Effect of Heparin II Domain of Fibronectin on Actin Cytoskeleton and Adherens Junctions in Human Trabecular Meshwork Cells

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PURPOSE. To determine whether the heparin II (HepII) domain of fibronectin previously shown to increase outflow facility affects the formation and assembly of actin cytoskeleton and adherens junctions in human trabecular meshwork (TM) cells.

METHODS. Normal HTM cells and two transformed HTM cell lines were treated for 24 hours with increasing concentrations of the HepII domain. Disruptions in adherens junctions and the actin cytoskeleton were determined using immunofluorescence microscopy and Western blot analysis of immunoprecipitated cadherin/catenin complexes. Actin filaments were detected with phalloidin. Catenin (α and β) and cadherin antibodies were used to detect adherens junctions.

RESULTS. Treatments of cultures with the HepII domain caused cadherin/β-catenin complexes in adherens junctions and actin filaments to disassemble. The disruption of adherens junctions and actin filaments occurred in a dose-dependent and temporal fashion. The disassembly of actin filaments occurred first, followed by the disassembly of adherens junctions. Dissociation of adherens junctions, but not actin filaments, was reversible if the HepII domain was removed. Reassembly of actin filaments required the addition of serum. Serum, however, could not trigger the reassembly of actin filaments if the HepII domain was present, suggesting that the HepII domain acted downstream of the serum stimulated RhoA activity.

CONCLUSIONS. The exposure of HTM cells to the HepII domain triggers the disassembly of actin filaments and the subsequent destabilization of adherens junctions in HTM cells. This suggests that the HepII domain may increase outflow facility in cultured anterior segments by altering the organization of the TM cytoarchitecture. (Invest Ophthalmol Vis Sci. 2006;47:2924–2931) DOI:10.1167/iovs.06-0038

Regulation of outflow facility through the trabecular meshwork (TM) involves several biological processes, including phagocytosis, turnover of the extracellular matrix, and gene expression. Actin-mediated processes are also thought to regulate outflow facility by either altering the contractile properties of the TM cytoarchitecture or weakening the assembly of cell–cell and cell–matrix contacts used to maintain the integrity of the TM. Thus, agents that disrupt the actin cytoskeleton have been shown to increase outflow facility in enucleated human and bovine eye organ perfusion cultures as well as in live monkey eyes.¹ In contrast, agents that support actin cytoskeleton assembly reduce outflow facility.²

In many cell types, organization of the actin cytoskeleton by Rho GTPases is controlled by cell–matrix interactions.³,⁴ These interactions are mediated by transmembrane receptors that interact specifically with extracellular matrix proteins and cytoskeletal components within specialized membrane sites called focal adhesions. One family of receptors that regulates the activity of the actin cytoskeleton is integrins. Integrins are transmembrane receptors composed of α- and β-subunits covalently associated into a heterodimer. Another family that regulates the activity of the actin cytoskeleton is syndecans. Syndecans are transmembrane heparan sulfate proteoglycans that bind to extracellular matrix proteins, typically through interactions with their heparan sulfate chains. Both integrins and syndecans have been found in the TM. In human trabecular meshwork (HTM), the integrin subunits α1, α5, α6, αv, β1, β2, β4, and β5 and the αvβ3 and α5β1 integrins have been found.⁵ Syndecans found in the human trabecular meshwork include syndecan-3 and -4 as well as low levels of syndecan-1 and -2.⁶ Both integrins and syndecans use members of the Rho family of GTPases (Rac1, RhoA, Cdc42) to control the assembly and contractility of the actomyosin network as well as the maturation of cell–matrix and cell–cell adhesions.⁷–⁹ Rho GTPases play an important role in regulating outflow facility. Inhibition of RhoA activity with a dominant Rho kinase,⁹ an exoenzyme from Clostridium botulinum called C3 transferase or the Rho kinase inhibitor, Y27632,¹⁰–¹² increased outflow facility in organ-cultured porcine and human eyes and in rabbit and monkey eyes in vivo. This suggests that cell–matrix interactions with either syndecans or integrins that control the activity of Rho GTPases and/or the actin cytoskeleton could be involved in the regulation of outflow facility.

Results of recent studies support this idea and have shown that the actin cytoskeleton is necessary for cell–matrix adhesions.¹³,¹⁴ Fibronecetin is closely associated with cells in the juxtanacalicular tissue (JCT) in the anterior segment and changes in the expression of fibronecetin have been reported to occur with aging and in some glaucomatous eyes.¹⁶ The domain in fibronecetin that regulates outflow facility is called the heparin II (HepII) binding domain. This domain contains binding sites for syndecans-1, -2, -3, and -4 and αvβ1 integrin.¹⁷ In most cell types, the HepII domain regulates the organization of actin filaments via cooperative signaling between α5β1 integrins and either syndecan-4¹⁸ or αvβ1 integrin.¹⁹
In cultured human trabecular meshwork (HTM) cells, soluble HepII domain uses cooperative signaling events with α4β1 integrin to regulate the organization of the actin cytoskeleton during cell spreading. Activation of this cooperative integrin signaling pathway by the HepII domain resulted in the phosphorylation of FAK (focal adhesion kinase), a kinase that mediates the formation of focal adhesions and actin stress fibers and was RhoA dependent because the Rho kinase inhibitor Y27632 prevented cell spreading. This suggests the HepII domain activates a RhoA-mediated signaling pathway in the TM and hence could regulate aqueous humor outflow by altering actin cytoskeleton-mediated processes. In this study, we have examined the ability of the HepII domain to affect the actin cytoskeleton and the biological consequences on adherens junctions in confluent cultures.

**Material and Methods**

**Cell Culture**

A human TM cell strain (A7-1) and an immortalized human TM-1 cell line were grown in low-glucose DMEM (Sigma-Aldrich, St. Louis, MO) with 10% fetal bovine serum (Atlanta Biologicals, Inc., Norcross, GA), 2 mM l-glutamine plus antibiotics (2.5 μg/mL amphotericin B and 25 μg/mL gentamicin) as previously described. A7-1 cells were derived from a 30-year-old donor as previously described and were used at passage 6. The immortalized human TM-5 cells were grown in high-glucose DMEM (Sigma-Aldrich) with 10% fetal bovine serum (Atlanta Biologicals, Inc.), 2 mM l-glutamine plus antibiotics (100 U/mL penicillin G and 5 μg/mL streptomycin sulfate). TM-3 cells were a gift from Abbot F. Clark (Alcon Laboratories, Fort Worth, TX). Cultures were used 1 week past confluence to allow time for a stable morphology to be established.

For experiments, confluent cultures grown on glass coverslips or plastic tissue cultures dishes were incubated in serum-free DMEM containing 2.5 μg/mL amphotericin and 25 μg/mL gentamicin in the absence or presence of 125, 250, or 500 μg/mL HepII domain for various times. Serum was removed from the media to avoid interactions between the HepII domain and serum factors such as plasma fibronectin.

**Preparation of Recombinant Proteins**

The recombinant HepII domain (type III 12–14 repeats of fibronectin) and the recombinant heparin-binding domain from tenascin-C (TN fn6-8) were made as described previously.

**Immunofluorescence Microscopy**

HTM cells were permeabilized with 0.5% Triton X-100 and fixed with 4% paraformaldehyde, as previously described. Cells were then incubated with 1% bovine serum albumin (BSA) in 20 mM sodium phosphate (pH 7.4) 150 mM NaCl (PBS) to block nonspecific labeling. Blocked cells were labeled for 1 hour with anti-β-catenin monoclonal antibodies (Chemicon International, Inc., Temecula, CA) diluted 1:500 in 0.1% BSA in PBS. Cells were then incubated simultaneously with Alexa 568-conjugated goat anti-rabbit secondary antibody (5 μg/mL; Invitrogen, Eugene, OR) and Alexa 488-conjugated phalloidin (0.67 μg/mL; Invitrogen) in 0.1% BSA/PBS for 1 hour. Coverslips were mounted onto slides (Immuno-mount; Shandon Lipshaw, Pittsburgh, PA), and images were acquired with a camera (AxioCam HRm; Carl Zeiss Meditec, Inc., Thornwood, NY) mounted on a fluorescence microscope (AxioImager 2 Imaging together with AxioVision version 5.1 software; Carl Zeiss Meditec, Inc.).

**Immunoprecipitation and Immunoblot Analysis**

Confluent cell layers were washed with PBS and lysed for 15 minutes at 4°C with 1% Nonidet P-40, 0.1% Triton X-100, 30 mM sodium phosphate (pH 7.4) containing 1 mM sodium orthovanadate, 2.5 mM Tris-HCl (pH 7.5), 100 mM NaCl, and 10 μg/mL of pepstatin, leupeptin, and aprotinin. Lysates (4 μg/mL) from the control and treated cells were incubated with an anti-β-catenin monoclonal antibody (1:250 dilution; Upstate Group, Inc., Charlottesville, VA) for 1 hour at 4°C. The lysate was then incubated with protein G Sepharose beads (GE Healthcare, Piscataway, NJ) for 1 hour at 4°C. Beads were collected by centrifugation and bound proteins were eluted with Laemmli buffer for 5 minutes at 70°C.

Immunoprecipitates as well as total cell lysates (10 μg of total protein) from the control and treated cells were separated by 10% SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Immobilon-F; Millipore, Billerica, MA) as described. The membrane was blocked in 5% nonfat dry milk, 0.5% Tween 20 in 20 mM Tris-HCl (pH 7.5) containing 150 mM NaCl (TBS) and then incubated with an anti-phosphorylated tyrosine antibody (clone 4G10; 1 μg/mL; Sigma-Aldrich), as described earlier. All labeled membranes were washed with 0.1% Triton X-100 in TBS and incubated for 1 hour with either a horseradish peroxidase–conjugated goat anti-rabbit secondary antibody or a horse-radish peroxidase–conjugated goat anti-mouse secondary antibody (1:5000 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Bound antibody was detected with a chemiluminescence detection kit (ECL Plus; GE Healthcare), according to the manufacturer’s instructions.

**Subcellular Fractionation**

Confluent cultures were incubated in the absence or presence of 500 μg/mL of the HepII domain for 24 hours. Cells were lysed with cold hypotonic buffer containing 10 mM Tris-HCl (pH 7.5), 5 mM MgCl2, 1 mM dithiothreitol, 10 μg/mL of pepstatin, 10 μg/mL of leupeptin, and 10 μg/mL of aprotinin, as described. The cells were scraped, placed in test tubes and then lysed by snap freezing in liquid nitrogen. The lysates were then thawed at 65°C and centrifuged at 600g for 5 minutes at 4°C. The supernatants were decanted into fresh test tubes and the N-cadherin in the soluble compartment was analyzed by Western blot.
The heparin-binding domain of fibronectin and tenascin-C. Fibronectin monomer highlighting the type III repeats (numbered oval) and the HepII domain. The recombinant HepII domain consists of the 12th to 14th type III repeats plus the first 10 amino acids of the IIIICS domain (shaded rectangle). The heparin-binding domain in tenascin-C, which is indicated by the bar, consists of the 6th to 8th type III repeats.

The effect of the HepII domain is dose dependent. The effect of the HepII domain on cell morphology is not specific for the TM-1 cell line, since similar changes in cell morphology were observed in monolayers of normal HTM cells (Figs. 2A, 2B) and transformed TM-3 cells (data not shown) treated with the HepII domain.

To demonstrate the specificity of the effect, TM-1 and HTM cultures were also incubated with a heparin-binding site (TNfn6-8) from tenascin-C (Fig. 3C). Like the HepII domain, this domain is composed of three type III repeats (Fig. 1) and is from an extracellular matrix protein found in the trabecular meshwork. In contrast to the effect in cultures treated with the HepII domain, when monolayers of TM-1 cells were exposed to an equivalent amount of TNfn6-8, β-catenin staining remained localized at the cell–cell borders (Fig. 4G, arrow) and the F-actin cytoskeleton was indistinguishable from that of the control.

**Time Course of Actin Filaments and Adherens Junctions Disassembly**

Because the organization of actin filaments is important for maintaining the integrity of the adherens junctions, the disassembly of adherens junctions in the presence of the HepII domain may be the result of the HepII domain triggering the disassembly of the actin cytoskeleton. To determine whether the effect on adherens junctions was due to the HepII domain first triggering a disassembly of cortical actin filaments, a time course study was performed. As shown in Figure 5F, the F-actin staining of TM-1 monolayers exposed to the HepII domain appeared diminished and disorganized as early as 6 hours. In contrast, cadherin staining at the cell–cell borders was unchanged until the 12-hour time point (cf. Figs. 5A, 5C, 5E, 5G). Taken together, these data suggest that exposure to the HepII domain first triggers the disassembly of F-actin and this disassembly is then followed by a disruption of adherens junctions at the cell surface.

**Effect of the HepII Domain on the Association between β-Catenin and Cadherin**

Western blot analysis of β-catenin immunoprecipitates further verified that the HepII domain disrupts adherens junctions. In untreated TM-1 cultures, cadherin coprecipitated with β-catenin, indicating that cadherin is associated with β-catenin in a complex and that this complex is intact (Fig. 6A). However, when TM-1 cells were treated with the HepII domain for 24 hours, cadherin did not coprecipitate with β-catenin, suggesting that the association of β-catenin to cadherins is disrupted (Fig. 6A). Interestingly, Western blots of β-catenin immunoprecipitation indicated that the interaction between α-catenin and β-catenin was not lost (Fig. 6A) when the HepII domain was present. This finding suggests that the interaction respon-
sible for the loss of adherens junctions may be caused by a disruption between the cadherin and β-catenin interaction and not a disruption between α-catenin and β-catenin.

The disruption of adherens junctions is not due to a disruption in cell–matrix contacts. As shown in Figure 6B, when TM-1 cell layers were lifted up with a nonenzymatic cell dissociation buffer, immunoprecipitation of β-catenin from these nonadherent cells indicated that cadherin was still associated with β-catenin, despite the fact that attachment to the underlying matrix was disrupted by the buffer. Furthermore, cell binding assays with HTM cells indicated that the concentration of the HepII domain necessary to disrupt TM cell attachment to the underlying matrix far exceeded (>1.0 mg/mL) the concentration needed to disrupt adherens junctions in these experiments (data not shown).

Finally, to further demonstrate that dissociation of adherens junctions is due to the treatment with the HepII domain and

**FIGURE 3.** TNf68 did not promote cell rounding and separation in TM monolayers. TM-1 cells were grown to confluence on glass coverslips and then incubated in the absence (A) or presence of 500 μg/ml HepII domain (B) or 500 μg/ml TNf68 (C) in serum-free media for 24 hours. Images were captured with a Nikon Coolpix 8800 VR camera.

**FIGURE 4.** HepII domain disrupted β-catenin containing adherens junctions and actin cytoskeleton organization of TM cells. Cultured TM-1 cells were grown to confluence on glass coverslips and then treated with 0 (A, B) or 250 (C, D) or 500 (E, F) μg/ml of the HepII domain or 500 μg/ml of the TNf68 (G, H) in serum-free medium for 24 hours. Cells were permeabilized, fixed, and labeled with a polyclonal anti-β-catenin antibody (A, C, E, G) or Alexa 488-conjugated phalloidin (B, D, F, H). β-Catenin localized to cell borders (A, C, G, E, arrows) or as punctate aggregates (E). Inset: higher magnification of the area indicated by (✱). Bar, 25 μm.

**FIGURE 5.** Time course of actin cytoskeleton and adherens junction disruption. Monolayers of TM-1 cells were exposed to 500 μg/ml HepII domain for 0 (A, B), 4 (C, D), 6 (E, F), or 12 (G, H) hours. Cells were permeabilized, fixed, and labeled with a monoclonal anti-N-cadherin (A, C, E, G) antibody and Alexa 488-conjugated phalloidin (B, D, F, H). N-cadherin localized to cell borders (A, C, E) or as punctate aggregates (G). F-actin appeared organized in cortical actin bundles at hour 0 (B). At 4 (D) and 6 (F) hours, actin appeared less polymerized. At 12 hours (H), actin filaments were entirely absent, and the staining localized in a punctate pattern beneath the cell surface. Cell–cell contacts are indicated with arrows. Asterisk in (G) indicates area shown in inset. Bar, 25 μm.
not cell rounding, we compared the levels of soluble N-cadherin found in confluent cultures of TM-1 cells treated with the HepII domain to that observed in cells treated with EGTA. Treatment with EGTA should cause the cells to round, because it leads to a disruption in cell–matrix contacts. If a disruption in the linkage between adherens junctions and cortical actin filaments occurs under these conditions an increase in soluble cadherin should be observed.26,28 As shown in Figure 6C, when serum-starved cells were exposed to EGTA, the amount of soluble N-cadherin remained the same as the untreated serum-starved control cultures. However, if confluent cultures of TM-1 cells are exposed to the HepII domain for 24 hours, an increase in soluble N-cadherin was observed. Thus, the changes in the adherens junctions were specific to treatment with the HepII domain rather than simply a consequence of cell rounding and may reflect alterations in linkage of cadherins to the actin cytoskeleton.

Recovery of Adherens Junctions of TM Cells in the Absence of the HepII Domain

To determine whether the effect of the HepII domain on adherens junctions is reversible, TM-1 cells were exposed to the HepII domain for 24 hours. The media were then changed, and adherens junctions were allowed to reform in the absence of the HepII domain. As shown in Figure 7, in the presence of the HepII domain, β-catenin staining around the periphery of the cells was absent compared with TM cells never exposed to the HepII domain for 24 hours (Fig. 4A) and most of the staining appeared to be in a punctuate pattern beneath the cell surface (cf. Fig. 7A and Fig. 4A). In addition, there was a lack of cortical actin filaments (Fig. 7E). On removal of the HepII domain, β-catenin staining around the periphery of the cell reappeared, suggesting that adherens junctions reform (Fig. 7B). Surprisingly, the actin cytoskeleton was not reorganized in these cultures (Fig. 7F) indicating that, whereas disassembled adherens junctions are capable of reforming when the HepII domain is removed, the actin cytoskeleton is not.

Effect of Serum on the Reorganization of the Actin Cytoskeleton

Because serum factors have been shown to regulate actin polymerization,29,30 the experiment was repeated but this time in the presence of serum to see whether actin polymerization

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**Figure 6.** Effect of HepII domain on β-catenin associations in cultured TM cells. Cultured TM-1 cells grown to confluence were treated with or without the HepII domain in serum-free medium for 24 hours. Cadherin/β-catenin complexes were immunoprecipitated (IP) from cell lysates with an anti-β-catenin monoclonal antibody and analyzed by Western blots (A, B). In some experiments, cadherin/β-catenin complexes were immunoprecipitated from cell lysates obtained from cells serum-starved for 24 hours and lifted from plates with a cell dissociation buffer. (C) Levels of soluble N-cadherin were determined in cells treated with or without HepII domain in serum-free medium for 24 hours or from cells serum-starved for 24 hours and then exposed to 0.1 mM EGTA for 25 minutes. Equivalent protein concentrations (10 μg) of the hypotonic buffer lysates were loaded on the 10% SDS-PAGE and analyzed by Western blot using an anti-N-cadherin monoclonal antibody.

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**Figure 7.** Recovery of adherens junctions and actin cytoskeleton of TM cells exposed to HepII domain. Cultured TM cells were exposed to the HepII domain in serum-free media (A, E) for 24 hours. The cells were washed twice and then exposed to serum-free media alone (B, F), 10% fetal bovine serum (C, G) or 10% fetal bovine serum with 500 μg/ml of the HepII domain (D, H) for an additional 24 hours. Cells were permeabilized, fixed, and labeled with a polyclonal antibody against β-catenin (A–D) and Alexa 488-conjugated phalloidin (E–H). Arrows: cell–cell contacts. Bar, 25 μm.
could be recovered. As shown in Figures 7C and 7G, on removal of the HepII domain, β-catenin and cortical actin labeling around the periphery were observed when serum was present, indicating that both the adherens junctions and actin filaments reformed. Not surprisingly, the adherens junctions formed in the presence of serum were more pronounced and showed the more traditional “zipper-like” pattern (Fig. 7C), probably due to the assembly of cortical actin filaments stabilizing and enhancing the formation of adherens junctions at these sites. However, if the HepII domain was still present when serum-containing media were added back to the monolayers, the recovery that occurred with just serum alone was inhibited (Figs. 7D, H). That is, cells exposed to both serum and the HepII domain for 24 hours exhibited a level of β-catenin localization around the periphery of the cell similar to the HepII observed in the absence of serum, and cortical actin filaments were not observed around the periphery of the cells. Thus, the presence of the HepII domain appears to prevent the reassembly of cortical actin filaments.

DISCUSSION

In this study, we have shown that the HepII domain of fibronectin promotes the disassembly of the actin cytoskeleton and adherens junctions in confluent monolayers of TM cells. The effect was time and dose dependent and occurred in three different TM cell lines, indicating that it was not dependent on the cell lines used. The disassembly of actin filaments preceded the disruption in adherens junctions suggesting that the former mediated the latter and was not triggered by a disruption in cell adhesion to the underlying substrate. Disruption of adherens junctions was specific for the HepII domain, because another heparin-binding domain from an ECM protein, tenasin-C, did not have any effect, and the effect was reversible if the HepII domain was removed. This demonstrates for the first time that specific cell-matrix interactions can regulate the assembly of adherens junctions and the actin cytoskeleton in the TM.

The disassembly of adherens junctions appeared to be due to a disassembly of actin filaments, because the disassembly of actin filaments temporally preceded the disruption of adherens junctions in cultures treated with the HepII domain. This is not surprising, because actin filaments have been shown to play a critical role in the formation and stability of adherens junctions. Furthermore, the disruption of actin filaments had previously been shown to lead to the disassembly of adherens junctions in HTM cells exposed to latrunculins A and B.

The mechanism by which the HepII domain can regulate the actin cytoskeleton in TM cells remains to be elucidated. The studies presented herein suggest that the signaling events mediated by the HepII domain block signaling events upstream of RhoA activation because serum could not mediate the reformation of actin filaments when the HepII domain was present. In keratinocytes, inactivation of RhoA also causes a decrease in the accumulation of actin filaments and cadherin at sites of cell-cell contacts. Thus, as shown in Figure 8, one hypothesis is that the HepII domain triggers the disassembly of actin filaments by inhibiting a Rho-mediated pathway and this in turn leads to the loss of adherens junctions in TM cells.

At first, the effect of the HepII domain on actin filaments and adherens junctions appears to be a paradoxical finding. Previous studies by Peterson et al., showed that the HepII domain activated a cooperative α/β1/α5β1 integrin-signaling pathway that increased stress fiber formation in a RhoA-dependent manner in HTM cells. Thus, the HepII domain would be expected to activate RhoA and hence promote actin polymerization and adherens junction formation. One plausible explanation for this discrepancy is that the effect of the HepII domain on actin polymerization may be cell-cycle dependent. In contrast to the previous studies by Peterson et al., which were performed in proliferating HTM cells plated on the cell-binding domain of fibronectin, the experiments presented herein were conducted with confluent monolayers of quiescent normal trabecular meshwork cells and TM-1 cells embedded in an extracellular matrix of laminin, type IV collagen, and fibronectin. Thus, differences in either the expression and/or activity of cell surface receptors for the HepII domain, or signals emanating from other matrix proteins could be affecting the events triggered by the HepII domain. Alternatively, the activities of RhoA and Rac1 have been shown to be antagonistic and transient. Thus, whereas signaling from the HepII domain may trigger the activation of RhoA this could ultimately lead to the downregulation of Rac1 activity. Rac1 activity has been shown to play a critical role in regulating adherens junctions, especially involving N-cadherin, and downregulation of Rac1 would also lead to the disassembly of cortical actin responsible for stabilizing adherens junctions.

The disruption of adherens junctions appeared to involve a break in the association between β-catenin and the cytoplasmic tail of N-cadherin, because α-catenin was still associated with β-catenin in the immunoprecipitates. Thus, it is unlikely that the mechanism responsible for the disruption of the N-cadherin/catenin complex involved IQGAP1, because IQGAP1 disrupts adherens junction complexes by binding to β-catenin and severing its association with α-catenin when not bound to activated Rac1 or Cdc42. Whether, the disruption of adherens junctions could be mediated by tyrosine or serine/threonine phosphorylation of β-catenin or other adherens junction components or the endocytosis of the cadherin complex after tyrosine phosphorylation and monoubiquitination of cadherin remains to be determined.

Our immunofluorescence data suggest that exposure of TM cells to the HepII domain may cause the relocation of N-cadherin to intracellular compartments just below the cell surface. Cadherins are constantly removed from the cell surface by early or sorting endosomes so they can be recycled back to the cell surface or sent to lysosomes for degradation. As observed in vascular endothelial cells, localization of cadherins to subcellular compartments reflects a regulatory mechanism to control vascular permeability. Whether ad-
herens junctions in the trabecular cells on the corneoscleral trabecular beams and in the juxtacanalicular tissue in vivo are likely to contribute to aqueous humor outflow regulation by acting as a barrier to fluid flow. It is not known. The disruption of adherens junctions, however, is likely to disrupt the cytoarchitecture of the cells and hence could affect outflow facility by altering the structural integrity of the TM.

In summary, the ability of the HepII domain to disrupt actin filaments and adherens junctions could explain the ability of the HepII domain to increase outflow facility in cultured human anterior chambers. Actin cytoskeleton disrupting agents, including H-7, latrunculin A and B, and cytochalasin D, have been shown to increase outflow facility. The data presented herein suggest that the HepII domain belongs to this family of agents and that cell–matrix interactions in general should be explored for their potential to regulate the actin cytoskeleton of the trabecular meshwork and, thus, aqueous humor outflow facility.

References