MUC1 Mucin Inhibits Airway Epithelial Barrier Formation Through a β-Catenin Dependent Mechanism

Erik P. Lillehoj[1], Whitney Cabrera[1], and K. Chul Kim[2]

[1]University of Maryland School of Medicine, Baltimore, MD; [2]Lovelace Respiratory Research Institute, Albuquerque, NM

Abstract

Rationale: The cytoplasmic region of MUC1 mucin (Muc1 in nonhumans) binds to β-catenin, a cytosolic protein that also interacts with E-cadherin in the formation of intercellular adherens junctions that help to maintain the integrity of epithelial barriers (1, 2). This study was designed to test the hypothesis that MUC1/Muc1 competes with E-cadherin for binding to β-catenin, thus reducing epithelial barrier formation.

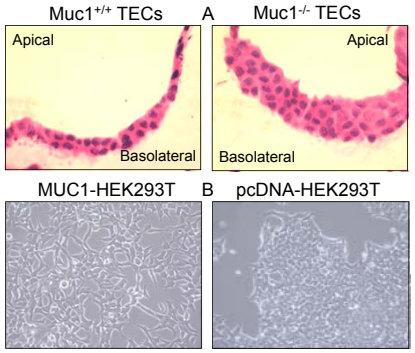
Methods: Primary cultures of tracheal epithelial cells (TECs) from Muc1$^{+/+}$ and Muc1$^{-/-}$ mice, and human airway epithelial cells (HAECs) transfected with a MUC1 siRNA or control RNA, were cultured on Transwell membrane supports as described (3) and analyzed for transepithelial electrical resistance (TER) and transepithelial flux of a fluorescently-tagged tracer protein (FITC-BSA) across cell monolayers. Finally, HAECs were treated with *Pseudomonas aeruginosa* flagellin to stimulate MUC1 phosphorylation and MUC1/β-catenin and E-cadherin/β-catenin coimmuno-precipitation (coIP), and TER were measured.

Results: Muc1/MUC1 expressing cells exhibited reduced intracellular contact formation compared with nonexpressing cells (Figure 1). Primary cultures of TECs from Muc1$^{-/-}$ mice displayed increased TER compared with Muc1$^{+/+}$ TECs (Figure 2). Transfection of HAECs with MUC1 siRNA significantly increased TER and decreased FITC-BSA flux compared with a non-targeting control RNA (Figure 3). HAECs treated with *Pseudomonas aeruginosa* flagellin to stimulate phosphorylation of MUC1 exhibited increased MUC1/β-catenin coIP, decreased E-cadherin/β-catenin coIP, and decreased TER compared with control-treated cells (Figure 4).

Conclusions: We conclude that MUC1 competes with E-cadherin for binding to β-catenin thereby disrupting adherens junctions thereby reducing airway epithelial barrier formation.

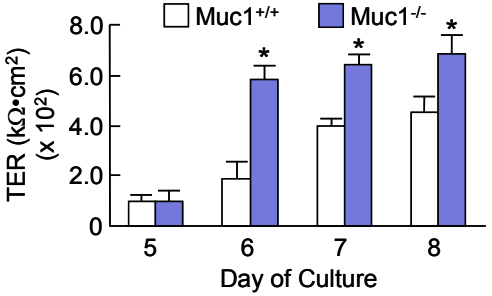
Results

Figure 1. Expression of Muc1/MUC1 Decreases Intercellular Contact Formation



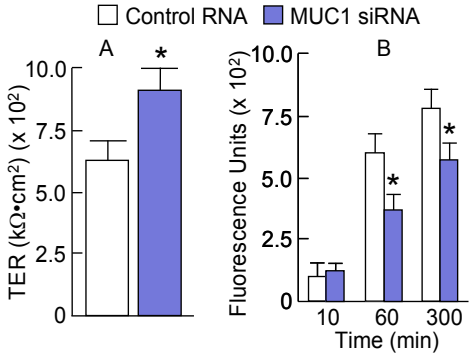
(A) Equal numbers of primary TECs from Muc1$^{+/+}$ and Muc1$^{-/-}$ mice were cultured at an air-liquid interface and transverse sections were examined by H&E staining. (B) Equal numbers of MUC1- or empty vector-transfected HEK293T cells were cultured for 24 hr and examined by phase contrast microscopy.

Figure 2. Expression of Muc1 Decreases TER



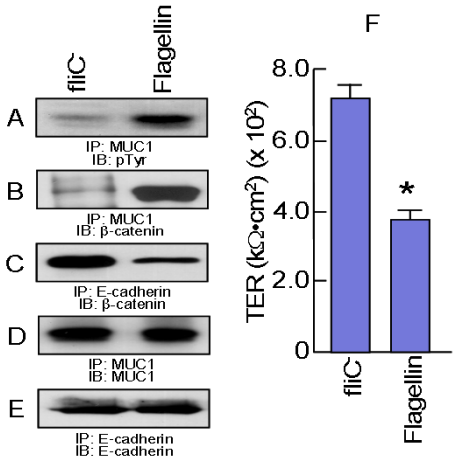
Equal numbers of primary TECs from Muc1$^{+/+}$ and Muc1$^{-/-}$ mice were cultured at an air-liquid interface for the indicated days and TER was measured. Values are means ± SEM (n=3). *, p < 0.05.

Figure 3. Expression of MUC1 Decreases Airway Epithelial Cell Barrier Formation



HAECs were transfected with a MUC1 siRNA or non-targeting control RNA, cultured for 24 hr, and TER (A) and transepithelial permeability of FITC-BSA (B) were measured. Values are means ± SEM (n=3). *, p < 0.05.

Figure 4. Phosphorylation of MUC1 Disrupts Adherens Junctions and Decreases TER



HAECs were treated with 10 μg/ml of flagellin or the fliC^{-} negative control, cell lysates were IPed with MUC1 Ab (A, B) or E-cadherin Ab (C), and analyzed by IB with β-catenin or pTyr Abs. (D, E) The IPed MUC1 and E-cadherin were IBed with the same IP Ab to verify equal sample loadings. (F) TER was measured. Values are means ± SEM (n=3). *, p < 0.05.

Summary

- HEK-293T cells over-expressing the full-length MUC1 protein exhibited reduced formation of intercellular contacts compared with MUC1 nonexpressing cells. Diminished cell-cell adhesion also was observed with primary TECs from Muc1$^{+/+}$ mice compared with Muc1$^{-/-}$ TECs.
- Muc1$^{-/-}$ TECs and human airway epithelial cells transfected with a MUC1 siRNA, demonstrated increased TER compared with the corresponding Muc1/MUC1 expressing cells.
- The MUC1 siRNA decreased transepithelial flux of FITC-BSA across HAEC monolayers.
- Tyrosine phosphorylation of the MUC1 induced by flagellin, increased MUC1/β-catenin coIP, decreased E-cadherin/β-catenin coIP, and diminished TER compared with cells treated with the fliC^{-} negative control.

References

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Acknowledgments

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