept that α-catenin directly linked cadherin–β-catenin to the cortical actin complex is incorrect. However, when α-catenin is released from β-catenin, it forms a dimer that competes with Arp2/3 for actin binding, thereby limiting lamellipodia formation and promoting actin bundling. IQGAP, a Ras-GAP-related protein that promotes Rac activity, competes with α-catenin for binding to β-catenin (68). This new role of α-catenin as an actin binding protein refocusses IQGAP to regulation of actin, a role consistent with other GTP binding proteins.

Regulation of Cadherin Adhesion

Overview. Under time-lapse photography of stable cultures of MDCK cells, sites of E-cadherin cell-cell adhesion are very dynamic, forming adhesions and remodeling many times over the course of a few hours (James Nelson, presentation to Biology Department at the University of Iowa, 2005). In the time-lapse images, the cell membranes at sites of cell-cell attachment appear to be in constant motion, continuously changing shape but at the same time apparently maintaining contact. In this context, the electron tomographic images of
cadherins in desmosomes is especially helpful, demonstrating a thick linear collection of interacting cadherins at sites of cell-cell contact and an even larger reserve of unengaged cadherin just inside the cell membrane. The density of the molecules is estimated at 17,000 cadherin molecules per square micrometer (49). This velcro-like collection of cell-cell adhesion molecules, with many more just below the cell surface ready to insert into the cell membrane, seems capable of moving like a caterpillar along the cell membrane as the membranes go through the complex changes in shape caught in the time-lapse images. To maintain persistent cell-cell adhesion, it is therefore necessary to coordinate the changes in cell shape and the linked sites of cell-cell adhesion. This would require precise communication between the cell-cell adhesion molecule complex and the cortical actin skeleton that is a major driver of cell shape. The dynamic nature of these sites of adhesion would also imply that persistent cell-cell adhesion involves constant interruption of adhesion between some molecules and establishing adhesion between different molecules. Consistent with this paradigm, Le et al. (74) observed that 35% of surface biotinylated E-cadherin on MDCK cells is internalized within 20 min and 80% within 2 h. This internalized E-cadherin is rapidly recycled to the cell surface so that only ∼13% of total surface labeled E-cadherin was inside the cells in steady-state conditions.

Actin, G proteins, and adherens junctions. Although there is still a dynamic debate about the details of the formation of adherens junctions, there is also general consensus that the G proteins Rap1, Cdc42, and Rac play critical roles in establishing actin-driven filopodia and lamelipodia that increase cell-cell contact and thereby enhance the formation of adherens junctions. Fukuyama et al. (37) have outlined a pathway in which nectin binding at sites of nascent cell-cell contact activates a cSrc-Crk-C3G-Rap1-Cdc42-Rac cascade, activating Cdc42 with consequent filopodia formation and then activating Rac with lamellipodia formation and formation of the zipper-like structures of adherens junctions. A somewhat different sequence was outlined by Hogan et al. (54) who reported that C3G bound to the E-cadherin complex and that ligation of E-cadherin itself was capable of activating Rap1 with similar effects on filopodia and lamellipodia formation. Lampugnani et al. (72) observed that homophilic ligation of VE-cadherin induced localization of the Rac-specific GEF, Tiam, to sites of cell-cell adhesion and inhibition of Rho, events that would be expected to enhance lamellipodia formation and limit stress fiber formation. Sakurai et al. (111) reported that MAGI-1, an additional GEF for Rap1, binds to β-catenin in the VE-cadherin complex and supports activation of Rap1 after homophilic ligation of VE-cadherin. The well-recognized effects of cAMP in enhancing endothelial barrier function are mediated, at least in large part, by activation of E-pac, a GEF for Rap1 (36).

A very recent report describes a cadherin-independent process for the development of the actin structures that are necessary for the stability of adherens junctions (101a). In Drosophila embryos, a synaptogamin-like protein, Bitesize, is necessary for stable E-cadherin-based adherens junctions. In the absence of Bitesize, E-cadherin localized to sites of cell-cell contact, but the organized actin structures usually associated with the adherens junctions did not, and E-cadherin was unstable in location. Bitesize itself bound moesin, a component of the ezrin-moesin-radixin complex that is essential to the organization of cortical actin. In the absence of E-cadherin, Bitesize and actin formed normal actin complexes at the apical sites of cell contact, and these actin complexes were more stable in the absence of E-cadherin than was E-cadherin in the absence of Bitesize. This new observation, along with the work of Drees et al. (30) and Yamada et al. (141), emphasizes a role for actin in stabilizing the adherens junction complex in space and at the same time emphasizes an independence of the elements of the cadherin complex from the actin complex. The details of how the actin and cadherin complexes interact to stabilize each other is an important direction for future investigations.

In an on-off model of G proteins, stable adherens junctions might be characterized as Rac on and Rho off. In the context of the dynamic and moving, but stable, epitheliod cell complex that is not making a mesenchymal transition, caught in James Nelson’s time-lapse images, there would be a balance, tilted towards Rac, but not without any Rho. This balance is clearly delineated in a review by Jaffer and Chernoff (63). Nectin and cadherin activation of Rac supports phosphorylation of p190RhoGAP with inhibition of Rho and consequent decreased tension and enhanced adhesion. However, Rho also supports adherens junctions via its effector Dia that promotes actin polymerization and microtubule organization. Inhibition of the actin polymerization function of Dia destabilized adherens junctions while inhibition of microtubules prevented Dia from enhancing localization of E-cadherin to new cell-cell contacts (109). Similarly, constant activation of Rac promotes a migratory phenotype and results in internalization of E-cadherin and loss of cell-cell adhesion (11). In the dynamic environment of cells changing shape and remodeling sites of cell-cell adhesion, actin plays an important but as yet unexplained role in stabilizing adhesion, and a balance of Rac and Rho effects is necessary for stability.

IQGAP, an F-actin crosslinking protein, is a Rho effector that binds the amino-terminal domain of β-catenin and competes for binding at this site with α-catenin (68). Cdc42 and Rac compete with β-catenin for binding IQGAP and prevent IQGAP from displacing α-catenin (35). Initial hypotheses suggested that IQGAP displaced α-catenin from the cadherin complex and weakened cadherin adhesion. However, in the context of the observations of Drees et al. (30) and Yamada et al. (141) that α-catenin does not link the cadherin complex to actin, the functional effects of IQGAP displacing α-catenin from the cadherin complex remain uncertain. More recent work with siRNA demonstrated that knockdown of IQGAP or Rac1 weakened cadherin adhesion, and the effects of reducing active Rac1 were overcome by overexpressing IQGAP, indicating that IQGAP supported cadherin adhesion and that this was probably through effects on actin polymerization (91). GTP-activated Rac binds IQGAP. IQGAP has anti-GTPase activity, sustaining the active state of Rac. IQGAP’s actin crosslinking activity may promote stability of cortical actin and cortical cell shape.

Other data support the concept that IQGAP interacting with Cdc42 and Rac enhances cadherin adhesion by crosslinking actin. Izumi et al. (62) observed that endocytosis of E-cadherin that was trans-engaged with E-cadherin was suppressed by a Cdc42, Rac, IQGAP pathway. The Cdc42, Rac, IQGAP pathway itself was activated by the transengaged E-cadherin or by...
trans-engaged nectin. The inhibition of E-cadherin endocytosis by activated Cdc42 and Rac was lost when an IQGAP mutant, deficient in the actin binding domain, was substituted for native IQGAP. In this context, IQGAP associated with activated Cdc42, and Rac stabilizes adherens junctions through effects on actin polymerization. IQGAP not linked to Cdc42 or Rac and free to displace α-catenin from β-catenin might destabilize actin by α-catenin’s inhibition of Arp2/3 bundling. This entire paradigm has not been tested, but the new data on IQGAP and α-catenin suggest it should be.

IQGAP binding is also affected by intracellular calcium concentration. At low, physiological intracellular calcium, IQGAP binds Cdc42 and Rac and stabilizes actin. High intracellular calcium displaces IQGAP both from Cdc42, Rac and from β-catenin to calmodulin. This repositioning of IQGAP would be expected to destabilize cortical actin (63).

The small G protein, Go12, has been reported to bind E-cadherin and to displace β-catenin with resultant interruption of cadherin adhesion (82). A more recent report found that Go12 interacted with α-SNAP and the SNARE complex, facilitating delivery of VE-cadherin to the cell membrane and stabilizing VE-cadherin adhesion (1). Overexpression of Go12 alone had no effect on VE-cadherin adhesion, but overexpression of Go12 and RNAi interference of expression of α-SNAP together decreased barrier function of endothelial cells. Hence, the details of Go12’s effects on cadherin adhesion continue to evolve.

*p120 and adherens junctions.* p120 is a 92-kDa protein and member of the armadillo family of catenins that binds to the juxtamembrane domain of VE- and E-cadherins, promotes adherens junction stability, and limits endocytosis of VE- and E-cadherins (60, 126, 140, 144). Deficiency of p120 in mice results in embryonic death and in *Xenopus* produces gastrulation defects not unlike those associated with E-cadherin deficiency. A conditional deficiency of p120 in submandibular glands caused aberrant acinar development with impaired cell-cell adhesion, absence of cell polarity, and ductal obstruction with tumoralike masses of cells (26). Binding of p120 to the E-cadherin juxtamembrane domain is essential for strong cell-cell aggregation (126). A mutation in the carboxy-terminal domain of p120 causes a malignant phenotype in SW48 colon carcinoma cells that is rescued by wild-type p120 (60). The mechanism of p120’s stabilizing effects is not yet clear. It may relate to p120’s role as a docking site for kinases that are essential for cadherin stability (see α- and β-catenin discussion that follows) or p120 may shield tyrosine sites in cadherin that when phosphorylated lead to cadherin ubiquitination and endosomal proteolysis (34, 135). Alternatively, p120 may be important in Rac activation triggered by ligation of E-cadherin, enhancing adherens junction stability through actin (43). In *Drosophila*, the Rho contribution to adherens junction stability was recently reported to be independent of p120 (33). However, the details of Rho and Rac regulation of adherens junctions may be different in different cell contexts. CHO cells were significantly different from endothelial cells (12). In our lab, L cells expressing cadherins have been very consistent with both endothelial cells and airway epithelial cells (137–139, 146).

α-Catenin, kinases, and adherens junctions. α-Catenin binds to the amino-terminal end of β-catenin. Fer and Fyn, tyrosine kinases that bind to p120, can phosphorylate Tyr142 in β-catenin, and phosphorylation of Tyr142 in β-catenin interrupts binding of α-catenin to β-catenin (101). Historically, α-catenin was thought to bind actin, and/or actin binding proteins, directly linking the cadherin complex to cortical actin. I have referenced a lot of material demonstrating an important role of cortical actin in the stability of adherens junctions. However, α-catenin’s role now needs to be defined in the context of the findings of Drees et al. (30) and Yamada et al. (141) that α-catenin cannot bind β-catenin, and actin or β-catenin and the actin binding proteins at the same time. Possible effects of α-catenin on Arp2/3, actin bundling and regulation of actin, were discussed above.

**β-Catenin, Kinases, Phosphatases, and Adherens junctions**

*β-catenin binding to cadherin.* The observation of Huber et al. (55, 56) that unless β- or γ- (plakoglobin) catenin binds to the cadherin binding domain, cadherins are unstructured and unstable, underlies the critical principle that a catenin must bind cadherin in the cadherin binding domain for stable cadherin adhesion. This position is strongly supported by the observation of Carmeliet et al. (14) that mice expressing a VE-cadherin lacking the β-catenin binding domain had a phenotype in mice very similar to mice deficient in VE-cadherin itself. The observation of Cattelino et al. (15) that conditional deletion of β-catenin in endothelial also caused aberrant angiogenesis and leaky immature vessels inconsistent with survival also underscores the critical role of β-catenin binding to cadherin for stable cadherin adhesion. In systemic venules, expression of VE-cadherin cytoplasmic domain that was able to compete with endogenous VE-cadherin for β-catenin binding disrupted the integrity of the adherens junctions and the endothelial barrier (47). In this context, the observations of Roura et al. (106), Piedra et al. (100), and Lilien et al. (78) that phosphorylation of tyrosine residues on β-catenin disrupts adhesion of β-catenin to cadherin are especially relevant to understanding regulation of cadherin adhesion.

The details of the binding of β-catenin to cadherin are best known for E-cadherin. The β-catenin binding domain encompasses the carboxy-terminal 100 residues of E-cadherin (56). This sequence interacts with all 12 of the arm repeats of β-catenin creating an extended interface that can be modulated. Phosphorylation of Ser684, Ser686, and Ser692 in E-cadherin increases the affinity of β-catenin binding. Binding of β-catenin at these sites in E-cadherin masks a PEST sequence that when uncovered targets the cadherin for degradation (55). β-Catenin binds to cadherin in the endoplasmic reticulum, emphasizing the importance of this binding to persistence of cadherin. Mutation to alanine of these serines that enhance β-catenin binding reduces cell-cell adhesion (55).

*Tyrosine phosphorylation of β-catenin.* In contrast to the increase in binding of β-catenin to cadherin that follows phosphorylation of serines in E-cadherin, phosphorylation of Tyr654 in β-catenin interrupts a hydrogen bond between β-catenin and cadherin and reduces binding affinity of β-catenin for cadherin sixfold (106). Tyr654 in β-catenin is phosphorylated by Src and the epithelial growth factor receptor. Expression of Src by VEGF results in phosphorylation of β-catenin, disruption of β-catenin binding to VE-cadherin, and disruption of VE-cadherin adhesion in vivo (134). Inhibition of Src prevents edema formation resulting from VEGF in vivo (134). Src activation by integrin engagement also activates...
tyrosine phosphorylation of β-catenin in endothelium and disrupts VE-cadherin adhesion (133). Ligation of the type I histamine receptor in HUVEC with histamine also stimulates phosphorylation of γ- and β-catenin, disrupts binding of catenin to VE-cadherin, and interrupts VE-cadherin adhesion (2, 116, 137, 138). Src is a critical kinase in the epithelial-mesenchymal transition, and loss of cadherin adhesion is fundamental to this transition (77).

Abelson kinase (Abl) is another tyrosine kinase that has been implicated in tyrosine phosphorylation of β-catenin. Rhee et al. (105) identified Abl as part of a signaling complex that resulted in tyrosine phosphorylation of β-catenin and disruption of N-cadherin adhesion. Lilien and Balsamo (77) have identified Tyr489 of β-catenin as an Abl site that when phosphorylated interrupts binding of β-catenin to N-cadherin.

Activation of the type I histamine or the type 2 PAR receptor interrupts VE-cadherin adhesion in endothelium and E-cadherin adhesion in respiratory epithelium (138, 139, 146). Activation of the H1 receptor or PAR2 receptor increased phosphorylation of β-catenin (116).

Kinasases and phosphatases. Tyrosine kinases and phosphatases are incorporated into the E- and VE-cadherin complexes (28, 77). The kinase Fer and the Src kinases Fyn and Yes bind to p120 and are present in immunoprecipitates of VE- and E-cadherin (28, 77, 116). In VE-cadherin immunoprecipitates of endothelial cells exposed to histamine, a major band of kinase activity was detected at a molecular weight of ~60 kDa. This band blotted with antibody to Src, and the band had activity that phosphorylated β- and γ-catenin in vitro (116).

The phosphatase PTP1B binds to cadherin in the β-catenin binding domain and maintains β-catenin in a dephosphorylated state. PTP1B is present in the VE-cadherin immunoprecipitate (127). Like all tyrosine phosphatases, there is a cysteine in the catalytic domain of PTP1B and it is susceptible to oxidation. This may be a mechanism by which oxidants contribute to phosphorylation of elements of the cadherin complex and disruption of cadherin adhesion (92).

Another nonreceptor tyrosine phosphatase, the Src-associated phosphatase, SHP2, has been reported to bind to β-catenin in VE-cadherin complexes. Thrombin stimulation of the PAR1 receptor was associated with displacement of SHP2 from the VE-cadherin complex and increased tyrosine phosphorylation of β-catenin (127). It was not evident what were the substrates for SHP2 in these events, since its classic activity is to activate Src.

Just as homotypic binding of cadherin extracellular domains initiates outside-in small G protein signaling that stabilizes adherens junctions, interruption of homotypic binding with antibody to VE-cadherin initiates outside-in signaling that initiates phosphorylation of β-catenin and disruption of the VE-cadherin complex. In their studies, van Buul et al. (129) found that function-blocking antibody to the extracellular domain of VE-cadherin initiated Rac-dependent oxygen radical production that activated the tyrosine kinase Pyk2 that initiated signaling that resulted in phosphorylated β-catenin. A dominant negative Pyk2 prevented β-catenin phosphorylation and disruption of the VE-cadherin complex even after antibody to VE-cadherin, suggesting that tyrosine phosphorylation of β-catenin was a necessary step for interruption of adhesion. These data are consistent with the fundamental role of β-catenin binding to cadherin in stabilizing cadherin adhesion.

Whether β-catenin is the substrate for Pyk2, or whether Pyk2 participates in a signaling cascade that includes another tyrosine kinase or phosphatase activated or inactivated, respectively, downstream of Pyk2 was not addressed. In prostate epithelium, Pyk2 was reported to stabilize cell-cell adhesion and preserve an epithelial phenotype, so a direct role in β-catenin phosphorylation seems less likely (27).

Not all tyrosine phosphorylation of VE-cadherin is associated with decreases in adhesion. Carboxy-terminal Src kinase, Csk, a negative regulator of Src, binds to VE-cadherin at phosphorylated Tyr685, a position approximately midway between the p120 and β-catenin binding domains (7). Csk binding increases as cell density increases. Mutation of Tyr685 to phenylalanine blocked Csk binding and prevented contact inhibition of cell proliferation. Overexpression of Csk limited cell proliferation, and RNA interference of Csk expression blocked contact inhibition of cell proliferation. This important role of a Src inhibitor emphasizes an important role of Src in VE-cadherin adhesion and signaling.

Two other receptor tyrosine kinases associate with the VE-cadherin complex, but their precise roles are not yet defined. VE-PTP is a receptor tyrosine kinase that binds to VE-cadherin in the most membrane proximal portions of both molecules’ extracellular domains. VE-PTP binding is associated with decreased tyrosine phosphorylation of VE-cadherin and enhanced adhesion, but the enhanced adhesion is independent of the catalytic activity of VE-PTP (89). Another receptor tyrosine phosphatase, PTPmu, is expressed on lung microvascular cells and immunoprecipitates with VE-cadherin. Overexpression of PTPmu is associated with decreased tyrosine phosphorylation of VE-cadherin and enhanced endothelial barrier function (119). While the catalytic activity of PTPmu was important for enhanced lung microvascular endothelium barrier properties, PTPmu enhanced E-cadherin adhesion in prostate epithelium independent of catalytic activity (51). In a more recent report, the same group demonstrated that PTPmu binds IQGAP and affects Cdc42, Rac, IQGAP-driven actin remodeling, and filopodia formation (99).

Summary

Cell-cell adhesion is fundamental to tissue integrity and normal organ physiology. Much of what I was able to review was not specific to the lung or even endothelium. The same cell-cell adhesion molecules are expressed in different organs but likely have distinct structural and regulatory partners that determine site-specific physiology. Hence, there is much to learn about cell-cell adhesion molecule expression and regulation in lung endothelium and epithelium. Parker and colleagues (96) at University of South Alabama and Mitzner and Wagner and colleagues (82a) at Johns Hopkins have illuminated important phenotypic distinctions among pulmonary and bronchial endothelium, respectively. The recent observations that pulmonary microvascular endothelium expresses E-cadherin is one example that some of these distinctions may derive from the physiology of cell-cell adhesion (96, 108).

Because the integrity of the lung’s barriers is so essential for normal physiology and the life of mammals, many of us strive to understand how inflammation alters them. Throughout the organism, epithelial (including endothelium) tissues respond to signals dictating change, whether it be injury, growth, or...
Invited Review

L604 CELL-CELL ADHESION IN LUNG ENDOTHELIUM

renewal, with the epithelial mesenchymal transition (EMT). During EMT, cell-cell adhesion molecules are inactivated, and some are destroyed, while cell-matrix adhesion increases, cells migrate and divide. Once the transition has established a new base, cell-cell adhesion resumes, cellular adhesion complex forms, and signals are sent to prevent apoptosis and preserve epithelial integrity. Our recent work leads us to believe that inflammation uses some of the infrastructure of EMT to cause a temporary decrease in the integrity of cell-cell adhesion in an attempt to bring important immune-competent molecules and cells to sites of infection and injury. In this setting, the signals regulating cell-cell adhesion may be important to limiting excessive inflammation and organ pathophysiolog.

REFERENCES


JAP-Lung Cell Mol Physiol • VOL 292 • MARCH 2007 • www.apjphonol.org

Downloaded from http://ajplung.physiology.org/ by 10.220.32.247 on April 15, 2017
L605

CELL-CELL ADHESION IN LUNG ENDOTHELIUM


128. Valyi-Nagy T, Sheth V, Clement C, Tiwari V, Sculand P, Kavouras JH, Leach L, Guzman-Hartman G, Dermody TS, Shukla D. Herpes simplex virus entry receptor nectin-1 is widely expressed in the murine simplex virus entry receptor nectin-1 is widely expressed in the murine