

Mature Adipocyte-Derived Cells, Dedifferentiated Fat Cells (DFAT), Promoted Functional Recovery From Spinal Cord Injury-Induced Motor Dysfunction in Rats

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Transplantation of mature adipocyte-derived cells (dedifferentiated fat cells) led to marked functional recovery from spinal cord injury (SCI)-induced motor dysfunction in rats. When mature adipocytes were isolated from rat adipose tissue and grown in ceiling culture, transformation into fibroblast-like cells without lipid droplets occurred. These fibroblast-like cells, termed dedifferentiated fat cells (DFAT), could proliferate and could also differentiate back into adipocytes. DFAT expressed neural markers such as nestin, β III tubulin, and GFAP. Allografting of DFAT into SCI-induced rats led to significant recovery from hindlimb dysfunction. Grafted cells were detected at the injection site, and some of these cells expressed β III tubulin. DFAT expressed neurotrophic factors such as BDNF and GDNF prior to transplantation, and grafted cells were also positive for these factors. Therefore, these neurotrophic factors derived from grafted DFAT might have contributed to the promotion of functional recovery. These findings also suggest that mature adipocytes could become a new source for cell replacement therapy to treat central nervous system disorders.

Key words: Mature adipocytes; Dedifferentiated fat cells (DFAT); Spinal cord injury; Transplantation; Neurotrophic factor

INTRODUCTION

Unfortunately, there are few effective treatments for neurodegenerative diseases affecting the central nervous system, including spinal cord injury (SCI). Cell replacement therapy is currently attracting attention as a potential strategy for the treatment of such conditions.

Embryonic stem (ES) cells have various advantages for transplantation because these cells can proliferate and differentiate into multiple lineages. Numerous researchers have demonstrated that ES-derived neuronal stem cells or neuroprogenitor cells can promote significant recovery from SCI-induced motor dysfunction in animal models (14,21,25,26). In order to apply this method clinically, however, several problems need to be overcome, including ethical issues and the risk of teratoma (11,23,38,43).

On the other hand, multipotential adult stem cells

also exist as another source of stem cells. In addition to endogenous neuronal stem cells, bone marrow mesenchymal stem/stromal cells (MSCs) and adipose-derived stem/stromal cells (ASCs) have been shown to differentiate into neuronal cells both in vitro (1,6,10,12,17,30,31,41,45) and in vivo (2,15,18,22). Therefore, MSCs and ASCs have become very attractive targets for the study of tissue regeneration because of their plasticity.

As a possible new donor cell source, we focused our attention on mature adipocytes. These cells can be obtained from adipose tissue as well as ASCs, and represent an autologous tissue with a relatively high availability that can be harvested with little discomfort. Mature adipocytes can be easily separated as a unicellular fraction, unlike ASCs that also contain fibroblasts, endothelial cells, and a high percentage of blood cells (27).

Mature adipocytes that contain unilocular lipid droplets have been previously reported to undergo a change

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of morphology during ceiling culture, becoming multicellular cells and finally fibroblast-like cells without lipid droplets (29,32,34,35,39,42). Planat-Benard et al. have already demonstrated that fibroblast-like cells derived from mature adipocytes have the potential to differentiate into endothelial cells (29). Kano et al. have called these fibroblast-like cells dedifferentiated fat cells (DFAT), and have shown that such cells have the potential for differentiation into other types of cells such as osteoblasts, chondrocytes, myoblasts, and endothelial cells (19). We hypothesized that mature adipocyte-derived DFAT could be useful as donor cells for the repair of SCI, as has already been reported for MSCs (13). To confirm this hypothesis, we examined the characteristics of DFAT, the effect of DFAT transplantation on rats with SCI, and the influence of the grafted cells on functional recovery.

MATERIALS AND METHODS

Animals

Sprague-Dawley (SD) rats (Charles River Laboratories Japan, Kanagawa, Japan), Wistar rats (Japan SLC, Inc., Shizuoka, Japan), and green fluorescent protein (GFP) transgenic rats (Crj: Wistar, CAG/EGFP, The YS Institute, Inc., Tochigi, Japan) were housed in an animal room at a constant temperature ($23 \pm 1^\circ\text{C}$) and humidity (50–60%) with a 12-h light/dark cycle, and were allowed free access to a standard diet and water. Experimental procedures were done in accordance with the St. Marianna University guidelines for the welfare of animals.

Cell Culture

Primary rat adipocytes were isolated according to the method of Yagi et al. (42). In brief, dorsal fat pads (0.5 g) were obtained from 5-week-old male or female rats under anesthesia. The adipose tissue was minced and then digested for 1 h in 0.1% collagenase (Invitrogen, Carlsbad, CA) at 37°C . After filtration and centrifugation at 1000 rpm for 5 min, the floating adipocyte fraction was obtained as the top layer in the tube. Then these cells (10^5 cells) were placed into a 25-cm² culture flask completely filled with Dulbecco's modified Eagle's medium (DMEM; Invitrogen) containing 10% fetal bovine

serum (FBS; MP Biomedicals, Aurora, OH). During culture, cells attached to the ceiling of the flask and began to proliferate. About 10 days later, the flask was turned upside down so that the cells could be cultured on the base. The growth medium [DMEM containing 20% FBS, 1% antibiotic-antimycotic (Invitrogen)] was changed every 4 days. Confluent cells (about 3×10^5) were detached with 0.25% trypsin/1 mM EDTA (Invitrogen) and cultured in 75-cm² flasks (passage 1). DFAT (passage 2) were used for all experiments, except that shown in Figure 1.

Lipid droplets in the adipocytes were visualized by staining with AdipoRedTM assay reagent (Cambrex Bio Science Walkersville, East Rutherford, NJ). Hoechst 33342 (Dojindo, Kumamoto, Japan) was used for the detection of nuclei.

Differentiation into adipocytes was performed by a modification of the previously described method (28,33). DFAT were grown to confluence and then were incubated in adipogenic induction medium [a 3:1 mixture of DMEM and Ham's F-12 medium (Invitrogen) containing 10% FBS, 1% antibiotic-antimycotic, 5 $\mu\text{g}/\text{ml}$ insulin (Invitrogen), 5 $\mu\text{g}/\text{ml}$ transferrin (Invitrogen), 0.25 μM dexamethasone (DEX; MP Biomedicals), and 500 μM 3-isobutyl-1-methylxanthine (IBMX; Wako, Osaka, Japan)] for 3 days, followed by culture in maintenance medium (induction medium without DEX and IBMX) for several days. After this procedure was repeated three times, the cells were cultured in maintenance medium for 7 days.

Analysis of mRNA Expression by Real-Time Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA (about 20 μg) was extracted from DFAT (1×10^6 cells) by using TRIzol[®] reagent (Invitrogen). After reverse transcription of 1 μg of total RNA, an aliquot of the cDNA thus obtained was amplified with a LightCycler system (Roche, Penzberg, Germany) by using LightCycler FastStart DNA Master SYBR Green I (Roche) according to the manufacturer's instructions. The sequences of the primers were as follows: peroxisome proliferator-activated receptor gamma 2 (PPAR γ 2) (forward: 5'-CGCTGATGCACTGCCTATGA-3', reverse: 5'-GGGCCAGAATGGCATCTCT-3', 70 bp), nestin

Table 1. Experimental Protocol for Cell Transplantation

Donors	Recipients (SCI Rats)	Evaluation
SD (2 donors): female, 5 weeks old, GFP(–)	SD ($n = 5$): female, 10 weeks old	Motor function, H&E staining
SD (1 donor): male, 5 weeks old, GFP(–)	SD ($n = 3$): female, 10 weeks old	Cell survival (gene expression)
Wistar (2 donors): female, 5 weeks old, GFP(+)	Wistar ($n = 6$): female, 10 weeks old	Cell survival (GFP fluorescence)

The same number of rats was used as SCI controls in each experiment for the evaluation of motor function.

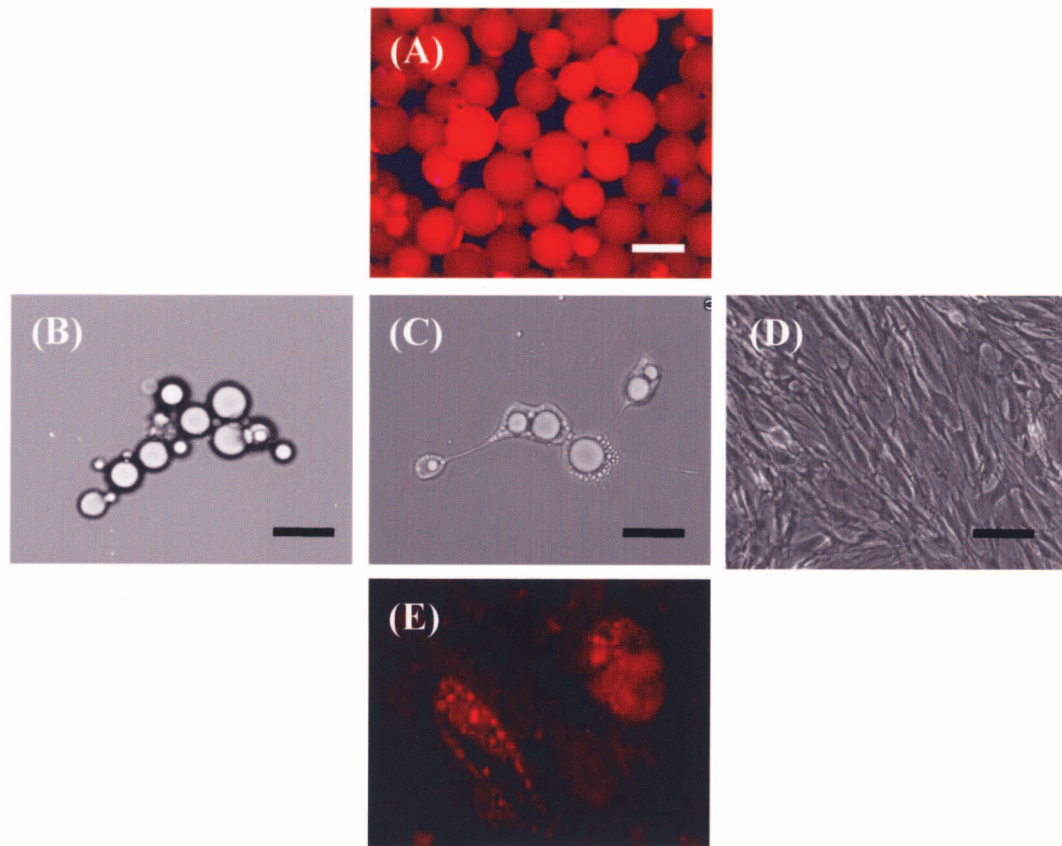


Figure 1. Mature adipocyte-derived fibroblast-like cells. (A) Mature adipocytes isolated from rat adipose tissue (AdipoRed™ and Hoechst 33342 staining). (B) Mature adipocytes with unilocal lipid droplets (day 0 of ceiling culture). (C) Mature adipocytes with multilocal lipid droplets (day 4 of ceiling culture). (D) Fibroblast-like cells without lipid droplets (dedifferentiated fat cells; DFAT). (E) Adipogenesis from DFAT (AdipoRed™ staining). Scale bars: (A, E) 50 μ m; (B–D) 100 μ m.

(forward: 5'-CAGGCTTCTCTTGGCTTTCTGG-3', reverse: 5'-TGGTGAGGGTTGAGGGTTGT-3', 159 bp), β III tubulin (forward: 5'-TGCGTGTGTACAGGTGAA TGC-3', reverse: 5'-AGGCTGCATAGTCATTTCCAAG-3', 200 bp), glial fibrillary acidic protein (GFAP) (forward: 5'-ACCTCGGCACCCTGAGGCAG-3', reverse: 5'-CCAGCGACTCAACCTTCCTC-3', 151 bp), brain-derived neurotrophic factor (BDNF) (forward: 5'-GGT TCGAGAGGTCTGACGAC-3', reverse: 5'-CAAAGG CACTTGACTGCTGA-3', 159 bp), glial cell line-derived neurotrophic factor (GDNF) (forward: 5'-CAAAAGAC TGAAAAGGTCACCAGAT-3', reverse: 5'-GCTTGC CGTTTCTCTCTCT-3', 69 bp), hypoxanthine phosphoribosyltransferase (HPRT) (forward: 5'-GCCCAA AATGGTTAAGGTTG-3', reverse: 5'-TCCACTTTTCG CTGATGACACA-3', 176 bp; internal control).

Flow Cytometry

DFAT were fixed and permeabilized by using a BD Cytofix/Cytoperm™ fixation permeabilization kit (BD Biosciences, San Diego, CA). Cells were incubated with

one of the primary antibodies for 30 min on ice, followed by incubation with the requisite secondary antibody for 30 min on ice. The following primary antibodies were used: nestin (mouse, Chemicon, Temecula, CA), β III tubulin (mouse, Promega, Madison, WI), GFAP (mouse, Sigma-Aldrich, St. Louis, MO), BDNF (rabbit, Chemicon), and GDNF (rabbit, Santa Cruz Biotechnology, Santa Cruz, CA). Allophycocyanin-conjugated rat anti-mouse IgG1 (BD Biosciences) and Alexa Fluor® 647 goat anti-rabbit IgG (Invitrogen) were used as secondary antibodies. Flow cytometry was performed by using a BD LSR II (BD Biosciences) and data were analyzed with FlowJo software (Tree Star, Ashland, OH). The number of cells per assay was at least 30,000.

Western Blot Analysis

An aliquot of DFAT lysate (20 mM Tris-HCl, 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, 5 μ g/ml aprotinin, 5 μ g/ml leupeptin) was subjected to electrophoresis on 5–20% gradient SDS/polyacrylamide gels. The protein concentration was deter-

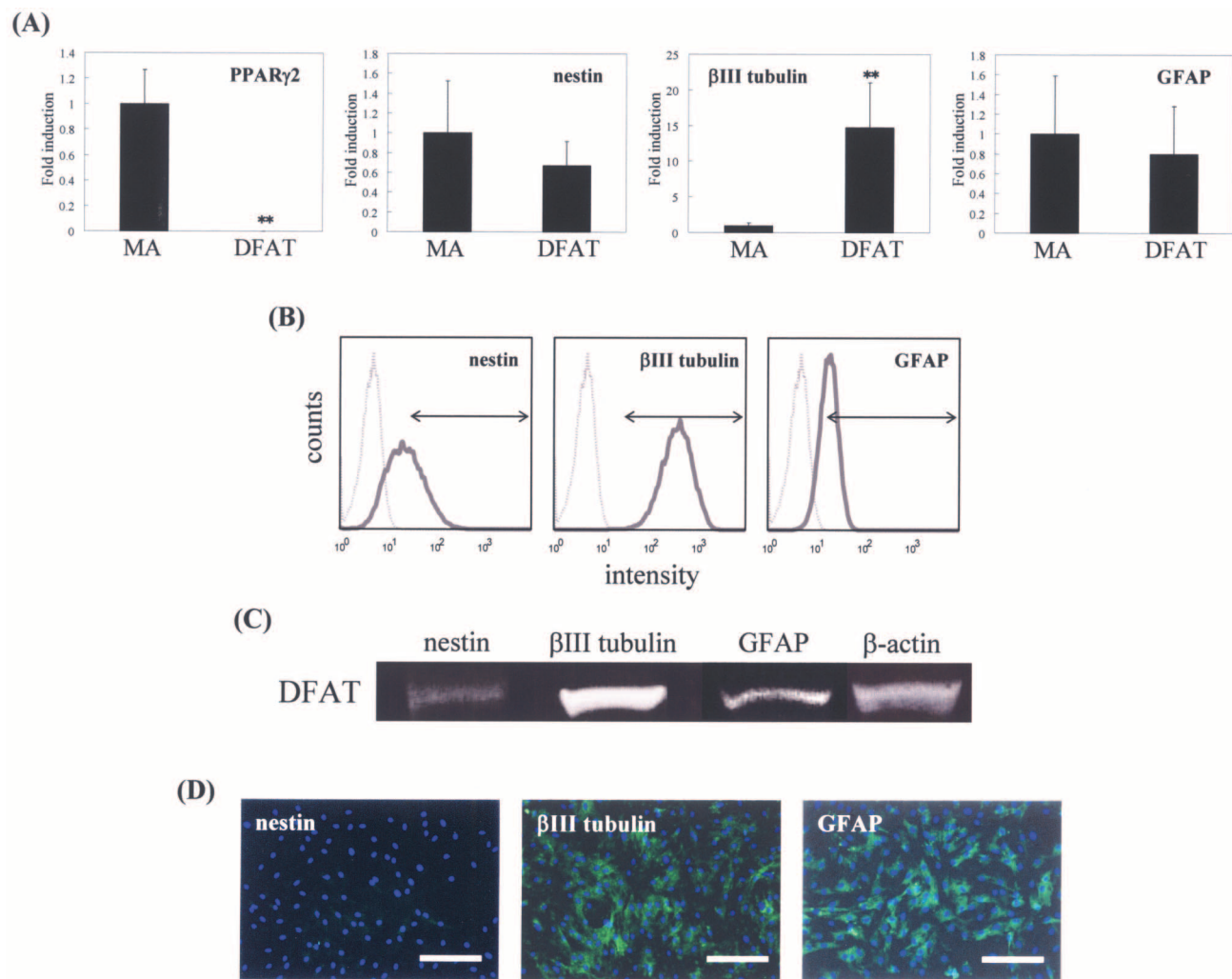


Figure 2. Expression of neural markers. (A) Expression of various mRNAs by DFAT. Data represent the mean \pm SD ($n = 6$) relative ratio, with the level of transcripts in mature adipocytes (MA) being arbitrarily set as 1. $**p < 0.01$ compared with MA. (B) Flow cytometric analysis of DFAT. Plots obtained after treatment with the respective antibodies are shown. The solid lines show positive cells and the dotted line shows the isotype control. Three separate assays were performed. (C) Western blot analysis of DFAT. Nestin (220 kDa), β III tubulin (55 kDa), GFAP (46 kDa), and β -actin (42 kDa). DFAT lysate (β III tubulin and β -actin: 10 μ g of protein; nestin and GFAP: 20 μ g of protein) was used for electrophoresis. Expression was confirmed by comparison with molecular markers and fetal rat brain lysate. (D) Immunohistochemical staining is shown after counterstaining with Hoechst 33342. Scale bars in (D): 100 μ m.

mined by using a BCA protein assay kit (Pierce, Rockford, IL). Proteins were transferred to PVDF membranes (Clearblot PTM, ATTO Corporation, Tokyo) and incubated with the primary antibodies for nestin (220 kDa), β III tubulin (55 kDa), GFAP (46 kDa), or β -actin (42 kDa, mouse, Sigma-Aldrich), followed by incubation with ECL anti-mouse IgG horseradish peroxidase-linked species-specific whole antibody (GE Healthcare, Buckinghamshire, UK). Finally, the membranes were reacted with ECL plusTM Western blotting detection re-

agents (GE Healthcare), and were exposed to Polaroid film.

SCI, Cell Transplantation, and Functional Evaluation

Impact injury of the spinal cord was induced with the weight-drop device developed at New York University (NYU impact model), as described previously (36). Adult female rats weighing 210–230 g (10 weeks old) were anesthetized, and dorsal laminectomy was performed at the T10 level. Then a 10-g weight was

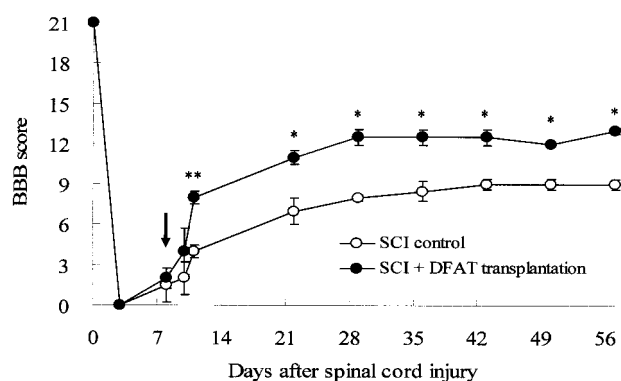


Figure 3. Time course of functional recovery from SCI-induced motor dysfunction. On day 8 after injury (arrow), DFAT (2.5×10^5 in $5 \mu\text{l}$) were transplanted into SCI rats. The baseline hindlimb locomotor function BBB score showed no significant difference between the control SCI group ($n = 5$) and the DFAT transplantation group ($n = 5$). BBB scores were determined before and after transplantation. Each median score was plotted, and the bars show QR. * $p < 0.05$ and ** $p < 0.01$ compared with the control group.

dropped onto the spinal cord from a height of 25 mm. On day 8 after SCI, cell transplantation was carried out (Table 1). In brief, DFAT were incubated with growth medium containing 10 ng/ml basic fibroblast growth factor (Invitrogen) for 20 h before transplantation. Treated cells were detached with trypsin/EDTA, and subsequently cells (2.5×10^5 cells in $5 \mu\text{l}$) were injected into the center of the damaged region of the spinal cord at the rate of $1 \mu\text{l}/\text{min}$ by using a stereotaxic microinjector (model 310, Muromachi Kikai Co., Ltd, Tokyo) with a Hamilton syringe. Ten seconds after the completion of transplantation, the syringe was gradually removed. DFAT obtained from one animal were transplanted into the damaged spinal cords of two or three SCI animals. Control SCI animals received an injection of $5 \mu\text{l}$ of physiological saline. The Basso-Beattie-Bresnahan (BBB) score, a locomotor rating scale developed by Basso et al. (3,4), was used to assess the effect of cell transplantation.

Detection of Transplanted Cells

SCI rats were perfused with saline and 4% paraformaldehyde (PFA; Wako)/phosphate-buffered saline (PBS) by intracardiac injection under anesthesia. For hematoxylin and eosin (H&E) staining, spinal cord specimens (15 mm long and containing the site of injury) were embedded in paraffin, cut into $4\text{-}\mu\text{m}$ sections, and placed on SUPERFROST® MAS-coated glass slides (Matsunami Glass Ind., Ltd., Osaka, Japan).

DFAT obtained from a male donor were transplanted

into female SCI rats (Table 1). The sex-determining region on the Y chromosome (Sry) gene was used for identification. Genomic DNA was extracted from the spinal cords of female rats by using a GenElute™ mammalian genomic DNA miniprep kit (Sigma-Aldrich) according to the manufacturer's instructions. The sequences of the primers were as follows: Sry (forward: 5'-CCCGCGGAGAGAGGCACAAGT-3', reverse: 5'-TAGGGTCTTCAGTCTCTGCGC-3', 146 bp) (20). PCR products were separated by electrophoresis on 2% agarose gel, stained with SYBR Safe™ DNA gel stain (Invitrogen), and detected with a BioDoc-It™ system UV transilluminator (UVP, Upland, CA).

DFAT obtained from GFP transgenic rats were transplanted at the site of SCI (Table 1). Then SCI rats were perfused with saline and 4% PFA/PBS by intracardiac injection under anesthesia. Resected spinal cord specimens (15 mm long) were fixed by immersion for 1 h, followed by cryoprotection with 10%, 20%, and 30% sucrose/PBS at 4°C . Tissue specimens were frozen after being embedded in Tissue-Tek® O.T.C. compound (Sakura Finetechnical, Tokyo, Japan), and then $10\text{-}\mu\text{m}$ sections were cut and placed on SUPERFROST® MAS-coated glass slides. Green fluorescence was used for identification of transplanted cells and fluorescent images were acquired by using a conventional microscope equipped with CCD camera (model IX70, Olympus, Melville, NY).

Immunostaining

Cells fixed with 4% PFA/PBS or spinal cord specimens were used for immunostaining. After being reacted with the primary antibodies for nestin, β III tubulin, GFAP, BDNF, or GDNF, incubation was done with rhodamine-conjugated anti-mouse IgG (ROCKLAND, Gilbertsville, PA), rhodamine-conjugated anti-rabbit IgG (MP Biomedicals), fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Sigma-Aldrich), or FITC-conjugated anti-rabbit IgG (Chemicon) as the secondary antibodies. Then the cells and sections were counterstained with Hoechst 33342.

Statistical Analysis

Data on mRNA expression were analyzed by Welch's *t*-test, and BBB scores were analyzed by Mann-Whitney *U*-test. Values are expressed as the mean \pm SD, and the median and quartile range (QR), respectively. In all analyses, $p < 0.05$ was considered statistically significant.

RESULTS

Characteristics of Cultured Rat DFAT

When culture was done in flasks completely filled with medium, mature adipocytes containing lipid drop-

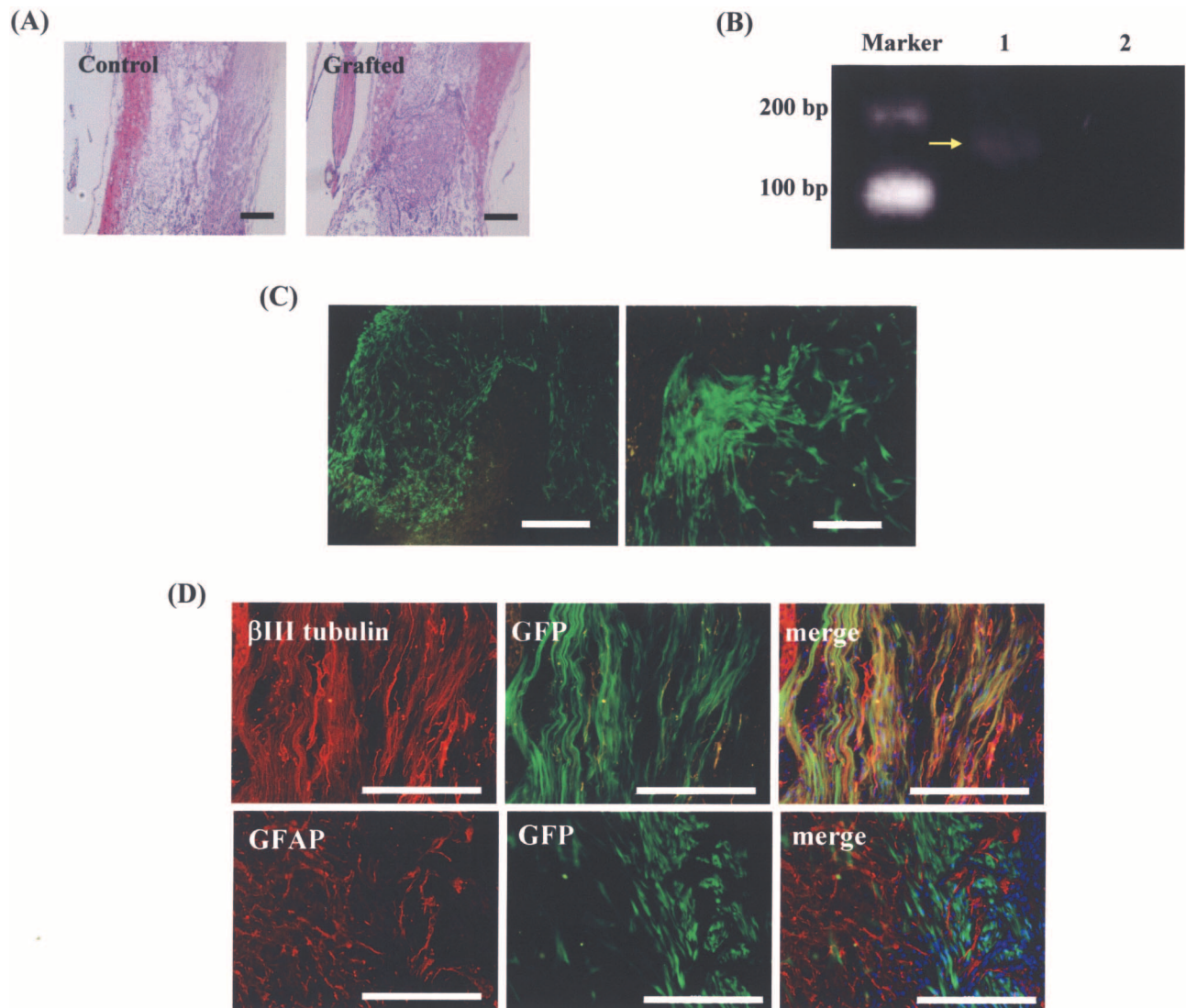


Figure 4. Identification of grafted cells in rat spinal cords. (A) H&E staining at 7 weeks after DFAT transplantation. The control spinal cord has a larger cavity than the DFAT-grafted cord. (B) DFAT obtained from a male donor were used for transplantation. When genomic DNA was extracted from spinal cords at 2 weeks after cell transplantation, the Sry gene (146 bp, arrow) was only detected in the spinal cords of DFAT-grafted females (lane 1). Lane 2: Spinal cord of a female without DFAT transplantation. (C) DFAT obtained from GFP transgenic rats were transplanted at the site of SCI. One week after transplantation, the presence of grafted cells was confirmed by green fluorescence. (D) Immunohistochemical staining was also performed to detect β III tubulin and GFAP expression. Merged photographs are shown after counterstaining with Hoechst 33342. Scale bars: (A, D) 200 μ m; (C) 500 μ m (left), 200 μ m (right).

lets (Fig. 1A) became adherent to the ceiling of each flask (Fig. 1B). During ceiling culture, these mature unilocular adipocytes changed into cells that contained multiple droplets (Fig. 1C). Approximately 10 days later, fibroblast-like cells without any lipid droplets (DFAT) were growing on the ceilings of the flasks (Fig. 1D). When confluent DFAT without lipid droplets were subsequently cultured in adipogenic medium, intracellular lipid droplets formed again (Fig. 1E).

As shown in Figure 2A, expression of PPAR γ 2 mRNA, a marker for adipocytes, was scarcely detectable in DFAT ($p < 0.01$). It should be noted that the expression of β III tubulin mRNA, a marker for neurons, was increased 14.7-fold in DFAT compared to that by mature adipocytes (MA) ($p < 0.01$). However, expression of mRNA for nestin (a neural stem cell marker) and GFAP (an astrocyte marker) showed no significant differences between MA and DFAT. More than 60% of

DFAT were positive for nestin by flow cytometry (Fig. 2B). It was also found that most DFAT were positive for β III tubulin and GFAP. Western blot analysis (Fig. 2C) and immunocytochemical analysis (Fig. 2D) confirmed their expression by DFAT, while the same amount of MA lysate was negative for β III tubulin by Western blot analysis (data not shown).

Cell Transplantation Into Injured Rat Spinal Cords

Next, we investigated whether DFAT were useful for improvement of SCI. As shown in Figure 3, SCI rats

grafted with DFAT showed improvement of hindlimb locomotor function from the early period after transplantation compared with control SCI rats. After 4 weeks, some animals in the grafted group could walk with forelimb–hindlimb coordination and could perform integrated voluntary motor movements. Control SCI rats also recovered, but some of them crawled persistently and moved without weight bearing. There was a significant difference between the two groups from day 3 after transplantation. The BBB score of all the SCI animals reached a plateau at 5 weeks after injury, when grafted

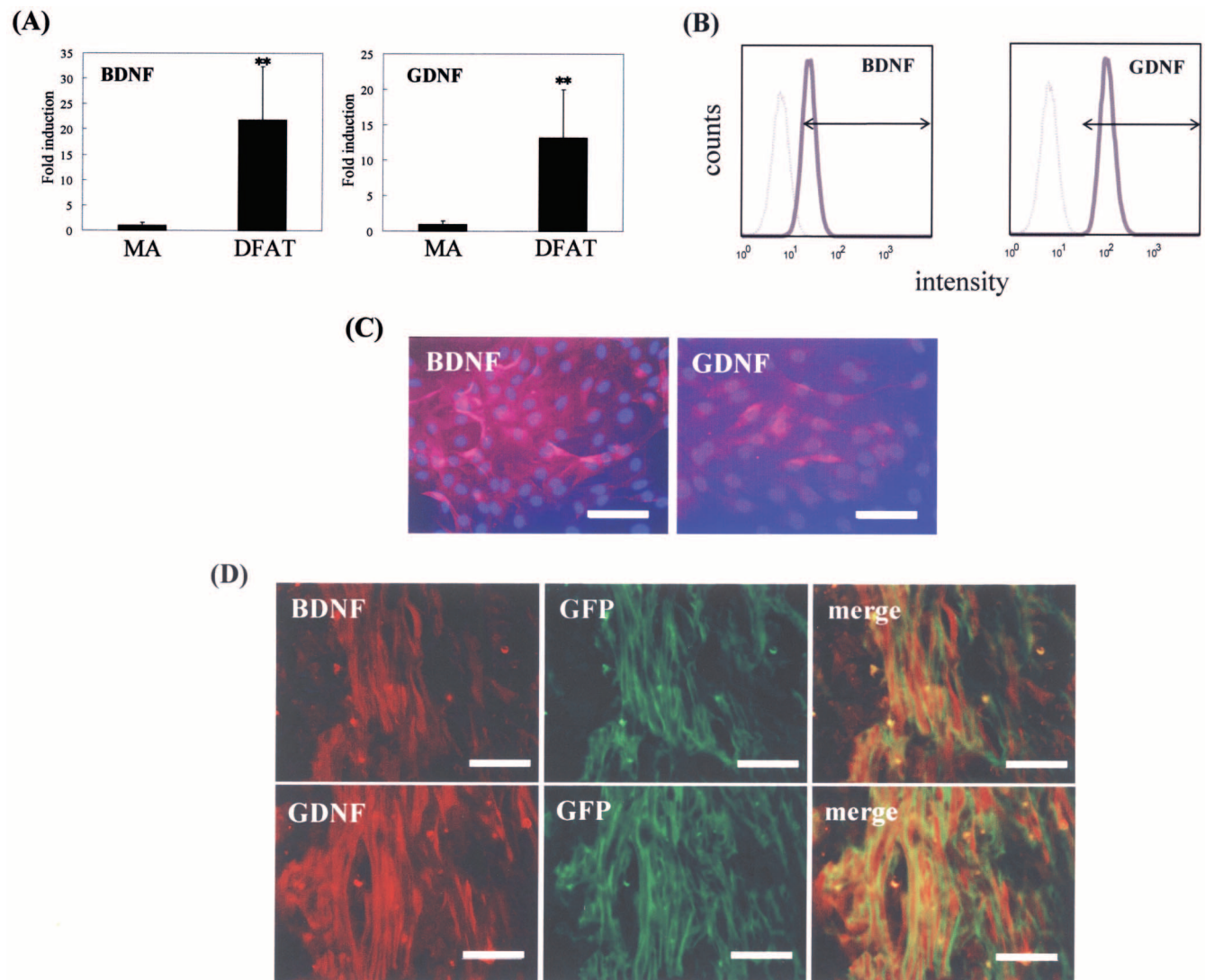


Figure 5. Expression of neurotrophic factors in vitro and in vivo. (A) Real-time RT-PCR analysis. Data represent the mean \pm SD ($n = 6$) relative ratio, with the level of transcripts in mature adipocytes (MA) being arbitrarily set as 1. $**p < 0.01$ compared with MA. (B) Flow cytometric analysis of DFAT. Plots obtained after treatment with each antibody are shown. The solid lines show positive cells and the dotted line shows the negative control without the primary antibody. Three separate assays were performed. (C) Immunocytochemical staining. DFAT are positive for BDNF and GDNF. Photographs are shown after counterstaining with Hoechst 33342. (D) GFP-expressing DFAT in the spinal cord were immunostained for BDNF and GDNF. Merged photographs are shown. Scale bars: (C) 100 μ m; (D) 50 μ m.

animals had significantly better locomotor function (median: 12.5; QR: 1.3) compared with the controls (median: 9.0; QR: 0.8) ($p < 0.05$).

Survival of Grafted Cells

We examined whether grafted cells survived at the site of injection. H&E staining showed that the cavity at the site of injury was much smaller in grafted animals than in controls animals at 7 weeks after transplantation (Fig. 4A).

The Sry gene on the Y chromosome was detected in the spinal cords of female rats injected with DFAT from a male donor, indicating that grafted cells existed at the site of injury 2 weeks after their injection (Fig. 4B). In contrast, this gene was not detected in the spinal cords of control females.

To investigate the persistence of grafted cells, DFAT harvested from GFP transgenic rats were used as donor cells. Fluorescence images revealed that GFP-positive cells survived and were not only localized to the injection site, but also spread into the white matter by day 7 after transplantation (Fig. 4C). Immunohistochemistry revealed that GFP-expressing cells were positive for β III tubulin, although only a few were positive for GFAP (Fig. 4D). GFP-expressing cells were also observed at 2 weeks after grafting (data not shown).

Expression of Neurotrophic Factors In Vitro and In Vivo

Finally, we examined the function of DFAT after grafting and their contribution to the recovery of motor function. To this end, we focused on neurotrophic factors. Expression of BDNF and GDNF mRNAs showed a 21.8-fold ($p < 0.01$) and 13.2-fold ($p < 0.01$) increase, respectively, compared to that by MA (Fig. 5A). It was obvious that cultured DFAT expressed BDNF and GDNF when analyzed by flow cytometry (Fig. 5B). These findings were also confirmed by immunocytochemical staining (Fig. 5C). Furthermore, spinal cords grafted with GFP-positive cells showed the expression of BDNF and GDNF (Fig. 5D).

DISCUSSION

This is the first study to demonstrate that mature adipocyte-derived cells, DFAT, may be promising as donor cells for the repair of SCI. Our findings also indicated that adipose tissue could be a new source for cell replacement therapy to treat central nervous system disorders.

Double staining with AdipoRedTM and Hoechst 33342 revealed that the vast majority (>99%) of the cells isolated from rat adipose tissue were mature adipocytes containing lipid droplets, as reported previously (29). When cultured in flasks completely filled with medium, these adipocytes gradually lost their lipid droplets and

became fibroblast-like cells attached to the ceiling of the flask. This process has been termed “dedifferentiation” (29,32,39,42). Based on the finding that exposure to adipogenic medium restored the cells to adipocytes, these “dedifferentiated” fibroblast-like cells (DFAT) were concluded to have the characteristics of preadipocytes.

DFAT had none of the features of adipocytes, such as accumulation of lipid droplets and PPAR γ 2 expression. It was noteworthy that nestin (neural stem cells), β III tubulin (neurons), and GFAP (astrocytes) were expressed without any stimulation. Nestin is not only a marker for neural stem cells, but also for endothelial progenitors. It has already been demonstrated that DFAT have the potential to differentiate into endothelial cells (19,29). Therefore, DFAT might have the dual potential for differentiation into the neuronal or glial lineages. MSCs have already been reported to express neural and glial markers without differentiation (5,9,40), and are useful donor cells for treating SCI. Further investigation is needed to clarify the similarities and/or differences of characteristics between DFAT and MSCs, but DFAT may also be useful as cell replacement therapy for SCI.

As expected, transplantation of DFAT significantly promoted the recovery of hindlimb dysfunction in SCI rats. During the observation period, the DFAT-grafted animals showed significantly better motor function than the controls. Survival of the injected cells was confirmed by detection of male Sry gene expression in female spinal cords and also by detection of GFP-positive cells. Some of the GFP-positive cells were also positive for β III tubulin. GFP-labeled DFAT were still found in the spinal cord at 2 weeks after transplantation (data not shown), but GFP fluorescence was not detected by 4 weeks after grafting. This study was done without any immunosuppressive therapy, so the grafted cells might have survived longer with immunosuppression.

There is a possibility that the grafted cells simply functioned as a matrix to support and promote network formation by residual endogenous neurons. Another possibility is that the grafted cells differentiated into neurons or glial cells that formed networks in cooperation with the residual host cells. DFAT were positive for nestin, GFAP, and especially β III tubulin in vitro, and some grafted DFAT also expressed β III tubulin, but it remains unclear whether grafted DFAT differentiated into the neuronal lineage. Additional studies, such as electrophysiological experiments and electron microscopic analysis of synapse formation, will be required to prove the differentiation of functional neurons from DFAT.

Neurotrophic factors are well known to play a key role in promoting repair after SCI (8,16,24,37,44), and cultured DFAT expressed neurotrophic factors such as BDNF and GDNF. MSCs have already been reported to promote functional recovery in SCI animals (13), and the mechanisms involved have been proposed to include

microenvironmental stabilization, activation of endogenous repair, or production of trophic factors (7). Some of the grafted DFAT expressed BDNF and GDNF, a finding that could lead to the conclusion that DFAT-derived neurotrophic factors contributed to promotion of functional recovery after SCI.

There have been no previous reports about the *in vivo* use of DFAT to promote regeneration in neurodegenerative conditions such as SCI. This is the first study to suggest that DFAT could be promising as donor cells for repair of SCI. Adipose tissue has several advantages as a donor source. (i) For example, autografting could be performed instead of allografting, and a study of this is now under way (DFAT are obtained from mature adipocytes isolated from the adipose tissue of SCI rats, and are transplanted at the site of injury in the same animals). (ii) Adipose tissue can be obtained in relatively large quantities with little discomfort. (iii) Adipose tissue can also be harvested safely from persons of any age.

This study showed that transplantation of DFAT into SCI rats significantly promoted the recovery of hindlimb dysfunction, along with survival of the grafted cells and expression of neurotrophic factors. These findings suggest that adipose tissue may be a potential new source for cell replacement therapy to treat central nervous system disorders.

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