CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

Vardhaan S. Ambati

Project Number

36706

Project Title

A Permutable Nanotherapeutic Using Engineered CRISPR/Cas9: A Personalized Treatment for Cancer Heterogeneity and Viruses

Abstract

Objectives/Goals

The goal is to create a specific nanotherapeutic consisting of engineered CRISPR/Cas9, quantum dot, and aptamer for treatment of cancer heterogeneity and rapidly mutating viruses at cellular level. In a cell, aptamer tethers nanotherapeutic to local structure, immobilizing it. Cas9 will only be released to cut genomic DNA in disease cells because the tether that binds Cas9 will be cut only in presence of proteins native to target cell.

Methods/Materials

Via inverse PCR, CRISPR/Cas9 fusion protein was produced from native Cas9 plasmid by inserting DNA sequences to code for thrombin (representing target cell protease) cleavage site and an E. coli biotinylation sequence at the C terminus of the Cas9 gene. Cas9 was His-purified from E. coli, and dot blot confirmed biotinylation. The Cas9 fusion protein was attached to a streptavidin coated Quantum dot by incubation using srreptavadin-biotin bond. A column chromatography proof of concept assay with biotin agarose representing structures in target cells and thrombin representing target cell protease that cuts Cas9 linker was conducted. The eluted Cas9 and a control was mixed with DNA and was run on agarose gel.

Results

PAGE gels confirmed CRISPR/Cas9 was expressed because there were clear band at 163 kDa, which is weight of Cas9. In the proof of concept assay, gel electrophoresis confirmed that DNA representing DNA of healthy cell (control) was not cut, indicated by one band at 2k base pairs. However, the DNA that represented the DNA of target cell was cut. There were two bands: 1st at 1.4k base pairs and 2nd at 600 base pairs. This assay showed that engineered Cas9 was released when column representing target cell was eluted with thrombin, which represents a target cell specific protease. Experiment repeated 20 times.

Conclusions/Discussion

In cancer, treatments are ineffective due to gene expression heterogeneity among cancer cells. Similarly, viruses mutate rapidly resulting in resistance. A therapy consisting of several such nanotherapeutics utilizing different proteolytic enzymes and targeting diverse DNA mutations can address cancer heterogeneity or viruses in a personalized manner because the cancer cell or virus cannot mutate fast enough to become resistant to all possible permutations of nanotherapeutic. The CRISPR/Cas9 acts as the active agent by cutting the DNA of the cancer or virus infected cell, preventing the cancer or virus from proliferating.

Summary Statement

A nanotherapeutic consisting of CRISPR/Cas9 and quantum dots designed to be easily permutable to facilitate the neutralization of escape mutations that are characteristics of cancer and viruses by targeting multiple genes and polymorphisms.

Help Received

Johan Sosa and Dr. Eric Espinosa for helped me design and conduct experiments. My teacher Dr. Thuy Anh Nguyen for being my advisor. BioCurious, a community lab, where I conducted my experiment. Eric Harness for helping me acquire quantum dots.