Improving gene therapy vector delivery in the lung by removing cell surface glycocalyx mucins

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Background

- The lungs are coated with a dual layer of secreted and tethered mucins produced by epithelial cells allowing them to be resilient against pathogens, toxins, and microorganisms constantly inhaled from the air. The ciliary proteins and tethered mucins form a brush-like structure, the periciliary layer (PCL).
- Mucin proteins, secreted and tethered, are characterized by extensive O-glycosylated serine and threonine (1).These highly glycosylated mucins orient themselves and fold to create the mucin "bottlebrush" structure protecting lung epithelial cells from foreign bodies (2).
- Currently, the focus is on identifying mucin-specific proteases which can cleave both secreted and tethered mucins, thereby allowing gene therapy vectors to penetrate these restrictive layers.
- StcE is a mucin-specific protease released from Escherichia coli O157:H7 (EHEC) with an ability to specifically cleave o-linked glycan-containing proteins.

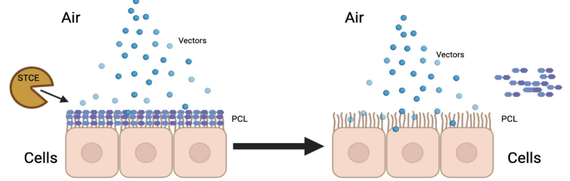
Methods

GalNaz Study- GalNaz was added to cell media, and cells were grown on 2x GalNaz Media for 2 weeks. Cells were then treated with StcE and stained using Click Chemistry.

WGA Study- Wheat Germ Agglutinin coupled to Alexa Fluor 488 was used to stain lectin sugars in the glycocalyx in StcE treated and untreated cell cultures.

StcE-E447D Dye Study- Well-washed airway cells were stained with a StcE-E447D dye made by adding StcE-E447D to Alexa Fluor 647 Succinimidyl esters in DMSO.

AAV Infection Study- Well-ciliated airway cells were treated with 40 μg/mL StcE overnight and subsequently infected with AAV-2 or coronavirus (NL63) at 10,000 MOI .



Results

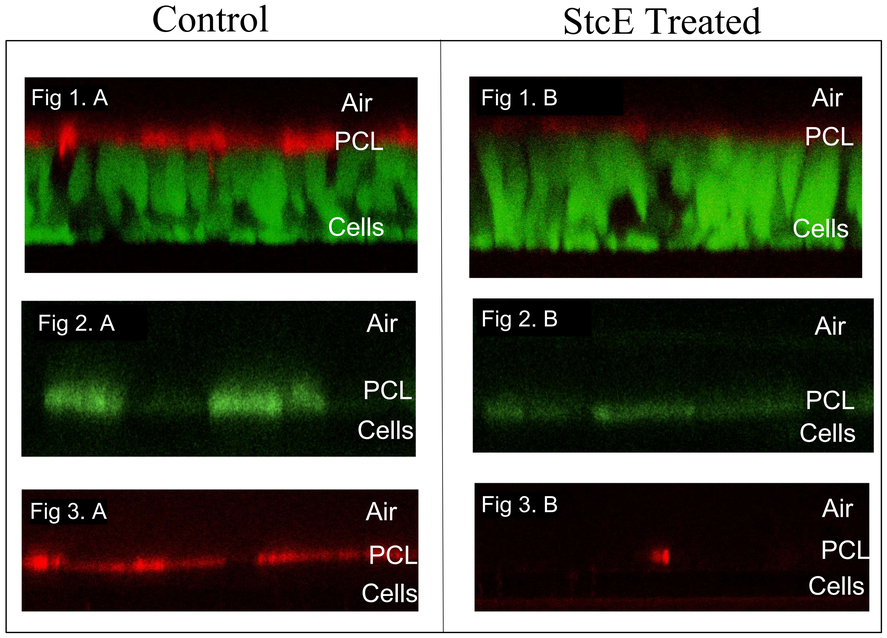
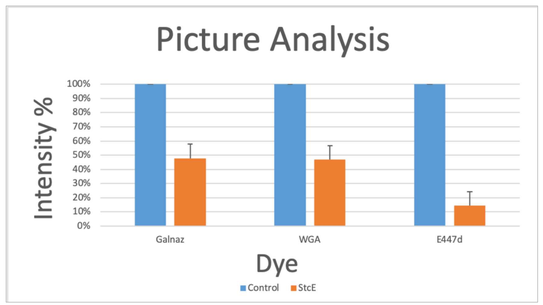


Figure 1. All o-linked glycan have a base structure beginning with a GalNac lectin. GalNac can be outcompeted with the sugar GalNaz and fluorescently labeled through Click Chemistry. The red fluorescently labeled GalNaz depicts the presence of a robust glycocalyx on top of the cells **(1A)**. After overnight StcE treatment, the glycocalyx is cleaved leaving no GalNaz glycocalyx proteins **(1B)**. **Figure 2.** The green fluorescently labeled WGA binds to lectin sugars in the glycocalyx **(2A)**. After overnight StcE treatment, the lectin sugars are cleaved, and the WGA signal is reduced **(2B)**. **Figure 3.** StcE-E447D, catalytically inactive StcE, binds to all sites where StcE will cleave. Before StcE treatment, the red staining indicates binding of the StcE-E447D to StcE cleavage sites **(3A)**. After overnight StcE treatment, there is minimal red staining showing cleavage of the glycocalyx leaving no available binding sites for StcE-E447D **(3B)**.



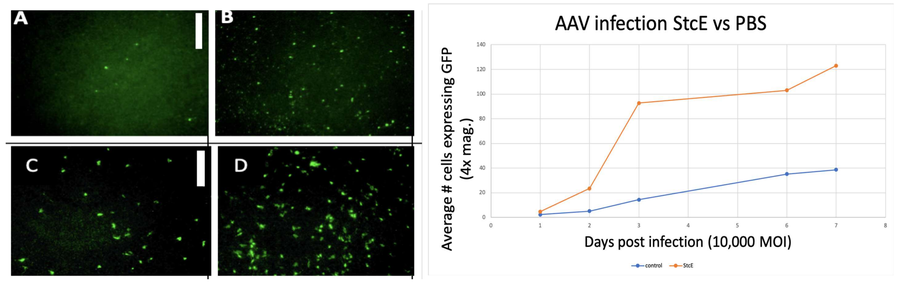


Figure 4. StcE increases viral infection in human airway cells. Fluorescent images of AA2-GFP infected cells 48 hours after **(1A)** vehicle or **(1B)** infection, and the same trend of increased infection is shown for coronavirus (NL63), **(1C)** vehicle and **(1D)** StcE. Bars= 200μm.

Discussion

- Tethered mucins form a barrier to prevent viral infection however specifically cleaving this barrier has not previously been studied in depth.
- By slightly modifying the glycocalyx, GalNac, the first sugar in o-linked gycans, was outcompeted by GalNaz, effectively adding a reactive azide group onto the first sugar of o-linked glycans allowing them to be fluorescently labeled. After StcE. treatment, the GalNaz dye intensity decreases indicating cleavage of o-linked glycans (Fig. 1A&B)
- To look at the unmodified glycocalyx, WGA staining was used to stain cell-surface lectins. The decreased intensity of WGA staining after StcE treatment (80 μg/mL overnight) highlights StcE's ability to independently cleave mucin proteins (Fig. 2A&B).
- The specificity and efficacy of StcE cleavage at StcE specific binding sites was observed with the StcE-E447D dye. StcE-E447D binds to StcE cleavage sites but is catalytically inactive, and after StcE treatment (80 μg/mL overnight), there is decreased StcE-E447D binding due to lack of binding sites indicating StcE's effective cleavage of accessible o-linked glycans (Fig. 3A&B).
- As StcE cleaves o-linked glycan-containing mucin proteins, it decreases the exclusion height of cells allowing particles, including viruses, to travel further into the periciliary layer before being trapped in the mucin and cilia mesh. This is visualized via increased AAV2 and Norovirus infection after StcE treatment (Fig. 4A, B, C, D).
- Currently, it is being studied how long the glycocalyx takes to regenerate itself to full capacity after StcE treatment, which has implications for how often and what does future medications will be administered. Future studies will investigate how the rate of regeneration of the glycocalyx can be manipulated through various means such as cytokine regulation, viral infections, and irregular metabolic conditions.

Acknowledgements and References

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2. Shon, D. J., Malaker, S. A., Pedram, K., Yang, E., Krishnan, V., Dorigo, O., & Bertozzi, C. R. (2020). An enzymatic toolkit for selective proteolysis, detection, and visualization of mucin-domain glycoproteins. *Proceedings of the National Academy of Sciences*, *117*(35), 21299–21307. https://doi.org/10.1073/pnas.2012196117