Human Airway Epithelia Express Catalytically-Active NEU3 Sialidase

Sang Won Hyun[1], Erik P. Lillehoj[1,2], Chiguang Feng[3], Lei Zhang[3], Anguo Liu[1], Wei Guang[2], Wenji Sun[4], Chinh Nguyen[1], Irina G. Luzina[5], Tonya J. Webb[4], Sergei P. Atamas[5], Antonino Passaniti[6], William S. Twaddell[6], Adam C. Puché[7], Lai-Xi Wang[8,9], Alan S. Cross[3,5], and Simeon E. Goldblum[1,5,6]

[1]Mucosal Biology Research Center, [2]Department of Pediatrics, [3]Center for Vaccine Development, [4] Department of Microbiology and Immunology, [5]Department of Medicine, [6]Department of Pathology, [7]Department of Anatomy and Neurobiology, [8]Institute of Human Virology, and [9]Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD 21201

Abstract

Sialic acids on glycoconjugates play a pivotal role in many biological processes. In the airways, sialylated glycoproteins and glycolipids are strategically positioned on the surface of epithelial cell (EC)s to regulate receptor-ligand, cell-cell, and host-pathogen interactions. The sialylation state of these glycoconjugates is dictated by the opposing catalytic activities of sialyltransferase (ST)s and sialidase/neuraminidase (NEU)s. While prior studies have established airway EC expression of STs, almost nothing is known about the expression and activity of airway NEUs, and in particular, nothing is known about NEU3, a plasma membrane sialidase with a substrate preference for gangliosides. Therefore, in the current study we asked whether airway epithelia express catalytically active NEU3 sialidase. Increasing numbers of alveolar A549 airway ECs and primary small airway ECs expressed increasing sialidase activity for a ganglioside mixture. ECs derived from human trachea, bronchus, small airways, and the alveolus, each contained sialidase activity for this ganglioside substrate. NEU3 was expressed at mRNA and protein levels at comparable levels in these diverse airway tissues. By immunoblot analysis, NEU3 protein was detected as a 55.0 kDa band that corresponded to its predicted molecular size of 51.7 kDa. In small airway and alveolar epithelia, NEU3 protein was immunolocalized to the plasma membrane, cytosolic, and nuclear subcellular fractions. To further characterize NEU3 expression and activity in airway ECs, hemagglutinin (HA)-tagged NEU3 was ectopically expressed through adenovirus (Ad) infection. Ad-NEU3 infection of A549 cells dose-dependently increased sialidase activity for the ganglioside substrate. NEU3 also was detected in the cadherin-containing plasma membrane fraction of these cells. To unambiguously establish siRNA-induced knockdown of NEU3, A549 cells overexpressing NEU3 were transfected with NEU3-targeting or control siRNAs, after which cell lysates were processed for HA immunoblotting. At 24, 48, and 72h, NEU3 levels were reduced by 67.1%, 60.0%, and 49.1% respectively, relative to control siRNA-transfected cells. The NEU3-targeting siRNA did not diminish expression of the lysosomal NEU1 sialidase, indicating efficient and selective siRNA-induced depletion of NEU3. Finally, we applied an immunohistochemical approach to determine whether NEU3 expression could be extended to normal, intact human airway tissues. In the trachea, intense NEU3 immunostaining was evident at the mucosal surface within the ciliated brush border. The subepithelial mesenchymal cells displayed diminished staining relative to epithelia. In the mainstem bronchus, NEU3 staining was most intense in the superficial portion of the EC cytoplasm, particularly the ciliated brush border. Again, the subepithelial mesenchymal cells were weakly stained, and goblet cell mucus granules were completely devoid of staining. In the segmental bronchi, strong NEU3 cytoplasmic staining was evident in the EC layer, whereas the subepithelial mesenchyma was stained weakly. In expanded alveoli, NEU3 cytoplasmic and nuclear staining of ECs was apparent. Clear differences between the distribution of NEU3 protein along the length of the human lower respiratory tract were not evident. Taken together, these combined results indicate, for the first time, that human airway epithelia express catalytically-active NEU3 sialidase.

Figure 1. Airway ECs express sialidase activity for a ganglioside mixture.

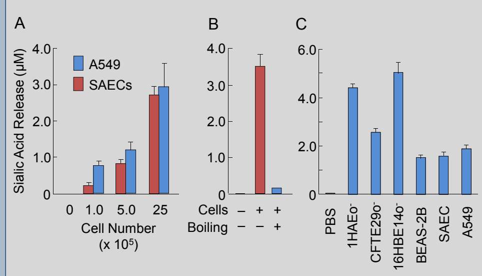

Figure 2. NEU3 mRNA expression in airway ECs.

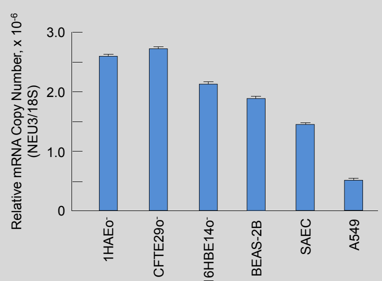

Figure 3. NEU3 protein expression in airway ECs.

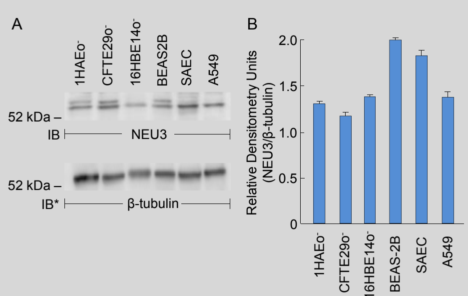

Figure 4. Overexpression of NEU3 in airway ECs increases sialidase activity.

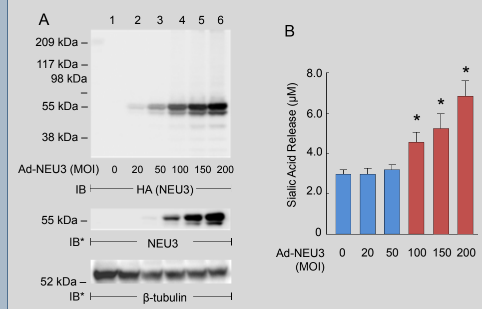

Figure 5. Silencing of NEU3 expression in airway ECs by siRNA.

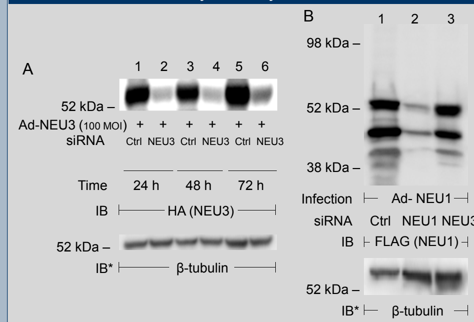

Figure 6. NEU3 siRNA, but not NEU1 siRNA, inhibits sialidase activity in airway ECs.

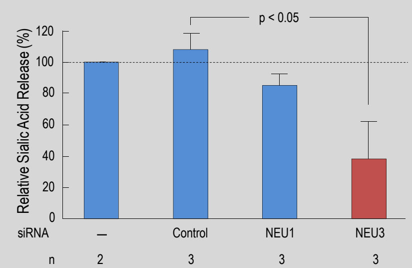

Figure 7. Subcellular localization of NEU3 in airway ECs.

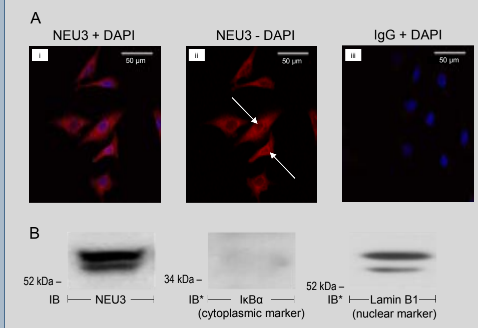

Figure 8. NEU3 immunostaining in airway epithelia of human tissues.

Summary

1) Overexpression of NEU3 increases sialidase activity for a ganglioside substrate in airway ECs.
2) NEU3 siRNA, but not NEU1 siRNA, reduces sialidase activity for the ganglioside substrate.
3) NEU3 was immunolocalized to to the plasma membrane, cytosolic, and nuclear subcellular fractions of airway ECs, and to the at the mucosal surface within the ciliated brush border of intact human airway tissues.

Acknowledgments

Funded by NIH grants HL084223 and HL070155 (SEG), AI072291 and AI083463 (EPL), and HL086933 (ASC).