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.

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(2-hydroxypropytriazolymethyl) (THFTA) 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.

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.

Results

Conjugation Reaction was Successful

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

CONCLUSIONS

• DES-fluorescein 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

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