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Laboratory Investigation

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Differentiation of Human Adult Adipose Derived Stem Cell in *vitro* and Immunohistochemical Study of Adipose Derived Stem Cell after Intracerebral Transplantation in Rats

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Objective : Adipose tissue is derived from the embryonic mesoderm and contains a heterogenous stromal cell population. Authors have tried to verify the characteristics of stem cell of adipose derived stromal cells (ADSCs) and to investigate immunohistochemical findings after transplantation of ADSC into rat brain to evaluate survival, migration and differentiation of transplanted stromal cells.

Methods : First, ADSCs were isolated from human adipose tissue and induced adipose, osseous and neuronal differentiation under appropriate culture condition in *vitro* and examined phenotypes profile of human ADSCs in undifferentiated states using flow cytometry and immunohistochemical study. Human ADSCs were transplanted into the healthy rat brain to investigate survival, migration and differentiation after 4 weeks.

Results : From human adipose tissue, adipose stem cells were harvested and subcultured for several times. The cultured ADSCs were differentiated into adipocytes, osteoctye and neuron-like cell under conditioned media. Flow cytometric analysis of undifferentiated ADSCs revealed that ADSCs were positive for CD29, CD44 and negative for CD34, CD45, CD117 and HLA-DR. Transplanted human ADSCs were found mainly in cortex adjacent to injection site and migrated from injection site at a distance of at least 1 mm along the cortex and corpus callosum. A few transplanted cells have differentiated into neuron and astrocyte.

Conclusion : ADSCs were differentiated into multilineage cell lines through transdifferentiation. ADSCs were survived and migrated in xenograft without immunosuppression. Based on this data, ADSCs may be potential source of stem cells for many human disease including neurologic disorder.

KEY WORDS : Adipose cell · Stromal cell · Stem cell · Brain .

INTRODUCTION

The nervous system, unlike other systems such as hematopoietic system and musculoskeletal system, has a limited capacity for self repair[8]. Recently, research on broad clinical applications using stem cells has shown prominent progress that stem cells are being used in trial for the treatment of refractory neurological disease such as stroke, spinal cord injury and parkinson's disease which cannot be treated with traditional methods. By definition, stem cells are able to self-renewal, exist in an undifferentiated or unspecialized state and are capable of differentiation or specialization along multiple lineages[5]. Among them, embryonic stem cells compared to adult stem cells, possess better multiplication and differentiation abilities. However, ethical restrictions are issues in clinical application of the embryonic stem cells[21].

Although neural stem cells (NSCs) are ideal candidate in treatment of refractory neurological disease, it is difficult to obtain neural stem cells which are located at the deep portion such as hippocampus, subventricular zone and dentate gyrus. Many studies have shown that bone marrow-derived cells can give rise to neuronal cells as well as many tissue-specific cell phenotypes, including hematopoietic, skeletal muscle, hepatocyte, heart and vascular endothelial cells[9]. Bone marrow-derived mesenchymal stromal cells have clinical importance in applications for tissue engineering. However, they may be obtained only by bone marrow biopsy, a potentially painful procedure.

Recently, mesenchymal stem cells (MSCs) have been isolated from several different tissues such as adiose tissue, muscle and dermis[11]. Because adipose tissue is readily accessible and

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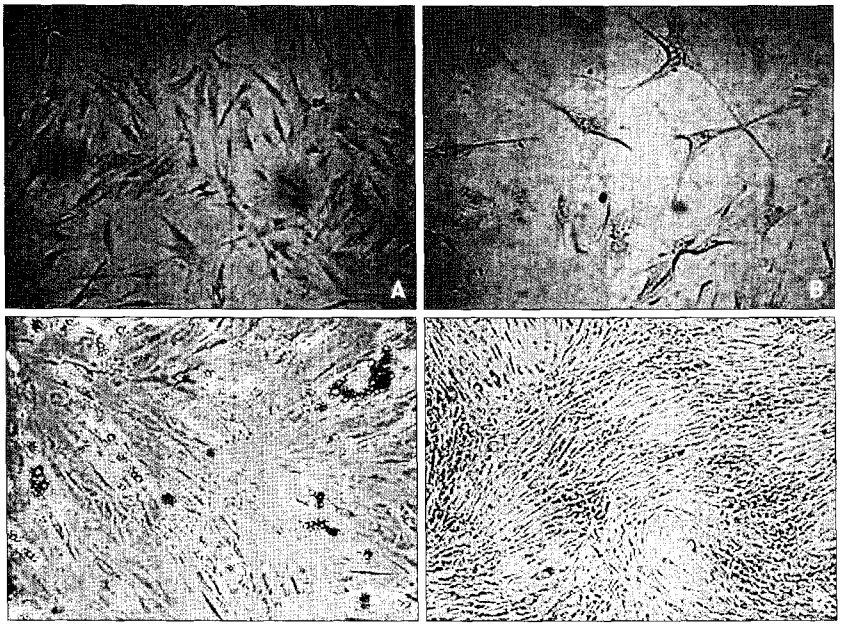

Fig. 1. A, B : Phase contrast photomicrograph showing morphological characteristics of human adipose derived stem cells (ADSCs) at tenth day of fifth passage cultures. Cells approached with confluency and show spindle shape-like fibroblastic morphology (A : Original magnification ×40, scale bar; 100 μm, B : Original magnification ×100, scale bar; 50 μm). C : At fourteenth day after differentiation into adipocyte lineage, differentiated human ADSCs are demonstrated by staining with Oil Red O (Original magnification ×200, scale bar; 150 μm). D : At fourteenth day after differentiation into osteocytes lineage differentiated human ADSCs are demonstrated by staining with ALP (Original magnification ×40, Scale bar; 300 μm).

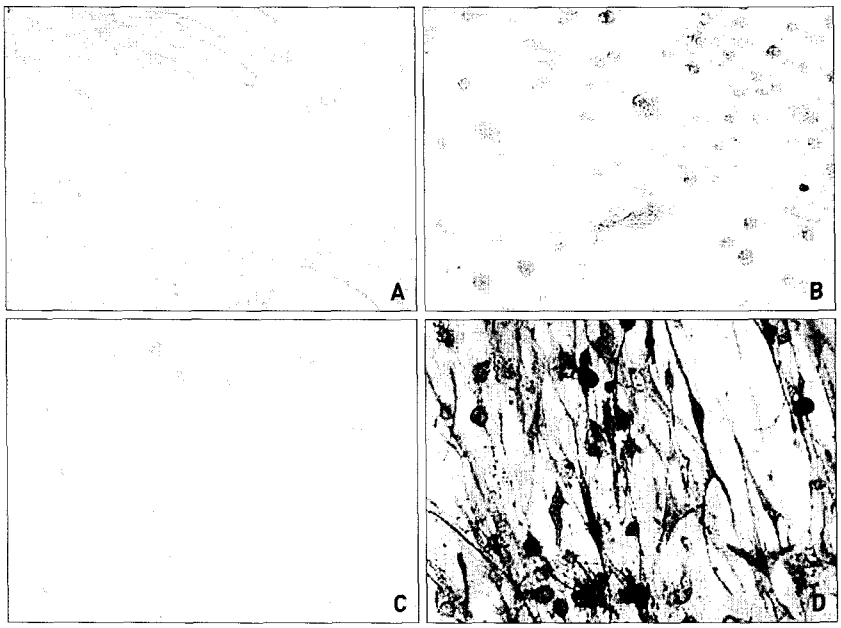

Fig. 2. Immunohistochemistry of (A) control, (B) trkA, (C) Tau and (D) NF-M. After 24 hours of neuronal induction, differentiated human adipose derived stem cell are labelled with anti-trkA (B), anti-Tau (C) and anti-NF-M (D) against mature neurons (Original magnification ×200, scale bar; 150 μm).

abundant, MSCs isolated from this tissue may be a better candidate for cell therapy and tissue engineering. In this experiment, we isolated adipose derive stem cells (ADSCs) from human adipose tissue and induced adipose, osseous and neuronal differentiation under appropriate culture condition in *vitro* and examined phenotypes profile of human ADSCs in undifferentiated states using flow cytometry and immunohistochemical study. Human ADSCs were transplanted into the healthy rat brain. We investigated survival, migration and differentiation of transplanted human ADSCs after 4 weeks.

MATERIALS AND METHODS

Isolation and culture of ADSC

Human adipose tissues were obtained from individuals undergoing elective mastoidectomy with patient's informed consent as approved by the Institution Review Board. For this experiment, tissues obtained from three different patients were used. Adipose tissues were transported to the laboratory in saline solution within 2 hours postsurgery. To isolate human adipose tissue stromal cells (ADSCs), adipose tissues were washed extensively with equal volumes of phosphate-buffered saline (PBS) and tissues were digested at 37°C for 30 min with 0.075% collagenase. Enzyme activity was neutralized with Dulbecco's modified Eagle's medium (DMEM), containing 10% fetal bovine serum (FBS) and centrifuged at 1,200 rpm for 10 min to obtain a pellet. The pellet was filtered through a 100-μm nylon mesh to remove cellular debris and incubated overnight at 37°C/5% CO_2 in control medium (DMEM, 10% FBS, 100 U/ml of penicillin, 100 μg/ml of streptomycin). Following incubation, the plates were washed extensively with PBS to remove residual nonadherent red blood cells. The resulting cell population was maintained at 37°C/5% CO_2 in control medium. The primary cells were cultured for 4-5 days until they reached confluence and were defined as passage "0". The cells were then harvested by digestion with 0.5 mM EDTA/0.05% trypsin, centrifuged at 1200 rpm for 5 min,

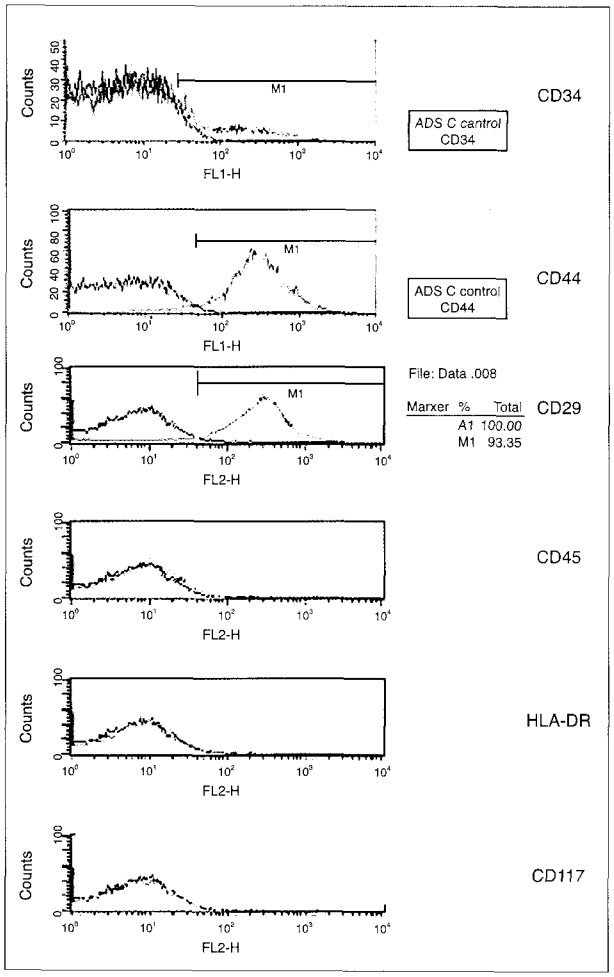

Fig. 3. Flow cytometric analysis of undifferentiated human adipose tissue-derived stromal cells (ADSCs). Undifferentiated ADSCs from individual donor are stained with monoclonal antibodies directed against either CD34, CD44, CD29, CD45, HLA-DR and CD117 and coupled to fluorescein isothiocyanate (FITC); these signals are indicated as green lines, An isotype matched monoclonal antibody served as a control; these signals are indicated as dotted lines. ADSCs were positive for CD29, CD44 and negative for CD34, CD45, HLA-DR and CD117.

resuspended in control medium and plated at a density of approximately 1,000 cells/cm^2. The cells were passaged and were used for in *vitro* differentiation and in vivo transplantation. The passage number of cells used in experiments was 5.

Characteristics of ADSCs

Osteogenic differentiation

To induce mineralization, the stromal cells were harvested using trypsin/EDTA and re-plated in multiple-well plates at 10,000 cells/cm^2 for 16 h to allow attachment in pre-adipocyte medium (Day 0). On Day 1, the medium was changed to medium supplemented with 10% FBS, 100 U penicillin/ml, 100 μg streptomycin/ml, 10 mMβ-glycerolphosphate and 50 μg/ml 2-phosphate ascorbate. Additional supplements included 10nM dexamethasone. Cultures were fed every 3rd day throughout the study. Culture-expanded cells were induced for 2 weeks

Adipogenic differentiation

To induce adipogenesis, the stromal cells were harvested using trypsin/EDTA and plated in multiple well plates at 10,000 cells/cm^2 for 16 h to allow attachment in preadipocyte medium (Day 0). On Day 1, the medium was then changed to Dulbecco's modified Eagle's-Ham's F-10 medium (vol/vol, 1:1) supplemented with 3% fBS 15mM HEPES (pH 7.4), biotin (33 μM), pantothenate (17 μM, simga), Human recombinant insulin (100 nM), dexamethasone (1 μM) and 1-methyl-3-isobutylxanthine (IBMX) (0.25 mM), BRL49653 (1 μM) for a 3-day period. From Day 4, the cells were fed every 3rd day with the same medium without IBMX and BRL49653 supplementation. Culture-expanded cells were induced for 3 weeks. Differentiation of preadipocyte was assessed by staining with triglyceride-specific dye Oil Red O.

Neurogenic differentiation

To induce neuronal differentiation, the adipose derived stromal cells were incubated in DMEM containing 20% FBS for 24 hours prior to neuronal induction. On Day 1, the medium was then changed to DMEM with butylated hydroxyanisole (BHA, 200 μM), KCl (5 mM), valproic acid (2 μM), forskolin (10 μM), hydrocortisone (1 μM) and insulin (5g/ml) for 3-day period. Neural differentiation was assessed by immunohistochemical staining using anti-NF-M, Tau, TrkA antibody.

Flow-cytometric analysis

Approximately 10^6 cells were harvested, washed in PBS and incubated with an appropriate amount of fluorescein conjugated primary antibody(1 ug/10^6 cells) in PBS buffer (supplemented with 1% bovine serum albumin in pH 7.5 PBS) in for 30 min on ice. These cells were washed in PBs and fixed in 10% formalin prepared in PBS containing 2% FBS. Fluorescein-conjugated antibodies raised against CD29, CD44, CD34, CD45, CD117 and HLA-DR (BD Pharmingen, San Diego, CA, USA) were used to identity ADSCs. FACS (Fluorescence Activated Cell Sorter) analysis was done using a FACScan (Becton Dickinson Franklin Lakes, USA). We analyzed a minimum of 10,000 event/sample. Analysis was done by Becton Dickinson cell quest software.

Transplantation of ADSCs

Preparation for ADSCs transplantation

In order to label the cell derived from ADSCs, 5 μM bromodeoxyuridine (BrdU, Sigma, St. Louis, USA) was added to the cultured medium 72 hours before the trans-

plantation. Identification of BrdU labelled cells was done via immunohistochemical staining prior to transplantation. Cells were then washed three times with PBS and counted using cytometer to ensure adequate cell number for transplantation. Approximately 10^5 cells in 10 $\mu\ell$ total fluid volume were transplanted into the brain of each rat.

Cell transplantation procedures

Adult female Spraque-Dawley rats (SD rats) weighing 250-280 g were used in our experiments. All transplantation procedures were performed under aseptic conditions. Rats were initially anesthetized with ketamin (50 mg/kg) and xylazine (2.5 mg/kg) by intraperitoneal injection. Rats were transferred to a Kopf stereotactic frame (Stoelting instruments, Wood Dale, IL, USA). The right corpus striatum at coordinates calculated from bregma based on the rat brain atlas (with the toothbar set at -3.3 mm) : 1.0 mm rostral to the bregma, 3.0 mm lateral to the midline and 4.5 mm ventral to the dura was the injection site. After opening the dura mater a 26-gauge Hamilton syringe (Hamilton, Reno, NV, USA) was inserted to the target point and 10^5 cells in 10 $\mu\ell$ total fluid volume were injected for a duration of 30 min using the micro-infusion pump (KD scientific, New hope, PA, USA). To ensure the transplanted cells not to flow out the needle trauma path, the syringe was left in place for an additional 5 minutes before retraction. During the operation sterilized tissue paper was used to apply pressure on hemorrhage sites. Immunosuppressive agents were not used in any animals.

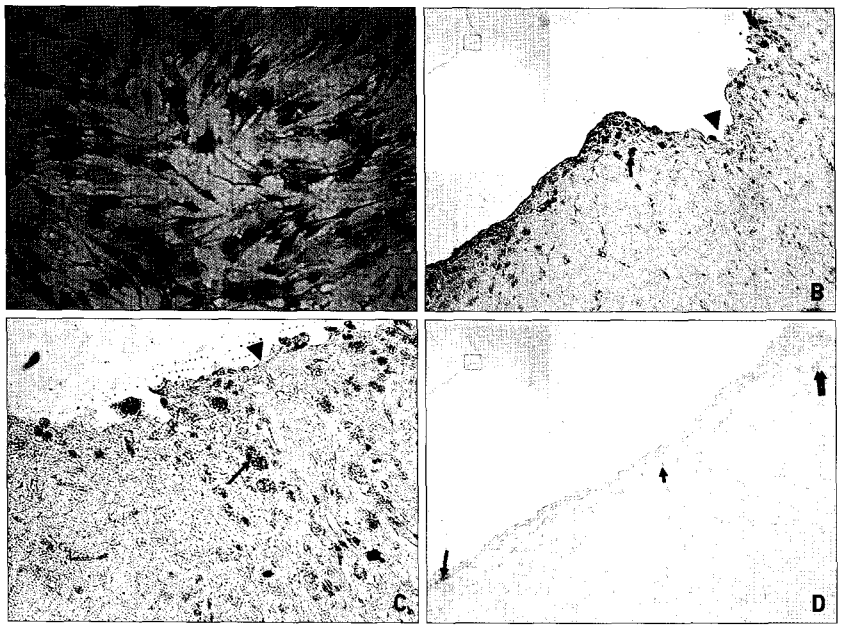

Fig. 4. A : Immediately before transplantation after fifth passage cultures, Photomicrograph showed BrdU labeled dark brown colored adipose derived stem cells (Original magnification ×100, scale bar ; 150 μm). B, C : At fourth week after transplantation, some BrdU reactive cells (thin arrow) are found around injection site (thick arrow head). (B : Original magnification : ×100, scale bar; 300 μm, C : Original magnification : ×400, scale bar; 60 μm). D : BrdU reactive cells (arrow) are found at a distance of 1 mm from injection site (double arrow) along the cortex (Original magnification ×100 & scale bar; 300 μm). Insets in B, D are the lower magnification of B and D.

Tissue preparation

Animals were allowed to survive for 4 weeks after transplantation at which each recipients rat was reanesthetized. Subsequently, the brain was fixed by transcardiac perfusion with saline, followed by 4% formalin. After transcardiac perfusion, the brain was detached by separating the cranial nerves from the basal surface. From 2.0 mm rostral to the bregma, the brain was severed from the coronal section and paraffin blocks were obtained. For detection of BrdU

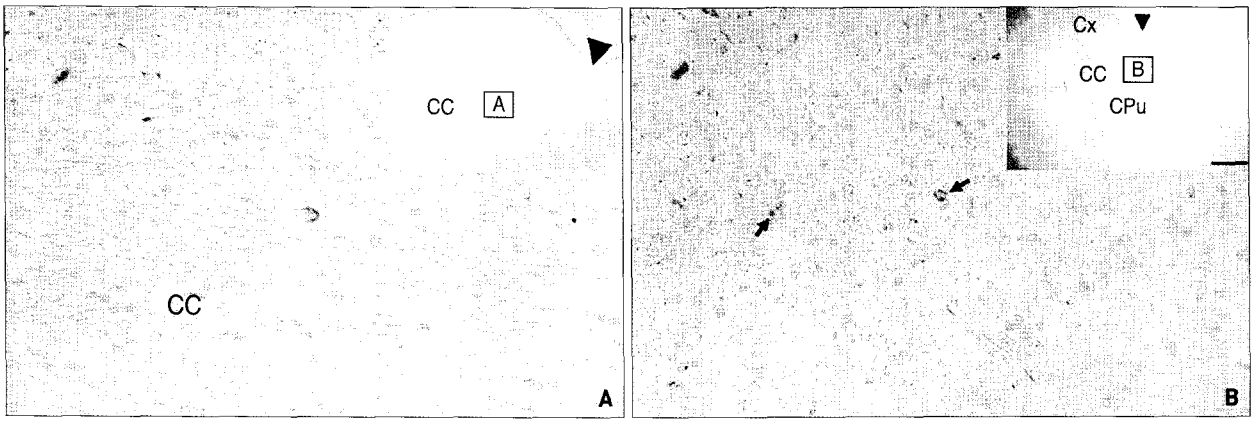

Fig. 5. A : A BrdU reactive cell is found at a distance of at least 1 mm from injection site (large arrow head) along corpus callosum (CC) (Original magnification : ×200 & scale bar; 150 μm). B : BrdU cells are found in the deep cortex adjacent to corpus callosum. Insets in A, B are the lower magnification of A and B. Inset A, B : the arrow head indicates injection site (Cx : cortex, CC : corpus callosum, CPu : caudate putamen).

labelled ADSCs, serial 10 ㎛-thick frozen sections of brain were adhered to poly-D-lysine-coated slides, allowed to dry in room air and fixed in 4% paraformaldehyde.

Immunohistochemical staining

For immunohistochemical staining, the sections were deparaffinized and then rehydrated. Brain sections were boiled in citrated buffer (pH 6) via autoclave (121°C) for 15 min. After sections were cooled in citrated buffer solution, sections were treated with peroxidase blocking agent (DAKO, Glostrup, Denmark) at 37°C for 5 min. 0.01 M PBS was used to rinse the sections and 2 x SSC (0.3 M NaCl, 0.03 M sodium citrate) was used twice to wash in 5 minutes. Following this, sections were put into 2N HCl at 37°C for 30 min. At room temperature 0.1 M boric acid was used to neutralized the HCl for 10 min. And then 0.01 M PBS was used to wash. After protein blocking in 1% BSA (bovine serum albumin) at 37°C for 30 min, section were treated with primary antibody, mouse anti-BrdU monoclonal antibody (DAKO, Glostrup, Denmark) diluted at 1:100 for 40 min. Once again, 0.01 M PBS was used to wash it out. Biotinylated secondary antibody and streptavidin-peroxidase (DAKO, glostrup, Denmark) were used for continued reaction. A mixture (Ultratech AEC 500-600 tests, Cat 2393, immunotech) of hydrogen peroxide and 3-amino-9-ethylcabazole was used as a chromogen for light microscopy. Double-labeled sections were treated according to the same procedure by using two different primary antibody, mouse anti-myelin basic protein (MBP) monoclonal antibody and anti-GFAP. Secondary antibody, alkaline phosphatase link anti-mouse and anti-rabbit IgG (DAKO, Glostrup, Denmark) were used for continued reaction. The stain was developed using fast red. Upon completion of staining, the coloring process was stopped with three washes of 0.05 Tris buffer solution. Hematoxylin was used for counterstaining.

Tissue examination

Immediately before transplantation, ADSCs were examined under the microscope (Olympus Bx, Tokyo, Japan) to determine what percentage of the cells derived from ADSCs were labeled with BrdU. In the rat brain, cells derived from ADSCs were identified using morphological criteria and brdU staining. The BrdU positive cells included small and round or oval nucleus with intensely stained chromatin.

RESULTS

Differentiation of ADSCs in *vitro*

Cell morphology did not differ from the first to subsequent cultures. At examination 10 days after passage of 5th passage cultures, ADSCs derived from human adipose tissues approached confluence and showed spindle-like fibroblastic morphology (Fig. 1A, B).

When ADSCs were cultured in adipocyte-specific induction media for 3 weeks, more than 50% of cells differentiated into lipid-laden cells, which was conformed with Oil Red O (Fig. 1C). When ADSCs were cultured in osteogenic medium for 2 weeks, more than 80% of cells could be induced into osteogenic lineage as demonstrated by alkaline phosphatase staining method (Fig. 1D).

Following neural induction, human ADSCs displayed changes in cellular morphology. The cytoplasm of ADSCs retracted towards the nucleus, forming contracted cell bodies with extended cytoplasmic extensions. Neuronal differentiation was conformed with anti-TrkA, anti-Tau and anti-NF-M using immunohistochemistry (Fig. 2). More than 75% of cells under neural induction were differentiated into neurogenic lineage.

Flow-cytometric analyses of undifferentiated ADSCs

The undifferentiated ADSCs were consistently positive for CD29, CD44 and negative for CD34, CD45, CD117 and HLA-DR that was HLA-class II antigen and was concerned in the rejection after transplantation (Fig. 3).

Histological and immunohistochemical assessment after transplantation of ADSCs into rat brains

After five passage cultures and 72 hours before the transplantation, 5 ㎛ BrdU was added to ADSCs and immunohistochemical staining of ADSCs against anti-BrdU was examined just before transplantation. More than 80% of the total cells were labeled with BrdU prior to transplantation (Fig. 4A). To investigate the survival and migration of transplanted ADSCs, 5 rats underwent ADSCs transplantation. Four weeks later, all 5 rats showed BrdU staining with the majority found around transplantation site (Fig. 4B, C). Transplanted ADSCs-BrdU reactive cells were found at superficial cortex at least 1 mm away from transplantation sites (Fig. 4D) and was found to move along corpus callosum in 2 rats (Fig. 5A). Also, BrdU reactive cell were found at deep cortex adjacent corpus callosum around transplantation route (Fig. 5B).

In double-labeled immunohistochemical stain to identify neuronal differentiation of transplanted ADSCs, any ADSCs was not immunostained with MBP and GFAP antibody.

DISCUSSION

The aim of the present study was to conform the characteristics of hADSCs as stem cell in *vitro* and to investigate

the survival, migration and differentiation of transplanted hADSCs into rat brain. Recent reports demonstrate adult stem cells are found in various organ. Mesenchymal stem cells exist in the central nervous system, which previously has been known not to be regenerative and recent experiments show that neural stem cells are able to undergo expansion and differentiation into neurons, astrocytes and oligodendrocytes[4,7,14,19]. Therefore, neural stem cells are the ideal potential cell sources of transplantation to treat refractory neurological diseases. However, because they are located at deep portion such as hippocampus, dentate gyrus and subventricular zone, they cannot be harvested without brain injury and used in clinical practices[4,7,14,19].

Controlled neural differentiation of mesenchymal adult stem cells might become an important source of cells for cell therapy of refractory neurological diseases, since autologous mesenchymal stem cells are more easily harvested and effectively expanded than corresponding neuronal stem cells. Adipose tissue like bone marrow is derived from the embryonic mesoderm and contains a heterogenous stromal cell population[12]. Primary cultures of adipose tissues are heterogenous population containing pericytes endothelial cell, hematopoietic cells and smooth muscle cells[22]. However, Gronthos et al.[6] reported that the frequency of these other cells appears to be diminished quickly through serial passages in culture and, in particular, hematopoietic markers as well as epithelial markers are not present by 2 to 3 passage of culture. We used human ADSCs of 5 passages for this study and observed that more than 90% of cells expressed CD29 and CD44, mesenchymal cell markers, not CD34 with, hematopoietic marker indicating that most cells are mesenchymal stem cells. Non expression of neuronal marker such as Tau, Trk-A and GFAP in undifferentiated ADSCs in our experiment are consistent with the previous reports in stromal cells of bone marrow[16]. After neuronal differentiation in *vitro*, human ADSCs revealed immunohistochemical characteristics of neuronal lineage. Like previous reports of BMSCs, our experiment demonstrated that ADSCs differentiate into adipocyte, osteocytes and neuron-like cells in *vitro*[5,15,16,20].

A recent report demonstrated that hMSCs from bone marrow express several neurotrophic factors that could participate in hMCS-mediated functional improvement[17]. Probably, functional improvements after transplantation were attributed to neurotrophic factors or angiogenic factors released from transplanted ADSCs rather than by neuronal differentiation of transplanted cells. Therefore, as shown in our results, only survival of transplanted hADSCs in recipient might be considered to expect good functional outcome in brain injury models.

The therapeutic use of ADSCs or BMSCs requires large quantities of cells for infusion or co-transplantation. A large quantity of ADSCs can be obtained by lipectomy, which is currently performed with local anesthesia on healthy people[1]. In addition to advantage in obtaining sufficient autologous stem cells, ADSCs from allogenic donor could constitute a valuable alternative source of stem cells for therapeutic use and the infusion of allogenic stem cells from a healthy donor, or even from a third party, in case of transplantation, could form the basis for new strategies in cell therapy[13]. A prerequisite when considering allogenic ADSCs for therapeutic use is the characterization of their immunological properties in allogenic conditions. Several studies reported on the in *vitro* and in *vivo* immunosuppressive properties of BM-MSC[2,3,10,18]. Furthermore, BM-MSC were shown escape to the immune system because they do not express major histocompatibility complex (MHC) class II or co-stimulatory molecule B7 and, consequently, they do not induce allogenic T-cell proliferative response[18]. In contrast, despite the increasing interest in the use of ADSCs in cell therapy, their immunosuppressive properties have not been studied yet. In our experiment, HLA-DR, one of the antigen of HLA class II involved in transplantation immune responses, was not expressed in undifferentiated ADSCs and human ADSCs transplanted into rat brain survived and migrated 4 weeks later without the use of immunosuppressive agent. The authors speculated that ADSCs might have similar immune suppressive mechanisms compared with BM-MSC. Yeu et al.[21] demonstrated a lot of transplanted allograft cells (rat-BMSC) into rat brain survived in injection site and migrated from the injection tract along the corpus callosum. But, our experiment showed a small number of injected xenograft cells (human ADSCs) in near injection route and migrated along corpus callosum and no hADSC was differentiated into neuronal lineage. Probably, this discrepancy might be due to the different growth factors and chemical mediators acting on the environment of cell growth between different species. Considering that survival, migration and differentiation of the transplanted cells were determined by the microenvironment of the recipient-the recipients used in our study did not undergo any procedures to induce ischemia or trauma prior to human ADSCs transplantation and were different species from human-it may be thought that transplanted human ADSCs into rat brain were rarely differentiated into neuronal cells.

CONCLUSION

The human ADSCs were successfully subcultured and differentiated into adipocyte, osteocyte and neuron like

cells in the laboratory and survived and migrated after transplantation into rat brain. It is suggested that ADSCs may play a significant role in the stem cell therapy in neurological diseases. Further research of the host's immune response to undifferentiated and differentiated ADSCs and in diverse host environments is warranted.

References

1. Aust L, Devlin B, Foster SJ, Halvorsen YD, Hicok K, du Laney T, et al : Yield of human adipose-derived adult stem cells from liposuction aspirates. **Cytotherapy 6** : 7-14, 2004
2. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, et al : Mesenchymal stem cells suppress lymphocyte proliferation in *vitro* and prolong skin graft survival in vivo. **Exp Hematol 30** : 42-48, 2002
3. Djouad F, Plence P, Bony C, Tropel P, apparailly F, Sany J, et al : Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogenic animals. **Blood 102** : 3837-3844, 2003
4. Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, Suhonen JO, et al : Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. **Proc Natl Acad Sci USA 92** : 11879-11883, 1995
5. Grimble JM, Guilak F : Adipose-derived adult stem cells : isolation, characterization and differentiation potential. **Cytotherapy 5** : 362-369, 2003
6. Gronthos S, Franklin DM, Leddy HA, Robey PG, Storms RW, Gimble JM : Surface protein characterization of human adipose tissue-derived stromal cells. **J Cell Physiol 189** : 54-63, 2001
7. Johansson CB, Momma S, Clarke DL, Risling M, Lendahl U, Frisen J : Identification of a neural stem cell in the adult mammalian central nervous system. **Cell 96** : 25-34, 1999
8. Kang SK, Lee DH, Bae YC, Kim HK, Baik SY, Jung JS : Improvement of neurological deficits by intracerebral transplantation of human adipose tissue-derived stromal cells after cerebral ischemia in rats. **Exp Neurol 183** : 355-366, 2003
9. Kang SK, Putnam LA, Ylostalo J, Popescu IR, Dufour J, Belousov A, et al : Neurogenesis of Rhesus adipose stromal cells. **J Cell Sci 117** : 4289-4299, 2004
10. Le Blanc K, Tammik L, sundberg B, Haynesworth SE, Ringden O : Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. **Scand J Immunol 57** : 11-20, 2003
11. Lee RH, Kim BC, Choi IS, Kim HN, Choi HS, Suh KT, et al : Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. **Cell Physiol Biochem 14** : 311-324, 2004
12. Pettersson P, Cigolini M, Sjoestroem L, Smith U, Bjoerntorp P : Cells in human adipose tissue developing into adipocytes. **Act Med Scand 215** : 447-451, 1984
13. Puissant B, Barreau C, Bourin P, Clavel C, Corre J, Bousquet C, et al : Immunomodulatory effect of human adipose tissue-derived adult stem cells : comparison with bone marrow mesenchymal stem cells. **Br J Haematol 129** : 118-129, 2005
14. Reynold BA, Weiss S : Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. **Science 255** : 1707-1710, 1992
15. Safford KM, Hicok KC, Safford SD, Halvorsen YD, Wilkison WO, Gimble JM, et al : Neurogenic differentiation of murine and human adipose-derived stromal cells. **Biochem Biophys Res Commun 294** : 371-379, 2002
16. Sanchez-fRamos J, Song S, Cardozo-pelaez F, Hazzi C, stedeford T, Willing A, et al : Adult bone marrow stromal cells differentiate into neural cells in *vitro*. **Exp Neurol 164** : 247-256, 2000
17. Tremain N, Korkko J, Ibberson D, Kopen GC, DiGirolamo C, Phinney DG : MicroSAGE analysis of 2,353 expressed genes in a single cell-derived colony of undifferentiated human mesenchymal stem cells reveals mRNAs of multiple cell lineages. **Stem Cells 19** : 408-418, 2001
18. Tse WT, Pendleton JD, Beyer WM, Egalka MC, Guinan EC : Suppression of allogenic T-cell proliferation by human marrow stromal cells : implications in transplantation. **Transplantation 75** : 389-397, 2003
19. Vescovi AL, Reynolds BA, Fraser DD, Weiss S : bFGF regulates the proliferative fate of unipotent (neuronal) and bipotent (neuronal/astroglial) EFG-generated CNS progenitor cells. **Neuron 11** : 951-966, 1993
20. Woodbury D, Schwarz EJ, Prockop DJ, Black IB : Adult rat and human bone marrow stromal cells differentiate into neuron. J **Neurosci Res 61** : 364-370, 2000
21. Yeu IS, Lee HJ, Yi JS, Yang JH, Lee IW, Lee HK : The survival and migration pattern of the bone marrow stromal cells after intracerebral transplantation in rats. **J Korean Neurosurg Soc 36** : 400-404, 2004
22. Zuk PA, Zuh M, Mizuno H, Huang J, Futrell JW, Katz, AJ, et al : Multilineage cells from human adipose tissue; implications for cell-based therapies. **Tissue Eng 7** : 209-226, 2001