Oligodendrocyte Progenitor Cells Derived from Induced Pluripotent Stem Cells Survive and Differentiate After Transplantation into the Injured Rat Spinal Cord

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Introduction

Spinal cord injury currently has no clinical treatment. One promising therapy is the transplantation of oligodendrocyte progenitor cells (OPCs) which can potentially remyelinate axons (1,2). We previously devised a protocol for directed differentiation of OPCs from mouse fibroblasts, via an induced pluripotent stem (iPS) cell intermediary. Here we show that such derived OPCs survive after transplantation in the injured rat spinal cord, and thus represent a new strategy for potential treatment of spinal cord injury.

Methods

IPS cells were generated from mouse fibroblasts expressing eGFP, using the Yamanaka method (3). These cells were subsequently cultured according to our protocol, established for generating OPCs from mouse iPS cells, as outlined in Figure 1. OPCs were characterized during the differentiation protocol via immunocytochemistry, RT-PCR marker analysis, and transplantation into dysmyelinated mouse brains, where their myelinating capacity was demonstrated (shown previously). Moderate spinal cord injury was induced in adult rats, using the NYU spinal cord impactor, and the OPCs were transplanted 9 days after the injury. The rats were sacrificed at two and three weeks following transplantation. Immunohistochemical studies were performed on the spinal cord tissue to look for the continued presence of transplanted cells.

OPC Differentiation Protocol

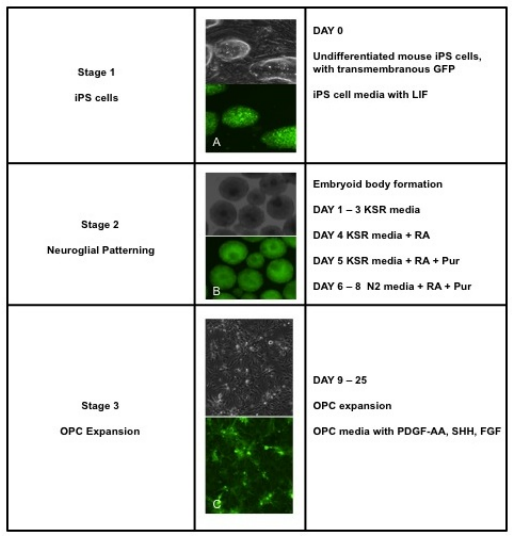

Figure 1

Results

OPCs were cultured to day 25 of the protocol, and then characterized by immunocytochemistry and RT-PCR, prior to being transplanted. Immunocytochemistry confirmed expression of OPC markers, such as Olig 1, Olig 2, PDGFRa, NG 2, Nestin, and A2B5, Figure 2. RT-PCR analysis demonstrated expression of genes characteristic of OPCs (Fig. 3B), absence of genes expressed in stem cells (Fig 3A), mature oligodendrocytes, and neurons (Fig. 3C). The percentage of cells in culture expressing OPC markers was greater than 80%. Such generated OPCs were injected into spinal cord of rats, 9 days after moderate spinal cord injury. Fluorescence microscopy demonstrated presence of eGFP positive cells in the injured rat spinal cord at two and three weeks following transplantation, Figure 4 - green:eGFP, red:Tuj 1. The eGFP signal was found to align with cells of the spinal cord. The transplanted cells migrated away from the injection sites, and developed elongated processes.

OPC Characterization

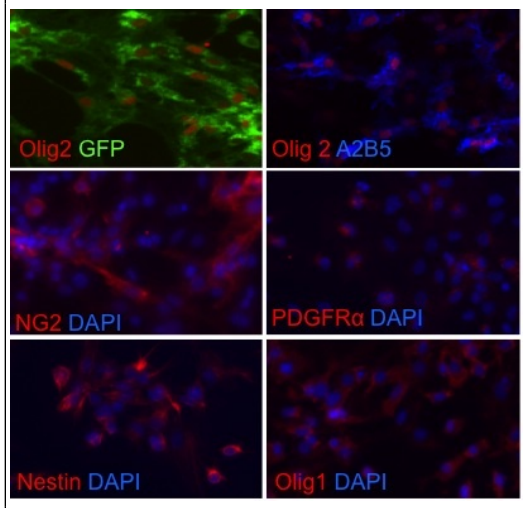

Figure 2

OPC Gene Expression Profiles

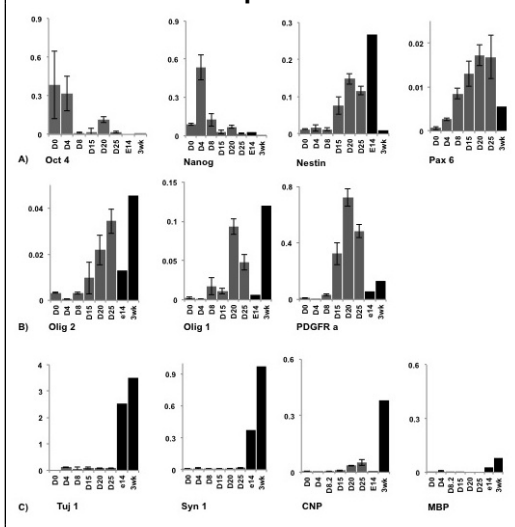

FIgure 3

Transplanted Cells in Spinal Cord

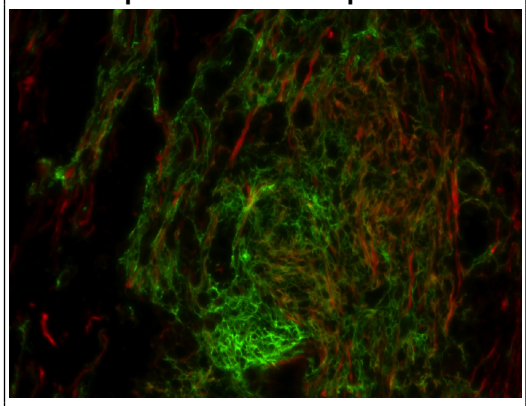

Figure 4

Conclusions

OPCs derived from mouse iPS cells survive transplantation into the injured rat spinal cord. They demonstrate ability to migrate away from the injection sites, and develop processes that align with cells of the spinal cord, suggestive of their differentiation into oligodendrocytes. iPS cells represent a new source of OPCs that can be utilized as a model to study OPC effects on the environment of spinal cord injury. Future studies include further characterization of the transplanted cells via electron microscopy, and long term functional analysis of injured rats after OPC transplantation.

Learning Objectives Following this presentation the audience should be able to recognize the potential for oligodendrocyte progenitor transplantation in spinal cord injury.

References 1. Sharp J, et al. Human Embryonic Stem Cell-Derived Oligodendrocyte Progenitor Cell Transplants Improve Recovery after Cervical Spinal Cord Injury. Stem Cells. 2010;28:152–163
2. Faulkner J, Keirstead HS. Human Embryonic Stem Cell Derived Oligodendrocyte Progenitors for the Treatment of Spinal Cord Injury. Transplant Immunology 2005; 15, 131-142.
3. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676.