NEU1 Sialidase Increases Shedding of MUC1 Ectodomain as a Decoy Receptor for *Pseudomonas aeruginosa*, Decreasing Bacterial Adhesion to Human Airway Epithelia

Erik P. Lillehoj[1], Wei Guang[1], Sang Won Hyun[2], Irina G. Luzina[2,4], Sergei P. Atamas[2,4], Avelino Verceles[2,4], and Simeon E. Goldblum[2,3,4]

Departments of Pediatrics[1], Medicine[2], and Pathology[3], University of Maryland School of Medicine, and Baltimore VA Medical Center[4], Baltimore, MD 21201

Abstract

Background: The full-length MUC1 protein is initially synthesized as a single polypeptide chain that is proteolytically cleaved at a Gly[316]-Ser[317] 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: We now have demonstrated and quantified shed MUC1-ED in the supernatants of EC cultures. Reciprocal co-immunoprecipitation experiments established that shed MUC1-ED was not associated with MUC1-CD, thereby ruling out nonspecific release of full-length MUC1 from dying ECs. NEU1 overexpression and flagellin treatment of airway ECs each increased MUC1-ED shedding. Prior NEU1 silencing abolished the flagellin-induced increases in MUC1-ED shedding. 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. Shed MUC1-ED levels in BALF were higher in ventilator-associated pneumonia patients with culture-confirmed Pa or *Stenotrophomonas maltophilia* lung infections, compared with patients who were culture-positive for *Klebsiella oxytoca*, *Candida parapsilosis*, or *Proteus mirabilis*. Preincubation of Pa with shed MUC1-ED isolated from supernatants of airway ECs, reduced bacterial adhesion to these same ECs.

Conclusions: (1) NEU1 regulates flagellin-dependent MUC1-ED shedding from airway ECs, (2) two established MUC1-ED sheddases, MMP14 and γ-secretase, are expressed in human airway epithelia, (3) MMP inhibition protects against MUC1-ED shedding, and (4) shed MUC1-ED acts as a decoy receptor to inhibit Pa adhesion to airway ECs.

Figure 1. MUC1-ED is Shed into Culture Supernatants of Human Airway ECs.

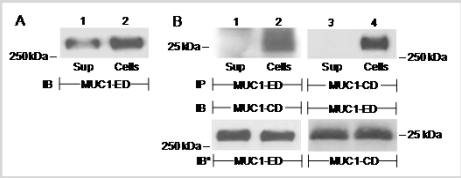


Figure 2. NEU1 Overexpression Increases MUC1-ED Shedding from Airway ECs.

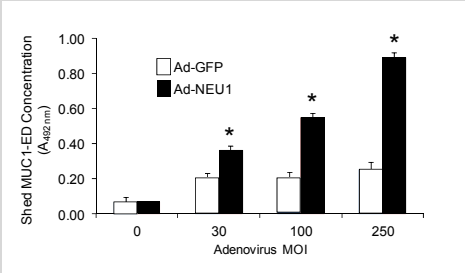


Figure 3. NEU1-Regulates Flagellin-Dependent MUC1-ED Shedding from Airway ECs.

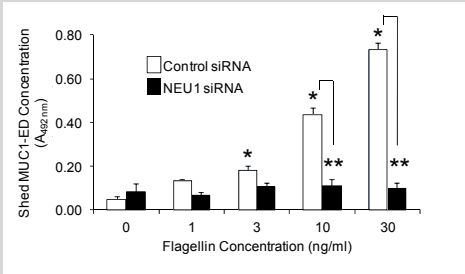


Figure 4. Airway ECs Express the MUC1-ED Sheddases MMP14 and γ-Secretase.

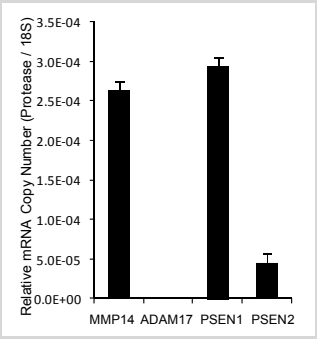


Figure 5. The Broad Spectrum Matrix Metalloprotease Inhibitor, Galardin, Inhibits MUC1-ED Shedding.

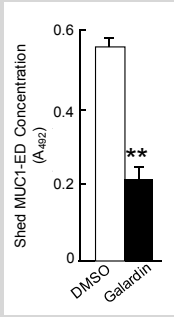
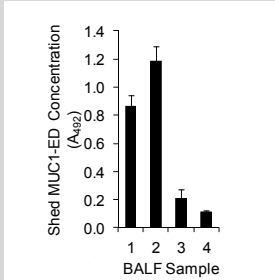


Figure 6. Pa Infection is Associated with Increased MUC1-ED Levels in Bronchoalveolar Lavage Fluid.



1, *Pseudomonas aeruginosa* (>10⁴ CFU/ml)
2, *Stenotrophomonas maltophilia*
3, *Klebsiella oxytoca*, *Candida parapsilosis*
4, *Proteus mirabilis* (>10⁴ CFU/ml)

Figure 7. Preincubation of Shed MUC1-ED with Pa Decreases Bacterial Adhesion.

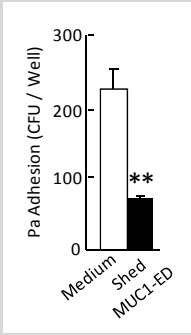
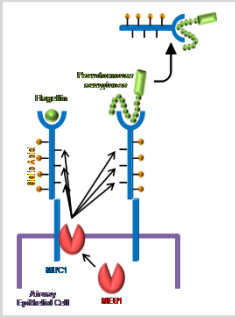


Figure 8. Hypothetical Model



References

1. Parry S, Silverman HS, McDermott K, Willis A, Hollingsworth MA, Harris A. *Biochem Biophys Res Commun* 283:715, 2001.
2. Lillehoj EP, Han F, Kim KC. *Biochem Biophys Res Commun* 307:743, 2003.
3. Thathiah A, Blobel CP, Carson DD. *J Biol Chem* 278:3386, 2003.
4. Thathiah A, Carson DD. *Biochem J* 382:363, 2004.
5. Julian J, Dharmaraj N, Carson DD. *J Cell Biochem* 108:802, 2009.
6. Hoskins LC, Zamcheck N. *Gastroenterology* 44:456, 1963.

Acknowledgments

Funded by NIH grants HL084223 and HL070155 (SEG), and AI072291 and AI083463 (EPL).