CALIFORNIA STATE SCIENCE FAIR
2002 PROJECT SUMMARY

Name(s)

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Project Number

22226

Project Title

Effects of UVB on p53 Wild Type and Null Cells

Abstract

Objectives/Goals

p53 is a tumor suppresser gene commonly mutated in human cancers. It is responsible for the regulation of cell cycle arrest and/or apoptosis when DNA damage occurs. The purpose of this work was to compare the effect of UVB irradiation on HCT cells that had p53 (p53+/+) versus cells lacking p53(p53-/-).

Methods/Materials

Wild Type and null cells were exposed to different doses of UVB light (312 nm), and cell death was measured using a Coulter counter to determine the correct amount of exposure for the microscopy experiments. Cells were grown in Lab Tec chamber slides. The cells received 0 or 750 mJ/m2 of UVB light. After exposure the cells were incubated for 24 or 48 hours. The cells were then staind with DAPI, a nuclear stain, and photographed.

Results

In the first part of the experiment, cell death in p53+/+ cells ranged from 34% at 250 mJ/m2 to 74% at 1000 mJ/m2. p53-/- cell death ranged from 0% at 250 mJ/m2 exposure to 82% at 1000 mJ/m2. In the microscopy experiment 24 hours after irradiation, very few p53 null cells appeared to be undergoing apoptosis whereas many p53 wild type cells were apoptotic. At 48 hours, all the surviving p53 +/+ cells appeared to be normal, however there were few mitotic cells suggesting that cells were in cell cycle arrest. In contrast, there were many mitotic p53 -/- cells, and many more cells with abnormally large nuclei or several nuclei were observed.

Conclusions/Discussion

The microscopy results at 24 hours indicate that +/+ cells do undergo apoptosis at high doses of UVB exposure. However, apoptosis was not apparent in the -/- cells, presumably because of the lack of p53, a regulator of apoptosis. In the -/- cells at 48 h, more mitotic cells were observed compared to +/+ cells and many more -/- cells with abnormally large nuclei or several nuclei were observed. Work by other groups have shown that p53-/- cells can go through mitosis after DNA damage, or that they arrest at the G2 phase, with multiple times the normal amount of DNA. Thus might explain the occurrence of oversized nuclei in the p53 null cells at 48 h. The results suggest that p53-/- cells respond incorrectly to UVB damage and can escape the cells cycle inhibition that occurs in cells with p53 present.

Summary Statement

This project studed the effect of UVB exposure of cells with and without a tumor suppressor protein, p53.

Help Received

Dr. Madeline Butler, Isis Pharmaceuticals, provided assistance and equipment