CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)
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Project Number
S0408

Project Title
Keratins Gone Bad: A Look at the Causes of a Rare Skin Disease

Abstract

Objectives/Goals

PURPOSE

My project is to test if a plasmid expressing K16 WT (wild type or normal) protein makes filaments (chains of keratin). In addition, I will test to see if a K16 mutant plasmid, that contains a mutation identified in a Pachyonychia Congenita patient, disturbs the making of filaments (making aggregates, broken filaments, instead).

HYPOTHESIS

I hypothesize that if I transfect K16 WT (wild type) fused to Yellow Fluorescent Protein (YFP) into human tissue culture cells, the cell will express strong, normal filaments. If I transfect K16 ÄHTM (mutant) fused to YFP into human tissue culture cells, the cells will form aggregates in place of filaments.

Methods/Materials

Overall objective: make a fusion between K16 (ÄHTM or WT) and a reporter protein, YFP. Therefore, because YFP can be seen using a fluorescence microscope, the K16 ÄHTM or WT fused to YFP can be seen.

1.Using Recombinant DNA Technology, use restriction enzymes and ligase to insert the K16 PCR fragment into an expression plasmid, which contains YFP.

2.Purify the K16 WT/YFP and the K16 ÄHTM/YFP expression plasmids, and transfect them into human tissue culture cells.

3.Incubate the cells for a few days to allow the cells to make K16 WT and K16 ÄHTM proteins.

4.Look under a fluorescence microscope for filaments and aggregates.

Conclusions/Discussion

My hypothesis was correct the K16 ÄHTM/YFP plasmids that were transfected into human tissue culture cells made aggregates and the K16 WT/YFP plasmids made filaments when transfected into human tissue culture cells. According to the results, it is possible that the broken filaments, or aggregates, cause people with the deletion mutation to have the symptoms of Pachyonychia Congenita patients.

The results are not as clear as I thought they would be. Although the difference between filaments and aggregates was clear, I was surprised that there was a mix of filaments and aggregates in the cells transfected with K16 ÄHTM (mutant) protein. Furthermore, there were some variations of the K16 WT (wild type) cells which contained a few aggregates.

Summary Statement

My project was to look at the effects of a rare skin disease mutation in keratin cells.

Help Received

Transderm, Inc. helped with project plan, supplies, and equipment; Somagenics, Inc. also procided necesary equipment; Dr. Francis Smith at University of Dundee, Scotland provided the K16 mutant and wildtype constructs; Dr. Michael Dalbey at the University of California Santa Cruz allowed me to use a