Axonal growth was promoted after transplantation of human peripheral blood derived CD133-positive cells via up-regulation of the VEGF mRNA expression in spinal cord tissue

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Introduction: Cell transplantation is a very promising therapeutic approach for the treatment of spinal cord injury (SCI). We focused on CD133+ cells isolated from human peripheral blood, they have been identified as endothelial progenitor cells (EPCs), for axonal regeneration in spinal cord. CD133+ cells in the peripheral blood also have the potential to differentiate into neuronal cells. We reported historical and functional recovery of the peripheral nerve and functional recovery after spinal cord injury of athymic rat with CD133+ cells. Therefore, transplantation of CD133+ cells derived from peripheral blood may be a hopeful approach for treatment of the injured central nervous system (CNS). In this study, we assessed the effect of transplanting CD133+ cells on corticospinal axon growth and the mechanism of CNS regeneration using the organotypic co-culture system [1-4], which is composed of brain cortex and spinal cord from neonatal rats and enables us to quantitatively evaluate the axonal regeneration and analyze the factors that regulate axonal growth.

Materials and Methods: Isolation and Preparation of Human CD133+ Cells: CD133+ cells were separated from total peripheral blood mononuclear cells of healthy adult fresh blood using CD133-bound microbeads and a magnetically activated cell sorter. Cells in the CD133+ fraction also expressed CD34. Organoctyptic Co-cultures and Tracing of Axonal Growth: Organotypic co-cultures of brain cortex and spinal cord were prepared as reported previously [2-4]. Brains and thoracic spinal cords were collected from rats on postnatal day 3. The dissected sensorimotor cortex and spinal cord were placed on separate membranes in serum-based medium in tissue culture plates. After one day incubation, the spinal cord pieces were aligned next to the white matter of the cortex (Fig. 1A). Then in the control group, 2 µl of phosphate-buffered saline (PBS) was added to the co-cultures. In the CD133 group, 1x10⁴ CD133+ cells in 2 µl of PBS per culture were applied. The co-cultures were incubated for up to 14 days. Axonal growth from the cortex to the spinal cord was assessed quantitatively using anterograde tracing with Dil.

Immunostaining of Co-culture Tissues: The co-culture tissues of the CD133 group were stained for human nuclear antigen (HNA), nestin, MAP2, GaLa, or GFAP at 7 days after transplantation. The immunostained cells were counterstained with DAPI. RT-PCR Analysis of Gene Expression in Isolated Human CD133+ Cells and Real Time RT-PCR Analysis of Co-cultured Spinal Cord Tissues: Expression of BDNF, NT-3, NGF and VEGF in isolated CD133+ cells before transplantation was evaluated using RT-PCR. Three days and 7 days after transplantation, we performed real-time quantitative RT-PCR for mRNA expression in spinal cord of BDNF, NT-3, NGF and VEGF. The mRNA expression levels were normalized to β-actin.

Results: Corticospinal Axonal Growth in Co-cultures with Transplanted CD133+ Cells: The average number of labeled axons in the CD133-treated cultures (n=15 cultures/group) extending 500, 1000, 1500µm or 2000 µm beyond the junction into the spinal cord was significantly greater than those in the control cultures (n=20 cultures/group) at all four distances (Mann-Whitney U test, p<0.01) (Fig. 1B). Differentiation and Distribution of CD133+ Cells Transplanted to Co-cultures: In the CD133-treated cultures 7 days after transplantation, the majority of HNA-positive cells were observed at the margins of the co-culture tissues. A few HNA-positive cells per culture were co-labeled with nestin or GFAP. However, we found no HNA-positive cells co-labeled with either MAP2 or with GaLa.

Analysis of mRNA Expression of Growth Factors in CD133+ Cells and in Co-culture Tissues: Specific bands of mRNA encoding BDNF, NGF, NT3 and VEGF were not observed in the CD133+ cells. In spinal cord tissues, real-time RT-PCR shows that at day 3 and day 7 after transplantation, the expression of VEGF derived from spinal cord was significantly higher in the CD133+ treated cultures than in the control cultures (Mann-Whitney U test, p<0.05), while no differences were seen for BDNF, NT-3 or NGF mRNA expressions at either time (Fig. 2).

Discussion: The current study showed that the transplantation of CD133+ cells significantly promotes axonal growth in organotypic co-cultures, and that the transplantation of CD133+ cells causes an up-regulation of VEGF mRNA in the spinal cord tissues. It was suspected that the human CD133+ cells affect the spinal cord immediately after transplantation and lead to increase in VEGF mRNA expression. Though the mechanism is not clear, CD133+ cells have a strong effect on axonal regeneration. CD133+ cells are easily and safely obtained from peripheral blood and can be autologously transplanted. CD133+ cells derived from bone marrow or peripheral blood are actually used for myocardial infarction, hepatic malignancy or acute myocardial leukemia treatment. They could be a promising cell source for spinal cord injury treatment in clinical setting.


Fig. 1 (A) The cortex/spinal cord organotypic co-culture system. (B) The average number of axons in CD133 group is significantly larger than that in control group. (* p<0.05)

Fig. 2 Quantitative assessment of mRNA expression in spinal cord assessed by real-time RT-PCR. After transplantation both day 3 and day 7, VEGF was significantly up-regulated in the CD133 cultures compared to control (* p < 0.05).