CALIFORNIA STATE SCIENCE FAIR
2002 PROJECT SUMMARY

Name(s)

Kedar Shah

Project Number

22350

Project Title

Identification of Single Amino Acid Mutations in the p53 Gene of Drosophila melanogaster

Abstract

Objectives/Goals

The goal of this study is to identify single amino acid mutations in the p53 gene of Drosophila melanogaster.

Methods/Materials

DNA from several fly strains, some with dominant negative p53 gene cDNA genomic insertions, was extracted. Primers were used to selectively amplify the gene of interest through PCR. After verifying th| success of the reaction through gel electrophoresis, the next step was to clone the gene into a vector. Using vector-specific primers, the gene from various strains was sequenced and mutations were identified| through computer analysis.

Results

The gels for each of the five attempts at DNA extraction and amplification either contained smeared band| or no bands at all. Therefore, recombination of the gene of interest into vectors was not attempted. Instead, plasmids containing the gene of interest were transformed into cells, which grew and multiplied| Large quantities of the plasmids were extracted from the cells using a Qiagen midi-preparation. A gel of| the plasmids after the midi-preparation showed bands at the correct size. PCR with a new set of primers was then run on the plasmids containing the gene of interest. A gel for this reaction showed multiple bands at 1025 base pairs for each sequence. Results of the sequencing are still pending.

Conclusions/Discussion

Five attempts to extract and amplify the mutated cDNA p53 sequences from the Drosophila genome were unsuccessful because the primers used were designed for a region outside the Dmp53 cDNA sequence engineered into the flies. A new set of primers was used for the PCR of the gene of interest contained i| plasmids. The success of this step verified that the same gene-specific primers could potentially be used to sequence the midi-prepared plasmids. If successfully found, the mutated sequences of the Dmp53 cDNA can be compared to the standard wild type sequence to identify single amino acid mutations. These single| amino acid mutations cause dysfunction in Drosophila melanogaster. Because Dmp53 is homologous to human p53, a gene whose mutations are the single most frequent cause of human cancer, understanding the cause and effect of single amino acid mutations in Dmp53 is instrumental in understanding the nuances of human p53.

Summary Statement

My project focuses on finding mutations in a strain of fruit flies that are genetically engineered with the p53 gene, which functions as a tumor suppressor in both fruit flies and humans.

Help Received

Received guidance from teacher Tanya Buxton and mentor Vikash Bhagawandin; Obtained fly strains from Mike Olmann of Exilixis Inc.; Obtained primers from Parkash Jhurani of Genentech Inc.; Sequencing performed by Dhaya Seshasayee at Genentech Inc. and Vikash Bhagawandin at UCSF.

Preliminary