CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

S0505

Project Title

Expression and Identification of N-acetylglucosamine-6-sulfatase (GNS): A Potential ERT Drug for Sanfilippo Syndrome D

Abstract

Objectives/Goals

There is currently no cure for Mucopolysaccharidosis IIID (MPS IIID or Sanfilippo syndrome D), which is a genetic lysosomal storage disorder caused by an absent lysosomal enzyme, N-acetyl-glucosamine-6-sulfatase (GNS). However, an enzyme replacement therapy (ERT) treatment may be a potential way to attenuate symptoms for MPS IIID patients. In order to obtain enough enzyme for the ERT, I isolated the highest GNS expressing Chinese hamster ovary (CHO) cells transfected with the human GNS gene.

Methods/Materials

The human GNS gene was transfected into Chinese hamster ovary (CHO) cell lines, and the protein was purified from the culture medium. A sulfatase activity assay was used to isolate the highest expressing clones out of 58 original clones, after which, the GNS fluorescent intensity assay was run to confirm the expression of these clones. After the highest expressers were isolated, they were re-cultured and their activities were monitored over two weeks. Then, two cell lines of MPS IIID fibroblasts were incubated with the CHO cell culture medium and tested for GNS uptake.

Results

Nine clones showed the highest sulfatase activity, and when monitored over two weeks, their activities all increased. After the screening with the GNS fluorescent intensity assay, however, these clones showed lower GNS activities than expected, suggesting that another CHO cell line needs to be transfected with the human GNS gene to acquire greater expression of the enzyme. After the fibroblasts were incubated with the CHO culture medium, the sulfatase activity inside the fibroblast cells was similar to that in the original clone culture medium.

Conclusions/Discussion

Although the ideal amount of GNS was not secreted by the transfected CHO cells, the optimization of the GNS assay and confirmation of a functioning GNS pure protein were achieved. Because the sulfatase activity following the uptake assay was similar to that of the original clones# culture mediums, it suggested that the cells contained similar amounts before and after the incubation. In the future, the GNS produced by the transfected CHO cells may be used in ERT to treat patients with Sanfilippo Syndrome D.

Summary Statement

I isolated the highest GNS expressing Chinese hamster ovary (CHO) cells transfected with the human GNS gene in order to obtain enough enzyme for future ERT treatments.

Help Received

Used Los Angeles Biomedical Research Institute facilities under the supervision of Dr. Patricia Dickson