Human CD133 positive cells with a magnetic delivery system promote functional recovery in rat spinal cord injury

INTRODUCTION:
Recently, many commonalities between angiogenesis and neurogenesis have been identified. We focused on the angiogenesis enhanced with cell transplantation as a factor for the spinal cord regeneration. We hypothesized that CD133+ cells, a rare fraction of endothelial progenitor cell from human peripheral blood, can provide both angiogenesis and neurogenesis in the damaged spinal cord.

CD133+ cells were isolated from peripheral blood by magnetic separation, with the cell-specific antibody coupled to the magnetic beads. Accordingly, CD133+ cells with magnetic beads can be controlled by magnetic force. We established delivery system of CD133+ cells using extra-magnetic device.

The purpose of this study was to clarify the therapeutic effects of CD133+ cells on spinal cord contusion by intrathecal administration via lumbar puncture, and to clarify the efficacy of delivery of magnetic labeled CD133+ cells.

METHODS:

Contusion SCI was induced by IH impacter (200 kdyne) at T10 level in athymic nude rats. Human peripheral blood derived CD133+ cells (CD133 group; 1x10^6 in 50 µl/rat, n=5) or phosphate-buffered saline (PBS group; 50µl/rat, n=5) was administered into the subarachnoid space at L4/5 level immediately after SCI. Animals were placed under the magnetic field, maximum 0.6T, generated from direct-current electromagnet (CD133M group; 1x10^6 in 50 µl/rat, n=5).

In vivo whole animal imaging was examined after luciferase expressing CD133+ cell transplantation. Distribution of grafted CD133+ cells was examined one hour after transplantation by counting the photons.

The hind-limb motor function of rats after spinal cord contusion injury was scored with the BBB locomotor rating scale on days 1 to 7 and every week up to the sixth week.

Motor evoked potentials (MEPs) were recorded in the hamstring muscles following transcranial stimulation of the cortex at sixth week after injury.

Double immunofluorescence study for cell marker in the endothelium induced from human derived CD133+ cells (von Willebrand factor (vWF) and human mitochondria (HuMit))were evaluated.

The mRNA expression analysis of angiopoietin-1 (Ang1), tumor necrosis factor-alpha (TNF-a), interleukin-1 (IL-1) and IL-6 in each group was performed by real-time polymerase chain reaction (PCR).

RESULTS:

In vivo imaging showed that CD133+ cells transplanted from lumbar portion were accumulated at the injury site by the magnetic force (Fig.1).

The BBB score of rats after spinal cord contusion injury was gradually improved in each group. After 3 week, the score of the experimental group demonstrates significant improvement compared with that of the other groups at every week up to the sixth week (Fig.2).

Base-to-peak amplitudes of MEPs in the experimental groups reveal significantly large than other groups.

Immunohistochemistry showed that intrathecally administered CD133 cells migrated into the injured spinal cord and were assimilated as vascular endothelial cells in the CD133 group (Fig.3).

DISCUSSION:

We demonstrated that the administration of human peripheral blood-derived CD133+ cells via lumbar puncture accelerated functional recovery of injured spinal cord in rat SCI model.Ang1 might be a key factor for the induction or enhancement of regeneration in the spinal cord through angiogenesis. Administration of CD133+ cells with magnetic delivery system has a therapeutic potential to a spinal cord injury model. That could be an optional less invasive treatment for spinal cord injury in the clinical settings.

Fig.1 In vivo whole animal imaging after CD133+ cell transplantation by lumbar puncture. Distribution of the grafted CD133+ cells was examined one hour after transplantation. In the CD133M group, the transplanted cells were densely accumulated in the magnetic targeted thoracic injury site, whereas cells still remained at the injected lumbar level in the CD133 group without magnetic delivery.

Fig. 2 Changes in the hind-limb motor function scored with the BBB locomotors rating scale. After 3 weeks, the BBB score of the CD133M group demonstrated significant improvement compared to that of the other groups at every week up to the sixth week (P < 0.05). There was no significant difference between groups CD133 and PBSM at each time point.

Fig. 3 Immunohistochemistry of the 3 days after the spinal cord injury and injection of CD133 cells. HuMit positive cells (green) and vWF (red). Intrathecally administered CD133 cells migrated into the injured spinal cord and were assimilated as vascular endothelial cells in the CD133M group. Arrowheads indicate double-positive cell.