Restorative Neurology and Neuroscience 21 (2003) 65–73
IOS Press

Abstracts of the 17th Annual Meeting of the Japanese Society for Neural Growth, Regeneration and Transplantation

1. Morphological and molecular biological analysis of the alteration of aged rat myelinate fibers

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Recent studies have revealed a significant decrease in white matter volume, including a loss of myelin, with age but minimal decrease in gray matter volume. Myelin is necessary for the rapid conduction of impulses along axons. Myelinated nerve includes various domains, the node of Ranvier, the paranodal region, the juxtaparanodal region and the internode. The paranodal junction may serve to anchor the myelin sheath to the axon. We analyzed the ultrastructure of the paranodal region in myelinated fibers from the aged rat brain. Severe alterations of myelinated fibers were observed in 31-month-old rats, resulting in the appearance of macrophages, splitting of the myelin sheath, myelin balloon formation and separation from the axon. Many paranodal retractions of myelinated axons occurred in the aged rats. It should be noted that the paranodal junction is functionally important serving to anchor the myelin to the axon and that there is a diffusion barrier in the paranodal region. Therefore, we analyzed myelin-related proteins from young and aged rat brains. There were no significant differences in the expression pattern of myelin proteins between young and aged rats. However, the 21.5-kDa isoform of myelin basic protein (MBP) almost disappeared in the 31-month-old rats. These results suggest that this isoform, a highly cationic charged major dense component protein that binds lipid bilayer in the membrane, may participate in the formation of a paranodal diffusion barrier at the myelin / noncompact membrane border.

2. Effect of retinoic acid on the neural differentiation of human neural stem/progenitor cells

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It has been reported that all trans retinoic acid (ATRA) has the ability to promote the efficiency of neural differentiation from neural stem/progenitor cells in rodent cells, but it is not clear whether ATAR has the same effect on human neural cells regarding differentiation. In the present study, we have isolated human neural stem/progenitor cells and examined the effect of ATAR on neural differentiation. With the approval from both the ethical committees of ONH and the TERC, human neural stem/progenitor cells were isolated from fetal forebrain tissues using the neurosphere culture method. Cultured neurospheres were differentiated with 1% FBS in presence or absence of

ATRA for 14 days. The differentiated cells were evaluated by triple immunocytochemistry using anti-tubulin β, anti-GFAP antibodies and nuclear staining with TO-PRO-3® iodide. The number of neurons was assessed by counting the cells labeled by an anti-tubulin β antibody. The percentage of tubulin β-positive cells almost 20% when differentiation was induced with only 1% FBS but when 1% FBS was supplemented with ATRA, the percentage of tubulin β-positive cells significantly increased to 30%. Our results suggested that ATAR can effectively promote neuronal differentiation from human neural stem/progenitor cells with the potential application for effective differentiation of human neurons from neural stem/progenitor cells.

3. Activation and proliferation of NG2 -positive oligodendrocyte progenitor cells after focal ischemia in the rat brain

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The present study examines the alteration of oligodendrocyte progenitor cells (OPCs) which express membrane NG2 chondroitin sulfate proteoglycan after focal ischemia in the rat brain. Adult male Sprague-Dawley rats were subjected to 90-min occlusion of the middle cerebral artery, followed by recirculation time of up to two weeks. The distribution and morphological changes in NG2-positive OPCs were immunohistochemically examined. Stellate-shaped OPCs with multiple branched processes were abundantly detected in both the gray and white matter of normal brain. After two weeks of recirculation, OPCs clearly showed enlarged cell bodies with hypertrophied processes in the area surrounding the infarction site (peri-infarct area). These stained strongly for NG2. The number of OPCs had significantly increased in the peri-infarct area, although it had markedly decreased in the infarct core compared to controls. Double immunostaining revealed that these OPCs were not astrocytes, microglia or mature oligodendrocytes, but NG2-positive OPCs. Phosphorylation of CREB (cyclic AMP response element binding protein) was clearly enhanced in these activated OPCs. In this peri-infarct area, the number of mature oligodendrocytes had significantly decreased at 48 hours of recirculation, but it clearly recovered to the control level at 2 weeks of recirculation. As such, this upregulation of NG2-positive OPCs may be an adaptive mechanism attempting to remyelinate or reorganize rat brain tissue after an ischemic insult.

4. Regeneration of hippocampal pyramidal neurons by recruitment of endogenous neural progenitors: An animal model for a neuronal replacement therapy for stroke

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The adult brain is extremely vulnerable to various insults such as ischemia, trauma and neurodegeneration. However, the recent discovery of neural progenitors capable of producing new neurons in adult mammals including humans raises the possibility of repairing damaged tissue by recruiting their latent regenerative potential. Here we show that activation of endogenous progenitors leads to massive regeneration of hippocampal neurons after transient global ischemia in the adult rat forebrain. Endogenous progenitors proliferated in response to ischemia, and subsequently migrated into the degenerated hippocampus to regenerate new neurons. Intraventricular infusion of growth factors markedly augmented these responses, and thereby induced significant recovery of hippocampal pyramidal neurons lost by ischemia. Electrophysiological and behavioral studies demonstrated that regenerated neurons were integrated into the existing brain circuitry, and contributed to ameliorating neurological deficits. Our results thus expand the possibility of novel neuronal regeneration therapies for ischemic brain injury and other neurological diseases.

5. Intermediate filament, nestin, expression in subpial astrocytes and ependymal cells after traumatic spinal cord injury in adult rats

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The present study used an antibody to the intermediate filament protein (nestin) as an immunohistochemical marker for neural stem cells and progenitor cells in a rat model of spinal cord trauma. Male Sprague-Dawley rats ($n = 30$) had a laminectomy at Th11/12, and spinal cord contusion was created by compression with 30 g of force for 10 min. The rats were sacrificed at 24 h, 1 week, 4 weeks and 12 weeks after injury. Time- and region-dependent alterations of nestin immunoreactivity were analyzed. At 24 h post injury, 5 mm rostral and caudal to the lesions, nestin expression was observed in ependymal cells and around the hemorrhagic and necrotic lesion located in the dorsal spinal cord, peaking at 1 week after injury. Moreover, nestin expression was also observed in the white matter of the ventral spinal cord, extending into the arborizing processes centripetally from the pial surface toward the central canal. At 4 weeks after injury, nestin expression in the ependyma decreased 10 mm from the injury site. On the other hans, nestin expression in the white matter increased dramatically with a one hundred-fold increase in nestin originating from the pial surface, and extension now to all the white matter. The latter was accompanied by GFAP positivity into very long arborizing processes, morphologically compatible with radial glia. The findings suggest two possible sources of precursor cells in the adult mammalian spinal cord; ependyma of the the central canal and subpial astrocytes. Subpial astrocytes may be associated with neural repair and regeneration after spinal cord injury.

6. Characterization of genes expressed in the choroid plexus

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The choroid plexus (CP) produces the majority of the cerebrospinal fluid (CSF) which provides the specialized environment of the central nervous system (CNS). Previously, we demonstrated that CP grafting facilitates axonal regeneration in the spinal cord injury model and that cultured ependymal cells from the CP can promote neurite outgrowth *in vitro*. We therefore postulate that the CP may produce certain factors that affect the CNS environment and regulate neural cell survival. To find molecules involved in supporting CNS function, we tried to identify genes which were distinctively expressed in the mouse CP using suppression subtractive hybridization (1) between the CP and the cerebral cortex from adult mice, and (2) between the CP from postnatal day-5 mice and that from adult mice. In addition, the preferential localization of the CP in the brain was confirmed by Northern blotting and in situ hybridization. In this study, we identified various CP-distinctive molecules including transthyretin (TTR), gelsolin, phospholipid transfer protein (PLTP) and ATP-binding cassette, sub-family A, number 8 (ABCA8). It is suggested that CP might be involved in the A clearance (TTR, gelsolin) and lipid metabolism (PLTP, ABCA8) for the maintenance of neural cells in CNS.

7. Na^+/myoinositol cotransporter-positive microglial cells infiltrate degenerating substantia nigra after intrastriatal hemorrhage in rats

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We studied the expression of an osmoprotective gene, Na^+/myoinositol cotransporter (SMIT) in intracerebral hemorrhage model rats by *in situ* hybridization and immunohistochemistry. Intracerebral hemorrhage model rats were made by the stereotaxic injection of saline containing 0.28 U collagenase type ? into left striatum. We immunohistochemically evaluated the substantia nigra area with an anti-TH antibody and the blood-brain barrier function with anti-rat IgG. The ipsilateral substantia nigra became gradually atrophic after hemorrhage (50% compared with the contralateral side at 42 days after hemorrhage). The expression of SMIT mRNA increased in the ipsilateral substantia nigra without blood-brain barrier disruption 7 days after hemorrhage and remained high until 42 days after hemorrhage. Immunohistochemistry revealed that many microglial cells were observed in the ipsilateral substantia nigra and that they were SMIT-positive. These results suggest that the expression of SMIT must be

regulated not only by osmosity but also by neural degeneration.

8. The functional analysis of nmda receptors in the early time of developing rat brain

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Although the expression of NMDA receptors is observed from the early stages of the developing brain, their function has not yet been fully elucidated. In this study, we examined whether sustained blockade of the NMDA receptors by AP-5, a specific antagonist of the NMDA receptors, would affect neural development. In cerebral cortex cultures from 17-day-old embryonic rats, the number of nestin-, GLAST-, or BrdU-positive cells was increased by the application of AP-5 (100 μM). On the other hand, there were no change in the number of TuJ-1-, or GFAP-positive cells compared with the cultures in the absence of AP-5. Using the calcium imaging method, we showed that the NMDA-responsive cells were MAP-2-positive cells, while the nestin-positive cells hardly responded to NMDA. These results suggest that the proliferation and differentiation of neural stem cells is regulated through the NMDA receptors expressed in neuron and/or neuronal progenitor cells.

9. Difference of the differentiation capacity between the neuroepithelial- and adult neural stem cell

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We have analyzed the feasibility of neuroepithelial stem cells (NESCs) for intracerebral grafting. The purpose of this study was to compare the differentiation capacity between NESCs and adult neural stem cells (NSCs) *in vitro*. The mesencephalic neural plates were removed from embryonic day 10.5 Wistar rats. Single cell cultures were examined after the dissociation of the mesencephalic neural plates. Neurosphere formation was observed in a medium containing 10 ng/ml basic fibroblast growth factor (bFGF), and was positive to anti-nestin antibody. Replaced on the poly-L-lysine (PLL) coated dishes in a medium containing serum, in two days the primary spheres differentiated into neurons extending long neuritis. bFGF responsive neurospheres derived from the ventricular wall in the striatum of adult Wistar rat (post-natal day 28) were also positive to anti-nestin antibody. On the PLL coated dishes, however, they predominantly differentiated into GFAP positive cells, and a few of them differentiated into neurofilament positive cells and gal-c positive cells. These experimental results suggested that NSCs were not always equal in their differentiation paths, and that the NESCs possessed a vigorous neuronal tendency. We concluded that NESCs could be a favorable donor material to repair damaged neuronal circuitry.

10. Evaluation of in vitro proliferative activity of human neural stem/progenitor cells using non-ri and indirect measurements of viable cells

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At present the most widely used culture technique for expanding neural stem/progenitor cells *in vitro* is the neurosphere method. With this technique, mammalian neural stem/progenitor cells are selectively expanded giving rise to floating spheroid cell aggregates called "neurospheres". This aggregating property of neural stem/progenitor cells does, however, make it difficult to estimate the total cells present within the neurospheres and to monitor the cell proliferation rates. In this study, we have examined the utility of two conventional and non-RI methods to estimate the cell proliferation rate and doubling time of the neural progenitor/stem cell indirectly within the intact neurospheres. With the approval from both the ethical committees of ONH and the TERC, human neural stem/progenitor cells were isolated from fetal forebrain tissues using the neurosphere method. Using the WST-8, ATP and BrdU incorporation assay methods, we estimated the population dou-

bling time (DT) of the human neural stem/progenitor cells forming neurospheres. Our results showed that the DT determined from the BrdU incorporation assay was almost identical to the DTs estimated by the WST-8 and ATP assays. These findings indicated that it is possible to determine the proliferative condition of human neural stem/progenitor cells indirectly using the WST-8 or ATP assay. In particular, the ATP assay was so convenient and sensitive that we believe it will become a popular method for rapidly estimating the numbers of viable human neural stem/progenitor cells in large-scale neurospheres cultures for clinical use.

11. Neuroprotection and regeneration through the use of a caspase-9 inhibitor in retinal explants tissue culture

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Purpose: To investigate the effect of caspase-9 on neuronal cell death and regeneration of retinal ganglion cells (RGCs) in retinal explants tissue culture. Methods: Mouse retinal explants were cultured in collagen gel at postnatal 8 $\sim$ 12 weeks and were incubated in 1) serum-free control media; 2) 100 ng/ml brain-derived neurotrophic factor (BDNF); 3) 100 ng/ml ciliary neurotrophic factor (CNTF); 4) 0.05 nmol/l $\sim$ 10 mmol/l caspase-9 inhibitors (Ac-LEHD-CHO); 5) 100 ng/ml BDNF and 10 mmol/l Ac-LEHD-CHO; and 6) 100 ng/ml CNTF and 10 mmol/l Ac-LEHD-CHO supplemented culture media. Quantitative analysis of TdT-dUTP terminal nick-end labeling (TUNEL) staining and the assessment of the number of regenerating neurites were performed. *Results:* The ratio of TUNEL positivity in 0.01 $\sim$ 10 moml/l Ac-LEHD-CHO incubated retina was very low throughout the period of culture. Furthermore, the number of neurites increased more than in the control. The same tendency was observed under conditions of BDNF and CNTF supplement. Furthermore, under the condition of a combination of CNTF and Ac-LEHD-CHO treatment, the greatest number of surviving cells and neurites were seen compared with all other conditions. *Conclusions:* Our findings suggest that caspase-9 has a critical role in protection and regeneration of damaged RGCs in adult retinal explants.

12. Potential use of embryonic stem cells for the treatment of mouse parkinsonian models

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The purpose of the present study was to examine the efficacy of transplantation of mouse embryonic stem (ES) cell-derived tyrosine hydroxylase (TH) positive cells into Parkinsonian mice by behavioral tests and immunohistochemical evaluation. Undifferentiated ES cells carrying the enhanced green fluorescent protein (EGFP) gene were differentiated into a cell population containing TH positive neurons by a 5-step *in vitro* differentiation method. These ES-derived cells were used as allografts in Parkinsonian mice, made by administering injections of 6-hydroxydopamine (6-OHDA). Fifteen hemiparkinsonian mice were divided into 3 groups. Four weeks after 6-OHDA injection, Groups 1, 2, and 3 mice received phosphate-buffered saline, 1x104 graft cells, and 1x105 graft cells, respectively, into the dopamine-denervated striatum. Improved rotational behavior was observed in the graft-transplanted groups (Groups 2 and 3) 2 weeks after transplantation. Mice in Group 2 displayed a continuous maintenance of reduced rotational behavior, while those in Group 3 showed ipsilateral rotation toward the lesioned side at 4, 6, and 8 weeks after transplantation. A few TH positive cells were found at the grafted sites 8 weeks after transplantation in Group 2 mice, and a larger number of TH positive cells in Group 3. Some of those TH positive cells were immunopositive to GFP, demonstrating the presence of dopaminergic neurons derived from the ES cells. Transplantation of *in vitro* differentiated ES cells improved rotational behavior in Parkinsonian mice. Our results suggest that an ES-based cell transplantation therapy is a promising treatment modality for Parkinson's disease.

13. Transplantation of dopaminergic neurons from ES cells through stromal cell-derived inducing activity

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A method of inducing dopamine (DA) neurons from mouse embryonic stem cells (ESCs) by stromal cell-

derived inducing activity (SDIA) was previously reported. When transplanted, SDIA-induced DA neurons integrate into the mouse striatum and remain positive for tyrosine hydroxylase (TH) expression. In the present study, to optimize the transplantation efficiency, we treated mouse ESCs with SDIA for various numbers of days (8–14 days). SDIA-treated ESC colonies were isolated by papain treatment, and then grafted into the 6-OHDA-lesioned mouse striatum. The ratio of the number of surviving TH-positive cells/ total number of grafted cells was highest when ESCs were treated with SDIA for 12 days before transplantation. This ratio revealed that grafting cell colonies was more efficient for obtaining TH-positive cells *in vivo* than grafting cell suspensions. When we grafted a cell suspension of 2 $\times$ 10^5, 2 $\times$ 10^4,or 2 $\times$ 10^3 cells into the 6-OHDA-lesioned mouse striatum, we observed only a few surviving TH-positive cells. In conclusion, inducing DA neurons from mouse ESCs by SDIA for 12 days and grafting cell colonies into the mouse striatum was the most effective method for the survival of TH-positive neurons *in vivo*.

14. Development of a cell processing technique of human neural stem/progenitor cells for clinical use

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Since the discovery of neural stem cells (NSCs), many researchers have been trying to improve the damaged mammalian CNS function by transplanting *ex-vivo* expanded immature neural stem/progenitor cells. In practical application, however, transplanting human neural stem/progenitor cells for the treatment of intractable CNS diseases requires large quantities of neural stem/progenitor cells which are very difficult to isolate from the fetus or adult human. It is therefore very important to develop techniques by which human neural stem/progenitor cells can be proliferated to form large quantities for clinical use. With the approval from both the ethical committees of ONH and the TERC, we have isolated neural stem/progenitor cells from the forebrain and spinal cords of total 12 human fetuses using the neurosphere culture technique and started to develop cell-processing methods of human neural stem/progenitor cells for clinical use. We have succeeded to maintain human neural stem /progenitor cells for more than one year without losing their proliferation capacity and these cells could be expanded in large volumes both for basic and clinical research. To apply our *ex-vivo* expanded cells for clinical use, now we are examining the suitability and functional ability of long-cultured human neural stem/progenitor cells as donor cells *in vivo*.

15. A novel MPTP primate model of parkinson's disease: Relationships between cell loss, clinical and neurochemical changes

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Monkeys treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have been used as animal models for various kinds of studies on Parkinson's disease (PD). This study focused on the rhesus monkeys produced in different grades of bilateral parkinsonism by a unilateral intracarotid (ICA) infusion, and subsequent intravenous infusions, of MPTP. In order to evaluate the potential of these monkeys for a PD model, we examined relationships between the behavioral, neurochemical and neuropathological changes. The monkeys were categorized as mild (stage 2) and moderate (stage 3) bilateral parkinsonism based on the PD rating scale. Postmortem biochemical analysis showed massive dopamine (DA) reduction equally in the caudate nucleus and putamen ipsilateral to the ICA MPTP infusion, with relative DA preservation in the contralateral hemisphere. Differences between stage 2 and stage 3 resulted from DA concentrations in the caudate nucleus and putamen of the contralateral hemisphere. A positron emission tomography study using [18F]6-fluoro-L-m-tyrosine showed that Ki values could be good indicators of the striatal DA concentration. By comparison with tyrosine hydroxylase (TH) immunohistochemistry, the midbrain dopaminergic cells, the

group A9, showed significant relationships with DA losses in the caudate nucleus and putamen. In conclusion, this PD model can play an important role for studies of a therapeutic basis in which different severities of symptoms may be required.

16. Dopaminergic neurons generated from embryonic stem cell were transplanted into monkey brain

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We previously reported success in generating dopaminergic neurons from embryonic stem cells (ESCs) by the SDIA (stromal cell-derived inducing activity) method. In this study, we transplanted dopaminergic neurons generated from ESCs into the monkey brain. Murine ESCs plated on PA6 stromal cells for 12 days were almost all positive for TuJ-1 which is a post mitotic neuronal marker. About 10% of these cells were positive for tyrosine hydrohylase which is a marker of dopaminergic neurons. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was intravenously administrated to *Macaca fascicularis* monkeys as a primate model of Parkinson's disease. Neurons generated from murine ESCs were transplanted into normal and MPTP-treated monkey brains. At 2 weeks, monkeys were transcardially perfused with 4% paraformaldehyde. Removed brains were sliced with a microtome and immunohistochemically analyzed. In the transplanted striatum, TuJ-1 and TH positive neurons were detected. In addition, we tested the SDIA method using *Macaca fascicularis* ESCs. At 2 weeks after plating on PA6, TuJ-1-positive and TH-positive neurons were detected. The SDIA method is a promising approach that brings stem cell therapy for Parkinson's disease closer toward the level of practical clinical application.

17. Differentiation from neural stem cells to dopamine secreting neurons by VHL gene induction and therapy for parkinson disease with grafting the VHL-inducted cells

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VHL gene induction causes neuronal differentiation in neuronal stem cells (NSCs). In contrast, a VHL mRNA antisense oligonucleotide inhibits the differentiation of NSCs and up-regulates their cell cycle. Some of the VHL-gene inducted NSCs show immunoreactivity to tyrosine hydroxylase (TH) suggesting dopamine secreting cells. An electrophysiological study with the patch clamp method reveal that the VHL-gene inducted NSCs show a sodium-potassium current equal to mature neurons. Grafting the VHL-gene induced NSCs intothe brain of Parkinson model rat markedly improves the behavioral symptoms. Most of the grafted cells differentiate into neurons showing immunoreactivity to both MAP-2 and TH. In conclusion, VHL-gene induced NSCs which are grafted into the central nervous system form a neuronal network and function as functional neurons. The grafting the cells would be clinically useful for intractable neuronal diseases such as Parkinson's disease.

18. Rate of axonal regeneration of cat retinal ganglion cells into peripheral nerve graft

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Although transected axons of retinal ganglion cells (RGCs) regenerate into a transplanted peripheral nerve (PN) segment, the numbers of RGCs with regenerated axons are fewer than 5,000 in the cat retina. Enhancing axonal regrowth is one of the promising methods to obtain more regenerated RGCs. We estimated the rate of regrowth of RGC axons regenerating in a PN graft as the control value. The left transected optic nerve (ON) of anesthetized cats was connected with a PN segment. After 4, 6, and 8 week survival, RGCs with regenerated axons were double-labeled with two fluorescent dyes injected at 10 mm (2 day before enucleation) and 20 mm (4 day before enucleation) from the surgical site. Numbers of single- and double-labeled

RGCs were counted. Proportions of alpha, beta, and not alpha/beta (NAB) cells were estimated with Lucifer yellow injections. From a scatter diagram of ratios of double-labeling vs surviving weeks, we obtained a formula: $Y = 82.7 \ln(X)$-93.2, where Y is the double labeling ratio expressed as a percentage, and X is the survival in weeks. The formula indicates that the fastest growing axon reached a length of 20 mm by 3.1 wk. Supposing a regrowth lag of 4 days, the fastest rate of axonal regrowth was 1.1 mm/day. Lucifer injections revealed that axonal growth rates of alpha, beta, and NAB cells were 1.4, 1.1, and 1.0 mm/day, respectively. The value of 1.1 mm/day in our cat model is close to that in hamster RGCs. Higher regeneration rates of alpha cells may reflect their greater regenerative ability than other cell types.

19. Schwann cell transplantation for contused spinal cord repair

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Although spinal cord injury results in progressive tissue damage, leading to permanent functional deficits, recent advances in cellular transplants strategies suggest that there may be a window of opportunity for spinal cord injury repair. With the potential prospect of a physical channel for axonal growth and myelination, populations of myelinating glial cells may provide a supportive microenvironment. In the present study, to clarify the ability of Schwann cell (SC) grafts to repair the damaged spinal cord, the effects of purified rat SC on spinal tissue preservation, axonal regeneration/sparing, axonal myelination and behavioral improvements were assessed in the moderately contused adult rat thoracic spinal cord. Cultured rat SCs were transplanted in the adult rat thoracic spinal cord one wk after a moderate contusion injury. At 12 wk after injury, SC grafts significantly reduced spinal tissue damage compared to controls. SC grafts promoted robust regeneration of mainly propriospinal axons. Electromicroscopic analysis revealed that SC grafts contained a higher number of myelinated axons of the peripheral type. Retrograde tracing demonstrated that the number of propriospinal and brainstem axons reaching 5–6 mm beyond the grafted area was significantly higher with SC grafts compared to controls. Corticospinal fibers terminated closer to the lesion epicenter in SC grafted animals. With SC grafts a modest but statistically significant improvement in hind limb locomotor performance could be detected at 8–10 wk after injury. Our results show that a SC graft is effective in limiting tissue damage and promoting axonal sparing/regeneration in the moderately contused adult rat thoracic spinal cord.

20. Bone marrow therapy for CNS diseases

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Transplantation of a cell fraction isolated from human bone marrow into damaged CNS lesions in animal models results in varying degrees of regeneration. The isolated cell fraction contains a variety of precursors and stem cells such as hematopoietic, nonhematopoietic, and unknown immature stem cells. A small population of bone marrow may not be restricted to produce tissue-specific cell types (those from the tissue in which they reside). Indeed, bone marrow cells can differentiate into cells of a neural lineage both *in vitro* and *in vivo*. In the present study, bone marrow cells were transplanted into several experimental animal models, and the potential use for regenerative medicine in CNS was examined. The characteristics of bone marrow stem cells (BMSCs) include a high proliferating potential, good multipotentiality, and they are present even in human adults. BMSCs showed good adaptation in the host CNS tissue, and migrated through the normal and damaged brain tissue following transplantation. They accumulated in and around the damaged lesion, and differentiated in the host microenvironment. These characteristics seem to be very useful to establish a cell therapy for CNS diseases. BMSCs can also be a potential donor source for auto-transplantation therapy, are easily accessible and appear to have a wider differentiation potential than previously thought, and may be a source for a cell therapy for treatment of CNS diseases.

21. Transdifferentiation of bone marrow stromal cells into neurons, and application in parkinson model rats

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Bone marrow stromal cells (MSCs) are well known as multi-potential stem cells that under specific experimental conditions differentiate into several types of cells, for example, osteoblasts, adipocytes and chondrocytes. In the present study, MSCs are demonstrated to be induced into cells with neuronal characteristics, which may well be applicable to the treatment of Parkinson model rats. MSCs which differentiated into neuronal phenotypes were immunopositive for neuronal markers MAP-2, Tuj-1, beta-3-tubulin and neurofilament-M, and were positive to neurotransmitter related molecules such as vesicular tyrosine hydroxylase. By a certain procedure, percentage of tyrosine hydroxylase positive cells can be increased, and the effect of transplantation of these cells were investigated using model rats for Parkinson's disease, which resulted in a remarkable decrease in apomorphine induced rotation. When the corpus striatum of the brain of the experimental animals were investigated under a microscope, virtually all the GFP-labeled transplanted cells had differentiated into neurofilament-expressing neurons. In conclusion, differentiated MSCs are considered to be one of the strongest candidates for cell transplantation in the treatment of CNS disorders.

22. Transplantation of autologous bone marrow cells into ischemic lesions in the rat middle cerebral artery model

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It has recently been thought that bone marrow cells retain the potential for both neural production and differentiation. The bone marrow crude cell fraction contains several types of precursors and stem cells such as hematopoietic stem cells, nonhematopoietic stem, and other unknown precursor cells. Transplantation of bone marrow is reported to repair the CNS tissues in several animal models. The objective of this study was to study whether the intravenous administration of autologous bone marrow cells repairs the ischemic lesion in the mammalian brain. Acutely-isolated bone marrow cells were genetically marked with the LacZ gene, and were intravenously injected into ischemic lesions in the rat middle cerebral artery occlusion model. Histological examination of the ischemic lesion after transplantation revealed that the transplanted bone marrow cells had reconstructed neural tissue. The cells which had differentiated from the transplanted bone marrow cells showed both neuronal and glial phenotypes. Thus, bone marrow cells retain the potential for both neural production and differentiation in ischemic brain tissues, and intravenous administration of bone marrow cells have shown the potential to effect the repair of ischemic lesions in the mammalian adult CNS, at least in animal models. Intravenous administration of autologous bone marrow cells may be a new strategy for the treatment of cerebral infarction.

23. Intravenous administration of autologous bone marrow cells in the rat demyelinated model

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The regenerative potential of bone marrow cells was studied in the demyelinated rat model. Although both the focal injection and the intravenous administration of bone marrow cells isolated from bone marrow repaired the demyelinated spinal cord in the adult rat, the ideal protocol in terms of the administration method and the cell number remains unknown. This study was designed to examine how we should transplant the bone marrow cells and how many cells are required to establish sufficient numbers of remyelinated axons in the demyelinated rat spinal cord. A focal demyelinated lesion was created in the dorsal columns of the rat spinal cord using X-irradiation and ethidium bromide injection (EB-X). A suspension of bone marrow cells ($1 \times 10^2 - 1 \times 10^8$) collected from the same rat was directly transplanted into the middle of the EB-X-induced lesion or was injected into a femoral vein 3 days after the EB injection. Lesions were histologically examined 3 weeks after transplantation. Light microscopic examination revealed the demyelinated axons were extensively repaired by autologous bone marrow cells. The numbers of the repaired axons following bone marrow transplantation were in proportion to those of the transplanted cells. In addition, the effectiveness of the focal injection was 100 times greater than the intravenous administration. These results demonstrate that the intravenous administration of the autologous bone marrow cells may be a better strategy for treatment of the injured CNS.