CALIFORNIA STATE SCIENCE FAIR
2008 PROJECT SUMMARY

Name(s)

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Project Number

S0410

Project Title

Get1 Transactivator Regulates Epithelial Barrier Protein Upk2 in a Cell-based Luciferase Assay

Abstract

Objectives/Goals

The purpose of this project was to design a cell-based assay investigate the particular interaction between a transcriptional regulator Get1 and Uroplakin 2 (Upk2). The Grainyhead-like epithelial transactivator (Get1) is a transcriptional regulator linked to epithelial differentiation in the bladder. Uroplakin 2 is an essential bladder structure protein found to be lacking in Get1 knockout mice.

Methods/Materials

A prospective Get1 binding site was identified upstream of the Upk2 gene. This 2.8kb Get1 binding site was ligated into a pGL3 luciferase assay expression vector. The Get1 was ligated into a pcDNA plasmid. Both ligated pGL3 vector and pcDNA plasmid were co-transfected into HaCat cell, a human keratinocyte cell line. Luciferase assay was performed to demonstrate the relationship of Get1 and Upk2 gene products.

Results

The luciferase assay results show that the 2.8kb pGL3 plasmid produce more luciferin than its mutated counterpart. The 2.8kb plasmid is consistently more productive, often significantly so, than the negative control 2.8kb mutated plasmid. Furthermore, the testing of various relative concentrations of pGL3 and pcDNA plasmid DNA showed that the highest luciferin productions were the range of 0.005ìg, 0.01ìg, and 0.05ìg pcDNA to 1.6ìg of pGL3, making these the most effective concentrations.

My data indicated that the tested binding site did show up regulation in the presence of Get1.

Conclusions/Discussion

In conclusion, my data show that the 2.8kb pGL3 plasmid produce more luciferin than its mutated counterpart, leading to the conclusion that the prospective Get1 binding site was the actual, functional binding site, indicating a direct correlation between Get1 and the uroplakin 2 protein. The data support the hypothesis that the tested Get1 binding site was the actual connection between the presence of Get1 and Upk2.

The three data sets given by my three repetitions of the procedure all have some consistent positive trends, but also show enough variation to demand further experimentation and more results.

Summary Statement

My project is designing a cell-based assay to demonstrate Get1 as the transcriptional regulator of Uroplakin 2, an essential structural protein for bladder epithelial differentiation.

Help Received

I would like to thank Dr. Andersen, my mentor, Amy Soto, my supervisor, and my mother. I am also very thankful for Dr. Andersen#s guidance in terms of research direction and experimental design. I thank Amy Soto, a graduate student in Dr. Andersen#s lab, for supervising my work, educating me in laboratory