

CALIFORNIA STATE SCIENCE FAIR 2011 PROJECT SUMMARY

Name(s)

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Project Number

S0519

Project Title

Optimization of Transfection Efficiency and Cell Viability in Various Cancer Cell Lines for Gene Therapy

Objectives/Goals

Abstract

Gene therapy involves delivering therapeutic genes to diseased cells in order to introduce a new function or correct a genetic abnormality. Gene therapy can be used as a promising alternative in the treatment of cancer, such as the introduction of the p53 tumor suppressor gene in certain cancerous tissues. However, the potential of gene therapy is limited due to delivery barriers; for example, nucleases exist in circulation to degrade free nucleic acids. Nonviral vectors, such as polyethylenimine(PEI), offer a solution to these barriers by protecting DNA and increasing cellular uptake. In this study, transfection efficiency and cytotoxicity utilizing different concentrations of PEI-derived polyplexes were evaluated by delivering GFP-encoding DNA into four cancer cell lines. The goal was to optimize the transfection efficiency while minimizing cytotoxic effects. This library will be useful as a model for subsequent studies.

Methods/Materials

.Biosafety cabinet; .gloves; .Eppendorf tubes; .Pipette and pipette tips; .Guava Flow Cytometer; .Polyethyleneimine (PEI); .GFP-encoding DNA; .Dulbecco's modified Eagle's medium (DMEM); .centrifuge; .tissue culture dishes; .inverted microscope; .MTT solution; .DMSO; .Glycine buffer; .HeLa cells; .MCF7WT cells; .22RV1 cells; .T98G cells; .Fetal Bovine Serum; .Penicilin streptomycin; .0.5 Trypsin solution; .Hemocytometer; .De-ionized water.

Recults

By using flow cytometry and MTT Assay, I was able to gather quantitative data regarding transfection efficiency and cell viability respectively. By using fluorescence microscopy and bright sield microscopy, I was also able to gather visual representative data. At the end of data gathering, the average data showed that the optimized concentration was at $1.6 \,\mu\text{g/mL}$ for all the cell lines. However, all the cell lines also show distinct profiles regarding transfection efficiency and cytotoxicity.

Conclusions/Discussion

Increasing polyplex concentrations above a threshold will lead to high cytotoxicity levels but not necessarily guarantee the highest transfection rate. The optimized values for transfection rate and cell viability for these cancer cell lines would be at $1.6~\mu g/mL$ DNA when delivered with polyplexes at N/P ratio of 10. Although an optimized range was observed, it was also clear that different cancer cell lines had different transfection and cytotoxicity profiles.

Summary Statement

The goal is to optimize transfection efficiency and cell viability in various cancer cell lines for the advancement of polymer vectors in gene therapy.

Help Received

Used lab equipment at University of California, Irvine. Two students in the lab, Shirley Wong and David Nguyen helped train me in cell subculturing and basic lab procedure. Mother drove me to UCI.