CALIFORNIA SCIENCE & ENGINEERING FAIR
2019 PROJECT SUMMARY

Name(s)

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Project Number

S0513

Project Title

Epigenetic Therapy for Liver Cancer: The Effect of 5-Azacytidine on the Expression of Tumor Suppressor Genes

Abstract

Objectives

This study examines epigenetic therapy as a treatment for slowing the growth of cancerous liver cells. In addition to having genetic and environmental causes, cancer can be considered an epigenetic disease. More specifically, epigenetic changes such as the abnormal patterning of DNA hypo- and hypermethylation are known to be common characteristics of cancerous cells and play critical roles in the regulation of the disorder. Hypermethylation, in particular, in the promoter regions of tumor suppressor genes is a hallmark of human tumors and leads to the transcriptional silencing of critical defense proteins responsible for tumor cell invasion, cell cycle control, DNA repair and other processes where silencing would lead to the spread of cancer. Thus in investigating epigenetic therapy as treatment for cancer, this experiment tested the effect of 5-azacytidine (a DNA methyltransferase inhibitor on the growth of liver cancer by comparing cell counts, cellular viability rates, and examining the expression of tumor suppressor genes p15INK4b, p16INK4a, and SOCS-1 through RT-PCR and gel electrophoresis. The results of this experiment, seen through cell counts and gel images, show 5-azacytidine (at 1.5uM = 3uM dosage) does not serve an important role in minimizing the growth of liver cancer cells, as there was no statistically significant difference between treated and control cell counts and viability rates. Coupled with this, the gel electrophoresis displayed the overall expression of p15INK4b and p16INK4a in the WB311 treated and control cells as absent except for the presence of few random, faded bands. Bands from the SOCS-1 gene (both primers), though, appeared clearly with the treated DNA band appearing thicker than the control. We believe that 5-azacytidine (at 1.5ul = 3uM dosage) may hold the potential to increase the expression of certain tumor suppressor genes but does not heavily influence the translation process whereby these genes produce proteins for cancer defense.

Methods

Materials used for this experiment include 5-azacytidine (1.5 uL dosage), WB311 (Rat cancerous liver) cell line, primers for p15INK4b, p16INK4a, and SOCS-1, and 2 cell plates (12 wells), and materials for cell counting with Trypan Blue. On day 3, RNA concentration from the treated wells and the control wells was measured. On Day 4, I performed a reverse transcriptase procedure to obtain cDNA. On Day 5, after the primers for p15INK4b, p16INK4a, and SOCS-1 were diluted, five master mixes were made (1 primer for p15INK4b, 2 primer for p16INK4a, and 2 primers for SOCS-1) and four samples created (treated +RT, control +RT, treated -RT, control -RT), resulting in 20 PCR tubes for electrophoresis. Gel electrophoresis was performed on the PCR products to measure the expression of the treated and untreated p15INK4b, p16INK4a, and SOCS-1 genes. I also used cell counting to measure cellular viability rates.

Summary Statement

I examined epigenetic therapy as a treatment for slowing the growth of cancerous liver cells.

Help Received

Dr. Jennifer Sanders, Amanda Dombroski, Valerie Zabala, Jessica Zambuto