CALIFORNIA STATE SCIENCE FAIR
2010 PROJECT SUMMARY

Name(s)

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Project Number

S0419

Project Title

In vitro Analysis of a Synthetic Protein: A Model for Enzyme Replacement Therapy

Abstract

Objectives/Goals

My first objective was to determine if a glycine serine linker could be used to attach an Insulin-like Growth Factor 2 (IGF2) tag to the enzyme alpha-N-acetylglucosaminidase (NAGLU) to create a NAGLU-IGF2 fusion protein that retains NAGLU's enzymatic activity. My second objective was to determine if the IGF2 tag improves uptake of synthetic NAGLU by cells.

Methods/Materials

A plasmid coding for the production of the NAGLU-IGF2 fusion enzyme with a glycine serine linker was created, amplified in E. coli cells, extracted and screened by gel electrophoresis. DNA sequencing verified correct construction of the plasmid. The plasmid was transfected into Chinese hamster ovary (CHO) cells to express the NAGLU-IGF2 enzyme. CHO cells were subcloned. The CHO cell growth medium was collected and assayed using Western blots and enzymatic activity assays. NAGLU-IGF2 was purified from the CHO cell growth medium using Concanvalin-A and c-Myc affinity columns. Human fibroblasts deficient in NAGLU were treated with purified NAGLU-IGF2 and lysed open to measure cellular uptake of the fusion enzyme.

Results

Western blots confirm that NAGLU-IGF2 is successfully produced and purified. Enzymatic activity assays reveal that NAGLU-IGF2 has enzymatic activity. Uptake assays show that NAGLU-IGF2 can enter human fibroblasts.

Conclusions/Discussion

My previous two years of research showed that attaching the IGF2 tag directly to NAGLU caused NAGLU to lose its enzymatic activity. The glycine serine linker has allowed the IGF2 tag to be attached to NAGLU and preserve NAGLU's ability to bind with its substrate, as hypothesized. Furthermore, the IGF2 tag improves NAGLU's cellular uptake, in comparison to a negative control: synthetic NAGLU without the IGF2 tag. As created by my project, NAGLU-IGF2 could potentially be used as enzyme replacement therapy for Sanfilippo B syndrome, a genetic disorder resulting in the body's inability to produce NAGLU.

Summary Statement

My project uses recombinant DNA techniques to create a NAGLU-IGF2 fusion protein and characterizes it.

Help Received

Used lab equipment at Los Angeles Biomedical Research Institute with mentoring by Patricia Dickson, MD