Michelle Carter

Major: Biology
University: Truman State University
Faculty Mentor: Dr. Mark D. Kirk
Mentor Department: Biological Sciences
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Determining an efficient protocol for production of neural stem cells

Michelle Carter, Jessica Struckhoff and Mark Kirk

Mouse embryonic stem (ES) cells are pluripotent cells derived from the inner cell mass of the developing blastocyst. When cultured in non-adherent dishes, ES cells form free-floating embryoid bodies (EBs). Cells within the EBs can then be induced to form neural stem and progenitor cells. These 'neuralized' mouse ES cells have been used for therapeutic transplantation experiments in mouse models of human neurodegenerative diseases, including neuronal ceroid-lipofuscinoses (NCLs). This study focused on developing a more homogenous population of neural stem cells from ES cells for use in transplantation experiments. A homogenous population of neural stem cells could provide a renewable source of neural stem cells and thus a more consistent fate outcome for transplanted cells. We tested selected protocols for neural induction of mouse ES cells and compared their efficiencies in creating neural stem cells in vitro. Three previously developed protocols were tested in this study. The first induction protocol was specifically used to generate spheres of neural precursor cells, or neurospheres. It used a retinoic acid induction protocol followed by seeding dissociated EBs into neurosphere media. The second protocol involved growing neural stem cell colonies in astrocyte-conditioned media. The third protocol consisted of growing ES cells in flasks in neurosphere media (including FGF) without EGF for four days and then four days in neurosphere media plus EGF. Four variations on the last protocol were also tested. Preliminary results suggest that to produce a larger yield of neurospheres, the first protocol would need to be altered. The second protocol was time consuming and produced a small population of neural stem cells. The third protocol produced promising results with a larger yield of neurospheres than the first two protocols. Future studies will focus on the third protocol and define the optimal conditions whereby it will produce more neural stem cells.