Translational Research with Monkey Embryonic Stem Cells

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Since embryonic stem (ES) cells hold promise in the treatment of human disease, pre-clinical studies of ES cell mediated disease attenuation are highly desirable, if not absolutely essential, and we are using the rhesus monkey as a clinically relevant animal model. Several rhesus monkey ES cell lines have been derived and characterized and we have established an ES Cell Program along with a core laboratory at the ONPRC that provides services in the assisted reproductive technologies (ART) and in ES cell maintenance and propagation. Here we provide an overview of our ongoing interests along with the capabilities of the core laboratory. Our transplantation studies are designed to begin in rodents with the establishment of efficacy and safety before moving to the non-human primate.

Resource Management and Core Programs: The core laboratory at ONPRC conducts routine egg and sperm collection, *in vitro* fertilization, embryo production and embryo transfer. An ES cell component maintains, characterizes and distributes rhesus monkey ES cell lines. NIH approved human ES cell lines (H-1 from WiCell and BG02 from BresaGen) have also been acquired and will be maintained by the Core.

Morphological and Molecular Characterization of Undifferentiated Monkey ES Cells: Undifferentiated primate ES cells are routinely characterized by their morphology and expression of the markers SSEA-3, -4, TRI-1-60, -81, and Oct4. Low passage monkey ES cells are propagated by co-culture with mouse feeder cells. Manual selection is required to minimize spontaneous differentiation and feeder cell contamination. The propagation of monkey ES cells under a feeder cell-free culture system is being explored. Additionally, the expression of genes associated with pluripotency in monkey embryos and ES cells is under investigation.

Directed Differentiation: Monkey ES cell-derived nestin- and Musashi1-positive progenitor cells (ES-NPCs) are produced in approximately 80% homogeneity. Specific protocols have been established for the differentiation of ES-NPCs into several phenotypes: insulin-containing pancreatic beta cells, serotonin-containing cells, or glial and neuronal cells that are positive for tyrosine hydroxylase (dopaminergic) and choline acetyltransferase (cholinergic). Purification strategies for differentiated cell populations are under development.

Directed differentiation and purification of human ES cell progeny will also be developed.

Translational research of monkey ES cell derivatives: The ONPRC/OHSU ES Cell Program is currently focused on establishing animal models for transplantation of monkey ES cell derivatives the assessment of the feasibility of ES cell-based therapies for 4 human diseases.

[1] Parkinson's Disease (PD): Establishment of a hemiparkinsonism model by 6-OHDA administration in the rat and by MPTP in the monkey is underway, in association with the Parkinson Center of Oregon. We plan to determine the developmental fate of grafted ES cell derivatives (i.e., NPCs, dopaminergic cells) in the striatum of hemi-PD animals and assess the ability and duration of the engrafted cells to ameliorate PD symptoms. We also plan to transplant enriched monkey ES cell-derived dopaminergic neuronal phenotypes into these PD animal models .

[2] Diabetes Mellitus (DM): We have recently described the production of insulin-positive beta-like cell phenotype by incubation of ES cells with specific growth factors including the GLP-1 homologue, Exendin4. The ability of these cells, or further enriched populations, to alleviate symptoms in diabetic rodent and monkey models will be assessed both acutely and chronically. The primate model will be used to assess the safety and efficacy of stem cell derived therapies for DM.

[3] Stress/Drug Addiction: Serotonergic function is tightly associated with the development and response to stress or drug addiction. Currently, there are no primate serotonergic cell models for use in drug discovery and the study of cellular and molecular responses to drug treatments. Protocols have been established to differentiate monkey ES cells into serotonergic phenotypes that express and secrete serotonin. Continued characterization of these cells by gene expression profiling, electrophysiology, and by potassium-mediated serotonin release are ongoing.

[4] Multiple Sclerosis and Spinal Cord Repair: A population of monkey ES cell-derived, S-100-positive glial cells has been isolated (>80%) and subcloned. These cells also expressed Schwann cell markers (p75, O4, GFAP, SCIP) and myelin-associated proteins (MBP, PLP). A spontaneous MS monkey model exists at the ONPRC, and we intend to investigate the ability of these cells to enhance re-myelination and to promote nerve re-growth.

Future Research Plans: We will continue the definition of factors associated with pluripotency of primate ES cells using both conventional markers and microarray technologies, the enrichment of specific, functional progeny in transplantable quality using epigenetic and genetic approaches, and the establishment of rodent and monkey models of cellular degenerative disease for transplantation trials using primate ES cell derivatives.

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