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Short Review

Cell transplantation studies on the treatment of spinal cord injury using clinically relevant cells

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Abstract

Many different kinds of cells have been studied for transplantation in experimental animals including rats with spinal cord injury. This short review focused on adult somatic and umbilical cord cells to be used for therapy of spinal cord injury. Adult somatic cells have no inherent ethical problems in using for clinical application. Embryonic stem (ES) cells, neural stem cells, and induced pluripotent stem (iPS) cells were excluded. Bone marrow stromal cells and olfactory ensheathing cells have already been used clinically for transplantation to patients with spinal cord injury. Other cells dealt with in this review include dental pulp-derived, skin-derived, adipose-derived, and umbilical cord-derived stem cells, Muse cells, and choroid plexus epithelial cells.

Introduction

This short review focused on somatic and umbilical stem cell transplantation therapy for spinal cord injury (SCI). Adult somatic and umbilical stem cells have no inherent ethical problems. Some of them have already been applied for clinical use in patients with SCI, and others have the potential for clinical application in the near future. Embryonic stem (ES) cells and neural stem cells were extensively studied in previous decades. However, these cells were derived from embryonic cells or neural tissues of neo- or prenatal animals, possibly leading to ethical problems with their clinical application. Cell transplantation studies aiming at the repair and regeneration of host tissues are of significance only when the cells being studied are clinically applicable. This review focuses on the transplantation of cells that have no ethical or biological problems in clinical application. Induced pluripotent stem (iPS) cells have been studied as sources for cells of various organs and tissues in regenerative medicine. However, iPS cells have not been used as yet for clinical treatment.

Here, studies on treatment for SCI by the transplantation of cells listed as follows were reviewed:

1. Bone marrow stromal cell
2. Olfactory ensheathing cell
3. Dental pulp-derived stem cell
4. Skin-derived stem cell
5. Adipose-derived stem cell
6. Muse cell
7. Umbilical cord blood stem cell
8. Umbilical cord mesenchymal stem cell
9. Choroid plexus epithelial cell

1. Bone marrow stromal cell

Bone marrow stromal cells (BMSCs) are bone marrow-derived, adherent cells in culture. They are called bone marrow-derived mononuclear cells (BMMCs) before culture. BMSCs and BMMCs contain a small number of stem cells.

BMSCs have been extensively studied. Please refer to our recent study (Nakano et al., 2013) to read about the current trends in BMSC transplantation for SCI. Here, we would like to explain about BMSC transplantation based mainly on our own studies. BMSCs can be used for autologous transplantation. The transplantation of BMSCs is safe, with no ethical problems. The efficacy of BMSC transplantation for SCI has been established. The clinical application of BMSCs by our group is presently in Phase I/II.

BMSCs were transplanted via the cerebrospinal fluid (CSF) or directly into lesions of the spinal cord in our transplantation studies. The basic findings concerning spinal cord repair and locomotor recovery were compatible between the two transplantation techniques.

Before BMSC transplantation, the spinal cord of rats was crush-injured at the Th8-9 level by dropping a metal rod weighing 10 g from a height of 7.5 cm.

For acute SCI, BMSCs were transplanted via the CSF immediately after injury (Wu et al., 2003; Ohta et al., 2004). We examined cavity formation and the survival of axons in the host spinal cord lesion, and the BBB scores evaluating locomotor improvement. A small number of transplanted cells survived within the lesion, or on the surface of the spinal cord. However, they disappeared from the spinal cord at least 3 weeks after transplantation. Cavity formation was reduced, and axonal outgrowth in the spinal cord was promoted in BMSC transplantation. BBB scores were 10-14 in the BMSC-transplanted rats, and 5-10 in the control rats. This study was the first to demonstrate the effects of BMSC transplantation via the CSF. From the finding that transplanted BMSCs disappeared from the spinal cord within 2-3 weeks after transplantation, we hypothesized that BMSCs had effects not by integrating into host tissue, but by secreting some molecules into the CSF. There was no finding suggesting that BMSCs serve as a scaffold for the outgrowth of regenerating axons. It is suggested that mesenchymal BMSCs are not integrated into the nervous tissue.

Next, we examined the effect of BMSC transplantation on sub-acute SCI in rats (Ide et al. 2010). BMSCs were injected directly into the spinal cord lesion. Cell transplantation started 2 weeks after spinal cord injury (sub-acute phase). Although engrafted BMSCs did not survive for longer than 2 weeks after transplantation, BMSC transplantation was effective. It was notable that numerous regenerating axons extended in astrocyte-devoid areas in the lesion. Astrocyte-devoid areas are filled with tissues including axons, Schwann cells, and vessels. It appeared that astrocytes did not invade or spread into such connective-like tissues occupied with extracellular matrices. Axons were surrounded by Schwann cells, a finding indicating that regenerating axons are peripheral nerves in the normal state. Cavity formation was markedly reduced in the BMSC-transplanted spinal cord. BBB scores were approximately 10 points in rats with BMSC transplantation, and below 6 points in the control at 4 and 8 weeks after transplantation. These results show that the transplantation of BMSCs into lesions of subacute SCI promoted tissue repair, axonal regeneration, and locomotor improvement of rats.

Recently, we examined the effect of three injections of BMSCs on subacute and chronic spinal cord injury (Nakano et al. 2013). BMSCs were injected via the CSF (through the 4th ventricle) 1, 2, and 4 weeks after SCI. BMSCs were injected three times weekly for three weeks. In this study, extensive axonal outgrowth was observed throughout the astrocyte-devoid areas (Figs. 1, 2). Axons extended throughout the length of the astrocyte-devoid areas and further extended into the host spinal cord tissue. Regenerating axons showed no findings suggesting the blockade of extension at the border of the lesion. BBB scores were 9-11 points in cell transplantation groups, and 3-6 points in the control. We also found in this study that the CSF of rats that had been injected with BMSCs 2 days before harvesting had an effect on neuronal survival and neurite extension in vitro.

Based on these results, we examined the effect of BMSCs on clinical application. Firstly, we began clinical application in 5 patients in Kansai Medical University (Saito et al. 2012). Next, we performed the transplantation of BMMCs into 10 patients with SCI in Kitano Hospital (Suzuki et al. 2014). A basic study on the clinical application of BMMCs was published several years ago (Yoshihara et al. 2007). BMSCs and BMMCs have an advantage in that they can be transplanted autologously. So far, there have been no adverse effects reported on the clinical usage of BMSC or BMMCs.

2. Olfactory ensheathing cell

Olfactory ensheathing cells (OECs) are a type of Schwann cell associated with olfactory nerves extending from the olfactory epithelium to olfacto-

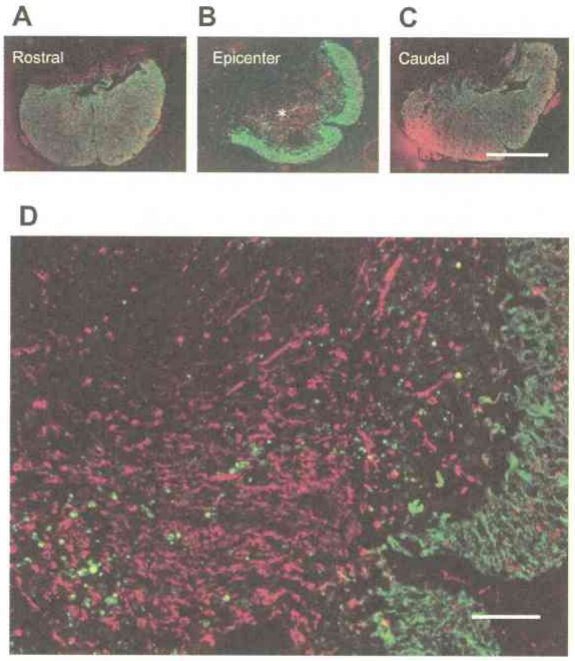

Figure 1 Spinal cord of a BMSC-transplanted rat. 2-w post-injury. Immunostaining for axons (red) and astrocytes (green). Transverse section. This rat had received BMSC injections starting at 2 weeks after contusion injury and was fixed 4 weeks following the initial cell injection. BBB points were 3 before BMSC injection and 9 at fixation.
The panels (A), (B), and (C) show the cross sections of the spinal cord 1-mm rostral to the epicenter, epicenter of the lesion, and 1-mm caudal to the epicenter, respectively. The spinal cord at the epicenter (B) has, on the right margin of the spinal cord, a part of the white matter (shown in green) remaining after injury, with numerous axons extending in the central area of the lesion. A part of the epicenter (*) in (B) is enlarged in (D), showing numerous axons and a small number of astrocytes remaining on the margin of the spinal cord. Scale: 1 mm for (A), (B), and (C); 125 μm for (D). From Nakano et al. PLoS One (2013)

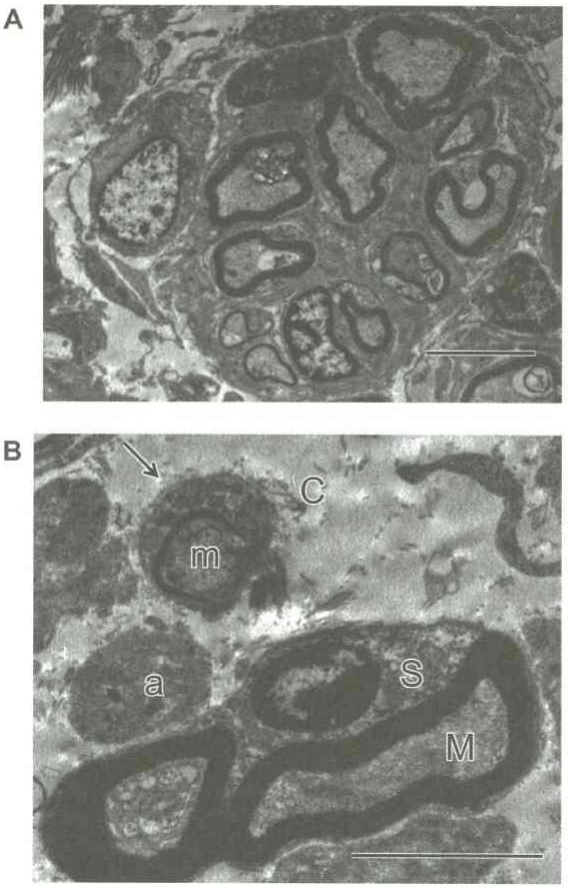

Figure 2 Electron microscopy of regenerating nerve fibers
These micrographs were obtained from the spinal cord epicenter of the rat that had received BMSC transplantations starting at 4 weeks after contusion injury and was fixed 4 weeks following the initial cell injection (4-w post-injury). BBB points were 4 before cell injection and 10 at fixation.
(A) Myelinated nerve fibers are grouped in a small fascicle, which is surrounded by a clear, cell-free space. Scale: 5 μm
(B) Part of the nerve fascicle is enlarged. There are thick-myelinated (M) and thin-myelinated (m) axons. The former is surrounded by a Schwann cell (S), and the latter is associated with a thin Schwann cell cytoplasm, the basal lamina of which is clearly seen (arrow). An unmyelinated fiber (a) is also seen near these myelinated axons. These nerve fibers are located in the space, through which collagen fibrils (C) are distributed, partly in association with Schwann cells. Scale: 5 μm. From Nakano et al. PLoS One (2013)

ry bulb. Olfactory cells are continuously supplied from basal cells of the olfactory epithelium: basal cells develop into olfactory cells, which, as neurons in nature, project regenerating axons through OECs to the olfactory bulb in adults. OECs surround bundles of axons in the same fashion as unmyelinated nerves. OECs guide those regenerating axons to enter the olfactory bulb in adults. OECs are characterized by this unique property.

Schwann cells in peripheral nerves have no such property; regenerating axons' growth is blocked at the transition from the peripheral to central nervous system, for example, regenerating sensory axons' growth from the periphery is interrupted at the entry zone of the dorsal root. OECs transplanted into the injured spinal cord are considered to promote outgrowth of regenerating nerves through the lesion into the host tissue in the spinal cord.

The autologous transplantation of OECs into patients with SCI is now under clinical trial.

The experimental transplantation of OECs was first conducted in 1994 (Ramon-Cueto and Nieto-Sampedro, 1994). They purified OECs from the olfactory bulb, and implanted them into the spinal cord of rats with rhizotomy of the dorsal root at T10. This study demonstrated that dorsal root axons extended through the PNS-CNS border into the spinal cord. This finding is important, as explained above. Since then, many studies have been conducted to examine the effectiveness of

OECs for SCI; a large number of studies reported the effective transplantation of OECs for SCI, while others presented unsupportive findings. Effective transplantation studies claim that OECs support the outgrowth of regenerating axons, and form a myelin sheath around naked axons. It appears that OECs do not migrate within the spinal cord after transplantation.

Li et al. (1998) reported successful results on transplantation into spinal cord lesions. They created unilateral electrolytic lesions of the corticospinal tract at C1-2, and transplanted OECs obtained from the olfactory bulb. They showed that corticospinal axons traversed the lesion site and reentered the caudal spinal cord. Regenerating axons were wrapped by a peripheral myelin sheath. Furthermore, they showed improvement of the forelimb function in a well-controlled system.

Lu et al. (2002) transplanted pieces of the lamina propria of the olfactory mucosa into a rat spinal cord, in which the spinal cord had been completely transected at Th10 4 weeks prior. The scar tissue was removed to create a 3-4-mm gap before transplantation. Ten weeks later, the locomotor activity of rats had significantly improved. Serotonergic axons regenerated and extended across the transplanted site to the host spinal cord tissue caudally. They concluded that the nasal olfactory lamina propria has the ability to promote spinal cord regeneration 4 weeks after complete transection.

Steward et al. (2006) recreated the experiment by Lu et al. (2002). This study was part of the NIH "Facilities of Research- Spinal Cord Injury" contract to support the independent replication of published studies. This replication study system has been established to formally examine whether the excellent results of clinically relevant studies can be replicated.

Steward et al. replicated the experiment by Lu et al. The hind limb motor function was estimated using the BBB scale. There was no significant recovery after olfactory lamina propria transplantation, and no significant difference between the OLP and RLP (respiratory lamina propria containing no olfactory ensheathing cells). Fluorogold, a neural tracer, was injected into the distal segment to retrogradely label proximally located neurons. There were no neurons labeled with Fluorogold in the rostral part of the spinal cord. Serotonergic axons were found within the transplants, but a few serotonergic axons were present in the sections caudal to the injury in 2 animals that received OLP transplant and 1 animal that received RLP transplant.

They concluded that, although OLP transplant may stimulate regeneration under some circumstances, the effect is not so robust. Similarly, Pearse et al. (2006) reported negative results. They transplanted purified olfactory ensheathing cells obtained from the olfactory bulb into the rat spinal cord at Th8 with moderate contusion injury. The results of their study were as follows: poor survival and no migration of OECs, and no axonal regeneration into the injury site. No major locomotor improvement was seen.

The effectiveness of the transplantation of OECs has been controversial. The survival, migration, and promotion of axonal regeneration of OECs after transplantation are major topics of debate. Myelination by transplanted OECs is another major problem to be addressed. Axons seen in the spinal cord lesion are usually myelinated by Schwann cells. In OEC transplantation experiments, it has yet to be determined whether such Schwann cells are derived from OECs or from other sources, such as nearby spinal root fibers.

In the study comparing the effects of OECs and BMSCs on spinal cord injury, both OECs and BMSCs disappeared from the spinal cord 2 weeks after transplantation, but provide tissue protection even in delayed transplantation. BMSCs improved the locomotor function. BMSCs seem to be a more favorable option than OECs for SCI treatment (Torres-Espin et al. 2014).

OECs have the ability to interact with astrocytes, remyelinate axons, and contribute to angiogenesis. Since they are able to interact with astrocytes, OECs can interact with scar tissue cells in the spinal cord (Franssen et al. 2007). This is an important difference from Schwann cells. Schwann cells and astrocytes are not able to be in direct contact with each other, because basal laminae are formed on both types of cell.

Raisman (2007) reported that grafted OECs encouraged the growth of cut nerve fibers, and that grafted cells adopt an elongated shape and align to form a bridge between the ends of the cut fiber tract. The regenerating nerve fibers enter the graft and cross the graft to re-enter the host spinal cord.

Clinical application

In a preclinical experiment, OECs from a single monkey olfactory bulb were cultured for 3 weeks, providing a sufficient number of OECs for autograft transplantation, indicating that human olfactory bulbs are reliable sources of OECs for cell transplantation (Rubio et al. 2008). Iwatsuki (2011)

reported experimental and clinical studies of OECs. Tabakow et al. (2014) conducted OEC transplantation for patients with complete SCI at the thoracic level. OECs were grafted into the lesions of the spinal cord. There were no adverse findings. Neurological improvement was observed in transplant patients. Two patients improved from ASIA A to ASIA B and C. The third patient, even though remained ASIA A, showed improved motor and sensory functions of the spinal cord segments below the level of the injury.

3. Dental pulp-derived stem cells

The dental pulp consists primarily of odontoblasts, fibroblasts, and nerves. Odontoblasts are arranged beneath the dentin, being involved in the production of tooth dentin. There are many capillaries with and around the dental pulp. In vitro experiments have shown that dental pulp cells differentiate into osteoblast, chondrocytes, and adipocytes (Gronthos et al. 2002; Balic et al. 2010). In addition, dental pulp cells have the capacity to differentiate into neurons (markers: MAP2, neurofilament, and βIII-tubulin), astrocytes (marker: GFAP), and oligodendrocytes (marker: CNPase). Neurospheres are produced on dental pulp cell culture.

Dental pulp-derived stem cells (DPSCs) produce neurospheres on non-adhesion culture in rats (Takayasu et al. 2006; Sasaki et al. 2008) and humans (Govindasamy et al. 2010; Arthur et al. 2008). DPSCs were first studied on transplantation in rats with SCI in 2012. Sakai et al. (2012) isolated and digested dental pulp cells from human dental pulp tissues to obtain single cell suspensions. From these cell suspensions, DPSCs with mesenchymal stem cell markers such as CD90, CD73, and CD105 were separated and harvested by flow cytometry. Cells with endothelial/hematopoietic markers such as CD34, CD45, CD11b/c, and HLA-DR were excluded. The rat spinal cord was completely transected with a surgical blade at the Th9-11 level, and DPSCs were transplanted at the site 2 mm from the lesion in the rostral and caudal stump using a Hamilton syringe. In addition, DPSCs in fibrin glue were implanted into the transection gap to fill the lesion in the severed spinal cord. Rats were given cyclosporine every day after surgery to prevent immunorejection of the transplanted DPSCs. There was marked locomotor recovery in cell-transplanted rats. DPSCs regenerated the transected corticospinal tract and raphespinal serotonergic axons. In vitro, conditioned medium of cultured DPSCs promoted neurite extension activity under the severe condition of CSPG (chondroitin sulfate proteoglycan) or MAG (myelin-associated glycoprotein)-mediated neurite growth inhibition. In this study, the authors emphasized that DPSCs exhibit three major neuroregenerative activities. Firstly, they inhibit the SCI-induced apoptosis of neurons, astrocytes, and oligodendrocytes. Secondly, they promote the regeneration of transected axons by directly inhibiting multiple axon growth inhibitors, including CSPG and MAG, via paracrine mechanisms. Thirdly, they differentiate into oligodendrocytes in the severed spinal cord. The differentiation of DPSCs into neurons has not been identified in the spinal cord.

From these results, they concluded that DPSCs may provide therapeutic benefits for treating SCI through both cell-autonomous and paracrine neuroregenerative activities. Sugiyama et al. (2011) suggest that the recovery effects of DPSC transplantation are mediated through the release of trophic factors from DPSCs. In line with this, Arthur et al. (2009) suggest the release of CXCL12 chemokine from transplanted DPSCs, based on the finding that axons grow toward DPSCs implanted into the hindbrain of chicken embryos. Young et al. (2013) reviewed the potential roles of DPSCs in CNS regeneration and repair.

4. Skin-derived stem (precursor) cells

Keratinocytes of the epidermis are continuously supplied from the basal layer where new epidermal cells are produced by the mitosis of basal cells in adults. In addition, a hair follicle, involving the anagen-catagen cycle of the hair shaft is a site of active cell proliferation. The hair follicle has two sites with an abundance of immature cells: the dermal papilla at the bottom of the hair follicle, and hair bulge situated at the level of the sebaceous gland.

Hunt et al. (2008) demonstrated the presence of stem cells in the dermal papillae of adult skin. On development, skin-derived stem cells are readily obtained from the human neonatal fore skin. This means that stem cells are not restricted to hair follicles, but distributed throughout the skin during development and growth. However, stem cells become limited to hair follicles in adults. Skin-derived stem cells are of neural crest origin. Mice in which neural crest cells with the Wnt-1 gene are genetically engineered (Wnt1-Cre/R26R) to express β-galactosidase, were used for the visualization of neural crest cells. Dermal papillae show an intense reaction for β-

galactosidase, indicating that neural crest cells reside in the dermal papillae. Some neural crest cells are found at other sites of both pelage and whisker hair follicles. Dermal papillae are enriched with sphere-forming cells. Cell spheres (papillasheres) are formed from dissociated dermal papilla cells in floating culture. Papillaspheres contain neural crest transcription factors such as Pax3 and Slug. Immunohistochemistry demonstrated that papillaspheres are composed of nestin$^+$ and fibronection$^+$ immunoreactive cells.

Papillaspheres generate neuronal cells by treatment with neural differentiation medium containing neurotrophic factors (NT3, BDNF, and NGF), bFGF, and retinoic acid. Treatment with forskolin and neuregulin produced Schwann cells.

Papillaspheres can be generated from human facial skin dermal papillae.

Human papillaspheres differentiate into neuronal and Schwann cells. For neuronal differentiation, papillaspheres are cultured in the medium containing neurotrophic factors and retinoic acid, as in the case of rat papillapheres. Human papillaspheres also produce Schwann cells in medium containing forskolin and neuroregulin.

Liu et al. (2011) showed that bulge areas of the mouse vibrissal follicle contain nestin-expressing cells. Cell spheres are formed from both bulges and dermal papillae of mouse vibrissal hair follicles. However, cells from the bulge showed higher sphere-forming efficiency than those from the dermal papillae. These nestin-expressing cells differentiate into Tujl- (neuronal cell) or GFAP-expressing (astrocyte) cells in medium lacking bFGF and EGF.

In an experiment in which spheres plus Gelfoam were transplanted into the spinal cords of nude mice, nestin-expressing sphere cells survived for over 100 days post-surgery. Cells from both the bulge area and dermal papillae migrated toward adjacent spinal cord segments. Axons grew into the transplant in the lesion. Seven to 8 weeks after transplantation, transplants showed Tuj-1-, and GFAP-positive immunoreactions, indicating that some fractions of transplanted cells differentiated into neurons and astrocytes in the injured spinal cord.

Sieber-Blum et al. (2006) studied the effect of the transplantation of epidermal neural crest stem cells into a lesioned spinal cord. Epidermal neural crest cells are derived from the bulge area of a hair follicle. They demonstrated that bulge cells have the ability to differentiate into all major neural crest derivatives (Sieber-Blum et al. 2004). Transplanted cells survived in the spinal cord lesion. They did not migrate or proliferate. There was no tumor formation. Many cells demonstrated neuronal and glial markers in the spinal cord. Neuronal markers include βIII tubulin and GAD67 (glutamate decarboxylase 67), and glial markers include RIP and MBP (myelin basic protein). There were no GFAP-immunoreactive cells in the transplants.

These data indicate that cells derived from hair follicle bulges differentiate into neurons and oligodendrocytes after transplantation into an injured spinal cord.

5. Adipose-derived stem (mesenchymal) cell

Embryonic fetal stem cells (ES cells) and neural stem cells have drawn attention due to their high pluripotency and viability; however, they are associated with ethical, regulatory, and availability concerns regarding clinical application. Adult adipose-derived stem cells have no ethical problem. Adipose-derived stem cells can be harvested from subcutaneous fat tissue, presenting no limitation in terms of availability for clinical use. Bone marrow-derived stem cells are another type of mesenchymal stem cell. Mesenchymal stem cells are often called stromal cells (adipose-derived stromal cells or bone marrow-derived stromal cells).

Adipose tissue is a useful source of stem cells, since it is abundant in the subcutaneous zone, and can vigorously proliferate in vitro. Rhu et al. (2009) reported the isolation, culture, and characterization of adipose stem cells, and their transplantation into a canine model of acute SCI. The description below is based on their report:

Adipose tissue is collected from subcutaneous fat. Fat tissues are minced, digested with collagenase, and centrifuged. Pellets of the cellular fraction are re-suspended, filtered through nylon mesh, and incubated in culture medium (DMEM) containing 10% fetal calf serum. The non-adherent cells are removed, and cells adherent to the dish are further cultured in the medium supplemented with epidermal growth factor (EGF), bovine pituitary extract, N-acetyl-L-cysteine, ascorbic acid, insulin, and hydrocortisone. These cells are characterized by CD 44(+), CD 90(+), CD105(+), CD14(−), CD34(−), and CD45(−). These adipose-derived stem cells can differentiate into adipocyte, chondrocyte, myocyte, osteoblast, and neural lineages. For neurogenic differentiation, isolated adipose stem cells are preincubated in DMEM low-glucose medium supplemented

with FBS and mercaptoethanol. After preincubation, the cells are incubated in neurogenic medium composed of DMEM supplemented with butylated hydroxyanisol and DMSO. For the differentiation of other cell types, culture media specific for each cell type are used. A dog spinal cord was compression-injured at L1 by applying an epidural balloon catheter for 12 hours. A cell suspension containing 1×10^6 of cells in 150 μL of PBS was injected into the lesion at 3 sites (epicenter, and rostral and caudal borders of the lesion). Cells were transplanted 1 week after SCI. Dogs showed significant behavioral improvement at 4-8 weeks after transplantation. Eight weeks after cell transplantation, immunohistochemistry showed that engrafted adipose-derived stem cells differentiated into astrocytes (marker: GFAP), neurons (Tuj-1), and oligodendrocytes (MAB 5540). This study indicates that engrafted cells that survived in the spinal cord and differentiated into neuronal cells, promoted the behavioral as well as histological recovery of injured spinal cord. On the other hand, cytoplasmic extracts of adipose tissue stromal cells have effects on enhancement of survival of cultured spinal cord-derived neural progenitor cells.

Arboleda et al. (2011) studied the involvement of predifferentiated adipose-derived stromal cells (pASCs), comparing it with that of naïve adipose-derived stromal cells (ASCs). Adipose tissue was harvested from inguinal pads of GFP-transgenic SD rats. Cultured ASCs were plated in 10 cm^2 Petri dishes and induced to form spheres by replacing the proliferation medium with sphere-induction medium consisting of DMEM/F12 (+Glutamax), B27 supplement, EGF, and bFGF. After 4 days of culture, the formed cell spheres were collected, plated on laminin-coated dishes, and then placed for 6 days in differentiation medium consisting of Neurobasal media supplemented with B27, NGF, and bFGF.

A total of 5×10^5 cells suspended in 5 μL of PBS were injected into the rostral, central, and caudal parts of the SCI lesion of Wistar rats. All rats were immunosuppressed by cyclosporine injection throughout the experiment.

Both types of transplanted cell were detected in the host spinal cord at 8 weeks after transplantation. ASCs did not exhibit morphological changes from those seen in culture before transplantation. There was no finding suggesting interactions of transplanted cells with host neuronal cells. On the other hand, pASCs extensively migrated and showed interaction with axons. Although they resemble Schwann cells or oligodendrocytes associated with axons in appearance, electron microscopy showed that they are perineurial cells surrounding nerve fibers. pASCs or ASCs did not exhibit any neuronal markers suggesting differentiation into neurons, astrocytes, or oligodendrocytes. The fact that functional improvements were comparable in both groups indicates that spinal cord repair is induced mainly through paracrine mechanisms.

Kingham et al. (2014) stimulated ASCs in medium containing β mercaptoethanol and retinoic acid for 24 hours, and then in medium containing neuregulin 1, bFGF, PDGF, and forskolin for 72 h. Stimulated ASCs secrete an increased amount of BDNF, GDNF, VEGF and angiopoietin-1.

Both ASCs and pASCs have common CD antigens such as CD29, CD 90, CD 105, and CD13. They are negative for CD 11b, CD19, CD 34,and CD 45 (Tapp et al. 2009).

Although adipose-derived stem cells have been reported to survive for several weeks after transplantation, there has been no definite finding suggesting the close interaction of engrafted adipose-derived stem cells with the host spinal cord tissue. Several studies have indicated that ASCs have paracrine effects by secreting many trophic factors and cytokines. This characteristic is the same as that of BMSCs. It has been reported that ASCs secrete granulocyte macrophage-colony stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), brain derived neurotrophic factor (BDNF), and insulin-like growth factor-1 (IGF-1). Wei et al. (2009) demonstrated that the conditioned medium of ASCs can protect against apoptosis induced by serum and potassium deprivation. This effect is mainly modulated by IGF-1

6. Muse cells

Muse (multilineage-differentiating stress-enduring) cells are stem cells derived from mesenchymal populations such as dermal and bone marrow tissues (Kuroda et al. 2013). The first step is to culture mesenchymal populations from BMSCs or dermal fibroblasts. The second step is to isolate Muse cells by FACS. Muse cells are immunolabeled for stage-specific embryonic antigen-3 (SSEA-3), and isolated by FACS. These Muse cells form cell spheres in suspension culture. Spheres contains cells positive for alkaline phosphatase, and pluripotency markers, including Sox2, Oct3/4 and Nanog. These cells can undergo

triploblastic differentiation (mesodermal, endodermal, and ectodermal lineages). They actually differentiate into osteocytes, adipocytes, hepatocytes, and neural cells. It has been suggested that Muse cells are a primary source of induced pluripotent stem cell in human fibroblasts (Wakao et al. 2011). A transplantation study using Muse cells is now in progress.

7. Umbilical cord blood-derived stem cells

It is known that human umbilical cord blood contains stem cells (UCBSCs).

Dasari et al. (2007) showed, based on study of a transplantation of UCBSCs into spinal cord-injured rats, that UCBSCs transdifferentiated into neurons and oligodendrocytes. Remyelination occurred on the transplantation of UCBSCs into shiverer mice (Veeravalli et al. 2011). The question of whether the transplanted UCBSCs can differentiate into neuronal cells after transplantation is a critical problem. The findings of previous studies should be carefully re-examined in experiments. On the other hand, UCBSCs have a neuroprotective potential to protect against neuronal apoptosis in the injured spinal cord. UCBSCs have the ability to secrete many kinds of trophic factor such as IL-10, GDNF, and VEGF.

The latest study (Cui et al. 2014) on the transplantation of UBSCs into SCI rats reported the behavioral improvement of rats as estimated by BBB scores, and histological repair. In this study, 1.0×10^7 human UBSCs were injected into spinal cord lesions, and behaviors of rats were observed 1, 2, and 4 weeks after transplantation. BBB scores were higher in the cell-transplanted group than the control group. The authors stated that transplanted UBSCs differentiated into neurons and astrocytes in the spinal cord lesions based on immunohistochemical images of neurons and astrocytes within the spinal cord. However, these images do not provide evidence for the engrafted UBSCs differentiating into neuronal cells. It is probable that UBSCs have neurotrophic effects on the histological recovery of spinal cord injury.

Allogenic transplantation is a problem inherent to UCBSCs. This problem should be addressed before the clinical application of UCBSCs.

8. Umbilical cord matrix (Wharton's jelly) cells

Umbilical cord mesenchymal stem cells (UMSCs) are obtained from the umbilical cord. Mesenchymal cells in the umbilical cord are considered developmentally primitive. Therefore, it is reasonable to use these mesenchymal cells for transplantation into SCI. They cannot be used for autologous transplantation.

Yang et al. (2008) cultured mesenchymal stem cells from human Wharton's jelly, and transplanted then into the transection-injured spinal cord of rats. BBB scores were elevated. Axons regenerated into the lesion. Engrafted human UMSCs survived for 16 weeks. It has been reported that, in the CNS, allogeneic transplants can survive for a long time without immunosuppressants. In addition, they secrete neurotrophic factors such as NT-3 and bFGF.

9. Choroid plexus epithelial cells

Choroid plexus epithelial cells (CPECs) are a type of glial cell, and they produce cerebrospinal fluid (CSF) at the blood-CSF barrier. Ide et al. (2001) first demonstrated that transplantation of minced choroid plexus tissues enhanced axonal outgrowth in the injured spinal cord of rats. Thereafter, CPECs have been studied from the point of view of cell transplantation. CPECs contain stem cells that differentiate to neurons, astrocytes, and oligodendrocytes (Kitada et al. 2001; Itokazu et al. 2006). Furthermore, the transplantation of cultured CPECs via the CSF can rescue ischemic tissues in the rat brain to which blood supply from the middle cerebral artery has been interrupted (Matsumoto et al. 2010). The transplantation of CPRCs through the CSF revealed that CPECs were not integrated into the host CNS after grafting, suggesting that CPECs secrete trophic factors effective for rescuing ischemic cerebral tissues. This has been supported by in vitro studies (Kimura et al. 2004; Chakrabortty et al. 2005; Watanabe et al. 2005). From this standpoint, viable CPECs are transplanted in an immunoprotected, encapsulated form directly into the site of injury (Skinner et al. 2006). Futhermore, CPECs have been proposed to prmote the neuroprotection in Huntington's disease (Emerich and Borlongan. 2009). It has been reported that cell proliferation and differentiation occurred in choroid plexus epithelium in response to stroke in rats (Li et al, 2002). CPECs undergo increases of IL-1β, NTF-α, and 70 in response to acute spinal cord injury in dogs (Moore and Oglesbee. 2012). These studies indicate that CPECs may play an important role in regulating and recovering normal functions following CNS injury.

References:

Arboleda D, Forostyak S, Jendelova P, Marekova D, Amemori T, Pivonkova H, et al.: Transplantation of predifferentiated adipose-derived stromal cells for the treatment of spinal cord injury. Cell Mol Neurobiol 31: 1113-1122, 2011

Arthur A, Rychkov G, Shi S, Koblar SA, Gronthose S: Adult human dental pulp cells differentiate toward functionally active neurons under appropriate environmental cues. Stem cells 26: 1787-1795, 2008

Arthur A, Shi S, Zannettino AC, Fujii N, Gronthos S, Koblar SA: Implanted adult human dental pulp stem cells induce endogenous axon guidance. Stem Cells. 27: 2229-2237, 2009

Balic A, Aguila HL, Caimano MJ, Francone VP, Mina M: Characterization of stem and progenitor cells in the dental pulp erupted and unerupted murine molars. Bone 46: 1639-1651, 2010

Chakrabortty Sl, Kitada M, Matsumoto N, Taketomi M, Kimura K, Ide C: Choroid plexus ependymal cells enhance neurite outgrowth from dorsal root ganglion neurons in vitro. J Neurocytol. 29: 707-17, 2000

Cui B, Li E, Yang B, Wang B: Human umbilical cord blood-derived mesenchymal stem cells transplantation for the treatment of spinal cord injury. Exp Ther Med 7: 1233-1236, 2014

Dasari VR, Spomar DG, Cady C, Gujrati M, Rao JS, Dinh DH: Mesenchymal stem cells from rat bone marrow down-regulate casase-3-mediated apoptotic pathway after spinal cord injury in rats. Neurochem Res. 32: 2087-2093, 2007

Emerich DF, Borlongan CV, Kingham PJ, Kolar MK, Novikova N, Novikov LN et al.: Potential of choroid plexus epithelial cell grafts for neuroprotection in Huntington's disease: what remains before considering clinical trials. Neurotox Res. 15: 205-211, 2009

Franssen EH, De Bree FM, Verhaagen J: Olfactory ensheathing glia: their contribution to primary olfactory nervous system regeneration and their regenerative potential following transplantation into the injured spinal cord. Brain Res Rev 56: 236-258, 2007

Govindasamy V, Abdullah AN, Ronald VS, Musa S, Che AB, Aziz ZA et al.: Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. J Endodont 36: 1504-1515, 2010,

Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A et al.: Stem cell properties of human dental pulp stem cells. J Dent Res 81: 531-535, 2002

Hunt DPJ, Morris PN, Sterling J, Anderson JA, Joannides A, Johoda C et al.: A highly enriched niche of precursor cells with neuronal and glial potential within the hair follicle dermal papilla of adult skin. Stem cells 26: 163-172, 2008

Ide C, Kitada M, Chakrabortty S, Taketomi M, Matsumoto N, Kikukawa S et al.: Grafting of choroid plexus ependymal cells promotes the growth of regenerating axons in the dorsal funiculus of rat spinal cord: a preliminary report. Exp Neurol 167: 242-251, 2001

Ide C, Nakai Y, Nakano N, Seo T-B, Yamada Y, Endo K et al.: Bone marrow stromal cell transplantation for treatment of sub-acute spinal cord injury in the rat. Brain Res 1332: 32-47, 2010

Itokazu Y1, Kitada M, Dezawa M, Mizoguchi A, Matsumoto N, Ide C et al.: Choroid plexus ependymal cells host neural progenitor cells in the rat. Glia 53: 32-42, 2006

Iwatsuki K: Olfacotry mucosa transplantation for spinal cord injury. J Stem Cells Med. 7: 92-, 2011

Kimura K, Matsumoto N, Kitada M, Mizoguchi A, Ide C: Neurite outgrowth from hippocampal neurons is promoted by choroid plexus ependymal cells in vitro. J Neurocytol. 33: 465-476, 2004

Kingham PJ, Kolar MK, Novikova N, Novikov LN, Wiberg M: Stimulating the neurotrophic angiogenic properties of human adipose-derived stem cells enhances nerve repair. Stem Cell Dev 23: 741-754, 2014

Kitada M, Chakrabortty S, Matsumoto N, Taketomi M, Ide C: Differentiation of choroid plexus ependymal cells into astrocytes after grafting into the pre-lesioned spinal cord in mice. Glia 36: 364-374, 2001

Kuroda Y, Wakao S, Kitada M, Murakami T, Nojima M, Dezawa M: Isolation, culture and evaluation of multilneage-differentiating stress-enduring (Muse) cells. Nature Protcol 8: 1391-1415, 2013

Li Y, Chen J, Chopp M: Cell proliferation and differentiation from ependymal, subependymal and choriod plexus cells response to stroke in rats. J Neurol Sci 193: 137-146, 2002

Li Y, Field PM, Raisman G: Regeneration of adult rat corticospinal axons induced by transplanted olfactory ensheathing cells. Neurosci 18: 10518-10523, 1998

Liu F, Uchugonova A, Kimura H, Zhang C, Zhao M, Zhang L et al.: The bulge area is the major hair follicle source of nestin-expressing pluripotent stem cells which can repair the spinal cord compared to the dermal papilla. Cell Cycle 10: 830-839, 2011

Lu J, Feron F, Mackay-Sim A, Waite PM: Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. Brain 125: 14-21, 2002

Matsumoto N, Taguchi A, Kitayama H, Watanabe Y, Ohta M, Ide C et al.: Transplantation of cultured choroid plexus epithelial cells via cerebrospinal fluid shows prominent neuroprotective effects against acute ischemic brain injury in the rat. Neurosci Lett 469: 283-288, 2010

Moore SA, Oglesbee MJ: Involvement of the choroid plexus in the inflammatory response after acute spinal cord injury in dogs: an immunohistochemical study. Vet Immunol Immunopathol 148: 348-352, 2012

Nakano N, Nakai Y, Seo T-B, Yamada Y, Ohno T, Ide C et al.: Characterization of conditioned medium of cultured bone marrow stromal cells. Neurosci Lett 483: 57-61, 2010

Nakano N, Nakai Y, Seo T. B, Homma T, YamadaY, Ide C et al.: Effects of bone marrow stromal cell transplantation through CSF on the subacute and chronic spinal cord injury in rats. PLoS ONE 8(9): e73494. doi: 10.137/journal.pone.0073494, 2013

Ohta M, Suzuki Y, Noda T, Ejiri Y, Dezawa M, Ide C et al.: Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. Exp Neurol 187: 266-278, 2004

Pearse DD, Sanchez AR, Pereira FC, Andrade CM, Puzis R, Pressman Y et al.: Transplantation of Schwann cells and/or olfactory ensheathing glia into the contused spinal cord: survival, migration, axon association, and functional recovery. Glia 55: 976-1000, 2007

Raisman G: Repair of spinal cord injury by transplantation of olfactory ensheathing cells. C. R. Biologies 330: 557-560, 2007

Ramon-Cueto A, Niero-Sampedro M: Regeneration into the spinal cord of transected dorsal root axons is pro-

moted by ensheathing glia transplants. Exp Neurol 127: 232-244, 1994

Rhu HH, Lim JH, Byeon TE, Park JR, Seo MS, Lee YW et al.: Functional recovery and neural differentiation after transplantation of allogenic adipose-derived stem cells in a canine model of acute spinal cord injury. J Vet Sci 10: 273-284, 2009

Rubio MP, Munoz-Quiles C, Ramon-Cueto A: Adult olfactory bulbs from primates provide reliable ensheathing glia for cell therapy. Glia 56: 539-551, 2008

Saito F, Nakatani T, Iwase I, Maeda M, Murao M, Ide C et al.: Administration of cultured autologous bone marrow stromal cells into cerebrospinal fluid in spinal injury patients: A pilot study. Rest Neurol Neurosci Res 30: 127-136, 2012

Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T et al.: Neurosphere generation from dental pulp of adult rat incisor. Eur J Neurosci 27: 538-548, 2008

Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M et al.: Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest 122: 80-90, 2012

Sieber-Blum M, Grim M, Hu YF, Szeder V: Pluripotent neural crest stem cells in the adult hair follicles. Dev Dyn 231: 258-269 l, 2004

Sieber-Blum M, Schenll L, Grim M, Hu TF, Schneider R, Schwab ME: Characterization of epidermal neural crest stem cell (EPI-NCNC) grafts in the lesioned spinal cord. Mol Cell Neurosci 32: 67-81, 2006

Skinner SJM, Geaney MS, Rush R, Rogers NM-L, Emerich DF, Thanos CG et al.: Choriod plexus transplants in the treatment of brain disease. Xenotransplantation 13: 284-288, 2006

Steward O, Sharp K, Selvan G, Hadden A, Hofstadter M, Au E et al.: A re-assessment of the consequences of delayed transplantation of olfactory lamina propria following complete spinal cord transection in rats. Exp Neurol 198: 483-499, 2006

Sugiyama M, Iohara K, Wakita H, Hattori H, Ueda M, Matsushita K et al.: Dental pulp-derived $CD31^-$ / $CD146^-$ side population stem /progenitor cells enhance recovery of focal cerebral ischemia in rats. Tissue Eng Part A. 17: 1303-1311, 2011

Suzuki Y, Ishikawa N, Omae K, Hirai T, Ohnishi K, Ide C et al.: Bone marrow-derived mononuclear cell transplantation in spinal cord injury patients by lumbar puncture. Restor Neurol Neurosci. Mar 26. [Epub ahead of print], 2014

Tabakow P, Jarmundowicz W, Czapiga B, Fortuna W, Miedzybrodzki R, Czyz M et al.: Transplantation of autologous olfactory ensheathing cells in complete human spinal cord injury. Cell Transp 22: 1591-1612, 2013

Takayasu M, Nozaki T, Daito M: Differentiatio of dental pulp stem cells into a neuronal lineage. Peditr Dent J 16: 154-162, 2006

Tapp H, Hanley EN Jr, Patt JC, Gruber HE: Adipose-derived stem cells: characterization and current application in orthopaedic tissue repair. Exp Biol Med 234: 1-9, 2009

Torres-Espin A, Redondo-Castro E, Hernandez J, Navarro X: Bone marrow mesenchymal stromal cells and olfactory ensheathing cells transplantation after spinal cord injury — a morphological and functional comparison in rats. Europ J Neurosci 39: 1704-1717, 2014

Veeravalli KK, Dasari VR, Fassett D, Dinh DH, Rao JS: Human umbilical cord blood-derived mesenchymal stem cells upregulate myelin basic protein in sheverer mice. Stem Cells Dev 20: 881-891, 2011

Wakao S, Kitada M, Kuroda Y, Shigemoto T, Matsuse D, Akashi H et al.: Multilineage-differentiting stress-renduring) Muse) cells are orimary soure of pluripotent stem cells in human fibroblasts. PNAS 108: 9875-9880, 2011

Watanabe Y, Matsumoto N, Dezawa M, Itokazu Y, Yoshihara T, Ide C: Conditioned medium of the primary culture of rat choroid plexus epithelial (modified ependymal) cells enhances neurite outgrowth and survival of hippocampal neurons. Neurosci Lett. 379: 158-63, 2005

Wei X, Zhao L, Zhong J, Gu H, Feng D, Johnstone BH et al.: Adipose stromal cell-secreted neuroprotective media against neuronal apoptosis. Neurosci Lett 462: 76-79, 2009

Wu SF, Suzuki Y, Noda T, Bai H, Kitada M, Ide C et al.: Bone marrow stromal cells enhance differentiation of co-cultured neurosphere cells and promote regeneration of injured spinal cord. J Neurosci Res 72: 343-351, 2003

Yang CC, Shih YH, Ko MH, Hsu AY, Cheng H, Fu YS: Transplantation of human umbilical mesenchymal stem cells from Wharton's jellyafter completetransection of the rat spinal cord. PLoS ONE 3: e3336 PMID: 18852872 doi: 10.1371/journal.pone 0003336, 2008

Yoshihara T, Ohta M, Itokazu Y, Matsumoto N, Dezawa M, Ide C et al.: Neuroprotective effect of bone marrow-derived mononuclear cells promoting functional recovery from spinal cord injury. J Neurotrauma 24: 1026-1036, 2007

Young F, Sloan A, Song B: Dental pulp stem cells and their potential roles in central nervous system regeneration and repair. J Neurosci Res. 91: 1383-1393, 2013