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Human embryonic stem cell-derived neural precursor transplants in collagen scaffolds promote recovery in injured rat spinal cord

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Background aims

Several studies have reported functional improvement after transplantation of in vivo*-derived neural progenitor cells (NPC) into injured spinal cord. However, the potential of human embryonic stem cell-derived NPC (hESC-NPC) as a tool for cell replacement of spinal cord injury (SCI) should be considered.*

Methods

We report on the generation of NPC as neural-like tubes in adherent and feeder-free hESC using a defined media supplemented with growth factors, and their transplantation in collagen scaffolds in adult rats subjected to midline lateral hemisection SCI.

Results

hESC-NPC were highly expressed molecular features of NPC such as Nestin, Sox1 and Pax6. Furthermore, these cells exhibited the multipotential characteristic of differentiating into neurons and glials in vitro. Implantation of xenografted hESC-NPC into the spinal cord with collagen scaffold improved the recovery of hindlimb locomotor function and sensory responses in an adult rat model of SCI. Analysis of transplanted cells showed migration toward the spinal cord and both neural and glial differentiation in vivo.

Conclusions

These findings show that transplantation of hESC-NPC in collagen scaffolds into an injured spinal cord may provide a new approach to SCI.

Keywords

collagen scaffold, human embryonic stem cells, neural progenitor cells, spinal cord injury

Introduction

Human embryonic stem cells (hESC) can provide a useful source of cells for basic developmental studies and cell-based therapies. So far, many promising studies have shown the therapeutic potential of differentiated derivatives of ESC ameliorating neurologic disease in animal models. For example, ESC-derived neural progenitor cells (NPC) transplanted into the brains of rats with Parkinson's disease generate functional dopamine neurons [1,2] and retinal pigment epithelium derived from monkey and human ESC appears to preserve visual function in a rat model of macular degeneration [3,4]. Moreover, grafting of ESC into animal models with spinal cord injuries (SCI) has been shown to improve motor function [5–8]. However, their potential for tumor and inappropriate tissue formation [9] remains a significant concern for clinical application. Therefore, in order to restrict the breadth of cell-type differentiation and inappropriate tissue formation, precursor cells with a limited potential to form only neural tissue will probably be the preferred source for central nervous system (CNS) transplantation.

ESC can be induced to generate NPC by (i) mimicking the environment that produces neuroectoderm in the embryo by providing appropriate cell-cell interactions and signals through embryoid body formation, and (ii) depriving the ESC of both cell-cell interactions and

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signals by low-density culture in serum-free medium, thus evoking a default mechanism for NPC differentiation. Some protocols combine aspects of both approaches by promoting cell-cell interactions to facilitate the formation of all three primary germ layers followed by neural lineage-specific selection under defined conditions [10]. To harness the potential of ESC as a tool for scientific exploration and a source for possible cell replacement, in addition to the degree of maturity of the transplanted cells (stem cell, precursor and/or mature cell), the form of cell transplantation (individual, clumps and/or combined with scaffolding biomaterials) should be considered.

Synthetic three-dimensional biodegradable scaffolds seeded with stem/progenitor cells provide one of the most interesting strategies in the field of biomaterials [11–14]. A scaffold seeded with NSC for repairing the CNS can provide a platform for the cells, thus enabling repair of large neural defects. Also, the scaffold may induce stem cells to differentiate. For example, hESC- NPC that are cultured in the form of neural-like tubes in a three-dimensional collagen scaffold display an ependymal-like layer and neural cells with typical synapses [15]. Furthermore, artificial scaffolds made of synthetic biodegradable polymers have shown potential in combination with NPC transplantation [16,17]. Interestingly, an artificial nanofiber scaffold selectively induced rapid differentiation of mouse NPC into neurons and not astrocytes [18], and a collagen sponge self-assembled peptide-amphiphile nanaofiber hybrid scaffold enhanced bone formation [19]. Therefore, the culture of hESC-NPC in a collagen scaffold might provide a new approach for the repair of SCI. Previously, we have developed an efficient differentiation of NPC and neuronal cells with typical cellular, molecular and ultrastructural markers from hESC using a defined adherent culture protocol [15]. We now report on the generation of NPC from hESC in a defined adherent culture and examination of whether implantation of xenografted hESC-NPC in the spinal cord with collagen scaffold could improve clinical behavior in adult rats subjected to SCI by midline lateral hemisection.

Methods

hESC culture

The hESC lines Royan H5 and Royan H6 were used in these experiments [20]. The cells were passaged and maintained under feeder-free culture conditions as described elsewhere [21]. Briefly, the cells were cultured in hESC medium containing DMEM/F12 medium (21331–020; Gibco Pailsley, Scotland, UK) supplemented with 20% knock-out serum replacement (KOSR; 10828–028; Gibco), 2 mM l-glutamine (25030–024; Gibco), 0.1 mM β-mercaptoethanol (M7522; Sigma, St Louis, MO, USA), 1% nonessential amino acids (11140–035; Gibco), 100 U/mL penicillin, 100 µg/mL streptomycin (15070–063; Gibco), insulin-transferrin-selenite (ITS; 41400–045; Gibco) and 100 ng/mL basic-fibroblast growth factor (bFGF; F0291; Sigma). The cells were grown in 5% CO_2 at 95% humidity and were further passaged every 7 days. For passaging, hESC were washed once with phosphate-buffered saline (PBS; 14287–072; Gibco) and then incubated with DMEM/F12 containing 1:1 collagenase IV (0.5 mg/mL; 17104–019; Gibco) and dispase (1 mg/mL; 17105–041; Gibco) or only collagenase IV (1 mg/mL) at 37°C for 5–7 min. When colonies at the edge of the dish dissociated from the bottom, the enzyme was removed and washed with PBS. Cells were collected by gently pipetting and replated on Matrigel-coated (E1270; Sigma) dishes and the medium changed every other day.

Neural differentiation

The differentiation procedure is outlined in Figure 1A and was divided into four stages as described elsewhere [15], with some modifications. Briefly, feeder-free hESC proliferated in hESC medium for 7 days (stage 1) and induced to neural ectoderm by bFGF (20 ng/mL), retinoic acid (RA; 2 µM; R2625; Sigma), noggin (500 ng/mL; 1967-NG; R&D, Minneapolis, MN, USA), Shh (50 ng/mL; 1314-SH; R&D) and leukemia inhibitory factor (LIF; 10 ng/mL; LIF1010; Chemicon, Temecula, CA, USA) to form rosettes for 9 days (stage 2). The cells grew without noggin, Shh and RA for an additional 6 days in the same medium to what appeared to be neural-like tubes (stage 3). These structures were separated manually from the surrounding flat cells with a sterile pulled-glass pipette under a phase-contrast microscope (10×; CKX41; Olympus, Tokyo, Japan). The neural-like tubes were dissociated into single cells by 0.008% trypsin (27250–018; Gibco) and 2 mM disodium EDTA (108454; Merck, Darmstadt, Germany) [22] and then replated on laminin (5 µg/mL; L2020; Sigma) and poly-L-ornithine (15 µg/mL; P4957; Sigma)-coated tissue culture dishes in neurobasal medium (21103–049; Gibco) supplemented with 2% N2 (17502–048; Gibco), 2% B27 (17504–044; Gibco), 2.5% fetal bovine