CALIFORNIA STATE SCIENCE FAIR
2004 PROJECT SUMMARY

Name(s)

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Project Number

S0422

Project Title

The Effectiveness of RNA Interference at Inhibiting the Proliferation of HSV-1 by Silencing ICP27

Abstract

Objectives/Goals

Determine what sequence of siRNA is most effective in targeting ICP27.
Compare the amount of viral proteins between cells with and without RNAi.
Determine whether RNAi can inhibit HSV-1 infection.

Methods/Materials

1) Synthesize siRNA corresponding to desired genomic sequence of ICP27 of Herpes Simplex Virus Type 1 (HSV-1).
2) Determine which strands of siRNA are most effective via gel electrophoresis (SDS PAGE).
3) Transfect HeLa cells with siRNA and Oligofectamine Reagent.
4) Infect HeLa cells with HSV-1, harvest after 5-6 hrs.
5) Collect samples and run SDS PAGE; continue with a Western Blot.
6) Proceed with antibody staining and develop onto film.
7) For HSV-1 infected cells, label in vivo with S35-methionine to measure protein production and run SDS PAGE.
8) Stain gel with Coomassie blue for visualization.
9) Collect infected cells for a plaque assay count.

Results

Results confirm that siRNA is extremely effective in knocking down ICP27, with knockdowns upwards of 84% and statistical significance at the P=0.00446 level. Differences among siRNA sequences were not statistically significant (P=0.2112). The silencing of ICP27 shown by Western blots resulted only in minor differences in the amount of viral proteins between normal cells infected by HSV-1 and those with siRNA, shown by autoradiography and Coomassie blue staining. The plaque assays show siRNA#2 is more effective than siRNA#3 in inhibiting the proliferation of HSV-1. The number of plaque forming units (pfu) shows that HSV-1 cannot reproduce without ICP27. The difference in number of pfu in the mock and siRNA wells is statistically significant at the P=0.08987 level. A comparison of the siRNA wells shows that siRNA#2 worked better than siRNA#3 at bringing down the infection rate, statistically significant at the a=0.05 level with a p-value of 0.023.

Conclusions/Discussion

Our hypothesis was partially correct. Reduction of the viral protein ICP27 was successful, but a complete knockdown may be necessary to stop the proliferation of the virus. Further research includes using multiple siRNAs simultaneously or targeting several important proteins at once. Our research serves as a

Summary Statement

In this study, RNAi is used to silence ICP27, a crucial protein that exports intronless viral mRNA in Herpes Simplex Virus (HSV-1), in hopes of inhibiting the proliferation of HSV-1 in HeLa cell culture.

Help Received

Used lab equipment at the University of California, Irvine under the supervision of Dr. Rozanne Sandri-Golden and Santos Rojas; Santos Rojas did infections for us.