Mini Review

Regenerative medicine for spinal cord injury: Current status and open issues

Masaya Nakamura[1,*], Narihito Nagoshi[1,2], Kanehiro Fujiyoshi[1,2], Shinjiro Kaneko[1], Yoshiaki Toyama[1], and Hideyuki Okano[2]

[1] Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan
[2] Department of Physiology, Keio University School of Medicine, Tokyo, Japan

Spinal cord injuries result in devastating loss of function, because spinal cord of human beings never regenerates after injury. People believed in this dogma for a long time. There is an emerging hope for regeneration-based therapy of the damaged spinal cord due to the progress of neuroscience and regenerative medicine including stem cell biology. In this review, we have summarized recent studies aimed at the development of regeneration-based therapeutic approaches for spinal cord injuries, including therapy with transplantation of neural crest stem cells and induction of axonal regeneration, and the establishment of new method for evaluating injured and regenerated axonal fibers by MRI.

Rec.12/24/2008, 1/16/2009, pp198-203

* Correspondence should be addressed to:
Nakamura M, Department of Orthopaedic Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan. Phone: +81-3-5363-3812. Fax: +81-3-3353-6597. e-mail: masa@sc.itc.keio.ac.jp

Key words spinal cord injury, neural crest stem cell, chondroitin sulfate proteoglycan, semaphorin 3A, diffusion tensor tractography

Introduction

Neural stem cell transplantation is a promising regenerative medicine strategy for the treatment of spinal cord injury (SCI). We previously investigated the optimum timing of neural stem cell transplantation from the perspective of microenvironments within the injured spinal cord[1-3], and successfully transplanted rat neural stem cells into the injured spinal cord of adult rats[4] and human neural stem cells into the injured spinal cord of common marmosets[5], thereby promoting functional recovery. We believe these findings represent a significant step toward the clinical application of neural stem cell transplantation. However, because of various problems, it has not yet been possible to use neural stem cells clinically. Herein, we present basic studies that have been conducted to address various barriers against the realization of regenerative medicine for SCI. These problems include: (1) ethical issues related to the use of aborted fetal tissues, (2) axonal growth inhibitors within the injured spinal cord, and (3) insufficient methods for evaluating damaged and regenerated axons within the spinal cord.

Ethical issues related to the use of aborted fetal tissues

Aborted fetus-derived cells were used in our above-mentioned studies on neural stem cell transplantation for treating SCI. Ethical issues related to the clinical use of such cells have long been discussed at the relevant councils of the Ministry of Health,

Labour and Welfare. However, even the guidelines on the use of human stem cells for clinical research, published in 2006, do not include a definite stance on the validity of using aborted fetus-derived cells (Fig.1). Because of these problems and uncertainties, we have recently focused on neural crest cells derived from the patient's own tissue as a source of somatic stem cells, rather than on stem cells from aborted fetal tissue.

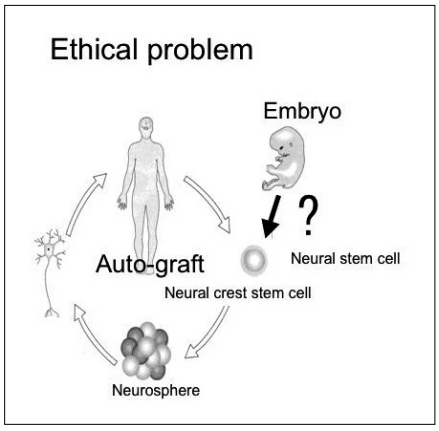

Fig.1 Avoidance of ethical problems associated with aborted tissue-derived neural stem cells by using neural crest cells

1) What is a neural crest cell?

Neural crest cells, which are induced at the border of the epidermal ectoderm and neural plate during development, move to the surrounding tissue immediately after closure of the neural tube. The migratory neural crest cells have diverse differentiation potentials, and are able to differentiate into neurons and glia of the sensory and autonomic systems, adrenal medulla, pigment cells, cranial skeleton (bone and cartilage), teeth (odontoblasts), arterial smooth muscle, and other cell types. Neural crest cells play an important role in many aspects of organ development and therefore are called the "fourth germ layer"[6]. Migratory neural crest cells differentiate into diverse tissues, depending on their environment. However, a portion of these cells remains undifferentiated and latent within various tissues while retaining their multipotential nature, even in adult organs. These neural crest stem cells have recently been attracting close attention as a potential cell source for autologous transplantation, because of their capacities of self-renewal and multipotential[7].

2) Isolation and identification of neural crest stem cells

Neural crest stem cells exist in many tissues, including the skin, intestine, heart, and corneas of adult mice[8-11]. Using transgenic PO-Cre and Wnt1-Cre/FLoxed-EGFP mice[12-14], we demonstrated that neural crest stem cells are also present in the dorsal root ganglia and bone marrow of adult mice[15]. Although the presence of stem cells (serving as a source of neural cells) in bone marrow has been shown in many reports, their embryological origin and differentiation potentials have been regarded as questionable. There are no reports that explain how the bone marrow-derived cells, of mesodermal origin, can differentiate into neural cells, which are of ectodermic origin. In addressing these questions, we found that neural crest stem cells move via the blood into the bone marrow during early embryonic development and remain latent in the bone marrow until adulthood, when they can produce neurons and glia. Furthermore, when we compared the properties of neural crest cells derived from different tissues (i.e. dorsal root ganglion, skin, and bone marrow) of adult mice, we found striking differences in their differentiation potential and gene expression profile . This result indicated that the neural crest stem cells latent in each adult tissue are not uniform, but rather retain properties that depend on their tissue of origin.

3) Transplantation of neural crest stem cells

Several recent transplantation studies have used skin-derived neural crest stem cells as a cell source. Miller et al. reported that skin-derived neural crest stem cells transplanted into demyelinated regions of central and peripheral axons differentiate into Schwann cells, which subsequently engage in remyelination[16]. Furthermore, these authors reported that skin-derived neural crest stem cells transplanted into injured spinal cords also differentiate into Schwann cells, and their differentiation is followed by axonal growth and the accumulation of endogenous Schwann cells, leading to a recovery in locomotor function[17]. However, in all these reports, the neural crest stem cells were obtained from neonatal mice. There are significant differences in the properties of neonatal and adult neural crest stem cells[18].

To achieve the goal of clinical application, it is important to evaluate the potential usefulness of neural crest stem cells derived from various adult tissues. In another study, neural crest stem cells derived from adult mouse skin were transplanted into injured mouse spinal cord. Some of the transplanted cells survived, but no improvement in locomotor function was described[19]. Further studies are needed to determine the effectiveness of this approach. Since neural crest stem cells are found in a variety of adult tissues, and since their characteristics depend on their tissue of origin, it will be essential to select the type of neural crest

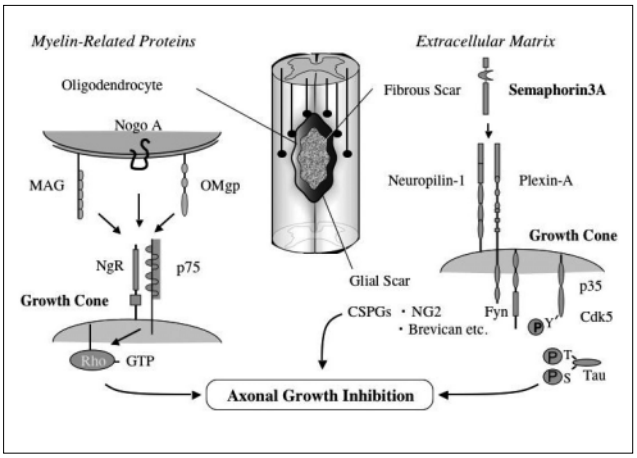

Fig.2 Axonal growth inhibitors in the injured spinal cord

stem cell for transplantation that will yield optimal results. In any event, neural crest stem cells are somatic stem cells that can be used for autologous transplantation. Given these features, in terms of both ethics and safety, neural crest stem cells are a promising source for transplantation in clinical cases.

Overcoming axonal growth inhibitors

Axonal growth does not occur in the injured central nervous system, although it can take place in the injured peripheral nervous system. One explanation for this is the presence of factors that inhibit axonal regeneration in the central nervous system. Even if effective stem cell transplantation for acute or sub-acute SCI can be achieved, it will still be difficult to establish valid regenerative treatments for chronic SCI unless the effects of the axonal growth inhibitors in the central nervous system can be overcome.

The axonal growth inhibitors found to date in the central nervous system can be roughly divided into myelin-associated proteins present in the myelin sheath (Nogo, MAG, and OMgp), and extracellular matrix components present in glial scar tissue, such as chondroitin sulfate proteoglycan (CSPG) and semaphorin 3A (Sema 3A) (Fig.2). In recent studies, animal models of SCI were treated with Nogo receptor antagonists (NEP1-40)[20], chondroitinase ABC (an enzyme involved in the degradation of CSPG)[21], and Rho signal- suppressing drugs (C3 and Y-27632)[22,23]. These methods are anticipated to be of value in treating spinal cord injuries.

We developed a Sema 3A inhibitor and applied it to the subarachnoid cavity of rats for 4 weeks after complete transection of the thoracic spinal cord. This agent stimulated axonal regeneration, induced vascularization, and promoted the migration of Schwann cells into the injured area, thus facilitating the recovery of leg locomotor function in the rats[24]. We also induced a thoracic contusive SCI in rats and administered chondroitinase ABC into the subarachnoid cavity of each rat for one week, beginning one week after injury. The CSPG level in the injured spinal cord decreased to a normal level after this treatment. In the same study, neural stem cell transplantation, applied in combination with chondroitinase ABC, exerted synergistic effects, and induced more marked axonal regeneration than either treatment given alone[25]. These results indicated that the regeneration of injured axons can be induced by combining the use of axonal extension inhibitors with neural stem cell transplantation. This important finding opens the door for effective treatments for chronic SCI.

Establishment of a method for evaluating spinal cord regeneration

The realization of regenerative medicine for the spinal cord requires the establishment of an evaluation method. Needless to say, axonal regeneration in the spinal projection tract is important for achieving spinal cord regeneration. However, the absence of an established method for evaluating axonal regeneration has made it clinically difficult to evaluate the responses of the injured spinal cord to cell transplantation.

To address this need, we have focused on a particular imaging technique, diffusion weighted imaging (DWI), which yields images based on the diffusion of water molecules. Two DWI methods, diffusion tensor imaging (DTI) and diffusion tensor tractography (DTT), have especially attracted our attention. We have applied these methods to the visualization of long tracts within injured spinal cords.

1) Anisotropy and the FA map

How water molecules diffuse in the living body varies depends on the nature of the local environment, and this variation is called, "anisotropic diffusion." For example, the white matter fibers constituting the spinal cord are highly anisotropic, and visualization of their anisotropy should delineate axonal arrangements. FA (fractional anisotropy) provides an indicator of the magnitude of anisotropy. FA ranges from 0 to 1, where it is 0 in cases with isotropic diffusion, and approaches 1 as the diffusion becomes more anisotropic. An image representing anisotropy two-dimensionally is called an "anisotropy map" or an "FA map." In a color FA map, different colors are assigned to different axes; thus, fibers can be distinguished from each other by using dif-

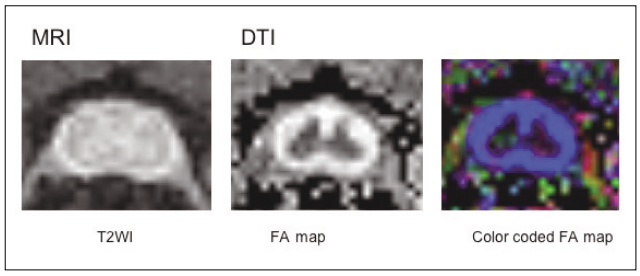

Fig.3 MRI and DTI of the normal spinal cord of a common marmoset

T2-weighted image (T2WI) and DTI (anisotropy map and colored anisotropy map) of the common marmoset spinal cord (cross-section). On the anisotropy map, white matter fibers, which have high anisotropy, are depicted as high signal areas. On the colored anisotropy map, different colors are assigned to different axes. White matter fibers are blue. The image shows the white matter to be composed of longitudinally arranged axonal fibers. (Reproduced from Reference 26)

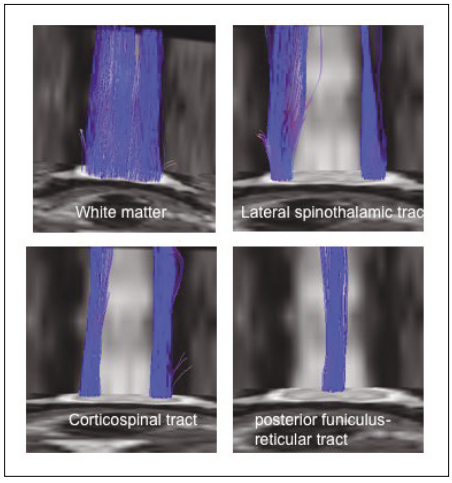

Fig.5 DTT of the normal spinal cord of a common marmoset (live model)

DTT clearly depicting fibers of the entire white matter were obtained by setting the regions of interest within the white matter of the cervical segment of the common marmoset spinal cord. By changing the regions of interest, it is possible to selectively depict by DTT, not only the corticospinal tract, but also the afferent fibers (lateral spinothalamic tract, posterior funiculus-reticular tract, etc.). (Reproduced from Reference 26)

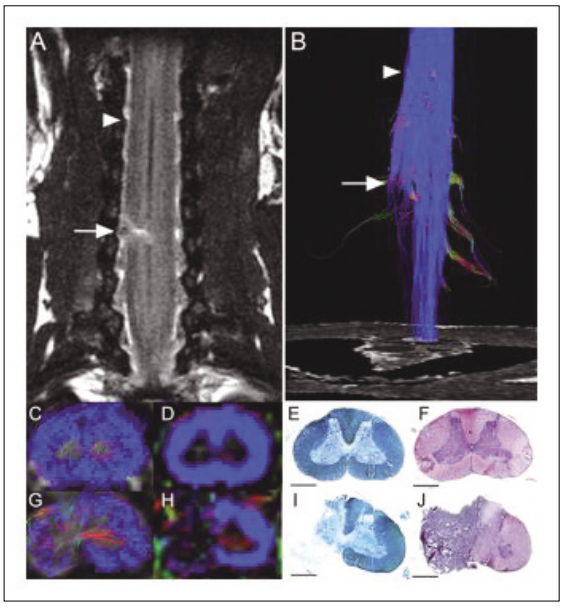

Fig.4 T2WI, DTT, and histological features of a common marmoset with half-cut spinal cord

Cervical segment of the common marmoset spinal cord 2 weeks after it was cut halfway through at the C5/6 level (a post-mortem model). A) MRI T2WI. B) Interrupted nerve fibers of the half-cut spinal cord are visible by DTT. C-F) Spinal cord 2-cm cranial to the half-cut level. G-J) Spinal cord at the half-cut level. Histological features of the HE- (F,J) and LFB- (E,I) stained specimens are well reflected by DTT (C,G) and the colored anisotropy map (D,H). (Reproduced from Reference 26)

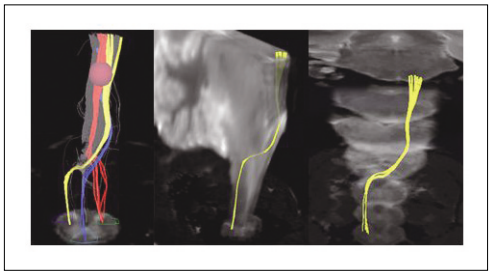

Fig.6 DTT of the corticospinal tract and pyramidal decussation of a common marmoset

The medulla oblongata-pyramidal decussation (an anatomical feature of the corticospinal tract) is clearly depicted in the DTT. In this area, it is known that 90% of corticospinal tract fibers pass through the pyramidal decussation, and a small percentage descend the ipsilateral lateral funiculus (red fibers) or the contralateral anterior funiculus (blue fibers). DTT clearly depicted even these fibers. (Reproduced from Reference 26)

ferent colors according to the direction of their arrangement. Usually, blue is assigned to fibers running longitudinally in the spinal cord, red to fibers running laterally, and green to fibers running dorsoventrally. Fig.3 shows a color FA map of the cross-section of a common marmoset spinal cord. In this figure, the blue represents white matter fibers running vertically[26].

2) Diffusion tensor tractography of the spinal cord

DTT (diffusion tensor tractography) is an imaging technique in which the direction of maximum anisotropy for each voxel is traced. Before spinal DTT can be applied clinically, it is indispensable to conduct detailed analyses to determine the extent to which DTT reflects each tissue type, and the reliability with which DTT depicts axonal information.

With this purpose in mind, we performed DTT for a common marmoset after SCI. Our results yielded the first, worldwide, clear DTT of the spinal cord of an experimental primate. In this experiment, the cervical segment of the spinal cord of a common marmoset was cut halfway through at the C5/6 level, and DTT was performed two weeks later (immediately following the sacrifice of the animal). Unlike MRI, which depicts the injured spinal cord only as changes in signal intensity on T1 and T2 weighted images, DTT allows visualization of the injury in the form of interrupted white matter fibers (Fig.4). The examination of histological specimens stained with HE and LFB confirmed that DTI and DTT precisely reflected the histological features of the injured tissue. By performing detailed post-mortem DTT analyses of this animal model, we devised various ways to minimize artifacts (e.g., movement caused by respiration, the beating of cerebrospinal fluid, etc.), which enabled us to perform spinal cord DTT in live common marmosets. Furthermore, by changing the regions of interest for DTT on the basis of our neuroanatomical findings, we obtained clear projection tract-selective DTT images in live animals (Fig.5). We have also obtained images of the pyramidal decussation, which was previously considered to be impossible (Fig.6)[26]. We have thus demonstrated that DTT is a very useful method of fiber tracking that may replace conventional tracers for monitoring SCI and repair.

Future perspectives

Basic research has been steadily advancing and overcoming the obstacles to the realization of regenerative medicine for SCI. Recently, induced pluripotent stem cells, developed by Yamanaka et al[27], have been attracting considerable attention as a cell source and are expected to provide significant advancements in regenerative medicine. To promote regenerative medicine in Japan and advance its techniques worldwide, further basic research aimed at ascertaining its safety and efficacy, followed by clinical trials, are essential.

References

1) Nakamura M, Bregman BS: Difference in neurotrophic factor gene expression profiles between neonate and adult rat spinal cord after injury. Exp Neurol, 169: 407-415, 2001.
2) Nakamura M, Houghtling RA, MacArthur L, et al: Differences in cytokine expression profile between acute and secondary injury in adult rat spinal cord. Exp Neurol, 184: 313-325, 2003.
3) Okada S, Ishii K, Yamane J, Iwanami A, et al: In vivo imaging of engrafted neural stem cells: its application in evaluating the optimal timing of transplantation for spinal cord injury. FASEB J, 19: 1839-1841, 2005.
4) Ogawa Y, Sawamoto K, Miyata T, et al: Transplantation of in vitro-expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats. J Neurosci Res, 69: 925-933, 2002.
5) Iwanami A, Kaneko S, Nakamura M, et al: Transplantation of Human Neural Stem Cells for Spinal Cord Injury in Primates. J Neurosci Res, 80: 182-190, 2005.
6) Le Douarin NM, Kalcheim C: The neural crest UK: Cambridge University Press, 1999.
7) Crane JF, Trainor PA: Neural crest stem and progenitor cells. Annu Rev Cell Dev Biol, 22: 267-286, 2006.
8) Fernandes KJ, McKenzie IA, Mill P, et al: A dermal niche for multipotent adult skin-derived precursor cells. Nat Cell Biol, 6: 1082-1093, 2004.
9) Kruger GM, Mosher JT, Bixby S, et al: Neural crest stem cells persist in the adult gut but undergo changes in self-renewal, neuronal subtype potential, and factor responsiveness. Neuron, 35: 657-669, 2002.
10) Tomita Y, Matsumura K, Wakamatsu Y, et al: Cardiac neural crest cells contribute to the dormant multipotent stem cell in the mammalian heart. J Cell Biol, 170: 1135-1146, 2005.
11) Yoshida S, Shimmura S, Nagoshi N, et al: Isolation of multipotent neural crest-derived stem cells from the adult mouse cornea. Stem cells, 24: 2714-2722, 2006.
12) Yamauchi Y, Abe K, Mantani A, et al: A novel transgenic technique that allows specific marking of the neural crest cell lineage in mice. Dev Biol, 212: 191-203, 1999.
13) Danielian PS, Muccino D, Rowitch DH, et al: Modification of gene activity in mouse embryos in utero by a tamoxifen-

inducible form of Cre recombinase. Curr Biol, 8: 1323-1326, 1998.

14) Kawamoto S, Niwa H, Tashiro F, et al: A novel reporter mouse strain that expresses enhanced green fluorescent protein upon Cre-mediated recombination. FEBS Lett, 470: 263-268, 2000.

15) Nagoshi N, Shibata S, Kubota Y, et al: Multipotent Neural Crest-Derived Stem Cells in Bone Marrow, Dorsal Root Ganglia and Facial Skin. Cell Stem cell, 2: 392-403, 2008.

16) McKenzie IA, Biernaskie J, Toma JG, et al: Skin-derived precursors generate myelinating Schwann cells for the injured and dysmyelinated nervous system. J Neurosci, 26: 6651-6660, 2006.

17) Biernaskie J, Sparling JS, Liu J, et al: Skin-derived precursors generate myelinating Schwann cells that promote remyelination and functional recovery after contusion spinal cord injury. J Neurosci, 27: 9545-9559, 2007.

18) Bixby S, Kruger GM, Mosher JT, et al: Cell-intrinsic differences between stem cells from different regions of the peripheral nervous system regulate the generation of neural diversity. Neuron, 35: 643-656, 2002.

19) Sieber-Blum M, Schnell L, Grim M, et al: Characterization of epidermal neural crest stem cell (EPI-NCSC) grafts in the lesioned spinal cord. Mol Cell Neurosci, 32: 67-81, 2006.

20) GrandPre T, Li S, Strittmatter SM: Nogo-66 receptor antagonist peptide promotes axonal regeneration. Nature, 417: 547-551, 2002.

21) Bradbury EJ, Moon LD, Popat RJ et al: Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature, 416: 636-640, 2002.

22) Ellezam B, Dubreuil C, Winton M et al: Inactivation of intracellular Rho to stimulate axon growth and regeneration. Prog Brain Res, 137: 371-380, 2002.

23) Fournier AE, Takizawa BT, Strittmatter SM. Rho kinase inhibition enhances axonal regeneration in the injured CNS. J Neurosci, 23: 1416-1423, 2003.

24) Kaneko S, Iwanami A, Nakamura M, et al: Axonal Regeneration and Functional Recovery by Administration of Strong and Selective Semaphorin3A Inhibitor into the Injured Spinal Cord. Nat Med, 12: 1380-1389, 2006.

25) Ikegami T, Nakamura M, Yamane J, et al: Chondroitinase ABC combined with neural stem/progenitor cell transplantation enhances graft cell migration and outgrowth of GAP-43-positive fibers after rat spinal cord injury. Euro J Neurosci, 2: 3036-3046, 2005.

26) Fujiyoshi K, Yamada M, Nakamura M, et al: In vivo tracing of neural tracts in the intact and injured spinal cord of marmosets by diffusion tensor tractography. J Neurosci, 27: 11991-11998, 2007.

27) Takahashi K, Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126: 663-676. 2006.