CALIFORNIA STATE SCIENCE FAIR
2009 PROJECT SUMMARY

Name(s)
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Project Number
S0407

Project Title
Using RNA Interference to Block HIV Entry into Cells

Abstract

Objectives/Goals
Human immunodeficiency virus (HIV) causes Acquired Immunodeficiency Syndrome (AIDS), an epidemic across the world that has no cure. HIV needs two receptors in order to enter a T- cell: CCR5 and CD4. Importantly, some people are born without CCR5 and are resistant to HIV infection. This project investigates the use of a recent genetic approach, RNA interference (RNAi), to reduce CCR5 RNA and thereby prevent HIV from entering target cells. The overall goal is to test several new RNAi reagents and to determine if combinations targeting different regions of CCR5 mRNA are more effective.

Methods/Materials
One form of RNAi called Short hairpin RNA (shRNA) was used to target the RNA for the CCR5 receptor. Rather than working directly with HIV or CCR5, which are technically more difficult (and dangerous), a fluorescent #reporter# was created in order to have an easier and more quantitative method of measuring the amount of CCR5 mRNA. DNA cotransfection was used to introduce shRNAs and the reporter into cells and the effects measured by microscopy and flow cytometry.

Results
A CCR5 reporter system was constructed using the red fluorescent gene, mCherry, and used to identify several new shRNAs that target CCR5 mRNA well. Two shRNAs appear to downregulate better in combination than each alone.

Conclusions/Discussion
These results show that potent shRNAs can be identified to downregulate the HIV receptor CCR5. Combinations of these shRNAs may be even better at downregulating CCR5. In further studies, the two shRNAs will be combined into a single plasmid to confirm their potency. In the future, combinations of shRNAs targeting CCR5 may be used by other researchers as a stem cell therapy to mimic a natural genetic deficiency of CCR5 and block HIV infection of T-cells.

Summary Statement
This study focuses on investigating one potential approach to eliminate the CCR5 receptor.

Help Received
Used laboratory equipment at David Geffen School of Medicine at UCLA; supervised and mentored by Dr. Masakazu Kamata.