

HUMAN EMBRYONIC STEM CELLS – A POTENTIAL PLATFORM FOR NEURONAL REPAIR

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Cell therapy is a novel approach with great promise for the treatment of neurodegenerative disorders. Human embryonic stem cells (hESC), which potentially can proliferate indefinitely in culture, and differentiate into any cell type, may serve as a renewable source of neural precursors (NPs) for the study of early human neurogenesis and for cell therapy of the CNS.

We have previously demonstrated that during spontaneous differentiation of hESC, in high density cultures, neural cells are formed within a mixture of other differentiated cell types (Reubinoff et al., 2000). Highly enriched cultures of NPs could be developed by selecting the NPs from the spontaneously differentiating hESC colonies, and culturing them under conditions that promote their proliferation. The neural progenitors could be propagated in culture for prolonged periods and could differentiate *in vitro* into astrocytes, oligodendrocytes and neurons (Reubinoff et al., 2001).

The developmental potential of the hESC-derived NPs was also studied *in vivo*. Following transplantation into the neonatal mouse brain ventricles, hESC-derived NPs incorporated in large numbers into the host brain parenchyma, demonstrated wide spread distribution and differentiated into neurons, astrocytes, and oligodendrocyte. The transplanted cells migrated along established host brain migratory tracks and differentiated in a region specific manner indicating their capability to respond to local cues and participate in the processes of host brain development. Teratoma formation was not observed in the recipient animals (Reubinoff et al., 2001).

Our initial approach for the development of highly enriched cultures of hESC-derived proliferating NPs was highly reproducible. However, it was depended on an initial phase of spontaneous disorganized multilineage differentiation, while controlled conversion into a homogeneous population of NPs would have been a more desirable approach for both basic and applied scientific research.

In the mouse system, undifferentiated ES cells autonomously acquire a neural fate upon removal of signals that prevent neural differentiation (Tropepe et al., 2001; Ying et al., 2003). Neuralization occurs following incubation in low-density suspension culture conditions in chemically defined medium without serum or added growth factors. It is blocked by bone morphogenetic proteins (BMPs) while BMP inhibitors such as noggin, promote neural differentiation (Tropepe et al., 2001). Initial evidences that

BMP antagonism may also have a potential role in neuralization of hESC was also reported (Pera et al., 2004).

Given these observations, we examined whether hESC, like their mouse counterparts, acquire a neural identity when cultured in a chemically defined culture system, and whether noggin promotes the process of neural differentiation.

When hESC were cultured and allowed to differentiate as floating multicellular aggregates, in defined serum-free medium, moderate enrichment for NPs was observed. However, inhibition of BMP signaling by supplementation of the medium with noggin resulted in the suppression of differentiation towards non-neural lineages and allowed the establishment of near homogenous cultures of NPs. The NPs could differentiate *in vitro* into astrocytes, oligodendrocytes, and mature electrophysiologically functional neurons. (Itsykson et al., 2005)

We further sought to determine whether hESC- derived NPs can induce functional recovery following transplantation to Parkinsonian rats. We also wished to study whether the striatal micro-environment would promote the differentiation of transplanted NPs towards a dopaminergic fate.

When hESC-derived NP were transplanted into the striatum of Parkinsonian rats, the NPs survived for at least 12 weeks, stopped proliferating, and teratomas were not observed. The grafted cells differentiated *in vivo* into dopaminergic neurons, though at a low prevalence similar to that observed following spontaneous differentiation *in vitro*. Transplanted rats exhibited a significant partial correction of behavioral deficits. (Ben-Hur et al., 2004)

The controlled establishment of NPs from hESC, under defined culture conditions, allows now the excess to study all stages of human neurogenesis *in vitro*. It may serve for the development of new drugs and *in vitro* models of human neurodegenerative disorders. The functional effect of the NPs in Parkinsonian rats encourages further developments for the potential use of hESC in transplantation for neurodegenerative disorders.

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