CALIFORNIA STATE SCIENCE FAIR
2006 PROJECT SUMMARY

Name(s)

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Project Number

S0401

Project Title

Preliminary siRNA Analysis of Genes Implicated in Neuronal Differentiation and Neurite Outgrowth

Abstract

Objectives/Goals

The focus of this study was to develop and validate a cell-based assay to screen previously identified candidate genes that may be essential in neuronal differentiation and neurite outgrowth.

Methods/Materials

In this study, nineteen genes which were previously shown by DNA microarray analysis to be up-regulated when mouse P19 cells were induced to differentiate into neuronal cells were selected for assay development and initial gene candidate validation. The mRNAs of candidate genes were targeted in differentiating P19 cells using Dicer-generated small interfering RNAs (siRNAs) to determine if a reduction in gene-specific mRNA levels interfered with differentiation and neurite outgrowth.

Results

Based upon statistically significant changes in cell body area and / or neurite length when differentiating P19 cells were targeted with gene-specific siRNA, three of the nineteen candidate genes evaluated appear to be important in neuronal development.

Conclusions/Discussion

As a preliminary step to test the effectiveness of Dicer generated siRNAs against specific genes, siRNAs were generated against MAP2 and b-III tubulin, two proteins found in neuronal P19 cells. When co-transfected with the NeuroD2 expression plasmid (to initiate neuronal differentiation) these gene-specific siRNAs were effective in greatly reducing the amount of MAP2 and b-III tubulin protein expressed.

To test the ability to detect phenotypic changes when genes known to be important in neuronal differentiation were targeted, siRNAs were used to inhibit the expression of AKT1 and AKT2. These experiments showed that inhibition of critical genes can result in a measurable change in cell body and neurite length.

Using this assay, nineteen genes that were previously determined to be potentially important in neuronal differentiation and neurite outgrowth were selected and siRNAs were generated for each candidate gene. The siRNAs were co-transfected with the transcription factor NeuroD2 to initiate neuronal differentiation. The knockdown of three candidate genes resulted in a statically significant change in either cell body size and / or neurite length. To further validate the importance of these candidate genes in neuronal development chemically synthesized siRNAs, which are more specific reagents for RNA inhibition, will

Summary Statement

To develop and validate a cell-based assay to screen previously identified candidate genes that may be essential in neuronal differentiation and neurite outgrowth.

Help Received

This study was conducted in the laboratory of Shelly Halpain, Ph.D. at the Scripps Research Institute in La Jolla, CA under the mentor ship of Leif Dehmelt, Ph.D.