CALIFORNIA STATE SCIENCE FAIR
2015 PROJECT SUMMARY

Name(s)

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Project Number

35906

Project Title

Examining the Role of CXCR4 on the Differential Migration of ESC-Derived Neural Stem Cells

Abstract

Objectives/Goals

The ultimate goal was to modify the ENStems for higher migratory capabilities, in turn making them more viable candidates for future methods of treatment for neurodegenerative diseases such as Alzheimer's and Parkinson's. For this purpose, much effort was devoted to investigate the impact of chemokine-receptor 4 "CXCR4" on the migratory capacities of ENStems when in contact with its ligand SDF-1a, and how CXCR4 levels differ between different generations of ENStems.

Methods/Materials

Methods: Trans-well Migration Assay: 50-100k cells were seeded to the top of trans-well plate filters and respective solutions were poured in specific areas in the wells. Cells were incubated, fixed, and stained, and visualized in a bright field microscope.

Brains: Inject ENStems into striatum of the mouse brain. After respective amount of time, take out brain place it into sucrose for dehydration. Then cut it at 40 microns. Analyze slices under fluorescence microscope. (NOTE! Student was not permitted to carry out culture or come into contact with live tissue. Student only worked with the fixed cells in the trans-well migration assay and only analyzed images obtained from confocal fluorescent microscope for in vivo assay.)

Materials: ENStems (CXCR4 overexpressed and normal), Trans-well dishes with polycarbonate filters, Microscope mounted camera, Cotton swab, Aspirator, PBS, NaN3, Basal medium.

Results

For the trans-well migration assays, P5 CXCR4 ENStems migrated successfully through the filter while P9 CXC4 and normal ENStems did not. For comprehensive measures, Western Blots were run for CXCR4 content analysis, and P5 ENStems had significantly higher levels of not only CXCR4 but also DCX, which is a microtubular transport protein for the cell. For the in vivo analysis, P5 CXCR4 ENStems were not only able to successfully survive but also to migrate and differentiate into neuronal cells.

Conclusions/Discussion

From the trans-well migration assays, I concluded that P5 CXCR4 ENStems have higher migratory capacities than their P9 and normal counterparts. Thus, from the Western Blot, due to lower levels of CXCR4 and DCX, gene silencing can thus be inferred. Finally, from the in vivo analysis, I concluded that P5 CXCR4 ENStems have the ability to migrate, survive, and differentiate, making them viable candidates for treatment of neurodegenerative diseases.

Summary Statement

The focus of the experiment was to find a way to modify ENStems so that they can migrate profusely throughout a diseased-brain environment.

Help Received

Unsafe laboratory protocols carried out by supervisor and assisting undergraduate student; used laboratory equipment at University of California, Irvine under the supervision of Dr. Mathew Blurton-Jones