Septin-2 Mediates Airway Epithelial Barrier Function in Physiologic and Pathologic Conditions

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Epithelial cells have the ability to regulate paracellular permeability dynamically in response to extracellular stimuli. With every respiratory effort, airway epithelial cells are exposed to both physiologic as well as pathologic stimuli, and regulation of the epithelial barrier in response to these stimuli is crucial to respiratory function. We report that increased membrane septin-2 localization mediates decreases in paracellular permeability by altering cortical actin arrangement in human airway epithelial cells. This phenomenon occurs in response to both physiologic levels of shear stress and a pathologic stimulus, particular matter exposure. The resulting changes in barrier function in response to septin-2 redistribution have a significant impact on the ability of the apical ligand, epidermal growth factor, to interact with its receptor, epidermal growth factor receptor, which is segregated to the basolateral side in airway epithelial cells. This suggests that the dynamic regulation of the epithelial barrier function is essential in regulating signaling responses to extracellular stimuli. These findings indicate that septin-2 plays a fundamental role in regulating barrier function by altering cortical actin expression.

Keywords: epithelial barrier function; paracellular permeability; septin-2; actin cytoskeleton

Airway epithelial cells form an important barrier that protects the subepithelial tissue from a wide array of noxious substances, including allergens, viruses, inhaled particles and irritants, and luminal microbial pathogens. The regulation of this epithelial barrier depends on a complex of proteins that compose distinct intercellular junctions, tight junctions, adherens junctions, and desmosomes, each of which exhibits cell type–specific regulation by various growth factors, agonists, and second messengers (1). In response to both physiologic and pathologic stimuli in the lumen, a coordinated epithelial response is necessary to regulate barrier function appropriately. Common to the regulation of the intercellular junctions is cortical actin. Modulation of cortical actin alters permeability in multiple cell types, from intestinal epithelial cell lines (2–4) to pulmonary endothelial cells (5, 6), and is intimately associated with regulation of the intercellular junctions (7).

Septin-2 is a member of a highly conserved GTPase family found in fungi and animals. Septins have been implicated in diverse cellular processes, including cytokinesis, formation of diffusion barriers, and vesicle trafficking. In yeast cells, septin-2 is responsible for separating the mother cell contents before division (8). In humans, 12 septin genes have been found so far, many of which also undergo alternative splicing to generate dozens of polypeptides. Septin-2 partially colocalizes with actin bundles in mammalian interphase, and is required for reorganization of the actin cytoskeleton in migrating or ruffling cells (9). Just recently, a new role for septin-2 has been identified in *Xenopus* oocytes where inhibition of septin-2 with forchlorfenuron (FCF) leads to defective gastrulation, and potential changes in cell shape (10, 11). It has been identified, in association with the protein, Fritz, to be involved in collective cell movement and ciliogenesis (10). However, in differentiated monolayers that are no longer dividing, the role of septin-2 has not been elucidated.

We have previously shown that, in response to physiologic levels of apical shear stress, the paracellular permeability of airway epithelial cells decreases, reflecting barrier enhancement. This barrier enhancement is associated with reorganization of the actin cytoskeleton with increased cortical actin ring (12). However, the molecular mechanisms mediating this cytoskeletal rearrangement are not known. In this study, we show that, in response to both low levels of shear stress and a pathologic stimulus, such as a single dose of particulate matter (PM) (13), the epithelial barrier is enhanced and septin-2 moves to the plasma membrane. In response to shear stress, this septin-2 redistribution is associated with increased septin-2–actin interactions and actin rearrangement, which is required to cause enhancement of the barrier in response to luminal stimuli.

CLINICAL RELEVANCE

This article shows a novel role for septin-2 in airway epithelial barrier function in response to both a physiologic level of shear stress as well as a pathologic exposure of particulate matter. In addition, it introduces the idea that in response to luminal stimuli, the airway epithelial barrier function dynamically changes and that can translate to changes in receptor–ligand interactions and resultant cell signaling.

MATERIALS AND METHODS

Materials

Antibody to septin-2 was a gift from Ian Macara (University of Virginia, Charlottesville, VA). Unless specified, all other reagents were purchased from Sigma (St. Louis, MO).

Cell Culture

Primary human bronchial epithelial (NHBE) cells (Lonza, Walkersville, MD) were grown on collagen-coated inserts (Falcon, Franklin Lakes, NJ) at 37°C with 5% CO_2 and maintained at an air–liquid interface for

(*Received in original form June 7, 2010 and in final form September 10, 2010*)

This work was supported by the Flight Attendant's Medical Research Institution Young Investigator Award and K08HL085763 (V.K.S.) and the Johns Hopkins Bayview Scholars Program (L.S.K.).

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Cell Mol Biol Vol 45. pp 120–126, 2011
Originally Published in Press as DOI: 10.1165/rcmb.2010-0235OC on September 24, 2010
Internet address: www.atsjournals.org

6–9 weeks before study. The transepithelial resistance was greater than 400 ohms when cells were used. Madin Darby Canine Kidney cells with and without yellow fluorescent protein septin-2 plasmid (gift of Elias Spiliotis, Drexel University, Philadelphia, PA) were cultured on inserts (transepithelial resistance $>$ 240 ohms). Human pulmonary artery endothelial cells (HPAECs; Lonza) were grown to confluence in gold microelectrodes containing polycarbonate wells (transendothelial electrical resistance [TER] $>$ 1,000), and TER was measured as previously described (14).

Cells were harvested and lysed in RIPA buffer (12). In certain experiments, membrane preparations were performed as previously described (12). Equal amounts of total protein (10–20 μg) in 1.5% (wt/vol) SDS were loaded per lane, as previously described (15).

Transfection

Cells were grown in chamber slides to 50–60% confluence and transiently transfected (1 μg/well) with YFP–septin-2 cDNA using FuGENE 6 (1.5 μl; Roche, Indianapolis, IN) or a control plasmid, according to the manufacturer's recommendations.

Confocal Imaging

Confocal laser microscopy (Leica SP5; Bannockburn, IL) was performed on cells grown on inserts using antibodies against septin-2 or actin and appropriate secondary antibodies (Alexa 488 or Alexa 555; Molecular Probes, Carlsbad, CA). Fluorescence resonance energy transfer (FRET) with acceptor photobleaching (AB) was performed on fixed samples. The donor fluorescence intensity in a region of interest before and after AB was compared. Positive FRET occurred if AB resulted in increased donor fluorescence, as described in the online supplement.

Immunoprecipitation

The crosslinking and immunoprecipitation (IP) was performed as previously described (16), where 2 mM Dithiobis[succinimidyl propionate] in PBS/25 mM Hepes were added to static and shear-exposed monolayers for 30 minutes at room temperature, after which the reaction was quenched with 20 mM Tris (pH 7.5) for 15 minutes. Co-IP of septin-2 and actin was performed, and the samples were analyzed by immunoblotting.

Lentiviral Transduction

Lentivirus expressing either short hairpin RNA directed against septin-2 or a nontargeting control (Sigma) (10^7 infectious particles/ml) was used at the lowest concentration required for protein knockdown. Cells were incubated with polybrene (8–16 μM) in 1 ml per insert. Apical media were removed, basal media changed, and cells were used 48–72 hours later.

Shear Stress

Fluid flow to generate shear levels of 1.5–3.0 dynes/cm^2 was applied as described previously (12). Fluid flow rates of 0.5–1.0 ml/min provide a shear stress consistent with this magnitude for airway epithelial cells *in vivo*.

Permeability Assay

Paracellular permeability of cells was assessed by the passage of 4-kD FITC-dextran across the monolayer, as previously described (12). The concentration was measured by fluorometry.

PM

Ambient Baltimore PM was collected using a high-volume cyclone collector with a cutoff point of 0.3-μm aerodynamic diameter when operated at a flow rate of 1 m^3/min. This has been used to collect Baltimore PM for studies of air pollution impact on airway hyperresponsiveness, and the PM has been well characterized, as previously described (17, 18).

Statistical Analysis

Statistical analysis was performed using STATA 9 (Stata Corporation, College Station, TX).

RESULTS

Airway Epithelial Barrier Enhancement Is Associated with Altered Septin-2 Expression

We previously demonstrated that low levels of shear stress lead to increases in airway epithelial barrier function, using both *in vivo* and *in vitro* models. Although the *in vitro* models have used only unidirectional shear stress, we have evidence of similar findings *in vivo* using both unidirectional (12) and bidirectional flow (unpublished data, V.S.). To dissect potential mechanisms mediating the shear-induced barrier enhancement that we have described (12), membrane preparations of both NHBE cells and 16HBE+adenoAQP5 cells under static and shear conditions were sent to the Johns Hopkins National Heart, Lung, and Blood Institute Proteomics Center for processing. In both of these cell types, we have shown that shear enhances barrier function. Although both shear and static conditions altered over 100 proteins, a comparison of the two conditions revealed only a limited number of proteins that exhibited consistent changes in membrane fraction abundance, one of which was septin-2. With enhanced barrier function, septin-2 increased in the membrane fraction. To confirm that shear increases in membrane expression of septin-2, immunoblotting of membrane fractions (Figure 1A) and immunofluorescence (Figure 1B) of NHBE cells were performed. Both revealed increased membrane expression of septin-2 in response to shear stress. Of note, there was no change in total septin-2 expression in the cells (Figure 1A), and the increase in membrane fraction was due to protein redistribution.

Septin-2 Alters Barrier Function in a Confluent Monolayer

To identify a potential role for septin-2 in regulation of barrier function, we used specific lentiviral shRNA to knock down endogenous septin-2 expression in MDCK cells (Figure 2A). Under static conditions, barrier function was not altered by septin-2 knockdown. In contrast to NHBE cells, shear stress increased paracellular permeability in MDCK cells (Figure 2B). To determine if septin-2 affects barrier function in MDCK cells, we infected MDCK cells with shRNA against septin-2 or control lentivirus. When septin-2 was knocked down, shear induced an even greater increase in paracellular permeability (Figure 2B). To test if overexpression of septin-2 enhanced barrier function, MDCK cells were transiently transfected with YFP–septin-2 (~40% transfection efficiency). After transient transfection, the majority of YFP–septin-2 was present on the membrane (Figure 2C, right). Cells expressing YFP–septin-2 had decreased paracellular permeability compared with cells transfected with a control plasmid (Figure 2C, left).

To assess if changes in septin-2 expression altered airway epithelial barrier function, NHBE cells were transduced with septin-2 shRNA or a nontargeting shRNA (Figure 3A). Under static conditions, expression of septin-2 shRNA or the nontargeting shRNA had no effect on barrier function. However, after exposure to shear stress, NHBE cells with septin-2 knockdown no longer exhibited shear-induced barrier enhancement; rather, paracellular permeability was significantly increased (Figure 3B).

To determine if septin-2 regulation of epithelial barrier function was epithelial cell specific, we assessed the effect of septin-2 on pulmonary endothelial barrier function. The role of cytoskeletal organization in pulmonary endothelial barrier function has been investigated extensively (14, 19–33). We measured TER as a reflection of endothelial monolayer permeability in control HPAECs compared with HPAECs treated with FCF (50 μm), which alters septin-2 assembly and organization

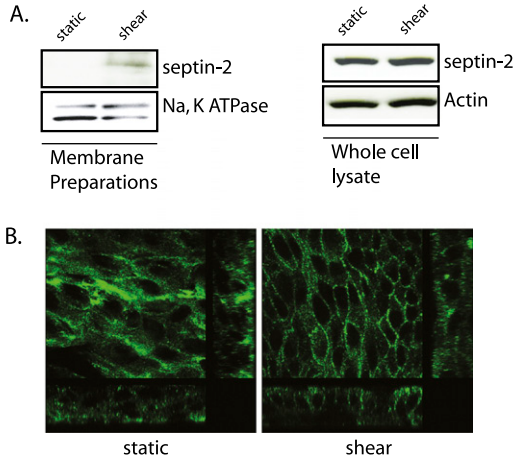

Figure 1. Shear stress leads to increased membrane-associated Septin-2 in primary human bronchial epithelial (NHBE) cells. (*A*) NHBE cells were placed under shear stress, as described in MATERIALS AND METHODS, for 2 hours and membrane preparations were compared with control cells under static condition. Immunoblotting shows that shear stress increases the membrane fraction of septin-2 in NHBE cells (*left*), whereas there is no change in septin-2 abundance in the whole-cell lysate (47). (*B*) Confirmation of shear-induced increases in membrane septin-2 expression is visualized using confocal immunofluorescence.

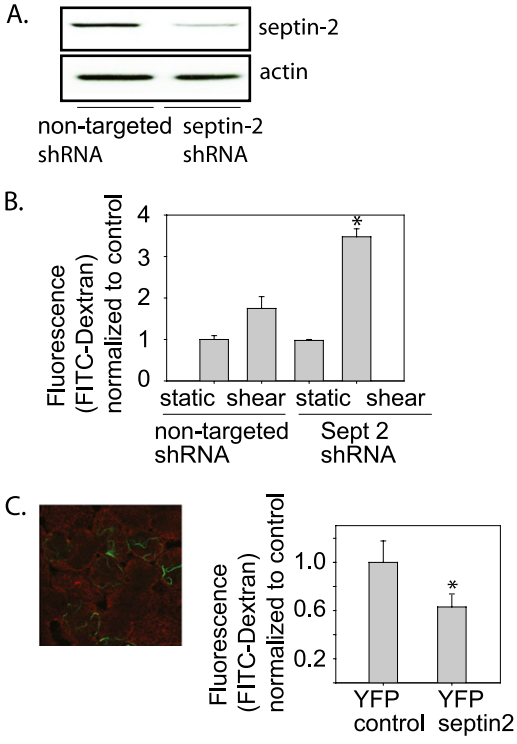

Figure 2. Septin-2 modulates barrier function properties in an Madin Darby Canine Kidney monolayer. (*A*) Using lentiviral delivery of short hairpin RNA directed against Septin-2, we have caused greater than 80% reduction in protein expression, as confirmed by immunoblotting. (*B*) After septin-2 knockdown, there is no significant change in barrier properties under static conditions, but when cells are placed under shear stress, there is a significant worsening in barrier function compared with cells transduced with a nontargeting construct. (*C*) (*Left*) Of note, when overexpressed, the majority of septin-2 is seen at the membrane (the membrane is shown in *red*, and yellow fluorescent protein–septin-2 is shown in *green*); (*right*) transient transfection of overexpression of yellow fluorescent protein–septin-2 in MDCK cells leads to barrier enhancement ($n = 3$; $^*P < 0.05$ with one-way ANOVA).

in vitro by stabilizing septin filaments (34). Treatment with FCF increased paracellular permeability of both MDCK and NHBE cells under shear stress (Figures 4A and 4B), similar to the effects of septin-2 knockdown on barrier function. Treatment with the septin-2 inhibitor, FCF, caused endothelial barrier dysfunction (Figure 4C).

Septin-2 Directly Binds to Actin and Mediates Cortical Actin Reorganization

Reduced paracellular permeability can result from cytoskeletal reorganization, and septin-2 expression is involved in actin rearrangements (9). We have shown that shear stress leads to actin rearrangement, contributing to the shear-induced barrier enhancement seen in NHBE cells (12). To determine if direct protein binding was responsible for the increased cortical actin observed in response to shear stress, we assessed for interaction between actin and septin-2. IP of actin under shear conditions also pulled down more septin-2 than observed in static conditions (Figure 5A). Similarly, IP of septin-2 pulled down more actin in shear than in static conditions (Figure 5A). To confirm protein–protein interaction further, we performed FRET AB in fixed static and shear samples probed for actin (Alexa488) and septin-2 (Alexa 555) to determine if the two proteins were spatially located close to one another under either condition. Excitation of one fluorophore results in excitation in the other, as long as the two fluorophores are in nanometer distance of each other, suggesting protein–protein interaction. In both static and shear conditions, the immunofluorescence of actin depicted corresponds specifically to the area that received photobleaching. Under static conditions after photobleaching, there was no increase in donor intensity (Figure 5B, left). However, after shear stress, AB resulted in increased donor intensity (Figure 5B, right). FRET maps were generated to visualize the interaction, with orange-red suggesting increased FRET, and blue with less FRET. After shear stress, there is a significant increase in the FRET sample. A total of 15 regions

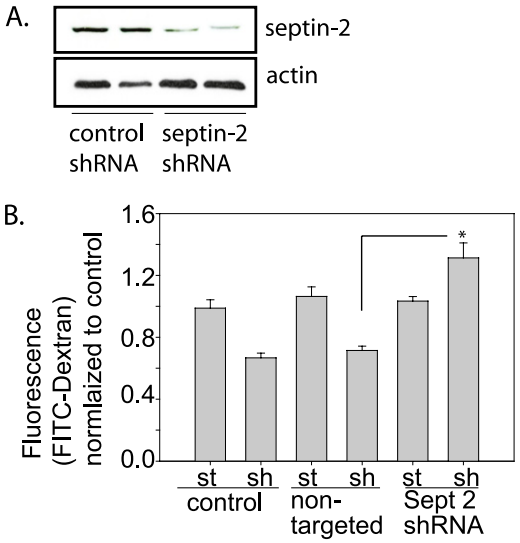

Figure 3. Septin-2 modulates barrier function properties in NHBE cells. (*A*) NHBE cells treated with lentiviral shRNA directed against septin-2 have more than 85% reduction in protein expression, as confirmed by immunoblotting. (*B*) After septin-2 knockdown, again there is no significant change in barrier properties under static conditions. In NHBE cells, shear leads to barrier enhancement, which is preserved in cells transduced with the nontargeting lentiviral construct, but exposure to septin-2 shRNA causes significant barrier disruption. (Sept, septin; sh, shear; st, static; $n = 4$; $^*P < 0.05$ with one-way ANOVA).

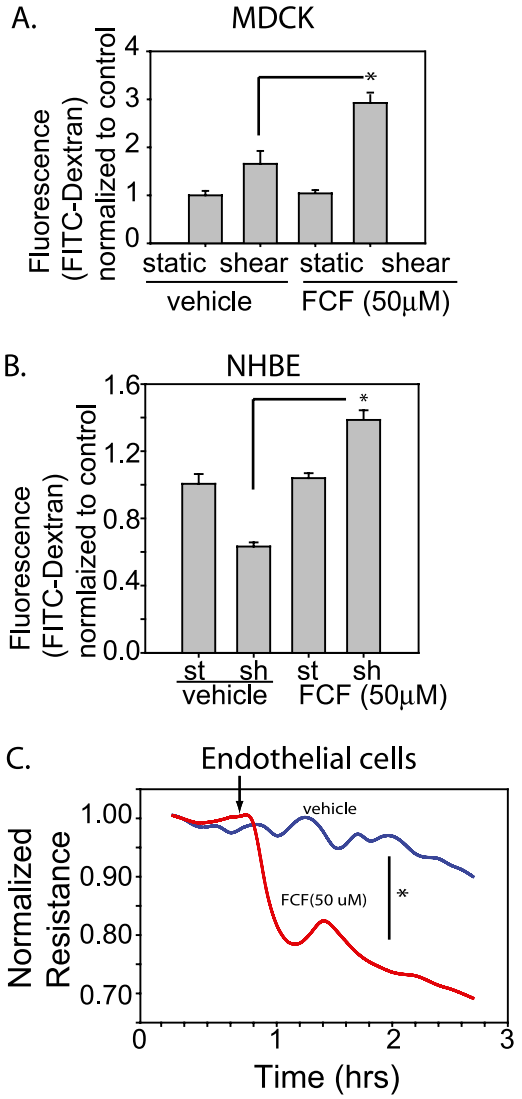

Figure 4. Treatment with the septin-2 inhibitor, forchlorfenuron (FCF), decreases barrier function. (*A*) MDCKs treated with FCF have increased permeability under shear conditions compared with vehicle-treated cells ($n = 3$). (*B*) NHBE cells also show evidence of increased permeability under shear conditions in FCF-treated cells ($n = 4$). (*C*) Human pulmonary artery endothelial cells (HPAECs) treated with FCF have a significant drop in resistance compared with vehicle control. *Arrow* indicates the addition of either the drug or vehicle to the cells ($n = 5$). *P < 0.05.

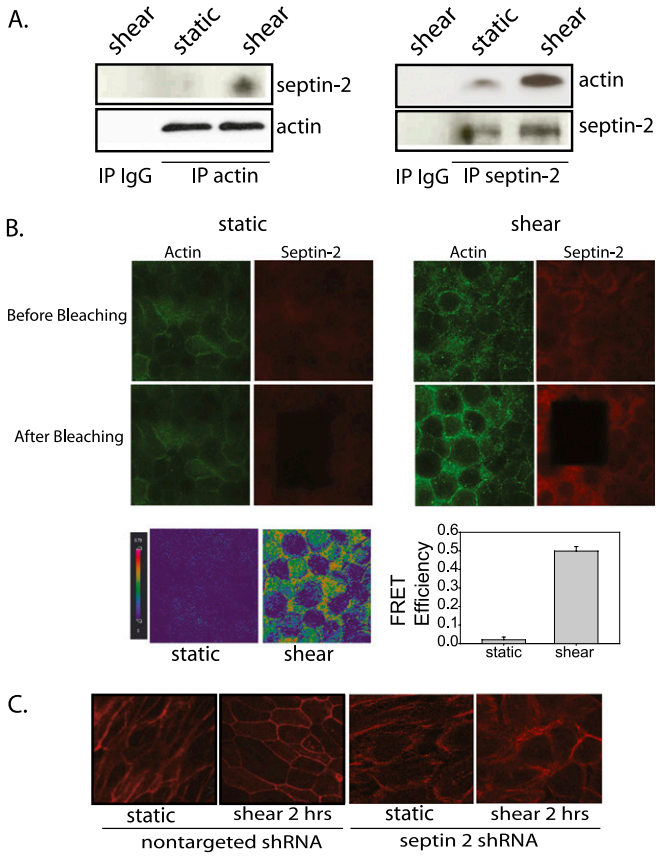

Figure 5. Septin-2 directly binds to actin under shear conditions, and is required for actin rearrangements in NHBE cells. (*A*) With immunoprecipitation (IP) of actin, there is increased pulldown of septin-2 under shear conditions (46). Similarly, immunoprecipitating septin-2 results in increased pulldown of actin under shear conditions compared with static (*left*). (*B*) Fluorescence resonance energy transfer (FRET) acceptor photobleaching (AB) was performed on fixed NHBE monolayers after exposure to static or shear condition. The areas of actin immunofluorescence depicted specifically correlates to the area that received photobleaching. Under static condition after AB (*red*), there is minimal change in the donor intensity (47). A FRET map (*bottom panel*) suggests minimal FRET efficiency. However, under shear conditions, there is an increase in donor intensity (47) after AB (*red*), and the FRET map suggests significant FRET efficiency on the cell boundaries. FRET efficiency was quantified in 15 preselected regions of interest in both static and shear cells with a significant increase in FRET AB between actin and septin-2 after exposure to shear stress ($P < 0.05$, one-way ANOVA; $n = 3$). (*C*) NHBE cells under specified conditions were fixed, permeabilized, and imaged using confocal immunofluorescence. Shear stress leads to actin rearrangement in NHBE cells. In cells treated with lentiviral septin-2 shRNA, there is a change in actin arrangement even under static conditions (*third panel from left*). In response to shear stress, cortical actin (*first and second panels from left*) is less well formed (*fourth panel from left*).

of interest was randomly preselected in both fields to quantify FRET efficiency. After shear stress, there was a significant increase in FRET efficiency, suggesting actin–septin-2 interaction under shear stress. Both biochemical methods of IP, as well as the fluorescent method of FRET, indicate that, in response to shear stress, there is a significant increase in the interaction of actin and septin-2, and the FRET AB data suggest that this is a direct interaction between the two proteins, and is unlikely to be via interaction with a third protein.

To determine if this interaction was necessary for the shear-induced actin reorganization, we examined the effect of septin-2 knockdown on cortical actin expression. After knockdown of septin-2, there was a decrease in shear-induced cortical actin (Figure 5C). Of note, changes in actin arrangement due to septin-2 knockdown were only associated with altered barrier function when the cells were exposed to a second stressor, such as shear, and not under static control conditions. When exposed to shear, however, the actin rearrangements that were evident in control cells and are associated with barrier enhancement were not seen in the cells treated with septin-2 shRNA (Figure 5C). In these cells, shear stress significantly increased paracellular permeability, as described previously here.

Altered Epithelial Barrier Function Changes Cell Signaling

Humlicek and colleagues (35) have described polarized epidermal growth factor (EGF) signaling in airway epithelial cells that

is affected by altered paracellular permeability in the context of a wound model or neutralizing antibody to E-cadherin. However, the effect of altering paracellular permeability on compartmentalization of signals in the context of a physiologic stress, or a less overt pathologic insult, is not known. To examine the functional consequences of altered epithelial paracellular permeability on cell signaling, we stimulated NHBE cells transduced with either septin-2 shRNA or a nontargeting shRNA with exogenous EGF on either the apical or basolateral surface. EGF receptor (EGFR) is localized to the basolateral surface of NHBE cells, and the application of shear stress in cells treated with a nontargeting shRNA or septin-2 shRNA does not change this distribution (Figure 6A). Without the addition of EGF ligand to the apical surface, no extracellular signal–regulated kinase (ERK) phosphorylation was observed in NHBE cells. Under static conditions, the addition of EGF to the basolateral chamber increased ERK phosphorylation both in cells transduced with septin-2 shRNA and a nontargeting control (Figure 6B). When control NHBE cells were exposed to shear, barrier function was enhanced, and the addition of apical EGF caused very little ERK phosphorylation; again, basolateral addition of EGF significantly increased ERK phosphorylation. In contrast, after septin-2 knockdown, epithelial barrier function decreased, and addition of EGF to either apical or basolateral surfaces produced high levels of ERK phosphorylation, indicating that the increased permeability resulting from septin-2 knockdown in sheared cells allows for an apically placed EGF to have increased access to the basolateral EGFR. Changes in ERK phosphorylation are quantified by densitometry in Figure 6C. To confirm that increased ERK phosphorylation was due to increased EGFR activation, we looked at the effects of the addition of EGF in cells under shear stress. After septin-2 knockdown, there was increased EGFR phosphorylation after apical exposure of EGF compared with cells treated with a nontargeting control (Figure 6B, right), confirming that the barrier disruption in sheared cells after septin-2 knockdown allowed for enhanced interaction between the apically placed EGF ligand and the basally positioned receptor.

PM Effects on Barrier Are Mediated by Septin-2

PM is known to produce airway inflammation and respiratory disease, as well as cardiovascular disease (13). PM has been well characterized, and has been reported to induce airway hyperresponsiveness and inflammation in mice (36), and induces a mucosal immune response in airway epithelial cells (37). In endothelial cells, a single exposure of PM has been reported to lead to barrier disruption (17, 38). In contrast, a single dose of PM (150 μg/ml) caused barrier enhancement (decreased paracellular permeability) in NHBE cells (Figure 7A). The initial barrier enhancement of NHBE cells in response to a single dose of PM is associated with increased membrane localization of septin-2, and a corresponding increase in cortical actin. A second exposure of NHBE cells to PM reduced membrane septin-2, decreased cortical actin, and increased paracellular permeability, suggesting that regulation of septin-2 could be responsible for pathologic changes in barrier function in airway epithelial cells after exposure to PM (Figure 7B). The effect of septin-2 on cortical actin was at least relatively specific, as B-catenin labeling in the membrane was not altered, also indicating that the cells were not overtly disrupted after multiple PM exposures (Figure 7B). To assess the role of septin-2 in PM-mediated barrier enhancement, NHBE cells treated with lentiviral septin-2 shRNA or a control nontargeted virus were exposed to a single dose of PM. A single dose of PM caused barrier enhancement in control cells expressing nontargeted virus. In contrast, after knockdown of septin-2, a single treatment of PM caused barrier disruption (Figure 7C), again indicating that septin-2 plays a necessary role in PM-induced barrier enhancement.

DISCUSSION

The airway epithelium senses both physiologic and pathologic stimuli in the luminal airstream, and dynamic responses to these exposures are part of the host defense system. The epithelial barrier is the first line of defense preventing access of inhaled particles to subepithelial tissues (39–42). In addition, the epithelium segregates the apical and basal compartments (35, 43),

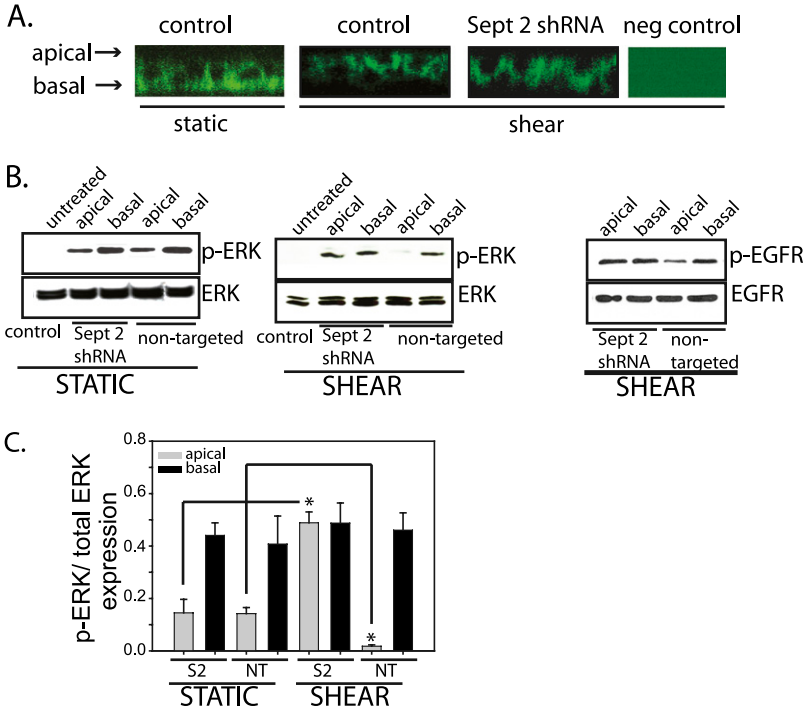

Figure 6. Epithelial barrier modulates cell signaling. (*A*) Epidermal growth factor (EGF) receptor (EGFR) is localized to the basolateral membrane of NHBE cells, and the application of shear stress or the knockdown of septin-2 does not alter its localization. (*B*) Under static conditions, placement of EGF in the basolateral chamber leads to more phosphorylated–extracellular signal–regulated kinase (ERK) than when placed on the apical membrane. Knockdown of septin-2 does not alter this. (*Left*) Under shear conditions, placement of EGF in either the apical or basolateral chamber leads to similar p-ERK after septin-2 knockdown (*middle*), as well as similar p-EGFR (46). However, in cells treated with a nontargeting construct, there is significantly less p-ERK (*middle*), as well as p-EGFR (46), in cells treated with apical EGF compared with cells treated with basolateral EGF. (*C*) Bar graph of densitometric analysis from three separate experiments of ERK phosphorylation (S2, septin 2 shRNA; NT, nontargeting shRNA; *shaded bars*, apically placed ligand; *closed bars*, basolaterally placed ligand; $n = 3$; $^*P < 0.05$ with one-way ANOVA).

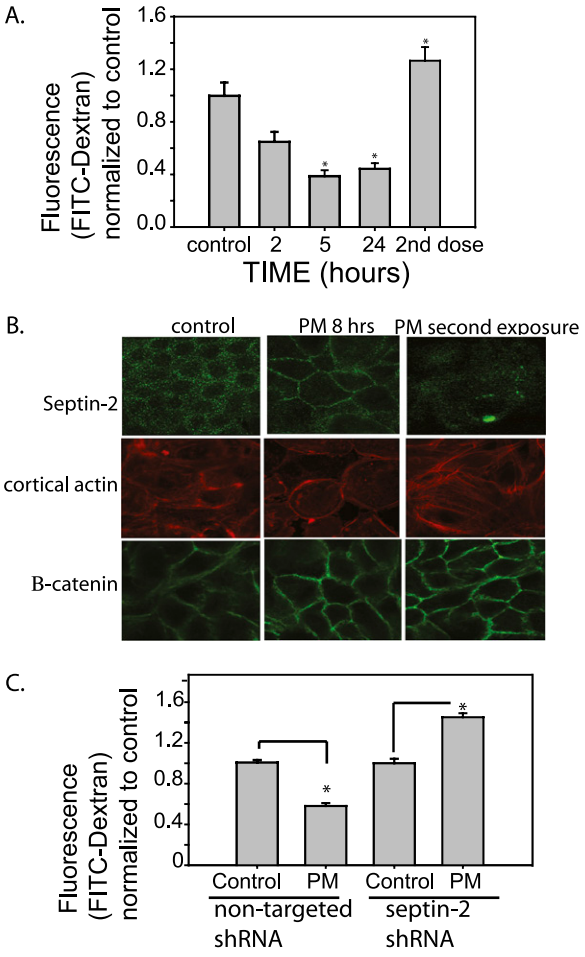

Figure 7. Particulate matter (PM) exposure alters NHBE barrier function and septin-2 translocation. (*A*) Single exposure of 8 hours to PM placed on the apical surface of NHBE cells causes barrier enhancement in NHBE cells, whereas a second treatment (8-h exposure, followed by washes and no treatment overnight, with a second 8-h exposure the following day) leads to barrier disruption ($n = 3$; $^*P < 0.05$ with one-way ANOVA). (*B*) Initial exposure to PM causes increases in septin-2 translocation to the membrane, as well as increased cortical actin. After a second exposure, very little membrane septin-2 is visualized, associated with a change in cortical actin organization. As a comparison, B-catenin is not altered by PM exposure with intact adherens junctions. (*C*) In cells transduced with septin-2 shRNA, there is no barrier enhancement seen after a single exposure to PM ($n = 3$; $^*P < 0.05$ with one-way ANOVA).

so that regulation of epithelial barrier properties can influence cell signaling (35). As an example, the EGFR, located on the basolateral membrane and the ligand, on the apical membrane, are spatially separated by the epithelial barrier. Overt disruption of the barrier by creation of a wound or treatment with antibodies that inhibit cell–cell contact eliminates the normal separation between receptor and ligand, allowing increased binding and activation of the EGFR (44). We propose that dynamic regulation of the epithelial barrier in response to luminal stimuli may be an integral part of normal epithelial function.

Although a potential role for septin-2 regulation of barrier function has been hypothesized since its discovery in separating the mother–daughter contents in yeast (8), to our knowledge, this is the first description of septin-2 regulating monolayer barrier properties, both in epithelial cells and in endothelial cells. Septin-2 regulates the actin cytoskeleton and cellular morphology in HeLa cells, where septin-2 depletion significantly alters stress fiber orientation and leads to disordered clusters (45). We find that knockdown of septin-2 in the absence of other exposures does not alter barrier function. However, in response to shear stress, there is increased interaction between actin and septin-2 that regulates the rearrangement of cortical actin, and decreases paracellular permeability of NHBE cells. Pharmacologic intervention to inhibit septin-2 function similarly alters regulation of barrier function in HPAECs, suggesting a more general role for septin-2 in cytoskeletal remodeling and barrier regulation.

We have previously shown that shear generated by airflow over the epithelium is a homeostatic mechanism that enhances the epithelial barrier (12). Constituents of the airstream can also provoke epithelial responses, including transient and dynamic change in barrier function. We found that an initial exposure to PM enhances the barrier function, whereas additional exposure causes barrier disruption. This potentially is a protective mechanism to prevent access of the PM to the vasculature, where it has known inflammatory effects (17, 38). In our model, this barrier enhancement was not maintained after additional doses of PM. This could be a maladaptive response, allowing exposure of subepithelial tissues to PM, but could also mediate altered cell signaling, as increased permeability would increase receptor–ligand access across the epithelium. Although our study focused on EGF ligand–receptor separation, Humlicek and colleagues (35) described that the IFN-α, -β, and -γ, as well as IL-4 ligands and receptors are all also spatially separated. Increases in permeability resulting from multiple exposures to PM (or other luminal stimuli) could facilitate generation of inflammatory signals. Dynamic regulation of the epithelial barrier in response to luminal stimuli may play a key role in regulating host defenses, and our data indicate that septin-2 localization is important in this barrier regulation.

Collectively these studies suggest that septin-2 localization and its interaction with actin allows for dynamic modulation of airway epithelial barrier function in response to both physiologic and pathologic luminal stimuli.

Author Disclosure: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgments: The authors thank Dr. Rachel Damico and Dr. Mahendra Damarla for the HPAECs, and for their technical assistance with ECIS (electrical cell substrate impedance sensing). In addition, they thank the Johns Hopkins National Heart, Lung, and Blood Institute Proteomics Center for technical assistance.

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