CALIFORNIA STATE SCIENCE FAIR
2002 PROJECT SUMMARY

Name(s)
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Project Number
22461

Project Title
Retroviral-Mediated Transfer and Expression of Neomycin and Hygromycin B Drug Resistance Genes

Abstract

Objectives/Goals
In this experiment, the Murine Leukemia Virus (MLV) pseudotyped with the Vesicular Stomatitis Virus glycoprotein (VSVg) was used to transfer the neomycin resistance gene and hygromycin B resistance gene within 293 cell line, human embryonic kidney cell line.

Methods/Materials
A 293T cell line, human embryonic kidney cell line, was cultured to package the pseudo-typed virus. 48 hours after transfection, the viral supernatant was harvested for the infection of the 293 cell line. Selection began 48 hours after infection and 500 ug/ml of G418 and 150 ug/ml of hygromycin B was added to 10% Fetal Bovine Serum (FBS) Dulbecco#s modified Eagle#s medium (DMEM). On a weekly basis, the number of cells were counted with the Trypan Blue Exclusion Assay.

Results
One week after selection, there were 46 x 105 cells/ml resistant to neomycin, 43.5 x 105 cells/ml resistant to both neomycin and hygromycin B (cells co-infected), and 6.25 x 105 cells/ml resistant to hygromycin B. Two weeks afterwards, the genes expressing hygromycin B and cells co-infected had become silenced. The neomycin drug resistance expressing cells, however, maintained a growth and had 61 x 105 cells/ml after two weeks, 78 x 105 cells/ml after three weeks, and 109 x 105 cells/ml after four weeks. Thus this system successfully transferred and expressed the drug resistance genes within 293 cells, but was not able to sustain the expression of hygromycin B resistance. Currently, the neomycin resistant cells are still being cultured and experimented with in order to determine the length of time 293 cells are able to express a transferred drug resistance gene before being silenced.

Conclusions/Discussion
In this experiment, the neomycin resistance gene was expressed in more cells than the hygromycin B resistance genes. An explanation for this may be that MND-EGFP-SN, providing resistance to Neomycin, was much smaller in genomic size. While the neomycin resistance gene was only 860 base pairs (bp), the hygromycin B resistance gene was more than twice as large, 1,800 bp. The silencing of the hygromycin drug resistance gene expression could have been caused by several factors: area of site integration, type of gene transferred, type of cell used as a host, an inefficient promoter, and DNA methylation. Since no hygromycin B resistance was found in 293 cells after ten days, the co-infected cells also died due to the application of Hygromycin B, not G418 (Neomycin).

Summary Statement
In this experiment, the MLV-VSVg retroviral vector transfers and expresses the neomycin and hygromycin B drug resistance genes in 293 cells.

Help Received
Used lab equipment at USC Medical School under the supervision of Dr. Michael Lai and Vicky Sung