FIRE: THE FIRST-YEAR INNOVATION & RESEARCH EXPERIENCE

MOLECULAR DIAGNOSTICS

Selecting DNA Aptamers Against Airway Mucin Proteins for Therapeutics and Diagnostics

Colin Savage, Zackary Shpilman, Siya Kothale, Joey Munyaneza, Defne Ustundag, Caroline Fuller, Charlotte Woodbury

Intent & Motivation

Intent: To select and characterize DNA aptamers against the mucin protein MUC5AC within mucus samples for therapeutic and diagnostic applications.

Motivation: Selected aptamers can be used in therapeutics to deliver engineered proteases to cleave mucin proteins and in diagnostics to tag mucin proteins in mucus.

Background

- Mucus is a protective function of the body made up of mucin proteins, inorganic salts, and water.
- MUC5AC contributes to making a thicker mucus layer, providing shelter for viruses or bacteria.[1]
  - Elevated MUC5AC levels are associated with impaired mucus clearance and development of chronic obstructive lung diseases, including Chronic Obstructive Pulmonary Disorder (COPD).[1,2,3]
- Current treatments that break down mucin proteins involve the use of enzymes or chemical treatments. Treatments are non-specific, result in adverse side effects, and can cleave protective mucins, leaving cells vulnerable to infection.[4]
- Aptamers are synthetic, single-stranded DNA or RNA oligonucleotides that are selected to bind strongly and specifically to target molecules.
  - Aptamers have been successful selected for similar targets such as MUC1, demonstrating viability of target

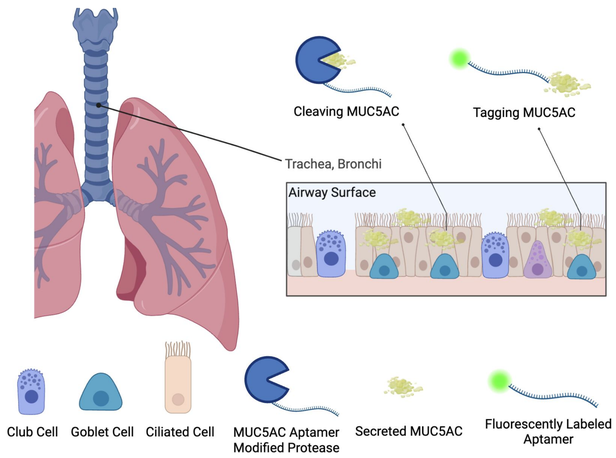

Figure 1. MUC5AC, secreted from goblet cells, makes up airway mucus that form hydrogel transported by cilia along airway surface.[1] Potential applications include fluorescently labelling MUC5AC with aptamer tags or directing engineered proteases for efficient cleavage.

Methods

In Tube ELISA Assay

- Confirms protein is immobilized on PCR tube and accessible to DNA library following immobilization on the SpeedVac Concentrator.
- Performed with Abcam Anti-Mucin 5AC and Goat Anti-Mouse IgG H&L (HRP) antibodies.

One-Pot Selection

- Mucus enriched with MUC5AC is immobilized on PCR tube using a SpeedVac Concentrator, drying for 4 hours.
- The immobilized protein is incubated with a Guanine and Cytosine enriched DNA library with a 63 nt DNA library with a 25 nt random region at room temperature for 30 minutes.[5]
- The unbound DNA is removed and PCR reagents are added to the remaining bound DNA.
- Counter selection is done by immobilizing MUC5B and incubating with ssDNA pool, removing unbound DNA and using for subsequent selection with target MUC5AC protein.

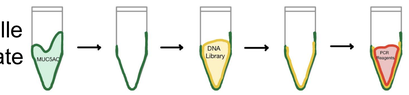

Figure 2. In-tube selection process from Scoville et. al. against semi pure MUC5AC to generate aptamer candidates after multiple rounds.[6]

Amplification of Single-Stranded DNA

- 10 cycles of symmetric PCR to amplify selection pool, cycle course PCR to determine number of cycles needed for large scale symmetric PCR.
- Large scale PCR is done with a 5' phosphorylated reverse primer.
- Lambda exonuclease digestion generates ssDNA for future rounds of selection and binding affinity assays.[8]

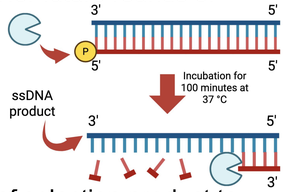

Figure 3. ssDNA is generated by lambda exonuclease digestion. Lambda exonuclease degrades dsDNA from 5' phosphorylated end.

Gel Shift Assay

- Binding affinity assay to gauge binding potential of selection product to protein target
- 2% agarose gel used to improve separation between unbound aptamer and aptamer-protein complexes
- Gels were post-stained with GelRed to avoid interference with protein during electrophoresis

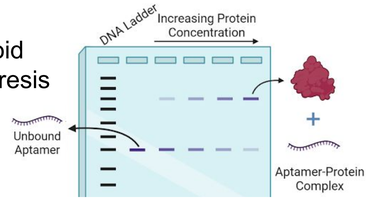

Figure 4. Gel shift assay. Selection product is run on a gel with increasing protein concentration. Band intensity shows binding strength.

References and Acknowledgements

QR code links to references. We would like to acknowledge our advisor, Dr. Catherine Spirito, the Whelan Lab at the University of Kansas, the First-Year Innovation and Research Experience (FIRE) program, and Grand Challenges collaborator faculty Dr. Louisa Wu, Dr. Gregg Duncan, and Dr. Phillip Bryan. Figures 1-4 were made using Bio Render.

Results & Discussion

In-Tube ELISA of MUC5AC

- Left tube: Positive samples containing MUC5AC protein immobilized via SpeedVac
- Right tube: Negative controls containing nuclease free water
- Blue color change indicates presence of MUC5AC protein

Figure 5. ELISA test with washes and a blocking agent to prevent nonspecific binding showed expected results

Isolating & Assessing ssDNA

- Gel of exonuclease digestion shows successful separation
- Qubit readings show consistent retention of DNA during selection and digestion

Table 1: Qubit fluorometry dsDNA & ssDNA concentrations of DNA aptamer pool during each round of aptamer selection.

Round	dsDNA Concentration	ssDNA Concentration
1	60.8 ng/µL	78.0 ng/µL
2	34.6 ng/µL	28.2 ng/µL
3	13.7 ng/µL	15 ng/µL
4	24.6 ng/µL	34.4 ng/µL
5	30.8 ng/µL	44.0 ng/µL

Lambda Exonuclease Digestion

- Amplified DNA library visualized on a GelRed pre-stained 4% agarose gel run at 90V for 45 minutes
- Incubation condition A produced the best results of ssDNA generation, outperforming conditions B & C

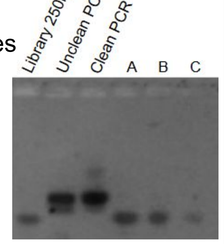

Figure 6. A) 60 minutes incubation, 0.21 U/µL enzyme. **B**) 2 hour incubation, 0.14 U/µL enzyme. **C**) 2 hour incubation, 0.21 U/µL enzyme.

Gel Shift Assay

- 3% agarose gel post-stained with GelRed nucleic acid stain
- Incubated MUC5AC with GC-enriched & Bowser library to observe aptamer-protein complex formation
- Results suggest assay requires further optimization

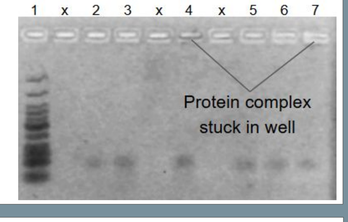

Figure 7. (**1**) Low MW Purple Ladder (**2**) Bowser 250 nM (**3**) 0 µM protein + Bowser (**4**) 0.708 µM protein + Bowser (**5**) G-Rich 250 nM (**6**) 0 µM protein + G-Rich (**7**) 0.708 µM protein + G-Rich

Future Work

One-Pot Selection:

- Finalize selected Aptamer Pool
- Run binding affinity experiments against MUC5AC target
- Counter select against MUC5B protein
- Learn how to incorporate selected aptamer in complex with Potomac Affinity Protein's engineered MUC5AC protease

Gel Binding Assays:

- Continue to optimize assay in order to visualize increased binding of aptamer to MUC5AC protein as its concentration increases
- Determine optimal protein concentration, electrophoresis voltage, gel dye, and TBE concentration