Selective cleavage and measurement of mucin with proteases: treatment and diagnostic.

Cadence Michels, Sahana Kumar, Phillip Bryan, Louisa Wu, Gregg A. Duncan
Fischell Department of Bioengineering and Department of Cell Biology and Molecular Genetics

Introduction

Airway epithelial mucus is a key component of pathogen clearance and immune response as mucin complexes entrap irritants within the mucus hydrogel and expel them through coughing (mucociliary clearance or MCC). For mucus to successfully function, it requires a proper ratio of human airway epithelial mucins MUC5AC and MUC5B within the hydrogel complex to form the ideal viscosity. The underlying pathology of asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis include significant overexpression of MUC5AC, causing highly viscous airway mucus and interfering with disease clearance and respiration [2].

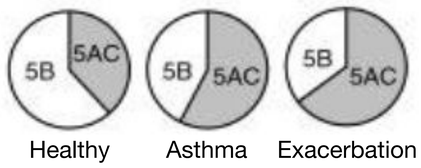

Figure 1: MUC5AC - MUC5B ratios in healthy mucus, asthmatic mucus and severe asthmatic mucus (exacerbation) [2].

Research suggests that selective mucin proteases may serve as useful treatments for unbalanced mucin expression in disease phenotypes [1, 3, 4]. Neither MUC5AC or MUC5B appear to be cleaved by the most well researched mucinase, StcE [3].

We have developed a potential MUC5AC-specific mucin protease. Furthermore, this protease has the capacity to form a substrate-protease complex after activation which triggers the release of fluorescent particles (AFC) thus providing a potential diagnostic tool for the measurement of MUC5AC within samples.

References

[1] Grys, T. E. et al. J Bacteriology, 4646–4653. (2006)

[2] Lachowicz-Scroggins, M. E. et al. Am J Respir Crit Care Med 194, 1296–1299 (2016).

[3] Lowery et al. Preprint (Unreviewed) (2024)

[4] Pedram, K. et al. Nat Biotechnol 42, 597–607 (2024).

Acknowledgments

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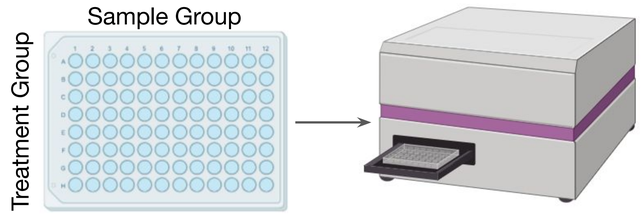
Preliminary data was sourced from the UMD Grand Challenges Project.

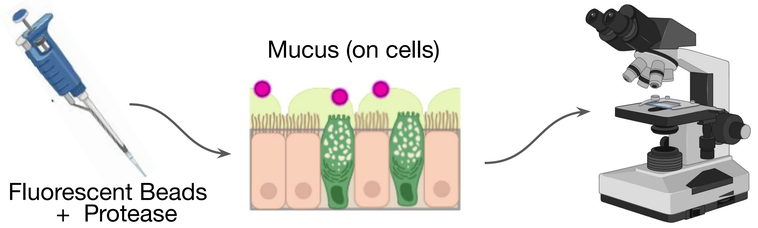
Methods

The Duncan Lab cultivates human airway epithelial (HAE) cell cultures to produce and collect mucus samples. The cell lines include healthy HAE cells, MUC5AC knock out cells, and MUC5B knock out cells.

- **Plate Reading Assay**: Records fluorescence of many treatment groups simultaneously.



- **Mucociliary Clearance Assay**: Records motion of mucus as cilia beat through movement of fluorescent beads.



Results and Conclusions

Figure 1: Absorbance from plate reader assay with substrate-protease complex at T= 0 to 60 minutes.

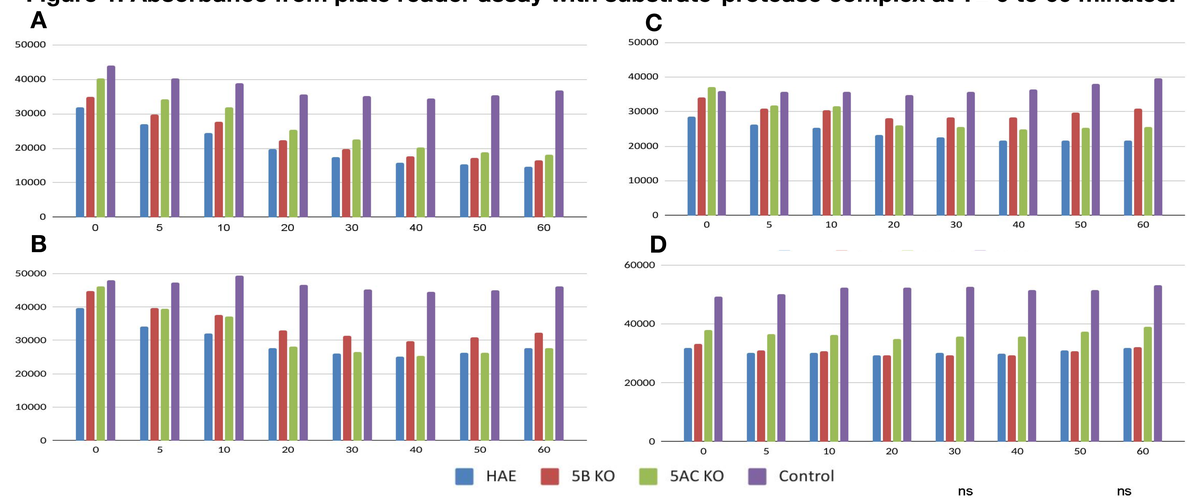


Figure 1: Absorbances from HAE (healthy), MUC5B and MUC5AC knockout mucus and a control without mucus at two mucus dilutions and two protease treatment concentrations. **(A)** 1X Mucus + 0.05uM pT2552 **(B)** 1:10X Mucus + 0.05uM pT2552 **(C)** 1X Mucus + 0.10uM pT2552 **(D)** 1:10X Mucus + 0.10uM pT2552

- The plate reader assay has yielded minimal conclusive data so far

Figure 2: *Preliminary data from another project, the Grand Challenges.* Successful MUC5AC selective protease in mucociliary clearance assay, but catalyst caused interference.

- Designed proteases can be effectively selective for MUC5AC
- NaNO2 may be too disruptive to function as a protease catalyst

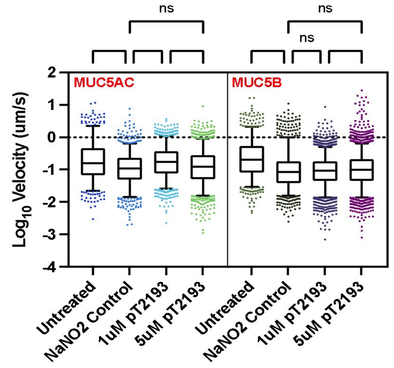


Figure 2: Mucociliary Clearance Assay in Knock-out Mucus

Future Directions

1: Confirm selective cleavage

- Verify that the fluorescent substrate-protease complex is selectively cleaving MUC5AC, as we saw with previous protease designs
- Determine other potential catalysts

2: Refine plate reader protocol

- Different concentrations, materials, time points, dilutions, and absorbances can be adjusted to determine the ideal experimental conditions
- The inconclusive results may have been due to the age of protease, using a new stock may yield better data

3: Analyze fluorescence levels

- Generate known MUC5AC concentrations using MUC5AC and MUC5B mucus stocks and gauge fluorescence patterns