Inhibition of RhoA Signaling with Increased Bves in Trabecular Meshwork Cells

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PURPOSE. Blood vessel epicardial substance (Bves) is a novel adhesion molecule that regulates tight junction (TJ) formation. TJs also modulate RhoA signaling, which has been implicated in outflow regulation. Given that Bves has been reported in multiple ocular tissues, the authors hypothesize that Bves plays a role in the regulation of RhoA signaling in trabecular meshwork (TM) cells.

METHODS. Human TM cell lines NTM-5 and NTM-5 transfected to overexpress Bves (NTM-w) were evaluated for TJ formation, and levels of occludin, cingulin, and ZO-1 protein were compared. Assays of TJ function were carried out using diffusion of sodium fluorescein and transcellular electrical resistance (TER). Levels of activated RhoA were measured using FRET probes, and phosphorylation of myosin light chain (MLC-p), a downstream target of RhoA, was assessed by Western blot analysis.

RESULTS. Overexpression of Bves led to increased TJ formation in NTM-5 cells. Increased TJ formation was confirmed by increased occludin, cingulin, and ZO-1 protein. Functionally, NTM-w cells showed decreased permeability and increased TER compared with NTM-5 cells, consistent with increased TJ formation. NTM-w cells also exhibited decreased levels of active RhoA and lower levels of MLC-p than did NTM-5 cells. These findings support a TJs role in RhoA signaling.

CONCLUSIONS. Increased Bves in TM cells leads to increased TJ formation with decreased RhoA activation and decreased MLC-p. This is the first report of a regulatory pathway upstream of RhoA in TM cells. In TM tissue, RhoA has been implicated in outflow regulation; thus, Bves may be a key regulatory molecule in aqueous outflow.

In the vertebrate eye, intraocular pressure (IOP) is a homeostatic balance between aqueous production and aqueous outflow. The conventional route of outflow, accounting for approximately 80% of the total drainage, is through the trabecular meshwork (TM), into Schlemm’s canal, and finally into the venous system. The inner TM is composed of collagen bundles and extracellular matrix covered by TM cells, resulting in large spacing between the trabeculae. The juxtacanalicular region of the TM is composed of layers of cells embedded in an extracellular matrix.1–3 Together, these regions create a network of porous structures with numerous openings through which aqueous can flow on its way to Schlemm’s canal and the venous system. The TM, however, is not merely a passive conduit for aqueous flow. Trabecular meshwork cells are thought to be the motor units that impart contractile capabilities to TM tissues. The contractile tone of TM modulates aqueous outflow with increased TM contraction leading to decreased aqueous outflow (increased IOP) and, conversely, TM relaxation, increasing outflow (decreased IOP).4–6

The state of TM cellular contraction is directly related to levels of phosphorylated myosin light chain (MLC-p), which is regulated by the Rho/Rho-kinase pathway. Briefly, activation of RhoA leads to activation of ROCK, a serine-threonine kinase, which, in turn, inhibits the activity of a myosin light chain phosphatase, leading to an accumulation of MLC-p (Fig. 1).7–12 The overall effect of increased RhoA signaling is increased MLC-p and increased TM contraction, resulting in decreased aqueous outflow. Studies also verify this regulatory pathway through pharmacologic inhibition of ROCK, which leads to increased aqueous outflow.9,11 However, little is known regarding the upstream regulation of Rho signaling in the aqueous outflow tract.

We have reported that a novel transmembrane adhesion molecule, Bves (blood vessel epicardial substance), regulates tight junction (TJ) formation in human corneal epithelial cells (HCECs).13–15 By altering levels of Bves expression, we observed the formation of TJs to be dependent on the levels of Bves in various epithelial cells—more Bves leads to more TJs, and less Bves leads to fewer TJs.13–15 TJs have traditionally been viewed as mechanical protein structures functioning in barrier formation, regulating paracellular flow of fluids and solute. However, through TJ-associated signaling pathways, TJs also play important regulatory roles in cellular differentiation, migration, and cell-cell communication.16–18 Recently, the laboratories of Balda and Matter described direct regulation of Rho signaling by TJ.19,20 As cells establish TJs, GEF-H1, an activator of RhoA, is sequestered within TJs, leading to an overall decrease in Rho signaling (Fig. 1). Thus, the overall effect of increased TJ formation is decreased Rho activation. These observations led us to suspect that through the regulatory role of Bves in TJ formation, Bves may play a direct role in modulating RhoA activation.

In vivo, isolated strands of TJs have been demonstrated in cells of the TM.21 The general consensus is that these TJ strands do not represent a significant barrier to paracellular passage of fluid.21,22 This is supported by observations that TJ strands in TM do not form complete zonula occludens.21 How-
However, cell culture studies suggest TM cells are capable of forming complete TJs. In primary cultures, TM cells cultured to full confluence on transwell filters exhibited significant resistance to transcellular fluid conductivity. Furthermore, this resistance is associated with increased TJ formation in cultured TM cells. These findings indicate that TM cells are capable of increasing TJ formation at sites of cell-cell contact in cell culture, raising the question of the in vivo role of TJ strands within trabecular meshwork cells. One possibility is that TJ strands may function more as signaling structures than as barrier structures.

Given that TJs are also regulators of Rho activation, we postulate that modulation of TJ formation in TM will lead to alterations in RhoA activation and subsequent regulation of myosin phosphorylation. In this study, we report that TM cells stably transfected to overexpress Bves exhibit an ability to form an increased number of TJs. Evidence of this includes increased TJ protein levels and increased electrical resistance, a functional assay of TJ formation, in cells overexpressing Bves. With increased TJ formation, TM cells exhibit decreased RhoA activation and a corresponding decrease in phosphorylated myosin. Our findings indicate that Bves regulates TJ formation in TM cells and may play a role in modulating RhoA activation and subsequent phosphorylation of myosin light chain. The effect of TJ formation on RhoA signaling has not been previously described in TM and represents a novel signaling pathway with the potential to affect aqueous outflow.

**Materials and Methods**

**Antibodies**

Polyclonal antibodies against ZO-1 and occludin were purchased from Zymed-Invitrogen. Polyclonal and monoclonal anti-cingulin antibodies were also purchased from Zymed. The polyclonal antibody against Bves (846) was obtained from David Bader’s laboratory (Vanderbilt University Medical Center, Nashville, TN). Monoclonal anti-GEF-H1 antibody was the gift of Karl Matter (Institute of Ophthalmology, University College, London). Monoclonal anti-phospho-myosin light chain 2 and polyclonal anti-myosin light chain 2 antibodies were purchased from Cell Signaling Technology, Inc. (Danvers, MA). β-Actin antibody was purchased from Sigma (St. Louis, MO). Rhodamine phalloidin and Alexa Fluor 488 and Alexa Fluor 680 secondary antibodies were purchased from Molecular Probes. For Western blot analysis, secondary antibodies were purchased from Rockland Immunochemicals (IRDye 800; Gilbert, PA).

**Cell Cultures**

Immortalized human trabecular meshwork cells from nonglaucomatous donors (NTM-5) and from glaucomatous donors (GTM-3) were obtained from Alcon Laboratories (Fort Worth, TX). The NTM cells were cultured in Dulbecco’s modified Eagle’s medium with high glucose 1× (Gibco-Invitrogen) and were supplemented with glutamate-penicillin-streptomycin solution (Sigma) and 10% FBS. Immortalized HCECs were a gift from Kaoru Araki-Sasaki. HCECs were cultured in a defined serum-free keratinocyte medium (Invitrogen-Gibco, Grand Island, NY).

**Stable Transfection**

To generate stable TM cell lines that overexpress Bves (NTM-w), NTM cells were transfected with reagent according to the manufacturer’s instructions (Lipofectamine 2000; Invitrogen) using full-length chicken Bves cDNA with a GFP sequence (NTM-wGFP) or FLAG tag (NTM-w plus the clonal isolate label) at the 3’ end of the coding sequence. Transfected cells were selected using medium containing G418 (Cellgro; 150 μg/mL). After antibiotic selection, the NTM-wGFP cells did not undergo clonal isolation or expansion and, therefore, were a mixed population. Resistant cells were maintained in medium containing 100 μg/mL G418.

**Western Blot Analysis**

Protein extraction and Western blot analysis were performed as previously described. Membranes were probed with antibodies against ZO-1, occludin, cingulin, Bves, and β-actin. Alexa Fluor 680 or infrared dye (IRDye 800; Li-Cor Biosciences, Lincoln, NE)-conjugated secondary antibodies were applied, and blots were scanned and analyzed (Odyssey Infrared Imaging System; Li-Cor Biosciences). For phosphorylated myosin light chain, the same blot was dual stained for phosphorylated myosin light chain and total myosin light chain. Lysates from primary human TM cells were the gift of Ted Acott and Kate Keller (Casey Eye Institute, Oregon Health & Science University, Portland, OR). Human TM cells were grown to confluence and cultured serum free for 72 hours. Extracts of the cell layer were obtained using 1 mL/T75 flask of RIPA with 1× Sigma proteinase inhibitor cocktail. The cells were scraped and spun at 14,000g for 2 minutes. The supernatant was collected and shipped on ice.

Densitometric measurements were made using Image Pro Plus. Each area of interest was background subtracted and normalized to β-actin. Phosphorylated myosin light chain was then divided by total myosin light chain. To account for exposure differences between

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**Figure 1.** RhoA regulates phosphorylation of myosin light chain. Activation of RhoA leads to increased levels of phosphorylated myosin light chain and is associated with increased cellular contraction (see pathway on the left). GEF-H1 activates RhoA by exchanging GDP with GTP to activate RhoA, which in turn activates ROCK, a serine-threonine kinase, which leads to phosphorylation and inactivation of myosin phosphatase and subsequent accumulation of MLC-p. MLC-p is associated with cellular contraction. Tight junctions (TJs) regulate RhoA activation by sequestering GEF-H1 (see pathway on the right). Because Bves regulates TJ formation, we hypothesize that Bves can also modulate levels of MLC-p.
blots, all samples were normalized to the NTM-5 samples. Normalized density values were compared using a one-way analysis of variance among all groups followed by an all pairwise multiple comparison ($P < 0.05$).

**Immunofluorescence**

Immunofluorescence staining was carried out using frozen sections of mouse eye and cultured TM cells fixed in 70% methanol, permeabilized in PBS with 0.25% nonionic surfactant (Triton X-100; Sigma), and blocked with PBS containing 2% BSA for 1 hour at room temperature. Primary antibodies were diluted in 1% BSA and incubated overnight at 4°C. The cells and sections were washed with PBS, and secondary antibodies were added for 3 hours at room temperature. After PBS and water washes, images were mounted in fluorescence mounting media and captured using a fluorescence microscope (Nikon, Tokyo, Japan).

**Monolayer Permeability**

TM cells were seeded at 5000 cells/cm² in six-well polycarbonate tissue culture inserts with 0.4-μm pores (BD Falcon, San Jose, CA). After cells reached confluence at approximately 7 to 9 days, permeability was measured. Culture inserts were rinsed with 37°C PBS. Inserts were placed in a six-well chamber containing 4 mL of 37°C PBS and a microstir bar on a heated stir plate. One milliliter of sodium fluorescein (NaFl) solution (5 × 10⁻⁴ mg/mL in PBS) was added to the insert. Six-microliter samples were removed from the lower chamber over a 10-minute interval. Fluorescence measurements from a fluorospectrometer (NanoDrop, Wilmington, DE) were converted to concentration, and concentration was plotted as a function of time. Permeability of the culture inserts was calculated using the method of Casnocha et al.²³ Permeability values were compared using one-way analysis of variance among all groups, followed by an all-pairwise multiple comparison ($P < 0.05$).

**Transcellular Electrical Resistance**

Electrical resistance was measured using the method of Oshima et al.²⁶ Briefly, TM cells were seeded as for the permeability assay. Cells were cultured for 8 days; control filters with media alone were incubated for the same period. Before measurement, inserts were washed with 37°C PBS with calcium and magnesium. Two milliliters of PBS was added to the chamber containing the lower electrode, the insert was placed in the chamber, 2 mL PBS was added to the insert, and the upper electrode was positioned in the PBS in the insert. Resistance of the culture insert was subtracted from the cell measurements. The background-corrected measurement was multiplied by the area of the filter. These resistance values were compared using one-way analysis of variance among all groups, followed by an all-pairwise multiple comparison ($P < 0.05$).

**RhoA Activation Detected by FRET Probes**

We evaluated the state of RhoA activation using FRET probes (fluorescence resonance energy transfer). RhoA-Raichu FRET plasmids (gift from Michiyuki Matsuda, Osaka, Japan) were transfected into NTM cells at 50% confluence. In addition, some NTM cells also received empty vector or FLAG-tagged full-length chicken Bves. Thirty-six hours later, whole lysates from the transfected cells were evaluated for FRET according to the method of Aijaz et al.¹⁹ Briefly, a fluorescence spectrophotometer (LS50B; PerkinElmer, Waltham, MA) was configured for 430-nm (CFP, FRET) and 517-nm (YFP) excitation and 475-nm and 530-nm emissions. Measurements were taken at (excitation/emission wavelength) 430/475 (CFP), 430/530 (FRET), and 517/530 (YFP). Non-transfected cells and cells transfected with CFP or YFP constructs only were used as background and single-channel cross-talk controls. Net FRET signal, including compensation for background and single-channel cross-talk, was calculated according to the method of Chusainow et al.²⁷ Net FRET efficiency values for the cell lines were compared by unpaired $t$ test ($P < 0.05$).

**Results**

**Bves in TM In Vivo and In Vitro**

Frozen sections of mouse anterior chamber and human TM cells were probed for Bves protein. Immunofluorescence localization studies of mouse anterior chamber with Bves antibodies exhibited staining in the TM region (Figs. 2A, B), suggesting the presence of Bves in vivo. The presence of Bves has been described in various ocular tissues including cornea and lens,¹⁵ and the patterns of Bves localization are similar in mouse and human corneal epithelia. To further investigate the presence of Bves in human TM, whole cell lysates of cultured human TM cells (NTM-5, GTM-3, and primary human TM cells) were subjected to Western blot analyses. When probed with Bves antibodies, the blots from all three human TM cell types exhibited bands just above 50 kDa, which correspond to endogenous Bves protein as seen in human corneal epithelial cells (Fig. 2E). These findings indicate that endogenous Bves protein is present in mouse and human TM tissues and cells.

For Bves to regulate cell-cell contact formation, it must be localized at the cell membrane. Immunofluorescence localization studies were performed on NTM-5 and GTM-3 cells to confirm endogenous Bves localization at the cell membrane (Figs. 2C, D). Dual immunolocalization staining shows Bves colocalizing with ZO-1 at the cell membrane in both NTM-5 (Fig. 3) and GTM-3 (results not shown). These findings indicate that the localization of endogenous Bves in TM cells is at the...
cell membrane and that it colocalizes with ZO-1. This pattern is similar to that of other cell types reported to express Bves.\textsuperscript{14}

**Cell-Cell Junction Localization of Bves-GFP Fusion Protein**

Because Bves colocalizes with ZO-1 in human TM cells, we postulated that Bves may play a role in regulating TJ formation in TM cells. In previous studies, the overexpression of chicken Bves with a FLAG tag in HCECs led to increased formation of TJs.\textsuperscript{14,15} Thus, Bves overexpression studies were carried out in NTM-5 cells using Bves-FLAG or Bves-GFP fusion protein constructs. Consistent with previous studies in human corneal epithelial cells, NTM-5 cells overexpressing Bves-FLAG (NTM-w) exhibited increased levels of Bves on Western blot analysis and a correspond-
Increased Tight Junction Formation in NTM-wBves Cells

There is increased prominence of immunofluorescence staining for endogenous ZO-1 in the NTM-wGFP cells (Fig. 3, red), which suggests that TJ formation may be increased. However, ZO-1 is not a precise indicator of TJ levels because of its association with adhesion junctions other than TJs. Alternatively, occludin is specific for TJs and is a good indicator of TJ levels. Western blot analyses were performed for ZO-1, cingulin, and occludin in NTM-5, NTM-GFP, and NTM-wGFP cells (Figs. 4A, B). We observed a significant doubling in the levels of ZO-1 and occludin protein in NTM cells with overexpression of Bves-GFP protein. We also detected a significant eightfold increase in cingulin protein in NTM-wGFP cells compared with NTM-5 cells, but there was not a significant change in junction protein levels in NTM-GFP control cells compared with NTM-5 cells. These biochemical findings indicate that increasing Bves expression in TM cells leads to increased expression of TJ proteins, including ZO-1, cingulin, and occludin.

Next, we evaluated the effects of Bves overexpression on TJ function by measuring the transcellular passage of small solutes and transcellular electrical resistance. The permeability of TM cells to fluorescein was determined using confluent cell cultures on porous tissue culture inserts. Overexpression of Bves in NTM-5 cells (Bves-wGFP) produced a significant decrease in the rate of transcellular passage of fluorescein compared with untransfected NTM-5 cells (Fig. 5A). There was not a significant difference in the permeability of NTM-GFP control cells compared with NTM-5 cells. Transcellular electrical resistance was also measured for confluent NTM cultures (Figs. 5, B). The transcellular electrical resistance of NTM-wGFP cells was 23 ohms-cm² compared with almost no measurable transcellular electrical resistance in either the NTM-5 or the control NTM-GFP cells. Increased Bves expression in NTM-5 cells leads to increased TJ function, as indicated by decreased permeability to fluorescein and increased transcellular electrical resistance.

Inhibition of RhoA Signaling in NTM-w Cells

Our findings indicate that Bves regulates TJ formation in TM cells consistent with previous studies in other cell types. Traditionally, TJs have been viewed as mechanical protein structures, playing a role in transcellular barrier formation and regulation of paracellular passage of fluids and solute. However, TJs are also viewed as regulatory complexes in cell signaling. The tight junction-associated protein cingulin has been shown to bind directly with the RhoA-specific guanine nucleotide exchange factor GEF-H1. As cells establish TJs, GEF-H1 is sequestered at the cell membrane. Because Bves appears to modulate TJ formation in TM cells, we performed immunofluorescence staining for GEF-H1 localization in TM cells overexpressing Bves.

In NTM-5 and NTM-GFP cells, GEF-H1 staining (Fig. 6, red) is seen primarily in the cytoplasm at the free edges of cells (Fig. 6, asterisk), not colocalizing with ZO-1 staining (green). The finding of GEF-H1 at free cell borders is similar to the original report identifying GEF-H1. In addition, in NTM-5 and NTM-GFP cells, ZO-1 staining is seen at cell-cell junctions (Fig. 6, arrows), as expected. When images of ZO-1 and GEF-H1 are merged, there is cell membrane colocalization of ZO-1 and GEF-H1 (yellow) only at sites of cell-cell contact. With overexpression of Bves, NTM-wGFP cells exhibit increased formation of cell-cell contacts, and this is associated with increased localization of ZO-1 and GEF-H1 at sites of cell-cell contact (Fig. 6). Interestingly, GEF-H1 localization is less prominent at free edges of cells overexpressing Bves. These findings suggest that with increased Bves expression, there is increased sequestration of GEF-H1 at TJs.
FRET efficiency of 1.1 significantly reduces RhoA activation.

These findings indicate that Bves regulates TJ formation in TM cells, with increased Bves leading to increased TJ formation (Fig. 5). This leads to the question regarding the role of Bves in adult ocular tissues. Evidence from this study using TM cells and previous studies using HCECs demonstrated that Bves regulates cell-cell contact formation in adult cells, specifically through the regulation of TJ formation. In both TM and HCECs, overexpression of Bves leads to increased TJ formation. It is also important to note that the fusion of GFP to Bves does not appear to interfere with the role of Bves in regulating TJ formation.

**DISCUSSION**

We hypothesize that Bves, by regulating TJ formation, may play a role in modulating RhoA signaling and the state of MLC phosphorylation in TM cells (Fig. 1). In this study, we have demonstrated the presence of endogenous Bves in TM tissues and cultured human TM cells (Fig. 2). Increased expression of Bves appears to lead to decreased levels of MLC-p (Fig. 8), which suggests that Bves plays a regulatory role in TM cells. Because overexpression of Bves leads to increased TJ formation in TM cells, this likely has a direct effect on the signaling role of TJs in TM cells. In this study, increased TJ formation was confirmed biochemically through the detection of increased levels of occludin message and protein (Fig. 4). In addition, cell culture-based assays for TJ function, transcellular passage of fluorescein, and electrical resistance further verified increased TJ formation with Bves overexpression in TM cells (Fig. 5). These findings indicate that Bves regulates TJ formation in TM cells, as was observed in human corneal epithelial cells. Because TJs have been shown to play a role in modulating RhoA activation, changes in the state of RhoA activation were evaluated in TM cells transiently overexpressing Bves. These cells exhibited decreased RhoA activation (Fig. 7). In addition, increased Bves expression is associated with decreased levels of MLC-p (Fig. 8), an end point target in the RhoA signaling pathway in TM. These findings indicate that Bves plays a role in regulating TJ formation in TM cells, with subsequent modulation of RhoA signaling.

Bves is a novel adhesion molecule originally isolated from a heart-specific subtraction screen. It was initially observed at the epithelial surface (epicardium) of the developing heart and was thought to be cardiac specific. Recent studies have expanded the role of Bves, and it is now thought to be involved in the development of a variety of organs including the eye. In addition, Bves appears to regulate cell-cell contact formation needed for coordination of cell migration in normal organogenesis. During the development of ocular structures, Bves localization is prominently seen at epithelial precursors of cornea, lens, and retina. The pattern of Bves localization follows the migration of these precursor cells, leading to the maturation of these ocular structures. Although we did not specifically examine Bves during the formation of the TM in our previous study, our current finding that Bves is present in adult TM tissues and TM cells led us to suspect that Bves may play a role in the embryonic formation of the TM.

**FIGURE 7.** Transient transfection of RhoA FRET probe in NTM cells led to FRET efficiency of 1.4 ± 0.2. Cells cotransfected with Bves and FRET (NTM+wBves) exhibit a FRET efficiency of 0.9 ± 0.6 (*P < 0.05, n = 6). NTM cells transfected with control vector and FRET exhibit a FRET efficiency of 1.1 ± 0.4, which is not significantly different from that of NTM-5 cells. This indicates that overexpression of Bves significantly reduces RhoA activation.

To determine whether these changes in GEF-H1 localization are associated with changes in the state of RhoA activation, FRET (fluorescence resonance energy transfer) probes were used to measure RhoA activation. NTM-5 cells were transiently transfected with RhoA-Raichu FRET plasmids or with RhoA-Raichu FRET plasmids plus Bves-FLAG or empty vector. The level of FRET activity directly correlates with the state of RhoA activation. Cells transiently overexpressing Bves exhibit a significant 35% decrease in FRET activity (*P < 0.05; Fig. 7), indicating that increased Bves expression leads to decreased levels of activated RhoA.

To further verify the state of RhoA activation, we evaluated levels of MLC-p, which is a downstream target of activated RhoA. It has been shown that decreased RhoA activity leads to decreased MLC-p. Western blot analyses indicated that NTM-5 cells overexpressing Bves (NTM-wGFP) exhibited decreased levels of MLC-p compared with NTM-5 cells after 6 days in culture (Fig. 8). MLC-p in NTM-GFP control cells was not significantly different from NTM-5 cells but was slightly increased. The observed decrease in MLC-p in Bves-overexpressing cells suggests that Bves modulates TJ-associated signaling, with increased Bves leading to decreased RhoA signaling and decreased MLC-p levels.

**FIGURE 8.** Quantification of Western blots dual stained for MLC-p and total MLC2 indicate that NTM cells overexpressing Bves (NTM-wGFP) have a lower percentage of phosphorylated myosin than do NTM-5 and NTM-GFP control cells (*P < 0.05).
Regulatory Effects of Bves on Tight Junction Formation

There is indirect evidence, in vivo, that Bves also plays a role in regulating TJ formation. In our previous study, immunofluorescence staining of the cornea suggested that the level of Bves parallels the levels of TJs. In the basal layer of cornea, Bves is seen in a speckled pattern outlining the borders of adjacent basal cells. The staining of Bves becomes more prominent and continuous at cell membranes in upper layers of the corneal epithelium, where there is increased TJ formation. There is also a gradient in the number and complexity of TJs along the conventional aqueous outflow tract. The lowest number of TJs and the least complex TJs are at the inner TM, and they increase toward the walls of Schlemm’s canal. Although our data in this study indicate that Bves is present in TM tissues and in primary TM cells, we cannot say whether the level of Bves follows the gradient of TJs in the TM. However, our cell culture studies indicate that Bves plays a role in regulating TJ formation in TM cells.

We have demonstrated in this study that the overexpression of Bves in TM cells (NTM-5) leads to increased TJ formation. The capacity for increased TJ formation does not appear to be isolated to the NTM-5 cell line. Findings by Alvarado et al. suggest that cultured primary human TM cells are also capable of increased TJ formation. In their studies, cultured primary human TM cells exhibited increased resistance to transcellular fluid movement when treated with glucocorticoids. They also demonstrated that levels of ZO-1, a TJ-associated membrane protein, increase with glucocorticoid treatment. ZO-1 is not a definitive marker for TJs, but their findings of increased ZO-1 and increased resistance to transcellular fluid movement are highly suggestive of increased TJ formation by primary TM cells. Given that endogenous Bves is observed in both primary and NTM-5 cells and that both TM cell types exhibit the capacity to modulate TJ formation in cell culture conditions, it would be reasonable to postulate that Bves plays a similar role in both primary TM cells and NTM-5 cells, a transformed human TM cell line.

Traditionally, TJs are viewed as multiprotein adhesion complexes important in the formation of transcellular barrier by limiting paracellular movement of fluid and small molecules. However, cells at the inner TM exhibit short, isolated stands of TJs that do not completely encircle the cell. Subsequently, TJs between TM cells are not significant barriers to paracellular flow. This raises the question of the role of the TJ strands within the trabecular meshwork. The findings in this study are consistent with the model proposed by Balda and Matter that TJ formation is the result of increased sequestration of GEF-H1 at the tight junction. The interplay between cell-cell adhesion and RhoA signaling. The capacity for increased TJ formation does not appear to be isolated to the NTM-5 cell line. Findings by Alvarado et al. suggest that cultured primary human TM cells are also capable of increased TJ formation. In their studies, cultured primary human TM cells exhibited increased resistance to transcellular fluid movement when treated with glucocorticoids. They also demonstrated that levels of ZO-1, a TJ-associated membrane protein, increase with glucocorticoid treatment. ZO-1 is not a definitive marker for TJs, but their findings of increased ZO-1 and increased resistance to transcellular fluid movement are highly suggestive of increased TJ formation by primary TM cells. Given that endogenous Bves is observed in both primary and NTM-5 cells and that both TM cell types exhibit the capacity to modulate TJ formation in cell culture conditions, it would be reasonable to postulate that Bves plays a similar role in both primary TM cells and NTM-5 cells, a transformed human TM cell line.

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