Development of a RNA-Guided Drug Delivery System

Jacob Stump[1], Guerini Paolo[1],David Smithrud, PhD[2], Pheruza Tarapore, PhD[3]

[1]University of Cincinnati, Cincinnati, OH; [2]Department of Chemistry, University of Cincinnati, Cincinnati, OH; [3]Department of Environmental Health, University of Cincinnati, Cincinnati, OH;

Abstract

Prostate cancer (PCa) is the second leading cause of cancer deaths among American men. One in 9 men will be diagnosed with PCa and one in 41 men will die from it. When caught early, PCa can be treated with androgen-deprivation therapy (ADT). However, most PCa's eventually progress to being hormone/castration-resistant PCa (CRPC) and are thus unaffected by ADT.

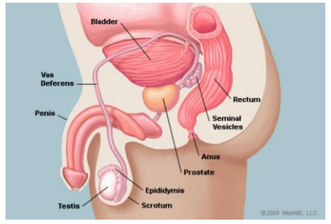

Figure 1: Location of the male prostate gland (circled). Its proximity to the urinary tract presents obstacles to surgical treatment of prostate cancer.

Diethylstilbestrol (DES), an agonist of the estrogen receptors was once used for treatment of CRPC. Although effective in this role, the dosage size required often lead to cardiovascular issues, hence limiting its usage. We hypothesize that by specifically delivering DES to CRPC cells, we can negate the off-target toxicity and thus lower the dose of DES used for treatments.

In this project, we utilize click chemistry to develop a conjugate between DES and a small RNA molecule called an aptamer. This aptamer binds specifically and tightly to prostate specific membrane antigen (PSMA), a protein overexpressed on CRPC tumors. DES-aptPSMA chimeras bind to CRPC cells rather than other cells that don't express PSMA.

Methods

The conjugation reaction between DES and the 24-mer is an example of "Click Chemistry", a class of organic reactions characterized by being easy to perform and high-yield, with only easily removed by products. The reaction itself is a copper-catalyzed cycloaddition between a butyne group on the fluorescein-DES molecule, and an azide group on the 24-mer.

4.2 micromoles of the 24mer oligonucleotide is combined with 42 micromoles of the fluorescein-DES molecule. Tris(3-hydroxypropyltriazolylmethyl) (THPTA) is combined with copper sulfate in a 2:1 molar ratio, incubating for several minutes before addition to the reaction. A schematic diagram of the reaction is shown below.

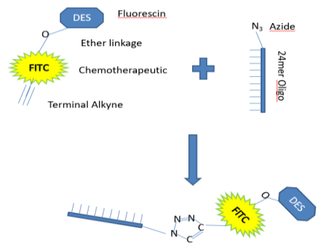

Figure 2: A schematic diagram showing the conjugation reaction between fluorescein-DES and the 24mer Oligo

Following conjugation, the DES-24mer conjugate is annealed with the A10 aptamer. The A10 aptamer is also annealed to the 24mer without the DES, for use as a control in cell trials. A schematic diagram of the annealing is shown below.

Figure 3: A schematic diagram showing the annealing of the DES-24mer conjugate to the A10 Aptamer. The DES-FITC molecule (in the dashed box) is absent in the control compound mentioned above.

Results

Conjugation Reaction was Successful

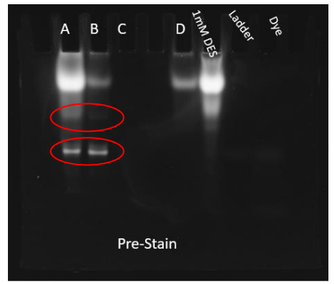

Figure 4: Gel electrophoresis performed on a microscale conjugation reaction. Lane B shows macroscale conditions. Notice the fluorescence of the DES-Fluorescein molecule in the circled bands in lanes A and B, indicating they are both product

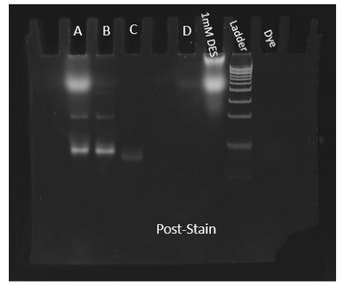

Figure 5: Gel from Figure 2, now stained in ethidium bromide

Lane	Details
A	10mM DES with 24mer
B	1mM DES with 24mer
C	24mer, no DES
D	1mM DES, no 24mer

Table 3: Lanes of gel depicted in Figure 2.

Cytotoxicity of Pure DES Determined on 22RV1 and C4-2 Cell Lines

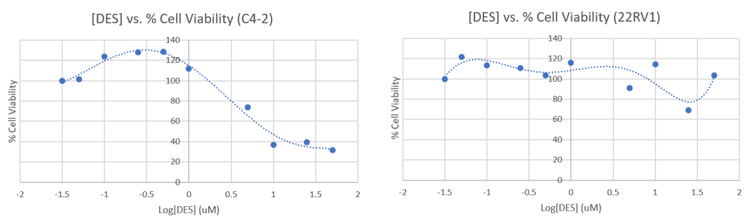

Figure 7: Log-scale graphs showing the effects of increasing DES concentration on cell viability for C4-2 and 22RV1 cell lines. DES concentrations range from 0 to 50 uM.

DES-24mer Conjugate Successfully Annealed to A10 Aptamer

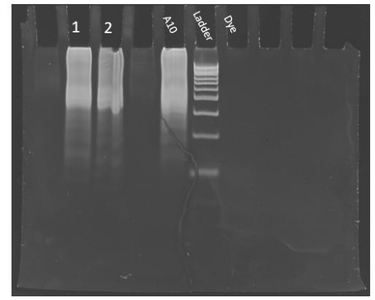

Figure 6: Gel for annealing. Streaks in lanes 1,2, and A10 are due to the folding of the A10 aptamer

Lane	Details
1	A10-24mer-DES
2	A10-24mer (No DES)

Table 4: Lanes of gel depicted in Figure 3. The numbering of the lanes aligns with the reactions detailed in Table 2

Conclusions

- DES-fluorescin can be successfully conjugated to a 24-mer oligonucleotide through a copper catalyzed cycloaddition reaction between a butyne group and an azide group.
- This conjugate can then be annealed to an RNA aptamer.

Future Studies

- Cell treatments utilizing the DES-aptamer chimera to determine its efficacy against cancer cells
- Animal trials to determine efficacy of chimera, as well as to determine if incidence rate of cardiovascular complications is lessened

References

Bosset et al., 2006; BJU Int. 110, E826-E829

Acknowledgements

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