CALIFORNIA STATE SCIENCE FAIR
2014 PROJECT SUMMARY

Name(s)

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Project Number

S0517

Project Title

A Novel Reporter System for Analyzing and Evaluating a Smart Bomb Approach to Annihilate HIV

Abstract

Objectives/Goals

The intent of this research is to create a reporter system to assess the effectiveness of a newly discovered smart bomb approach to combat HIV. If a plasmid composed of the glycoprotein-160 HIV gene, and the two reporter genes Green Fluorescence Protein (GFP) and Firefly Luciferase (Ffluc), is able to be successfully inserted into human T-cells to create cells that look like HIV, then data can be collected to analyze the efficacy of the therapeutic aptamer-small interfering RNA (siRNA) chimera for HIV cell destruction.

Methods/Materials

A plasmid that carries the 3 genes through molecular cloning was created and inserted into a human T-cell so that it would express the HIV-glycoprotein 120 (gp120) on the cell surface, thereby mimicking an HIV-infected cell. The plasmid was then transformed into bacteria to multiply. Taking the purified DNA plasmids, they were transfected into human cells through lentiviral packaging to create lentivirus. Human CD4+ T-cells were infected after the lentivirus multiplied. These infected cells were then tested by using Flow Cytometry and Luciferase Assays in order to analyze if the transgene in the cell was producing the HIV outer receptor protein, GFP, and Ffluc. Quantitative Real Time Polymerase Chain Reaction (qRT-PCR) was performed to measure and quantify the mRNA for GFP, Ffluc, and gp160.

Results

The results indicated that the GFP-P2A-Ffluc-T2A-gp160 lentivirus was successful in the infection and subsequent gene expression in the CEM T-cells. By performing flow cytometry and luciferase assays, the expression of GFP and presence of luciferase was confirmed respectively.

Conclusions/Discussion

Through data collection of the presence of the reporter genes, the functionality of the aptamer-siRNA in targeting the HIV look-alike cell could be determined. The siRNA attached to the aptamer silenced one or both of the reporter genes, so a dip in light emission or fluorescence after the aptamer was introduced has proved that the aptamer does indeed have an effect on HIV. In conclusion, the Ubiquitin promoter in the lentiviral vector provides evidence of strong expression of all three genes. The Ffluc signal is detectable while GFP signal in those transduced cells were 100 times brighter than the non-infected cells. Lastly, the HIV gp160 gene expression does not appear to be toxic to cells. This indicates that our construct is safe and usable for human and animal systems.

Summary Statement

I created a novel reporter system to assess the efficacy of a new approach against HIV by constructing a plasmid consisting of an HIV outer receptor gene and two reporter genes to create an HIV look-alike cell that luminesces and fluoresces

Help Received

My research was performed at Dr. John Rossi's lab at Beckman Research Institute at City of Hope under the supervision and great mentorship of Dr. John Burnett. I have obtained invaluable training as a student trainee of the Research Training Program hosted by Southern California Academy of Sciences.