Stem Cell Research (2013) 11, 758–771

Available online at www.sciencedirect.com

SciVerse ScienceDirect

www.elsevier.com/locate/scr

Bone morphogenetic proteins prevent bone marrow stromal cell-mediated oligodendroglial differentiation of transplanted adult neural progenitor cells in the injured spinal cord

Beatrice Sandner[a,c], Francisco J. Rivera[b], Massimiliano Caioni[a], LaShae Nicholson[c], Volker Eckstein[e], Ulrich Bogdahn[a], Ludwig Aigner[b], Armin Blesch[c,d,*], Norbert Weidner[a,c,*]

[a] Department of Neurology, University of Regensburg, 93053 Regensburg, Germany
[b] Institute of Molecular Regenerative Medicine and Spinal Cord Injury and Tissue Regeneration Center, Paracelsus Medical University, 5020 Salzburg, Austria
[c] Spinal Cord Injury Center, Heidelberg University Hospital, 69118 Heidelberg, Germany
[d] Department of Neurosciences, University of California, San Diego, CA 92093, USA
[e] Division of Hematology and Oncology, Department of Medicine V, University of Heidelberg, 69120 Heidelberg, Germany

Received 22 October 2012; received in revised form 2 May 2013; accepted 8 May 2013
Available online 16 May 2013

Abstract The loss of oligodendroglia and demyelination contributes to the lack of functional recovery after spinal cord injury. The transplantation of adult neural progenitor cells (NPCs) might be a promising strategy to replace oligodendroglia lost after injury, however only a very small proportion of grafted NPCs differentiate into oligodendroglia. The present study aimed to investigate whether co-transplantation of subventricular zone-derived NPCs with bone marrow stromal cells (BMSCs) will enhance oligodendroglial differentiation of NPCs. In vitro, oligodendroglial differentiation was strongly enhanced by co-cultivation of NPCs with BMSCs or BMSC-conditioned medium. For in vivo experiments, adult Fischer 344 rats underwent cervical dorsal funiculus transections, immediately followed by grafting of 5-bromo-2′-deoxyuridine (BrdU) pre-labeled syngeneic NPCs mixed with BMSCs isolated from adult bone marrow. Six weeks post-injury and grafting, BMSC-containing grafts filled the lesion cavity but did not enhance oligodendroglial differentiation of co-grafted NPCs. The failure of BMSCs to induce oligodendroglial differentiation in vivo coincided with a rapid upregulation of bone morphogenetic protein 2/4 (BMP2/4) around the injury site and in vitro data demonstrated that BMP2/4 can override the oligodendrogenic effects of BMSCs. Moreover, blocking BMP activity can rescue the effect of BMSCs on NPCs. Thus, neutralization of BMP or BMP signaling might be required to allow for BMSC-induced oligodendroglial differentiation of grafted NPCs in the injured spinal cord.

* Corresponding authors at: Spinal Cord Injury Center, Heidelberg University Hospital, Schlierbacher Landstrasse 200a, 69118 Heidelberg, Germany. Fax: +49 6221 5626345.
E-mail address: norbert.weidner@med.uni-heidelberg.de (N. Weidner).

1873-5061/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scr.2013.05.003

1. Introduction

Trauma-induced degeneration of white matter contributes significantly to the severe functional disability in individuals with spinal cord injuries. After spinal cord injury in rats and monkeys, oligodendroglial cells undergo apoptotic cell death in the immediate vicinity of the lesion site but also in remote white matter tracts (Casha et al., 2001; Crowe et al., 1997; Siegenthaler et al., 2007). Similarly, in individuals with severe spinal cord injury, a subpial rim of persisting axons can often be identified (Kakulas, 1999) but these axons are usually completely demyelinated. Neurophysiological studies also indicate that axons spared after injury do not recover proper conduction velocities (Nashmi and Fehlings, 2001a,b).

Following spinal cord injury, the proliferation of intrinsic spinal neural progenitor cells (NPCs) generates glial precursor cells (Horner et al., 2000; Scolding et al., 1998; Shibuya et al., 2002). However, spontaneous myelin repair is inadequate due to limited oligodendroglial differentiation and insufficient remyelination of axons (Sim et al., 2002; Wolswijk, 1998). Transplantation of cells such as NPCs, which can remyelinate axons, is one potential means to overcome the limited intrinsic remyelination capacity. NPCs are characterized by their potential to self-renew, to proliferate and to differentiate into all three major central nervous system (CNS) lineages: neurons, astrocytes and oligodendrocytes (Shihabuddin et al., 1997; Weiss et al., 1996). Interestingly, NPCs can follow an oligodendrogenic program resulting in a myelinating phenotype (Rivera et al., 2010) but following grafting to the acutely injured spinal cord, the majority of the cells differentiate into astroglia (Pfeifer et al., 2004). Previous studies have shown that oligodendroglial differentiation and remyelination of NPCs can be enhanced when combined with infusion of growth factors (platelet derived growth factor, basic fibroblast growth factor, epidermal growth factor) (Karimi-Abdolrezaee et al., 2006) or following genetic modification to express the transcription factor neurogenin-2 (Hofstetter et al., 2005).

We have recently shown that bone marrow stromal cells (BMSCs) secrete yet unidentified factors, which strongly promote oligodendroglial differentiation of hippocampal NPCs in co-culture conditions (Rivera et al., 2006; Steffenhagen et al., 2012) and in organotypic hippocampal slice cultures (Rivera et al., 2009). These data suggest that BMSC grafts might be one means for the robust induction of oligodendroglial differentiation of grafted adult NPCs.

The present study investigated whether BMSCs are sufficient to induce oligodendroglial differentiation of NPCs co-grafted into the injured rat spinal cord. In contrast to robust in vitro effects, in vivo data suggest that BMSC-derived factors are counteracted possibly by bone morphogenetic protein 2/4 (BMP2/4), strongly expressed at the spinal cord lesion site, thereby limiting BMSC-mediated effects on oligodendroglial differentiation.

2. Material and methods

NPC differentiation was first analyzed in vitro in NPC/BMSC co-cultures using immunocytochemistry. In vivo differentiation was examined after grafting to the lesioned spinal cord and effects of BMPs and BMP neutralization in vitro were quantified by qRT-PCR and immunocytochemistry.

2.1. Animal subjects

Adult female Fischer 344 rats (Charles River Deutschland GmbH, Sulzfeld, Germany) weighing 160–180 g were used for the isolation of syngenic cells (NPCs and BMSCs) and for all in vivo experiments. For some in vitro experiments NPCs were isolated from GFP-transgenic Sprague–Dawley rats (see below) (Lois et al., 2002). Experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and institutional guidelines. Animals had ad libitum access to food and water throughout the study.

2.2. Isolation of NPCs and BMSCs

For cell isolation, rats were deeply anesthetized using a cocktail of ketamine (62.5 mg/kg; WDT, Garbsen, Germany), xylazine (3.175 mg/kg; WDT, Garbsen, Germany) and acepromazine (0.625 mg/kg, Sanofi-Ceva, Düsseldorf, Germany) in 0.9% sterile saline solution and killed by decapitation.

Subventricular layers from the lateral wall of the lateral ventricle were aseptically removed and dissociated as described (Wachs et al., 2003). Cells were cultured in Neurobasal (NB) medium (Gibco BRL, Germany) supplemented with B27 (Gibco BRL, Germany), 2 mM L-glutamine (PAN, Germany), 100 U/ml penicillin/0.1 mg/l streptomycin (PAN, Germany), 2 μg/ml heparin (Sigma, Germany), 20 ng/ml FGF-2 and 20 ng/ml EGF (R&D Systems, Germany). Cultures were maintained as neurospheres in uncoated culture flasks at 37 °C in a humidified incubator with 5% CO_2 and cells were passaged weekly. Neurosphere cultures from passages 2 to 6 were used throughout this study.

For isolation of BMSCs, bone marrow was harvested from femurs and tibias of 2–4 month-old female Fisher 344 rats. Cells were mechanically dissociated in Minimum Essential Medium alpha (α-MEM) (Invitrogen GmbH, Germany) and recovered by centrifugation. Cell pellets were resuspended in α-MEM containing 10% Fetal Bovine Serum (αMEM-10% FBS) (PAN Biotech GmbH, Germany) and seeded at 1 × 10^6 cells/cm^2 in a humidified incubator at 37 °C with 5% CO_2. After 3 days, the medium was changed and non-adherent cells were discarded. Adherent cells were incubated in fresh αMEM-10% FBS until cells were confluent. Cells were trypsinized using 0.25% Trypsin (Gibco Cell Culture, Invitrogen GmbH, Germany), seeded in α-MEM-10% FBS at 8000 cells/cm^2. After 3–5 days of culture, the resulting monolayer of cells was trypsinized and frozen or cultured for further experiments. As demonstrated in our previous work, this cell culture preparation is highly enriched in multipotent BMSCs with virtually no hematopoietic contamination (Rivera et al., 2006).

2.3. Exposure of NPCs to BMSCs or BMSC-conditioned medium

NPCs and BMSCs were co-cultured as previously described (Rivera et al., 2006). Briefly, BMSCs (passage 2 to 6) were plated on polyornithine (100 μg/ml) and laminin (5 μg/ml)-coated dishes at a density of 2500–5000 cells per cm^2. Twelve to 24 h later, NPCs isolated from the SVZ of adult female transgenic