Generation of Dopaminergic Neurons from Human Embryonic Stem Cells

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Abstract

Since the first successful derivation of human embryonic stem cells (hESC), rapid progress has been attained in the development of strategies in differentiation of these cells into various neural lineages, with the fundamental objective of using these cells for replacement and repair of damaged neuronal circuits in the central nervous system (CNS). Of particular interest are midbrain dopaminergic (mDA) neurons, which play a central role in regulation of voluntary movement. Degeneration or loss of function of mDA neurons in the nigrostriatal pathway is associated with Parkinson disease (PD).

Stromal-Derived Inducing Activity (SDIA) is recognized as one of the most efficient methods in restricting ESC differentiation to a dopaminergic lineage, and refers to the property of mouse stromal cell lines such as PA6 or MS5 to cause ESC to differentiate to DA neurons. Although this strategy has been extensively used to generate mDA neurons from hESC, the biochemical nature of SDIA is yet unknown.

In the present study mDA neurons were generated from the BG01V2 hESC line by SDIA. To examine whether SDIA exerts its effect directly on hESC and is responsible for early dopaminergic induction, neural progenitor cells (NPC) were enzymatically isolated from the co-cultures and allowed to differentiate in feeder-free conditions. The isolated cells were committed to a mesencephalic neural lineage, and were capable of maintaining their phenotype and developing into postmitotic mDA neurons in feeder-free conditions. The mDA neurons showed neuronal excitability and dopamine transporter function. The in vitro proliferation and differentiation of the NPC was also investigated by a BrDU incorporation assay.

Next, the maintenance of cellular memory and capacity for proliferation of the mesencephalic NPC was assessed. The NPC could be expanded in vitro by five-fold as neurospheres for up to two weeks while retaining their DA differentiation potential, but did not retain a stable phenotype over extended periods of time. Preliminary transplantation experiments of neurospheres in striatal lesioned animals indicated, however, that these cells could survive and conserve their phenotype in vivo.

To gain additional insight into the biochemical role of SDIA in early dopaminergic induction of hESC, the separate contributions of cell surface activity and secreted factors were examined. The data revealed that the PA6 cell surface activity promoted cell survival and was mainly responsible for enhanced neurogenesis of hESC, whereas secreted factors provided DA lineage-specific instructions.

In order to identify the soluble factors responsible for the DA phenotype-inducing component of SDIA, the gene expression profile of PA6 cells was compared to that of cell lines lacking the DA-inducing property. A number of soluble factors known to be associated with CNS development that were
highly expressed in PA6 cells were identified as potential DA differentiation-inducing candidates. These differentially-expressed genes included stromal cell-derived factor 1 (SDF-1/CXCL12), pleiotrophin (PTN), insulin-like growth factor 2 (IGF2), and ephrin B1 (EFNB1). When these factors, termed SPIE, were applied to the hESC, they induced dopaminergic neuronal differentiation of hESC line, BG01V2 and other karyotypically normal hESC lines \textit{in vitro}. Thus, it appears that SPIE comprises the DA phenotype-inducing property of SDIA. This may provide a simple and direct means of differentiating hESC to form DA neurons in a single step, without a requirement for co-culture, animal cell lines, or animal products.

**Key Words**

Human, dopaminergic, embryonic stem cells, differentiation, stromal cells, PA6 cells, dopamine, neurons