Immunohistochemical Study of Fetal Stem/Progenitor Cells from Human Brain Transplanted into Traumatized Spinal Cord of Adult Rats

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Neural stem/progenitor cells from human fetal brain were grown in a tissue culture and transplanted into traumatized spinal cord of adult rats. The behavior and differentiation of transplanted cells were studied morphologically by means of histological and immunohistochemical methods and confocal microscopy. Human neural stem/progenitor cells were viable for not less than 3 months. They migrated and differentiated into neurons and glia in the traumatized spinal cord of adult rats.

Key Words: *human neural stem/progenitor cells; spinal cord; xenotransplantation; immunohistochemistry; confocal microscopy*

Neural transplantation holds much promise for the therapy of traumatic injuries in the brain and spinal cord. This method is based on substitution of lost cells and stimulation of compensatory and reparative processes [2,3,6,12]. Fetal stem cells (SC) of the brain are most convenient for transplantation. They serve as a potential source for various cells of the central nervous system in humans, including neurons, astrocytes, and oligodendrocytes [5,10,11]. Fetal neural SC maintained *in vitro* undergo differentiation in the tissue culture. Then these cells are used for transplantation to restore functions of the brain and spinal cord [1,14].

Here we studied the behavior and differentiation of cultured fetal stem/progenitor cells of human brain transplanted into traumatized spinal cord of adult rats.

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MATERIALS AND METHODS

Experiments were performed on 50 Wistar rats weighing 250-300 g. Trauma of the spinal cord in 30 animals was followed by transplantation of fetal SC from human brain. Transplantation was not performed in 20 rats with spinal trauma (control).

Laminectomy (Th8-9) was performed in rats intraperitoneally anesthetized with 2 ml 2% calipsol. Contusion of the spinal cord was produced with a weight of 10 g (height 6.5 cm). Fetal SC from human brain were transplanted into the spinal cord immediately after spinal trauma. The cells (6×10^5, 3 μl) were transplanted in stereotaxis using a Hamilton microsyringe. SC were introduced bilaterally at a depth of 1.5-2.0 mm (5 mm proximal and distal to the epicenter of injury). No immunosuppressive treatment was performed.

Isolation and differentiation of fetal SC from human brain were performed as follows. The cells isolated from the brain of human fetuses obtained after medical abortion (9-12 weeks' gestation) were dissociated and placed in NPBM growth medium (2×10^6 cells/ml; Neural Progenitor Medium,

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Clonetics).The medium contained human fibroblast growth factor, nerve cell survival factor, epidermal growth factor, and gentamicin/amphoterin (Clonetics). The cells suspension (1×10^5 cells/ml) was cultured in flasks for 7-14 days.

Before transplantation, SC were stained with nuclear luminescent dye bisbenzimide in a concentration of 20 μg/ml (Hoechst 33342, Serva). The suspension of single cells and small neurospheres served as the transplant.

The rats were narcotized with 500 mg/kg chloral hydrate 1 and 2 weeks and 1-2 and 4 months after transplantation. They were transcardially perfused with 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). The brain was removed and placed in fixative for 24 hour. Then it was maintained in 30% sucrose at 4°C overnight. Brain sections (20-40 μ) were prepared on a cryostat and mounted on glasses. The sections were selected by luminescence of transplanted cells stained with bisbenzimide. Some brain sections were stained with hematoxylin, eosin, and cresyl violet. Immunohistochemical methods involved primary antibodies against human cell nuclei (1:30, Chemicon), human cell nestin (1:20, Chemicon), gliofibrillar acid protein (GFAP, 1:250, DAKO), and β-tubulin III (1:200, Abcam). Samples were treated with secondary antibodies labeled with fluorescent dyes Texas Red and Cy-2 (Jackson). The sections were clarified with 50% glycerol in phosphate buffer and examined in the luminescent light under an Opton microscope and Leica laser scanning confocal microscope.

RESULTS

Morphological examination revealed transplants of cultured SC from human brain in the spinal cord in 25 of 30 recipient rats.

Examination of the spinal cord 1 week after surgery revealed regions of massive tissue injury and zones of hemorrhages, which spread in both directions from the area of trauma. Focal hemorrhages were found between degenerated neurons and broken fibers. The glial reaction and regions of newly formed vessels were revealed at the edges of mechanical injury.

Bisbenzimide-stained transplanted cells were present in the white and gray matter of the spinal cord and followed the needle track. The density of cells decreased with increasing the distance from the injection site.

Immunohistochemical staining with antibodies against human cell nuclei showed that not all bisbenzimide-stained cells are transplanted cells. Human cell nuclei-positive cells were localized between bisbenzimide-stained cells. These cells exhibited similar distribution, but their count was lower than that of bisbenzimide-stained cells. These findings illustrate partial redistribution of bisbenzimide in recipient cells that were adjacent to the transplanted cells.

Double immunohistochemical staining with antibodies against human cell nuclei and human nestin revealed the presence of true SC among transplanted cells. Single nestin-positive cells or groups of these cells were found at the boundary of damaged tissue.

The area of spinal cord injury increased 2 weeks after trauma and transplantation. The zone of degeneration of cells and fibers (1 cm) was distributed across the entire spinal cord. It included extended area of necrosis and focal hemorrhages. The density of vessels and capillaries was high in tissues around the traumatized area, which reflected induction of angiogenesis. The glial reaction was pronounced at the boundary of tissue injury. As differentiated from control rats, scar tissue and cysts were not found in animals with transplanted SC.

Immunohistochemical staining with antibodies against human cell nuclei revealed transplanted cells

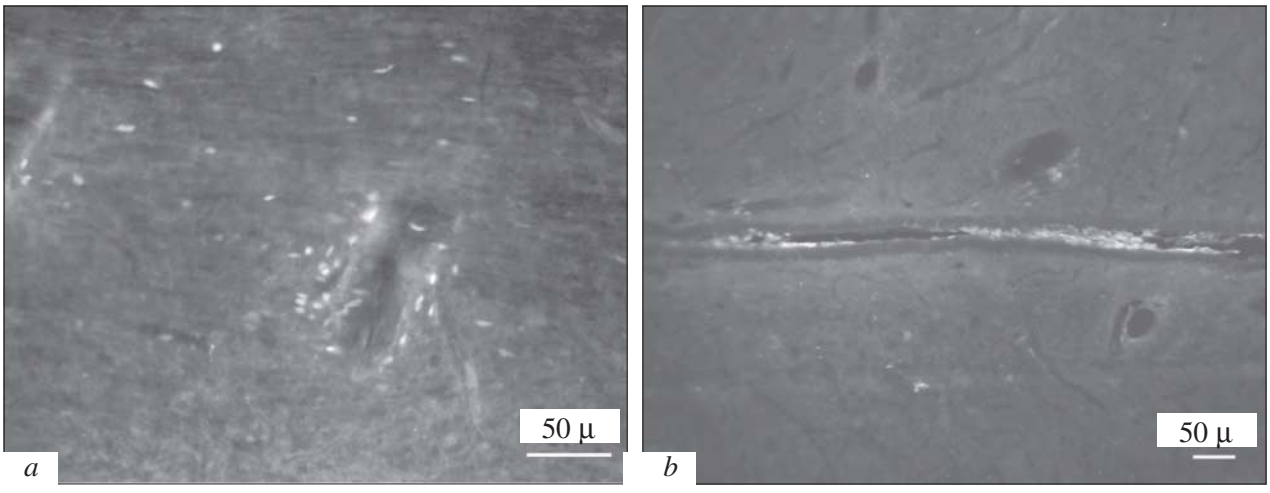

Fig. 1. Cultured neural stem cells of human brain after transplantation into the traumatized spinal cord of adult rats. Migration of cells along fibers and vessels (*a*) and in the recipient spinal cord (*b*). Staining with antibodies against human cell nuclei.

lying individually or arranged in groups. These cells migrated in the distal or proximal direction along fibers and vessels. Some cells were found in the zone of cells and tissue injury between blood cells. Many transplanted cells underwent degeneration in necrotic areas. Staining with antibodies against human nestin revealed the presence of SC among transplanted cells. Some nestin-positive cells migrated widely over the recipient brain tissue from the site of injection, while others were arranged in groups around vessels.

Histological examination of preparations stained with cresyl violet showed that the area of injury was 3-4 mm 1 and 2 months after trauma and transplantation of cultured SC. Tissue edema and cysts were not revealed. The glial reaction occurred in the zone of trauma, but was not accompanied by the formation of glial scars. A considerable number of small newly formed vessels were found in the zone of injury. Motor neurons and interneurons were localized distal and proximal to the zone of injury and retained normal morphological characteristics.

Immunohistochemical staining with antibodies against human cell nuclei revealed transplanted cells around the zone of trauma. Some cells were arranged in groups, while others migrated widely along vessels, in fibers and gray matter, and along or inside the spinal cord (Fig. 1). Double staining with antibodies against human cell nuclei and nestin revealed a considerable number of nestin-positive cells. These results indicate that some implanted cells were in the undifferentiated state. Nestin-positive SC were found between densely positioned transplanted cells, around vessels, and among cells migrating in the surrounding tissue. Human SC had long processes that were located along vessels or grew in the surrounding tissue (Fig. 2). Double staining with antibodies against human cell nuclei and GFAP or vimentin showed that some transplanted cells underwent differentiation into glial cells.

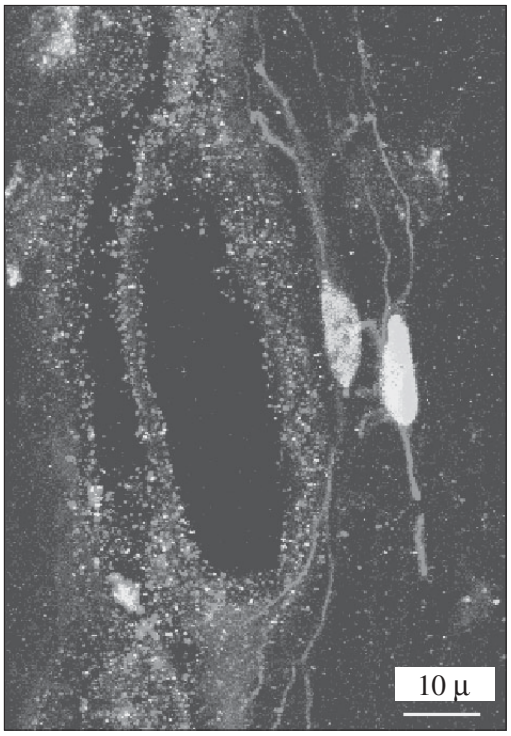

Fig. 2. Human brain stem cells migrating in the vessel and having long branching processes. Double staining with antibodies against human cell nuclei and nestin. Confocal microscopy.

Morphological study of spinal cord sections from experimental animals showed that the area of damage was 4-5 mm 4 months after transplantation. A moderate glial reaction in the zone of trauma was not accompanied by the formation of scars. We revealed no cysts in the zone of trauma. Nerve cells had normal morphological structure. Transplanted cells were found in the site of injection by luminescence of bisbenzimide and staining with antibodies against human cell nuclei. Nestin-expressing SC were absent. Some implanted cells differentiated into neurons, which was determined by staining with

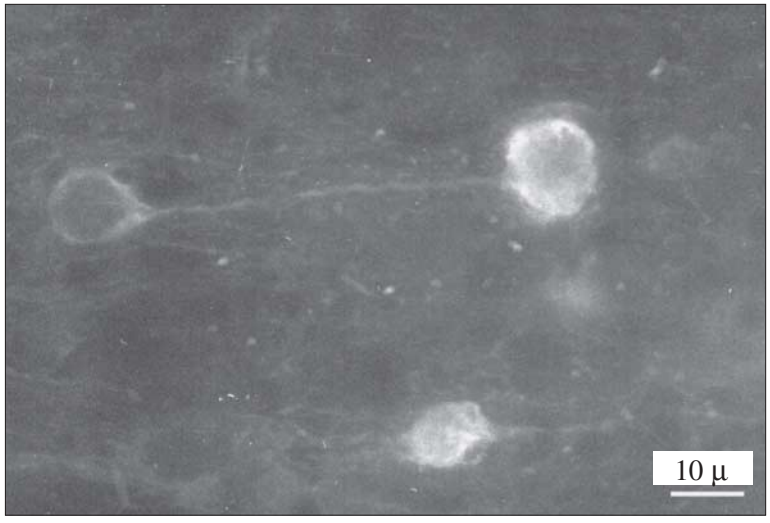

Fig. 3. Transplanted stem cells of human brain differentiating into neurons (growth bulbs). Three months after transplantation into the traumatized spinal cord of adult rats. Staining with antibodies against β-tubulin III.

antibodies against β-tubulin III (Fig. 3). Other cells differentiated into astrocytes and expressed GFAP.

Our results suggest that transplantation of cultured SC from human fetal brain into the spinal cord of rats with mechanical trauma was followed by revascularization without the formation of cysts and glial scar. These results are consistent with published data [13]. The protective effect of SC was probably related to secretion of various neurotrophic factors [7].

Immunohistochemical study showed that transplanted SC from human brain survive for 4 months and retain the ability to migrate and differentiate in the traumatized spinal cord. The behavior of human cells was similar to that of transplanted SC from rodents [4,8,914]. They migrated along fibers and vessels and were found in the zone of necrosis. Nestin-positive SC could be revealed up to the 2nd month after transplantation. They were localized between densely packed transplanted cells and migrated in the recipient brain tissue. Human SC had long processes that were elongated in the direction of migration. The main pathways of migration included vessels and fibers. Many cells were found in the neuronal layer and spinal cord channel of recipients. Some transplanted cells underwent differentiation into neurons and glia and expressed vimentin, β-tubulin III, and GFAP.

Our results suggest that cultured neural cells from human fetal brain can be used for transplantation during spinal trauma.

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