Tyrosine Phosphorylation of VE-cadherin Prevents Binding of p120- and β-Catenin and Maintains the Cellular Mesenchymal State*

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In several pathological conditions, epithelial cells demonstrate a breakdown of barrier function and acquire an invasive phenotype. Endothelial cells in particular are maintained in a mesenchymal state during the cell invasion phase of angiogenesis. We show here that tyrosine phosphorylation of the adherens junction protein VE-cadherin at two critical tyrosines, Tyr-658 and Tyr-731, via tyrosine kinase activation or phosphatase inactivation was sufficient to prevent the binding of p120- and β-catenin, respectively, to the cytoplasmic tail of VE-cadherin. In fact, phosphorylation at either site led to the inhibition of cell barrier function. Cells expressing wild-type VE-cadherin showed decreased cell migration compared with cells lacking VE-cadherin, whereas expression of VE-cadherin with a simple phosphomimetic tyrosine-to-glutamic acid mutation of Y658E or Y731E was sufficient to restore the migratory response. These findings demonstrate that a single phosphorylation event within the VE-cadherin cytoplasmic tail is sufficient to maintain cells in a mesenchymal state.

Cadherins are important regulators of a number of epithelial cell barriers (1). While allowing cells to maintain impermeable monolayers, cadherins also act to prevent epithelial cell motility. During the epithelial to mesenchymal transition (EMT), epithelial cells acquire an invasive phenotype characterized by breakdown of cadherin-mediated cell-cell junctions and increased cell invasive activity (2). EMT has been associated with changes in cadherin cell surface expression, adhesive activity, and/or cytoskeletal linkage (3, 4). The molecular mechanisms by which these processes occur are not entirely understood.

VE-cadherin is an endothelium-specific cell-cell junctional protein that plays a critical role in vascular barrier function and angiogenesis (5, 6). The breakdown of the endothelial cell barrier leads to vascular permeability and remodeling, which are associated with a number of disease processes including cancer, inflammation, and ischemic injury. Additionally, endothelial cells must be maintained in a mesenchymal state during the invasive stage of angiogenesis. Vascular endothelial cell growth factor (VEGF) is produced in response to ischemic injury and cancer and has a profound influence on VE-cadherin and its capacity to mediate cell-cell adhesion (7–9). Like all cadherin-mediated interactions, the integrity of the endothelial cell-cell junction depends on the adhesive function and cell surface expression of VE-cadherin as well as the ability of VE-cadherin to assemble with the underlying cytoskeleton. Cadherin-cytoskeletal interactions occur through a number of adaptor proteins that interact with the C-terminal portion of the cadherin cytoplasmic tail, including the α-, β-, and γ-catenin (6, 10). Additionally, VE-cadherin stability at the plasma membrane may be regulated by the binding of p120-catenin to the juxtamembrane region of the cytoplasmic tail (11–13).

Growth factor- and cytokine-mediated signaling has been linked to the regulation of cadherins and their adhesion mechanisms (14, 15). In fact, tyrosine phosphorylation of the cadherin cytoplasmic tail or its catenin binding partners is thought to regulate cadherin adhesion and cell surface expression (16–21). However, it remains unclear how this process is regulated and which tyrosine sites on cadherins are essential for the regulation of cell-cell junctional activity.

To begin to understand the molecular events involved in the regulation of cadherin function, we stably expressed either wild-type or various mutants of VE-cadherin in CHO cells lacking endogenous cadherins. We identified two critical regulatory phosphorylation sites within the tail of VE-cadherin that lead to the disruption of cell-cell junctions. Phosphorylation of Tyr-658 and Tyr-731 leads to uncoupling of p120- or β-catenin, respectively, from the cytoplasmic tail of VE-cadherin. In fact, phosphomimetic mutations of Tyr-658 to Glu-658 or Tyr-731 to Glu-731 are not only sufficient to uncouple cell-cell junctions, but they also promote focal contact assembly and lead to cell migration. This change in biological behavior could not be explained by a simple loss of functional cadherin from the cell surface. These findings demonstrate that phosphorylation of a single tyrosine in a cadherin cytoplasmic domain is sufficient to maintain cells in a mesenchymal, invasive phenotype.

EXPERIMENTAL PROCEDURES

Construction of Cell Lines Expressing VE-cadherin—cDNA of human VE-cadherin fused in-frame with GFP at the C terminus was a gift of Dr. Sunil Shaw (Harvard University). VE-cadherin-GFP was subcloned into pcDNA3.1 (Invitrogen). Y-E, Y-F, and Y-A mutants of VE-cadherin were constructed using the QuikChange site-directed mutagenesis kit (Stratagene). Plasmids were transfected into CHO cells using Lipofectamine PLUS (Invitrogen). CHO cells were cultured in complete Ham’s F12 medium containing 10% fetal bovine serum, 2 mM GlutaMax (Invitrogen), 100 units/ml penicillin, 100 µg/ml streptomycin, 250 ng/ml amphotericin B, and 50 µg/ml gentamycin. Stable cell lines were selected using 500 µg/ml G418. Resistant cells were pooled and sorted by flow cytometry based on both GFP fluorescence and VE-cadherin cell

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§ The abbreviation used are: EMT, epithelial to mesenchymal transition; CA, constitutively active; CHO, Chinese hamster ovary; FAK, focal adhesion kinase; GFP, green fluorescent protein; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; PTP, protein tyrosine phosphatase; PV, pervanadate; VEGF, vascular endothelial growth factor.

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surface expression. Because uniform flow cytometry gates were used to select the cells, all lines displayed approximately equivalent amounts of cell surface VE-cadherin.

Biological Characterization of VE-cadherin Complexes—CHO cells were washed twice with cold PBS and lysed in 1% Triton X-100, 10% glycerol, 20 mM Tris (pH 7.5), 157 mM NaCl, 2 mM CaCl2, 10 mM NaF, 1× complete protease inhibitor mixture (Roche Applied Science), and 100 μM pervanadate (PV; prepared according to Ref. 16). Protein concentrations of cell extracts were determined using the BCA protein assay (Pierce), and VE-cadherin was immunoprecipitated from 500 μg of extract using 2 μg of anti-VE-cadherin antibody (C-19, Santa Cruz Biotechnology) followed by 10 μl of UltraLink protein A/G beads (Pierce). Beads were washed four times using cold PBS containing 300 μM PV, boiled in SDS-PAGE loading buffer, and resolved by SDS-PAGE. Gels were transferred onto nitrocellulose, and immunoblotting was carried out using antibodies against a number of proteins including VE-cadherin (C-19), p120-catenin (S-19), phosphotyrosine (PY99), Src (GD11, Upstate), FAK (C-20), phospho- Src (Tyr-416, Cell Signaling Technology), phospho-ERK2 (Thr-202/Tyr-204, Cell Signaling Technology), and phospho-Src (Tyr-416, Cell Signaling Technology). The brightness and contrast of immunoblot images were adjusted using Adobe Photoshop 7.0. Where indicated, cells were treated prior to lysis with 100 μM PV for 20 min at 37 °C to inhibit tyrosine phosphatases and increase Src kinase activity. Where indicated, beads were washed three times with PBS and mounted using Vectashield (Vector Labs). Images were captured using a Bio-Rad MRC1024 confocal microscope with a ×40 objective lens and LaserSharp software. Images were adjusted for brightness and contrast using Adobe Photoshop 7.0.

Production of Recombinant VE-cadherin-Fc Fusion Protein—DNA encoding the extracellular portion of human VE-cadherin was placed in front of the Fc portion of human IgG1. Following subcloning into the expression vector pCEP4 (Invitrogen), DNA was transfected into 293-T cells using Lipofectamine PLUS. Stable transfectants were selected using 300 μg/ml hygromycin B. Following selection, cells were incubated in primary antibody (anti-FK-pY861 (BIOSOURCE) diluted to 2 μg/ml in blocking buffer) for 1 h at room temperature. Coverslips were washed three times in PBS and mounted using Vectashield (Vector Labs). Images were captured using a Bio-Rad MRC1024 confocal microscope with a ×40 objective lens and LaserSharp software. Images were adjusted for brightness and contrast using Adobe Photoshop 7.0.

Migration Assay—Transwell chambers (Costar; 6.5-mm diameter, 8.0-μm pore size, tissue culture-treated polycarbonate membranes) were pre-coated with 10 μg/ml collagen on the underside of the membrane and 1 μg/ml collagen on the upper side of the membrane. 3 × 105 cells, pre-cultured in complete Ham’s F12 medium, were then plated into the upper Transwell chamber. Following incubation at 37 °C for 4 h, the Transwell chambers were incubated for 15 min in 0.1% crystal violet and 20% methanol. Chambers were then rinsed repeatedly in water, and cells were removed from the upper side of the membranes using a cotton swab. Membranes were air-dried, and the crystal violet contained in the migratory cells on the underside of the membrane was extracted using methanol and quantitated by absorbance at 600 nm.

Immunofluorescence Staining—Cells expressing GFP-tagged VE-cadherin were grown on glass coverslips and fixed in 4% paraformaldehyde in PBS for 20 min at 4 °C. Coverslips were washed in 30 mM glycine in PBS for 5 min at 4 °C. Cells were permeabilized in 0.1% Triton X-100 in PBS for 15 min at 4 °C and then blocked in 3% bovine serum albumin in PBS for 30 min at room temperature. Coverslips were incubated in primary antibody (anti-FK-pY861 (BIOSOURCE) diluted to 2 μg/ml in blocking buffer) for 1 h at room temperature. Coverslips were washed three times in PBS and mounted with Alexa Fluor 660-conjugated goat anti-rabbit secondary antibody (Molecular Probes; diluted to 4 μg/ml in blocking buffer) and Alexa Fluor 588-conjugated phallolidin (Molecular Probes; diluted to 1 unit/ml in blocking buffer) for 30 min at room temperature. Coverslips were washed three times in PBS and mounted using Vectashield (Vector Labs). Images were captured using a Bio-Rad MRC1024 confocal microscope with a ×40 objective lens and LaserSharp software. Images were adjusted for brightness and contrast using Adobe Photoshop 7.0.
VE-cadherin Regulation by Tyrosine Phosphorylation

**RESULTS**

**Identification of Two Regulatory Tyrosines in the Cytoplasmic Tail of VE-cadherin**—Phosphorylation of cadherins or their associated proteins has been linked to changes in cadherin-mediated adhesive function. There are nine tyrosines in the cytoplasmic tail of VE-cadherin (Fig. 1A), but their specific function remains unclear. To determine whether phosphorylation of one or more of these sites might play a role in cadherin function, wild-type VE-cadherin and VE-cadherin containing phosphomimetic tyrosine-to-glutamic acid mutations were stably expressed in CHO cells lacking endogenous cadherins. Parental CHO cells are phenotypically mesenchymal, displaying high levels of focal contact formation and migration (data not shown). We hypothesized that introduction of wild-type VE-cadherin into CHO cells would force the cells to acquire an epithelial phenotype. Accordingly, we asked whether expression of mutant forms of VE-cadherin in these cells might disrupt the epithelial phenotype. To assess the functional consequences of the phosphomimetic mutations, cells expressing wild-type or mutant cadherins were grown to confluence in Transwell chambers, and the barrier function of the cell monolayer was determined, as measured by permeability to HRP-conjugated IgG (Fig. 1B). Cells expressing either the Y658E or Y731E mutations displayed little or no barrier function, and other mutations were without effect (Fig. 1B). These findings reveal that both Tyr-658 and Tyr-731 can disrupt VE-cadherin-mediated cell barrier function.

Cadherins interact with the actin cytoskeleton via catenins that bind to the cadherin cytoplasmic tail. Experiments were performed to determine whether the Y-E mutations that resulted in disruption of cell barrier function could influence the binding of one or more catenins to VE-cadherin within the cell. Expression of VE-cadherin containing the Y658E or Y731E mutations blocked p120- or β-catenin, respectively, binding to the cadherin, whereas all other Y-E mutations were without effect (Fig. 2). In addition, expression of the more dramatic structural mutations of VE-cadherin, Y658A and Y731A, also resulted in a complete loss of binding to p120- and β-catenin, respectively (data not shown). Together, our findings suggest that structural modulation of these sites (i.e. phosphorylation) can disrupt VE-cadherin junctional activity.

**Kinase/Phosphatase Regulation of Cadherin Phosphorylation and Function**—Previous studies suggest that Src kinases play a significant role in cadherin regulation (8, 22–25). To determine whether phosphorylation of the VE-cadherin sites Tyr-658 and Tyr-731 can disrupt VE-cadherin-mediated cell barrier function.

Cadherins interact with the actin cytoskeleton via catenins that bind to the cadherin cytoplasmic tail. Experiments were performed to determine whether the Y-E mutations that resulted in disruption of cell barrier function could influence the binding of one or more catenins to VE-cadherin within the cell. Expression of VE-cadherin containing the Y658E or Y731E mutations blocked p120- or β-catenin, respectively, binding to the cadherin, whereas all other Y-E mutations were without effect (Fig. 2). In addition, expression of the more dramatic structural mutations of VE-cadherin, Y658A and Y731A, also resulted in a complete loss of binding to p120- and β-catenin, respectively (data not shown). Together, our findings suggest that structural modulation of these sites (i.e. phosphorylation) can disrupt VE-cadherin junctional activity.

**Tyrosine phosphorylation is regulated by a dynamic balance between kinases and phosphatases. In fact, one mechanism of cadherin regulation is known to occur via tyrosine phosphatases (16, 26–30). Therefore we suppressed phosphatase activity in CHO cells expressing wild-type or mutant cadherin by treatment with pervanadate, a general tyrosine phosphatase inhibitor. Pervanadate treatment of cells led to phosphorylation of wild-type VE-cadherin (Fig. 4A), and this was significantly...
reduced in cells expressing the non-phosphorylatable mutants Y658F and Y731F or the dual Y658F,Y731F mutant (Fig. 4A).

Importantly, Src kinase activity was increased in cells treated with pervanadate as measured by Src phosphorylation on tyrosine 416 (Fig. 4A).

To confirm the identity of the β-catenin regulatory site on VE-cadherin, we developed a phosphospecific antibody to a peptide derived from this region of the cadherin, containing phosphotyrosine at position 731. The epitope of this antibody depended on phosphorylation at Tyr-731 (data not shown) and was specifically detected in immunoprecipitates from pervanadate-treated CHO cells expressing wild-type VE-cadherin and human umbilical vein endothelial cells (HUVEC) were treated without (−) or with (+) PV. VE-cadherin was immunoprecipitated from cell lysates, and phosphorylation levels were determined by immunoblotting (IB) with an anti-phosphotyrosine antibody. Immunoprecipitates were also probed with anti-VE-cadherin antibody to ensure equivalent expression and loading of VE-cadherin. Additionally, the ability of pervanadate treatment to cause phosphorylation of numerous cell proteins is exemplified by immunoblots of whole cell lysates for phospho-Src (pY416) and total Src. B, CHO cells expressing GFP, wild-type VE-cadherin, the Y731F mutant of VE-cadherin, and human umbilical vein endothelial cells (HUVEC) were treated without (−) or with (+) PV. VE-cadherin was immunoprecipitated from cell lysates, and phosphorylation levels were determined by immunoblotting with an anti-phosphotyrosine 731 (pY731) antibody. Immunoprecipitates were also probed with anti-VE-cadherin antibody to ensure equivalent levels of VE-cadherin. C, CHO cells expressing GFP, wild-type VE-cadherin, or the Y658F,Y731F double mutant of VE-cadherin were grown to confluence in Transwell chambers. Permeability of cell monolayers to HRP-conjugated IgG in the absence (−) or presence (+) of 100 μM PV was assessed 2 h following addition of the IgG. Abs, absorbance.

Three findings provide further evidence that Tyr-731 on endogenous VE-cadherin becomes phosphorylated in endothelial cells in response to phosphatase inhibition.

**Functional Regulation of VE-cadherin by Phosphorylation**—We next determined whether the Y658F and Y731F mutations would render VE-cadherin refractory to functional regulation by phosphorylation. CHO cells expressing GFP, wild-type VE-cadherin, or the Y658F,Y731F double mutant of VE-cadherin were grown to confluence on Transwell chambers, and the integrity of cell-cell junctions was assessed in response to phosphatase inhibition. Treatment of cells with pervanadate greatly decreased cell-cell junctional integrity among cells bearing wild-type VE-cadherin (Fig. 4C). In contrast, cells expressing the Y658F,Y731F double mutations maintained their cell-cell junctions in the presence of pervanadate, suggesting that the VE-cadherin in these cells is not subject to regulation by phosphorylation. These findings reveal that either activation of kinases such as Src and/or suppression of phosphatases leading to phosphorylation of VE-cadherin on residues Tyr-658 and/or Tyr-731 can disrupt VE-cadherin-mediated cell-cell junctions.
were coated on the upper side with 1 of VE-cadherin to migrate through Transwell chambers. Chambers expressing GFP, wild-type VE-cadherin, or Y658E and Y731E mutants nm. dye bound inside adherent cells was extracted and quantitated at 600 nm. Removed, adherent cells were fixed and stained with crystal violet, and bars quantitated at 600 nm. cr

\[C\] cells expressing either wild-type or mutant VE-cadherins were allowed to attach to immobilized recombinant VE-cadherin. Expression of wild-type VE-cadherin led to robust adhesion of cells to an immobilized VE-cadherin-Fc fusion protein, whereas control cells lacking cadherin failed to adhere (Fig. 5B). To our surprise, none of the Y-E or Y-F mutations suppressed the expression level of cadherin or the adhesivity of these cells to immobilized VE-cadherin-Fc (Fig. 5B). Thus, the phenotypic changes associated with VE-cadherin mutations cannot be attributed to decreased VE-cadherin adhesive function. However, our assay was not designed to measure the tenacity of the VE-cadherin-mediated adhesions and therefore cannot discriminate between subtle effects the mutations may have had upon VE-cadherin adhesive function.

Induction of Cell Migration by VE-cadherin Phosphorylation—Mesenchymal cells are characterized by their capacity to undergo migration/invasion. Based on the appearance of increased numbers of focal contacts in the cells expressing the Y658E and Y731E mutants, we suspected that these cells might display increased migration relative to cells expressing wild-type VE-cadherin. To investigate this possibility, cells were placed in migration chambers and allowed to undergo haptotaxis on a collagen substrate. Cells expressing wild-type VE-cadherin showed decreased levels of migration compared with control cells expressing GFP alone (Fig. 5C). Cells expressing either Y658E or Y731E showed a marked increase in migration relative to cells expressing wild-type VE-cadherin and displayed migration levels similar to control cells (Fig. 5C). These findings reveal that a single Y-E phosphomimetic point mutation in the tail of VE-cadherin, causing the simple uncoupling of either p120- or β-catenin with no change in cadherin adhesive properties or cell surface expression, is sufficient to initiate a migratory response consistent with the maintenance of a mesenchymal phenotype.

DISCUSSION

Cadherin-mediated cell-cell junctions play a critical role in maintaining the epithelial cell phenotype and regulation of cell barrier function. Accordingly, disruption of cell-cell junctions has been associated with the breakdown of cellular barriers and the initiation of EMT (33, 34). Growth factors, cytokines, or oncogenes are physiological regulators of cadherin function because they induce intracellular signals that disrupt cell-cell junctions, promote actin reorganization, and induce a migratory phenotype (35). Previous studies have demonstrated that disruption of cell-cell junctions depends on intracellular kinases and/or phosphatases that regulate the phosphorylation state of cadherins and their cytosolic binding partners including α-, β-, γ-, and p120-catenin (6, 10, 18, 19, 23).

Epithelial cells of different histological origins express a distinct repertoire of cadherins. For example, E-cadherin is expressed by a variety of epithelial cells, whereas VE-cadherin is restricted to endothelial cells where it regulates vascular

FIG. 5. Increased focal contact formation and migration in CHO cells expressing the Y658E and Y731E mutants of VE-cadherin (VE-Cad). A, CHO cells expressing wild-type (wt) VE-cadherin or the Y658E and Y731E mutants were fixed, and the expression patterns of GFP-tagged VE-cadherin (green), actin (red), and FAK-pY861 (blue) were determined by immunofluorescence. B, adhesion assay testing the ability of CHO cells expressing GFP, wild-type VE-cadherin, or Y-E and Y-F mutants of VE-cadherin to adhere to wells coated with IgG (white bars) or a VE-cadherin-Fc fusion protein (black bars). Following a 2-h incubation at 37°C, non-adherent cells were removed, adherent cells were fixed and stained with crystal violet, and dye bound inside adherent cells was extracted and quantitated at 600 nm. Absorbance. C, migration assay testing the ability of CHO cells expressing GFP, wild-type VE-cadherin, or Y658E and Y731E mutants of VE-cadherin to migrate through Transwell chambers. Chambers were coated on the upper side with 1 μg/ml collagen and on the underside with 10 μg/ml collagen; cells were added to the upper side of the chamber. Following a 4-h incubation at 37°C, non-migratory cells were removed from the upper side of the Transwell membrane, migratory cells on the underside of the membrane were fixed and stained with crystal violet, and dye bound inside migratory cells was extracted and quantitated at 600 nm.

Regulation of Cell Biology by Phosphorylation of VE-cadherin on Tyr-658 and Tyr-731—EMT represents a critical re-programming of the cell that occurs during tissue remodeling associated with wound healing, angiogenesis, and cancer (31). EMT has been linked to loss of cadherin adhesive function, reduction of expression from the cell surface, and/or disruption in its association with the cytoskeleton. In addition, EMT results in the gain of a migratory phenotype. CHO cells lack cadherin expression and thereby display a fibroblastic or mesenchymal appearance (data not shown). In contrast, CHO cells expressing wild-type VE-cadherin exhibited an epithelium-like appearance with intense cadherin expression at cell junctions and very few focal contacts (Fig. 5A, left panel). Cells expressing the VE-cadherin mutants Y658E or Y731E continued to express significant levels of cadherin at cell junctions, but interestingly, these cells displayed increased numbers of focal contacts consistent with a more migratory, invasive phenotype (Fig. 5A, middle and right panels). These focal contacts could be identified with the phosphospecific antibody to tyrosine 861 of FAK, a site normally phosphorylated in migratory cells (32).
permeability and angiogenesis (7, 23, 36). In fact, VEGF has been shown to disrupt endothelial barrier function via disruption of VE-cadherin-mediated cell-cell junctions (8, 22). This initial disruption of barrier function must then be maintained throughout the process of endothelial cell migration/invasion that takes place during angiogenesis. VE-cadherin becomes tyrosine-phosphorylated in response to VEGF (17), yet it remains unclear whether this phosphorylation contributes to loss of endothelial barrier function, induction of EMT, or maintenance of a mesenchymal state once angiogenesis is under way. There are nine tyrosines in the cytoplasmic tail of VE-cadherin, and we sought to understand whether one or several of these sites play a critical role in regulation of VE-cadherin function. To assess the role of phosphorylation in VE-cadherin function we expressed wild-type or mutant forms of VE-cadherin in CHO cells that lack endogenous cadherins. Specifically, each tyrosine was mutated to either glutamic acid (phosphomimetic), phenylalanine (non-phosphorylatable), or alanine (non-conservative). CHO cells expressing wild-type or mutant cadherins were evaluated for cell-cell junctional activity. Two of the nine Y-E mutations (Y658E and Y731E) not only revealed defects in cell barrier function while maintaining VE-cadherin cell surface expression but also failed to bind p120- and β-catenin, respectively. These tyrosines lie at the heart of regions of the protein shown previously to bind p120- and β-catenin (37–39). To our surprise, cells expressing either of these two mutants demonstrated additional properties consistent with a mesenchymal phenotype, as characterized by formation of focal contacts and a migratory response. Based on these findings, we conclude that either Tyr-658 or Tyr-731 in VE-cadherin can play a critical role in regulation of VE-cadherin function and that functional VE-cadherin junctions depend on interactions with both β-catenin and p120-catenin.

Src family kinase activation has been shown to promote uncoupling of VE-cadherin and E-cadherin. In fact, mice deficient in pp60-Src or pp62-Yes or wild-type mice treated with a Src inhibitor have profound defects in VEGF-mediated vascular permeability associated with the stabilization of VE-cadherin-mediated endothelial cell-cell junctions (8, 22, 23). Supporting a role for Src in the regulation of VE-cadherin, we found that VE-cadherin mutants containing Y658F and Y731F showed dramatically decreased levels of tyrosine phosphorylation in CHO cells expressing constitutively active Src. However, it remains to be determined whether the role of Src in this process is direct or indirect. In addition to kinases, phosphatases such as VE-PTP, PTP1B, PTPμ, and SHP-2 have been shown to influence cadherin function (26–30). For example, VE-PTP is known to associate with VE-cadherin and control its level of phosphorylation (26). Accordingly, we showed that phosphatase inhibition was capable of potentiating VE-cadherin phosphorylation at Tyr-658 and Tyr-731, leading to a disruption of cell-cell barrier function. In support of this finding, expression of phosphomimetic mutations also resulted in a loss of VE-cadherin-mediated barrier function. We contend that the observed loss in barrier function was due to the uncoupling of cadherin from the cytoskeleton via the dissociation of β-catenin or p120-catenin and not because of a simple loss of VE-cadherin adhesive function or expression on the cell surface.

Of interest, a comparison of cadherin cytoplasmic domains provides insight into the potential mechanisms by which other cadherin family members are regulated (Fig. 6). With the exception of E-cadherin, the Tyr-658 site is well conserved among cadherins, suggesting that phosphorylation of this residue may be a general mechanism to disrupt cell barrier function. Conversely, the Tyr-731 site is unique to VE-cadherin, suggesting that regulation of this residue may involve an endothelium-specific mechanism, as would be required for transient vascular permeability responses. Future experiments will aim to address such issues.

In addition to the role of p120-catenin in modulating cadherin adhesion and actin dynamics (12), p120-catenin has also been implicated in the regulation of cadherin expression at the cell surface. Using endothelial cells, several groups have reported that disruption of binding of p120-catenin to VE-cadherin can result in cadherin internalization and degradation (11, 13, 40). However, in our experimental system, VE-cadherin expression at the cell surface of CHO cells appeared constant, regardless of cytoplasmic tail mutations that affected binding to catenins. This could be explained by differences that likely exist between the internalization/degradation machinery of CHO cells compared with endothelial cells. Alternatively, as our cell selection procedures employed the use of flow cytometry to select for cells that did express VE-cadherin, it is possible that those cells with reduced cadherin cell surface expression levels were excluded from our analyses.

Together, our findings provide new insight into the regulation of EMT. We define an intracellular pathway that can regulate this process via phosphorylation of the cadherin cytoplasmic tail and thereby provide new insight into the role that cadherins and their associated molecules play in development, tissue remodeling, and various disease states. Furthermore, our findings indicate that kinases capable of phosphorylating specific sites within the cadherin C terminus can direct cells to maintain a mesenchymal phenotype.

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