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MOLECULAR DIAGNOSTICS

Selecting a DNA Aptamer Against CTLA-4 for Immune Checkpoint Therapy

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Background

Immune checkpoint therapies:
- Immune checkpoint therapies aim to treat cancer by mobilizing the patient's own immune system to attack it.
- Currently available immunotherapies are expensive and often cause severe side effects.[1,2]
- CTLA-4 is an immune checkpoint protein that helps regulate the immune system by acting as a brake.

Aptamer-based therapeutics:
- Aptamers are short, single-stranded nucleic acids that can bind to target molecules and proteins with a high affinity and specificity.[3]
- Due to their small size they penetrate tissues deeper than antibodies, are easier to modify, and cheaper.[3]
- They are non-immunogenic, preventing the adverse immune responses caused by antibody therapies.[3,4]
- DNA aptamers selected against murine CTLA-4 have shown antitumor effects in mice.[4]

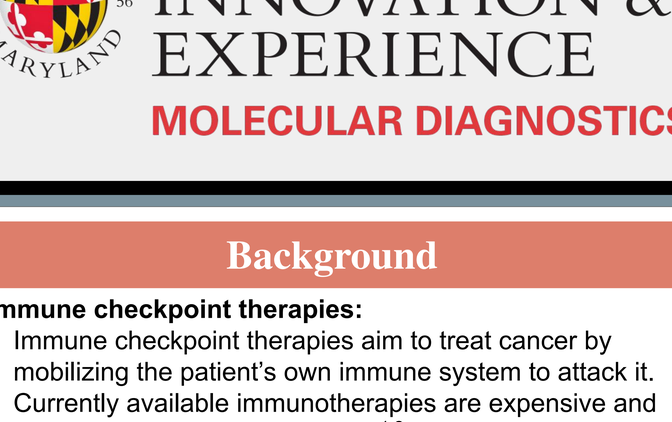

Figure 1: Binding of an aptamer to immune checkpoint proteins, such as CTLA-4, is inhibitory to deactivating cancer cell signaling.[3]

References & Acknowledgements

QR code links to references.

We would like to express our gratitude to Dr. Catherine Spirito for her guidance and the First-Year Innovation and Research Experience (FIRE) Program for their support of our project. Figures 1 and 2 were created in BioRender.

Intent & Motivation

Objective: To select and characterize a DNA aptamer that strongly and selectively binds to the immune checkpoint protein CTLA-4.

Significance: The development of more accessible, less immunogenic aptamer based cancer therapies can improve patient outcomes.

Methods

Figure 2: Cyclical process for SELEX against CTLA-4, or Systematic Evolution of Ligands by Exponential Enrichment. SELEX is commonly referred to as 'evolution in a test tube' since sequences with the highest binding affinity are enriched.[5]

SELEX begins with a DNA library, a pool of randomized sequences from which aptamer candidates will be selected through rounds of incubation with CTLA-4.

The DNA library is GC-enriched. Increased levels of guanine and cytosine increase the structural diversity of the library.[6]

Incubation with CTLA-4 protein target:
- CTLA-4 with a his tag is immobilized on Ni-NTA magnetic beads and incubated with library before washing and elution to obtain bound sequences. The sequence of the library is 63nt long and has a 25 nt random region.
- Rounds of negative selection are performed between rounds of positive selection to ensure specificity for the target protein.

Amplification of aptamer candidates:
- Cycle course PCR of bound pool is done to identify the appropriate level of amplification for aptamer candidates.
- Symmetric PCR with a 5' phosphorylated reverse primer, which tags strands for enzyme digestion.

Enzyme digestion to obtain single stranded DNA:
- Lambda exonuclease degrades dsDNA from the 5' phosphorylated end. The resulting ssDNA products can then undergo further rounds of selection.

Verifying the generation of ssDNA:
- Gel electrophoresis, DNA cleanup with spin columns, and Qubit fluorometer to quantify ssDNA.

Results & Discussion

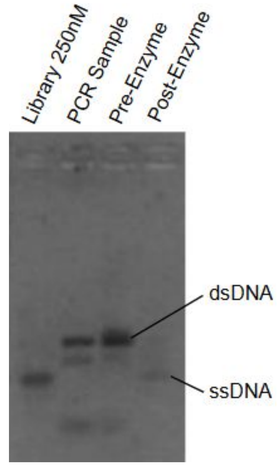

Figure 3 (Left): 4% agarose gel prestained with GelRed nucleic acid stain and run at 170V for 45 min. 45 min incubation with 0.4 U/uL of enzyme. Bound pool after 20 cycles of large scale symmetric PCR before and after lambda exonuclease incubation.

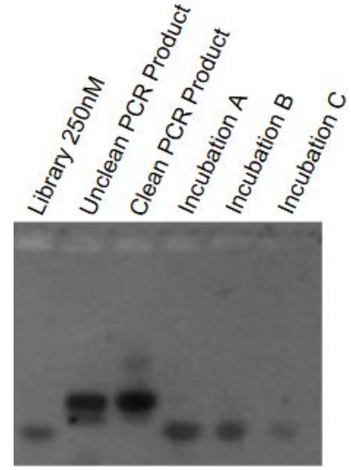

Figure 4 (above): 4% agarose gel prestained with GelRed nucleic acid stain and run at 90V for 45 minutes. Using amplified DNA library pre-selection. Conditions for Incubation A showed the best results. **A** - 60 min incubation, 0.21 U/ uL enzyme. **B** - 2 hr incubation, 0.14 U/uL enzyme. **C** - 2 hr incubation, 0.21 U/uL enzyme.

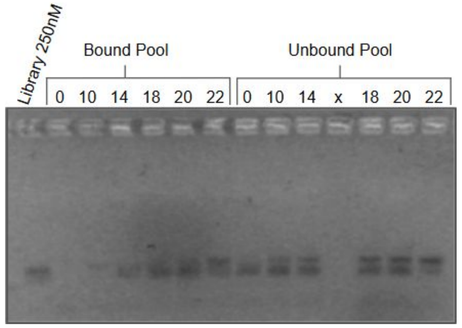

Figure 5 (above): 4% agarose gel prestained with GelRed nucleic acid stain and run at 90V for 40 minutes. Cycle course PCR of bound and unbound DNA pools. Progressive increase of amplification shown as number of cycles increase for both pools.

Summary:
- Successful generation of ssDNA using lambda exonuclease digestion after the identification of ideal concentration of enzyme and incubation time.
- Amplification of bound pool from first round of selection demonstrated the enrichment of sequences binding to CTLA-4.

Future Work

- Continue rounds of selection to select for aptamers with the highest affinity and specificity.
- We will perform binding affinity assays of aptamers determined with gel shift assays.
- We will sequence samples from the aptamer selection rounds to identify the most probable aptamer candidates.