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The effect of Camgaroo-2 incorporation on the differentiation potential of embryonic stem cells

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Embryonic stem (ES) cells are capable of differentiating into any cell type in the body and are a promising therapeutic agent. Our research focuses on the differentiation of ES cells into functional neurons and/or glial that can nurture host cells of the nervous system that are damaged due to disease. Cells must express the appropriate phenotype and perform the proper function after transplantation. Camgaroo-2 is a fluorescence protein that provides a basal fluorescence and responds to a rise in intracellular calcium by producing an increase in fluorescence emission. Our lab transfected a mouse ES cell line (GSI-1) with the Camgaroo-2 gene and is testing this fluorescence indicator to determine the physiological function of cells grown in vitro. There is concern that the incorporation of the Camgaroo-2 gene could alter the cell phenotype, potentially decreasing their differentiation potential. GSI-1 cells were plated on culture slides following a neural induction protocol that uses retinoic acid and allowed to proliferate. Immunohistochemisty of slides was performed to label for neural precursors, immature and mature neurons, astrocytes, and oligodendrocytes (anti-O4). GSI-1 labeling was compared to corresponding immunohistochemistry performed on another ES cell line that had also been 'neuralized' to determine if the differentiation potential of the GSI-1 cells was similar to that of the other ES cell line. Similar labeling was seen for all markers except O4 which did not label for the GSI-1 cells, indicating the GSI-1 cells have the potential to differentiate into all cells of neural lineage except possibly oligodendrocytes. GSI-1 cells retained the ability to differentiate post-transfection with the Camgaroo-2 gene. Because of their unique ability to respond to an influx of intracellular calcium, GSI-1 cells expressing Camgaroo-2 can be transplanted into rodent models for human disease, and can be tested post transplantation for their ability to function as neural cells.