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Research report

Neural precursor cells can be delivered into the injured cervical spinal cord by intrathecal injection at the lumbar cord

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Abstract

Neural precursor cells (NPCs) are promising grafts for treatment of traumatic CNS injury and neurodegenerative disorders because of their potential to differentiate into neurons and glial cells. When designing clinical protocols for NPC transplantation, it is important to develop alternatives to direct parenchymal injection, particularly at the injury site. We reasoned that since it is minimally invasive, intrathecal delivery of NPCs at lumbar spinal cord (lumbar puncture) represents an important and clinically applicable strategy. We tested this proposition by examining whether NPCs can be delivered to the injured cervical spinal cord via lumbar puncture using a mixed population of neuronal-restricted precursors (NRPs) and glial-restricted precursors (GRPs). For reliable tracking, the NPCs were derived from the embryonic spinal cord of transgenic donor rats that express the marker gene, human placental alkaline phosphatase, under the control of the ubiquitous Rosa 26 promoter. We found that mixed NRP/GRP grafts can be efficiently delivered to a cervical hemisection injury site by intrathecal delivery at the lumbar cord. Similar to direct parenchymal injections, transplanted NRP/GRP cells survive at the injury cavity for at least 5 weeks post-engraftment, migrate into intact spinal cord along white matter tracts and differentiate into all three mature CNS cell types, neurons, astrocytes, and oligodendrocytes. Furthermore, very few graft-derived cells localize to areas outside the injury site, including intact spinal cord and brain. These results demonstrate the potential of delivering lineage-restricted NPCs using the minimally invasive lumbar puncture method for the treatment of spinal cord injury.

Theme: Development and regeneration
Topic: Transplantation

Keywords: Neural stem cell; Spinal cord injury; Neuron; Glial cell; Transplantation; Neural progenitor

1. Introduction

Transplantation of neural precursor cells (NPCs) is a promising therapeutic strategy for the treatment of CNS injuries and neurodegenerative disorders [15]. Transplanted cells offer a number of possible therapeutic uses, including delivery of therapeutic factors to provide trophic support or missing gene products, mobilization of endogenous NPCs and replacement of lost or dysfunctional cells [36]. Traumatic spinal cord injury (SCI) can specifically benefit from the engraftment of NPCs. Transplanted cells may remyelinate denuded axons, decrease glial scar formation, prevent secondary cell loss, promote regeneration, form bridges and relays, and replace neural cells. A number of studies have demonstrated that transplanted NPCs promote anatomical plasticity and modest behavioral recovery in contusive and surgical lesion models of SCI [25,28]. The vast majority of studies have employed parenchymal injection of cells directly into the lesion site. This strategy is designed to efficiently deliver cells into the injury site; however, if transferred to the

Abbreviations: AP human placental alkaline phosphatase transgene; GRP glial-restricted precursor; NPC neural precursor cell; NRP neuronal-restricted precursor; SCI traumatic spinal cord injury

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clinic, this would be an expensive and invasive technique requiring general anesthesia and risking damage to the already injured spinal cord. In addition, many diseases that are candidates for NPC transplantation therapy are diffuse (e.g., multiple sclerosis) and would require delivery of engrafted cells over extensive, non-contiguous areas [34]. It is therefore important to develop alternative NPC delivery methods to direct and local parenchymal injections.

Transplantation of NPCs into the cerebrospinal fluid offers a potential alternative to intraparenchymal injection. Previous work has demonstrated that NPCs and bone marrow stromal cells can be delivered to SCI sites [5,29,30,41] and MS plaques [9,14,34] via intraventricular injection, and can promote behavioral improvement. Intrathecal injection [11,12] at lumbar levels (lumbar puncture; spinal tap) is a very promising strategy because of its minimal invasiveness, simplicity, and low cost, and because it would allow multiple injections at desired intervals. NPCs may be well suited for lumbar puncture delivery because of their responsiveness to signals expressed in the injured CNS. Previous work has shown that endogenous NPCs not only proliferate and differentiate following injury [18,20,42], but both endogenous [4,27,31,32] and grafted NPCs [2,37,38] can migrate to sites of CNS pathology.

The objective of the present study was to assess whether NPCs can be successfully delivered to the injured cervical spinal cord via lumbar puncture transplantation. This will aid in determining promising NPC engraftment protocols for the treatment of SCI. Specifically, a mixed population of neuronal-restricted precursors (NRPs) and glial-restricted precursors (GRPs) – NPCs with lineage restrictions for neurons and glia, respectively – was used in this study [24,35]. Transplanted cells were derived from embryonic day-13.5 fetal spinal cord of transgenic donor rats that express the marker gene, human placental alkaline phosphatase, under the control of the ubiquitous Rosa 26 promoter [22,26] for reliable graft tracking. Previous work has demonstrated that NRPs and GRPs, whether grafted individually or together, display robust survival, extensive migration, and differentiation following transplantation into the intact [10,17,19,23,43] CNS. However, while mixed NRPs/GRPs grafted into the injured spinal cord show the same properties, NRPs grafted alone show poor survival and differentiation [18,23]. Furthermore, NRP/GRP grafts promote recovery in motor, sensory, and autonomic functions in the thoracic contusion model of SCI (Mitsui et al., unpublished data). However, all these previous studies involved direct parenchymal transplantation of cells.

In this study, we report that mixed NRP/GRP grafts can be efficiently delivered to a cervical lateral funiculotomy injury site via intrathecal delivery at the lumbar cord. Similar to direct parenchymal injections, transplanted NRP/GRP cells survive in the injury cavity for up to 5 weeks post-engraftment (longest time point examined), migrate into intact spinal cord along white matter tracts, morphologically mature and differentiate into all three mature CNS cell types, neurons, astrocytes, and oligodendrocytes. Furthermore, very few graft-derived cells localize to areas outside the injury site, including intact spinal cord and brain. These results demonstrate that mixed populations of lineage-restricted precursor cells can be delivered to the injured spinal cord via clinically attractive delivery methods.

2. Materials and methods

2.1. Neural precursor cultures

2.1.1. Isolation and culturing of NRPs and GRPs

NRPs and GRPs were isolated from the embryonic day-13.5 spinal cord of transgenic Fischer 344 rats that express the marker gene, human placental alkaline phosphatase (AP), under the ubiquitous Rosa 26 promoter. The use of AP transgenic animals to derive NPCs allows for unambiguous detection of transplanted cells in the CNS. Briefly, embryos were isolated in a dish containing DMEM/F12. Trunk segments were incubated in a collagenase Type I (10 mg/mL)/dispase II (20 ng/mL)/HBSS solution for 8 min at room temperature to allow for peeling away of meninges from the cords. Cords were dissociated using a 0.05% trypsin/EDTA solution for 20 min at 37 °C. Cells were then plated in NRP complete medium [(DMEM-F12, BSA (1 mg/mL; Sigma; St. Louis, MO), B27 (Invitrogen), bFGF (20 ng/mL; Peprotech; Rocky Hill, NJ), Pen-Strep (100 IU/mL; Invitrogen), N2 (10 μL/mL; Invitrogen); bFGF (10 μg/mL) and NT-3 (10 μg/mL; Peprotech)] on poly-L-lysine-coated (13.3 μg/mL; Sigma) and laminin-coated (20 μg/mL; Invitrogen) dishes.

2.1.2. Preparation of cells for grafting

Although we have previously shown that NRPs and GRPs can be individually isolated, we reasoned that, for traumatic spinal cord injury, we will need replacement of both neurons and glia. Following embryonic dissection, NRPs and GRPs were co-cultured for 5–10 days prior to transplantation. The mixed population of NRPs and GRPs was dissociated from culture flasks using 0.05% trypsin/EDTA, washed and re-suspended at a concentration of 100,000 cells/μL (in basal media) for transplantation. Cells were placed on ice throughout the grafting session. After the completion of the grafting session, cell viability was assessed using the trypan blue assay. Viability was always found to be greater than 90%. The composition of the NRP/GRP cultures, with respect to the presence of undifferentiated NPCs and the absence of mature cells, was verified before grafting by staining for the immature neural marker nestin, for markers of mature neurons (NeuN), astrocytes (GFAP), and oligodendrocytes (RIP), as well as for markers of NRPs (E-NCAM) and GRPs (A_2B_5).