

NEU1 Sialidase Promotes MUC1 Ectodomain Release from Human Airway Epithelial Cells as a Soluble Decoy Receptor that Blocks *Pseudomonas aeruginosa* Adhesion

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Abstract

Background: The full-length MUC1 protein is initially synthesized as a single polypeptide chain that is proteolytically cleaved at a Gly³¹⁶-Ser³¹⁷ peptide bond in its ectodomain (MUC1-ED) (1). This allows for shedding of MUC1-ED from the airway EC surface (2). Three membrane-bound proteases have been reported as MUC1-ED sheddases, MMP14, TACE/ADAM17, and γ -secretase (3-5). In airway ECs, *Pseudomonas aeruginosa* (Pa) and its flagellin stimulate NEU1 recruitment to MUC1-CD and desialylation of MUC1-ED (see accompanying poster). Sialic acid residues are usually the terminal sugars on glycan chains attached to glycoproteins. Here, they are strategically positioned to mask protease recognition sites through protein conformational changes, electrostatic repulsion, and/or steric hindrance (6). Whether sialic acid on MUC1-ED similarly protects against proteolysis, and whether flagellin-stimulated, NEU1-mediated MUC1-ED desialylation regulates its shedding, has never been reported.

Results: Transcripts for MMP14 and γ -secretase (PSEN1, PSEN2), but not ADAM17, were found in airway ECs. Shed MUC1-ED levels were diminished in ECs treated with galardin (GM6001), a broad spectrum MMP inhibitor. NEU1 overexpression and flagellin stimulation of airway ECs each increased MUC1-ED shedding. Prior NEU1 silencing abolished the flagellin-induced increases in MUC1-ED shedding. Shed MUC1-ED levels in BALF were higher in ventilator-associated pneumonia patients with culture-confirmed Pa lung infections, compared with noncolonized patients or patients colonized with non-Pa microorganisms. MUC1-ED shed both in response to flagellin stimulation, and in BALF from Pa-colonized patients, was desialylated. Preincubation of Pa with shed MUC1-ED both in supernatants of flagellin-stimulated airway ECs, and BALF from Pa-colonized patients, competitively inhibited Pa adhesion to EC-associated MUC1-ED.

Conclusions: (1) Two established MUC1-ED sheddases, MMP14 and γ -secretase, are expressed in human airway ECs, (2) MMP inhibition protects against MUC1-ED shedding, (2) NEU1 regulates flagellin-dependent MUC1-ED shedding from airway ECs, (4) shed MUC1-ED levels are increased in the BALF of Pa-colonized patients, and (5) shed, desialylated MUC1-ED acts as a decoy receptor to inhibit Pa adhesion to airway EC-associated MUC1-ED.

Figure 1. Airway ECs Express the MUC1-ED Sheddases MMP14 and γ -Secretase.

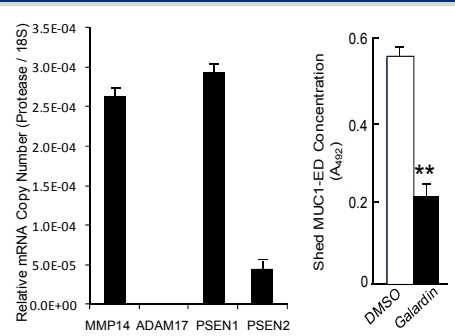


Figure 2. NEU1 Overexpression Increases MUC1-ED Shedding from Airway ECs.

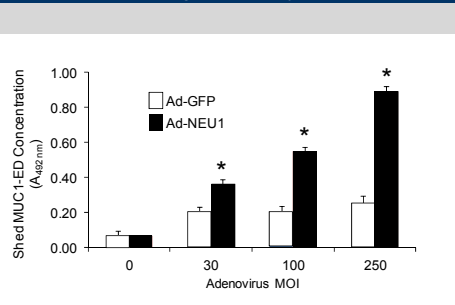


Figure 3. NEU1 Regulates Flagellin-Dependent MUC1-ED Shedding from Airway ECs.

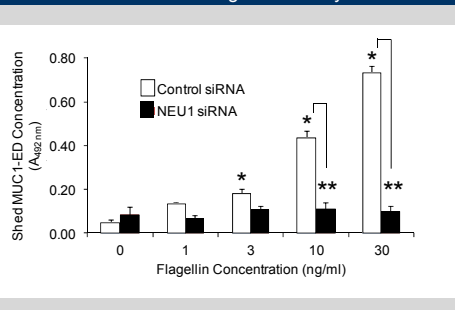


Figure 4. MUC1-ED Shed in Response to the Flagellin Stimulus is Desialylated.

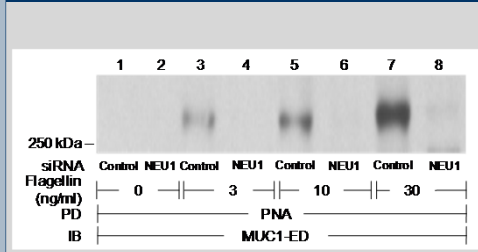


Figure 5. Desialylated MUC1-ED Shed in Response to Flagellin Competitively Inhibits Pa Adhesion to EC-Associated MUC1-ED.

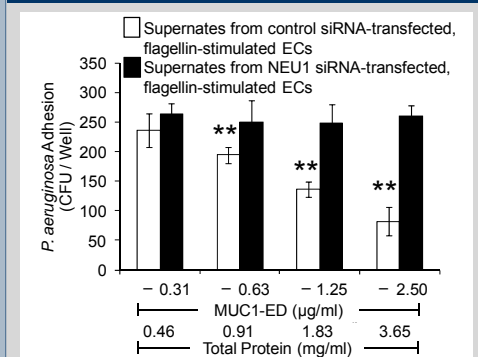


Figure 6. Pa Lung Infection is Associated with Increased MUC1-ED Levels in Bronchoalveolar Lavage Fluid.

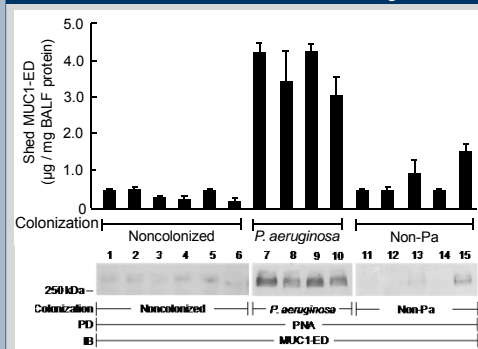


Figure 7. Desialylated MUC1-ED in BALF from Pa-Colonized Patients Competitively Inhibits Pa Adhesion to EC-Associated MUC1-ED.

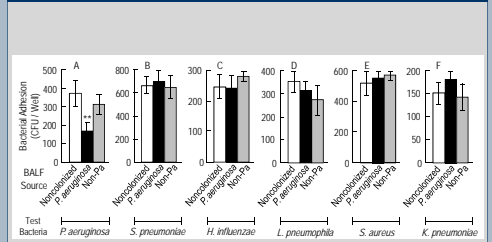
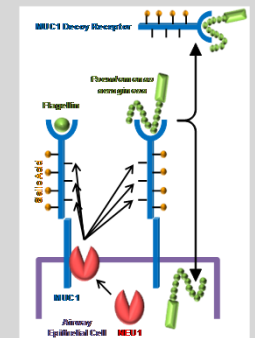


Figure 8. Hypothetical Model



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Acknowledgments

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