2002 TAIWAN INTERNATIONAL SCIENCE FAIR

CATEGORY：Biochemistry

PROJECT TITLE：Gene Targeting Using Chimeric RNA-DNA Oligonucleotide for Capase-10 in Various Cell Types

AWARD：First Award

SCHOOL：National Junior College

FINALISTS：Ong Mei Ci Victoria

COUNTRY：Singapore

ABSTRACT

Category: Biochemistry

Title: Gene Targeting Using Chimeric RNA-DNA Oligonucleotide for Capase-10 in Various Cell Types

Name: Ong Mei Ci, Victoria

Country: Republic of Singapore

Background:
The process of gene targeting via chimeraplasty is achieved by using a RNA-DNA oligonucleotide homologous to a gene of interest, to introduce a single base pair or frameshift mutation in genomic DNA. The extensive use of chimeraplasty is currently limited by wide variation in its gene conversion rates (.01-40%) and its mechanism of action remains to be fully understood. For cell studies, chimeraplasty is an alternative strategy to homologous recombination in generating gene knockout models.

Purpose of the research:
To further comprehend the process of chimeraplasty, I tested different cell lines using a Chimeraplast designed to disrupt Caspase-10 expression. Caspase-10 is a protease that is activated during programmed cell death (apoptosis). The physiological role of caspase-10 is not known since no knockout model exists for this gene.

Procedures:
In this study, the cell lines used were; Jurkat E6.1, a T cell leukemia, Nalm 6, a pre-B cell leukemia, DLD-1 and HCT116, colon carcinoma cells. To evaluate experiments, a PCR-RFLP protocol was set up and optimized to detect a single point mutation introduced by Chimeraplasts. Chimeraplasts for Caspase-10 gene (.25-1.25 ug) was introduced into 10^5 cells using Fugene transfection reagent. After 48 hours of exposure to chimeraplasts, cells were lysed and genomic DNA was extracted using DNAzol reagent and analyzed by PCR-RFLP. Results were analyzed using densitometry. To validate persistent transmission of the gene conversion to further descendants, I examined the transfected cells after 7 generations.

Results and Data:
Analyses of colon cancer cell lines DLD-1 and HCT116 transfected with Caspase-10 chimeraplast, exhibited 2-9% gene conversion. No gene conversion was observed in Nalm-6 and Jurkat E6.1 cells. Optimum gene conversion rates were achieved consistently with .25 ug chimeraplast/10^5 cells, suggesting that the result is not an artifact. Furthermore, analysis of a prolonged culture of transfected cells, show invariable gene conversion, thereby demonstrating that gene conversion via chimeraplasty is stable and inherited.

Conclusion:

These results show that the efficiency of chimeraplasty may be cell type dependent. Gene conversion was most significant in DLD-1 and HCT116 cell lines. Apparently, this is the first time that these 2-colon cancer cell lines have been demonstrated for chimeraplasty and may be superior to previously used cancer cell lines. Results obtained from DLD-1 and HCT116 cells can be a platform for further optimizing chimeraplasty as well as for generating Caspase-10 knockout cell lines.

評　　語

Using DNA-RNA hybrid Molecule as a vector for introducing site-directed mutagenesis into cells is highly innovative. The successfulness of this technique is well supported by the data provide. Additionally, the auother managed to provide data for studying cell dealth via the enzyme cascade that involved caspase ~10. This project is well designed and executed with great care. It is a “First place award” class of study.