CALIFORNIA STATE SCIENCE FAIR
2002 PROJECT SUMMARY

Name(s)

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Project Number

22683

Project Title

The Effect of Directing Ribozymes to Attack Proliferating Cell Nuclear Antigens and Inhibit the Growth of Cancer Cells

Abstract

Objectives/Goals

My project was to determine if by specifically directing a ribozyme to attack the Proliferating Cell Nuclear Antigen (PCNA mRNA), will the growth of a cancer cell be inhibited and thus kill the cell; and if the cells died, to determine the origin of their death.

Methods/Materials

A total of eight rat and human brain cancer cell cultures were treated with either ribozyme, ribozyme and lipid, dysfunctional ribozyme and lipid, c2 cerimide, or nothing. The treatments were cultured and WST-1 dye was added to be metabolized in the cells. The metabolic rate was determined by an ELISA plate reader, which read how much dye was in the cells. A different culture of cells had PI dye and ANNEXIN-5 dye added to the treatments. These were then read by flow cytometry to determine whether the cells died through necrosis or apoptosis.

Results

When compared to the control cells, the cancer cells which had the ribozymes and a specific lipid added seemed to have a reduction in growth, as much as 24%. However, the cells that had a dysfunctional ribozyme and lipid also had a reduction in growth, up to 18% when compared to the normal cells. In the second assay, when the mode of death was determined, our control worked. The cells with the c2 cerimide, a chemical know to induce apoptosis, did in fact die through apoptosis. The cells that had no treatment were still living, however the cells with all other treatments died through necrosis and not apoptosis.

Conclusions/Discussion

Although the first assay appeared to have succeeded in reducing the cancer cell count, the treatments that had the largest death rate were due to the toxic lipid that was added to them. Also, the naked ribozyme treatment did reduce the growth of the cancer cells, which means that although the RNA piece is small, it will be taken into the cell. Less exciting was the death of the brain cancer cells, which appeared to be mainly through necrosis. This means the cell lyses or bursts, which can cause severe swelling. However, the control c2 cerimide treatment induced apoptosis assuring me that the procedure I went through was correct and can be repeated in later experiments.

Summary Statement

My project involved using a particular known catalyst, called ribozyme, and directing it to attack PCNA, a transcription factor necessary for polymerase to proofread the mRNA, thus creating shorter mRNA fragments and cancer cell death.

Help Received

Dr. Joan Robbins and Mr. Eric Alspaugh, Immusol, Inc. for direction and assistance; Dr. Carol Kruse at Univ. of Colorado for consultation.