발생과 생식 제 12권 제 1호, 31~39 (2008)
Dev. Reprod. Vol. 12, No. 1, 31~39 (2008)

Differentiation of Dopaminergic and Cholinergic Neurons from Mesenchymal-like Stem Cells Derived from the Adipose Tissue

In-Kyung Hong, Na Hee Jeong, Ju Ran Kim, Byung-Rok Do[1], Hea Kwon Kim[2] and Sung Goo Kang†

School of Biotechnology and Biomedical Science, Inje University, Gimhae 621-749, Korea
[1]Bioengineering Institute, Hurim Biocell Inc., Seoul 153-803, Korea
[2]Dept. of Biotechnology, Seoul Women's University, Seoul 139-774, Korea

사람 지방 유래 중간엽 줄기세포의 도파민성 및 콜린성 신경세포분화

홍인경 · 정나희 · 김주란 · 도병록[1] · 김해권[2] · 강성구†

인제대학교 생명공학부, [1]휴림바이오셀 생명공학연구소,
[2]서울여자대학교 생명공학과

ABSTRACT : Neural tissue has limited intrinsic capacity of repair after injury, and the identification of alternate sources of neural stem cells has broad clinical potential. We isolated mesechymal-like stem cells from human adipose tissues (AT-MSCs), and studied on transdifferentiation-promoting conditions in neural cells. Dopaminergic and cholinergic neuron induction of AT-MSCs was also studied. Neural differentiation was induced by adding bFGF, EGF, dimethyl sulphoxide (DMSO) and butylated hydroxyanisole (BHA) in N2 Medium and N2 supplement. The immunoreactive cells for β-tubulin Ⅲ, a neuron-specific marker, GFAP, an astrocyte marker, or Gal-C, an oligodendrocyte marker, were found. AT-MSCs treated with bFGF, SHH and FGF8 were differentiatied into dopaminergic neurons that were immunopositive for TH antibody. Differentiation of MSCs to cholinergic neurons was induced by combined treatment with basic fibroblast growth factor (bFGF), retinoic acid (RA) and sonic hedgehog (Shh). AT-MSCs treated with DMSO and BHA rapidly assumed the morphology of multipolar neurons. Both immunocytochemistry and RT-PCR analysis indicated that the expression of a number of neural markers including neuro D1, β-tubulin Ⅲ, GFAP and nestinwas markedly elevated during this acute differentiation. While the stem cell markers such as SCF, C-kit, and Stat-3 were not expressed after preinduction medium culture, we confirmed the differentiation of dopaminergic and cholinergic neurons by TH/ β-tubulin Ⅲ or ChAT/ β-tubulin Ⅲ positive cells. Conclusively, AT-MSCs can be differentiated into dopaminergic and cholinergic neuronsand these findings suggest that AT-MSCs are alternative cell source of treatment for neurodegenerative diseases.

Key words : Human adipose tissue, Mesenchymal stem cells, Cholinergic neuron, Dopaminergic neuron.

요 약 : 손상된 뇌신경조직내에서 신경줄기세포로부터 새로운 신경세포로의 분화가 상당히 제한되어 있어 이것이 손상된 뇌신경조직의 복구가 잘 이루어지지 않는 원인이라 여겨지고 있다. 본 연구에서는 세포배양을 통해 지방조직 중간엽 줄기세포를 도파민성 신경세포와 콜린성 신경세포로 분화를 유도하였다. 중간엽 줄기세포를 신경세포로 분화시키기 위해 N2배양액에 bFGF, EGF, dimethyl sulphoxide (DMSO)와 butylated hydroxyanisole (BHA)를 첨가하여 유도하였다. DMSO와 BHA에 처리된 중간엽 줄기세포가 빠르게 신경세포 모양으로 분화하는 것을 관찰하였으며, 이것은 면역조직학적 염색에서 신경세포 특이 표지인 β-tubulin Ⅲ, 별아교세포에 대한 특이 표지인 GFAP, 흰돌기아교세포에 대한 특이 표지인 Gal-C에 대해 양성반응을 나타내었다. RT-PCR 분석에서 배양 단계에 따라 신경세포에 특이적인 표지 인자인 neuro D1, β-tubulin Ⅲ, GFAP, nestin 등의 발현을 통해, 중간엽 줄기세포가 신경세포로 분화됨을 확인하였다. 그러나 중간엽줄기세포가 신경세포로 분화된 이후에는 줄기세포 표지인 SCF, C-kit와 stat-3 등은 발현되지 않았다. 또한, 중간엽줄기세포에 bFGF, SHH와 FGF8 등을 처리하면 도파민 신경세포로 분화하였다. 중간엽 줄기세포에 bFGF, RA, Shh를 처리하여 콜린성 신경세포로 분화

† Correspondence: School of Biotechnology and Biomedical Science, Inje University, Gimhae 621-749, Korea, Tel: +82-55-320-3213, Fax: +82-55-336-7706, E-mail: biosgkan@inje.ac.kr

시켰을 때, 신경세포 특이 표지인 β-tubulin Ⅲ와 콜린성 신경 특이 표지인 ChAT에 양성반응를 보였다. 결론적으로 사람 지방조직의 중간엽 줄기세포가 도파민성과 콜린성 신경세포로 분화가 가능하고 이러한 잠재성을 가진 지방 유래 중간엽 줄기세포는 퇴행성 신경질환에 대한 세포 치료제로서 가능성을 제시한다.

INTRODUCTION

Adult stem cells including hematopoietic, neural, gastro-intestinal, epidermal, hepatic and mesenchymal stem cells exist in most tissues (Tuan et al., 2003). Recently, there are increasing body of evidences suggesting that adult stem cells can differentiate into lineages other than the origin and certain adult stem cell types are pluripotent (Dazzi & Horwood, 2007; Mays et al., 2007). MSCs are one of the well-studied adult stem cells. In recent years, great interest has been generated in MSCs, due to their ability to self-renew, proliferate, and differentiate into a variety of cell types. They can be reprogrammed by extracellular cues to alter their character in ways that could have important implications for cell therapy and other forms of regenerative treatment. Tissue damage in the central nervous system is generally thought to be incapable of self- repair because mature neurons lack the ability to regenerate, and the residing population of neural stem cells that is capable of neuronal differentiation is slow and limited in its ability to generate new functional neurons in response to injury. Therefore an alternative source of stem cell is needed for the therapy of the brain damage.

Adipose tissue may be a source of mesenchymal stem cells. These stem cells, termed human mesenchymal-like stem cells from adipose tissue (AT-MSCs), can be easily isolated in larger quantities compared with bone marrow (Zuk et al., 2002). In a recent study, we isolated human AT-MSCs and analyzed its characteristics (Park et al., 2007). Recently, a number of studies have shown that MSCs could differentiate into neuron and glial cells *in vitro* and *in vivo*. Ischemic rats that received human MSCs exhibited significantly improved functional performanceand histological analysis revealed that transplanted human MSCs expressed markers for astrocytes, oligodendrocytes, and neurons (Zhaoet al., 2002). When MSCs were injected into the Parkinson's disease model mice, the transplantation resulted in the significant improvement on the rotarod test and some of these cells showed immunoreactivity for tyrosine hydroxylase (TH) (Lindvall et al., 2004). Adult rat bone marrow cultured *in vitro* with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) gave rise to cellular spheres which differentiated into neurons and glia (Kabos et al., 2002). Human bone marrow stromal cells also differentiated into a neuronal phenotype, under asimple culture condition (Woodbury et al., 2000) or by co-culture with rat fetal mesencephalic or striatal cells (Sanchez-Ramos et al., 2001). These observations indicate that MSCs can differentiate into neural cells. Therefore MSCs could restore the damaged brain function and thus might be suitable for cell therapy.

The dopaminergic (DA) neurons play a key role in the function of a variety of brain systems, including motor control and reward pathways. The development of midbrain dopaminergic (DA) neurons requires a complex combination of transcriptional regulators and diffusible signals to control both the acquisition and maintenance of a neurotransmitter-specific phenotype. Their initial specification requires the patterning information that is provided by sonic hedgehog (SHH) and fibroblast growth factor 8 (FGF8). The Lmx1b and nurr1 transcription factors are essential for the DA differentiation. Many studies have shown that under appropriate culture conditions *in vitro*, stemcells can be differentiated into DA neurons. Human embryonic stem cells were differentiated into midbrain DA neurons by means of the sequential application of differentiation molecules (Perrier et al., 2004). Using similar protocols, Human mesencephalic mesenchymal stem cells was induced to

DA neurons (Guo et al., 2005).

The cholinergic system is implicated in functional, behavioraland pathological states of brain. Degeneration of the cholinergic system of the basal forebrain occurs in many neurodegenerative diseases including Alzheimer's disease (Arendt et al., 1983) and Parkinson's disease (Dubois et al., 1983). Cholinergic neuron differentiation involves several steps. ES cells generate early neuroectodermal cells, which are organized into rosettes and express neuroectodermal transcription factorPax6 but not Sox1, and then late neuroectodermal cells, which form neural tube-like structures and express both Pax6. Only the early, neuroectodermal cells are efficiently posteriorized by retinoic acid (RA) and in the presence of sonic hedgehog (Shh), differentiate into cholinergic neurons. Human ES cells differentiate cholinergic neurons by treatment with bFGF, RA, and Shh (Li et al., 2005; Shin et al., 2005). Cholinergic neuron differentiation from mouse ES cells were induced by coculturing with murine bone marrow-derived stromal feeder cell lines (Barberi et al., 2003). However, few studies on dopaminergic and cholinergic differentiation of AT-MSCs have done *in vitro*.

In the present study, we tried to induce neural cells from AT-MSCs and further differentiation to dopaminergic and cholinergic neurons *in vitro* whether AT-MSCs could be used as therapeutical cells for the brain damage.

MATERIALS AND METHODS

1. Isolation and Culture of AT-MSCs from Adipose tissue

Human adipose tissue was collected from healthy donor by liposuction. Adipose tissues were transported to the laboratory in saline solution within 2 h post-surgery. The tissues were washed at least three times with 2 vol of Ca^{2+}, Mg^{2+}-free Dulbecco's phosphate-buffered saline (DPBS, Gibco, Grand Island, NY) to remove blood. The tissue was then digested with 1 vol of 0.075% collagenase type I (Sigma, St Louis, MO) for 30 min at 37℃ with intermittent shaking. Enzyme activity was neutralized with 10% fetal bovine serum (Gibco), and the cells were centrifuged at 500×g for 5 minutes at 20℃. The resulting pellet, containing AT-MSCs, was resuspended in the Dulbecco's Modified Eagle Medium with low glucose (DMEM-LG, Gibco) supplemented with 100 U/mL penicilln, 100 μg/mL streptomycin, 3.7 mg/mL sodium bicarbonateand 10% fetal bovine serum (FBS, Hyclone, South Logan, UT). After cell counting, cell suspension was seeded in noncoated 75-cm^2 culture flasks with a density of approximately 2×10^3 cells/cm^2. A fresh complete culture medium was added every 3 days.

2. Induction of Neural Cells from AT- MSCs

AT-MSCs were induced to differentiate into neural cells by the modified method of Woodbury et al. (Woodbury et al., 2000). The cells were grown to 50 to 60 percent confluency, at which point neural differentiation was initiated. Cells were cultivated in DMEM supplemented with 20% FBS, 0.1% β-mercaptoethanol (BME, Sigma), 1X nonessential amino acid (Gibco), and 2 mM L-glutamine (Gibco). The cells are cultured for 2~3 days. Twenty four hours prior to neural induction, medium were substituted with preinduction medium consisting of 1×N2 supplement (Gibco), 20 ng/mL epithelial growth factor (EGF, Sigma) and 20 ng/mL basic fibroblast growth factor (bFGF, Sigma) in the N2 medium (Gibco). Neuronal differentiation was induced with 2% dimethylsulfoxide (DMSO, Sigma) and 200 μM butylated hydroxyanisol (BHA, Sigma) in N2 medium plus1X N2 supplement. After 5 h, the medium was modified for long-term induction by adding 25 mM KCl, 2 mM valproic acid (Sigma), and 1 μM hydrocortison (Sigma) and incubation for 1.5 days, the cells were subjected to immunocytochemistry.

3. Dopaminergic Neuron Differentiation of AT-MSCs

AT-MSCs were plated at 1×10^4 cells on 4-well cell culture plates in DMEM, 20% FBS, 1X non essential amino acid, 10 μg/mL L-glutamin, 0.1% BME. After 3

days in plain medium, culture medium was substituted with DMEM, 20% FBS, 20 μg/mL EGF, 20 ng/mL bFGF for 24 hours. Dopaminergic differentiation medium consists of 100 ng/mL FGF8, 200 ng/mL SHH, 0.2 mM ascorbic acid (AA), 20 ng/mL brain-derived neurotrophic factor (BDNF) in N2 medium (Perrier et al., 2004). After additional 7~9 days of culture, cells were cultured in the presence of 20 ng/mL BDNF, 20 ng/mL glial cell line-derived neurotrophic factor (GDNF), 1 mM dibutyryl cAMP, 0.2 mM AA for 3 days.

4. Induction of Cholinergic Neurons from AT-MSCs

Cholinergic neuronal differentiation was induced by modified method of Barberi et al. (Barberi et al., 2003). The AT-MSCs were cultured on cover slips coated with poly-D-lysine, 15 mg/mL polyornithine (Sigma) and 1 mg/mL laminin (Sigma) in tissue culture plate for 2~3 days for dedifferentiation. Then, the cells were cultured in the pre-induction medium for overnight. At day 4 of culture, 500 ng/mL Shh (R&D) and 1 μM RA (Sigma) were added to N2 medium and then the cultured was transferred to N2 medium supplemented with 10 ng/mL bFGF and 500 ng/mL Shh from day 8 to day 11. Differentiation was induced at day 11 in N2 medium supplemented with 200 μM ascorbic acid (AA: Sigma) and 20 ng/mL brain derived neurotrophic factor (BDNF; Gibco).

5. Immunocytochemistry

Immunocytochemistry was done by using immunofluorescence staining. For immunofluorescence, cells were washed with PBS and fixed by treating with 4 % paraformaldehyde at room temperature for 30 min. This was followed by washing with three changes of PBS and PBS plus 0.2 % Triton X-100. Slides were blocked with 10 % normal goat serum and normal rabbit serum (derived from the same species as the secondary antibody) in PBS for 1 hr, β-tubulin Ⅲ (1:400, Sigma) for 24 hr at 4℃ or for 3 hr at RT. The slides were then washed with PBS, and incubated with antibody for TH (1:250, Vector Lab.) or choline acetyltransferase (ChAT) (1:100, Vector Lab.) and washed for three times with PBS and incubated with a Cy3-conjugated rabbit anti-goat IgG (1:500, Chemicon) and FITC-conjugated goat anti-rabbit IgG (1:128, Sigma) antibody for 30 min. at 4℃. Mounted slides were observed with a fluorescence microscope (Carl Zeiss Axioskop2+, Germany) to visualize the cellular colocalization of ChAT- or TH- positive cell population in β-tubulin Ⅲ positive cells.

6. RNA Isolation and RT-PCR

Cells were harvested and washed once in PBS. Total RNA was extracted from cultured cells using (Sigma), and then 5 μg per condition reverse- transcribed first-strand cDNA using oligo-dT primer (Promega). Reverse transcription was carried out in the presence of reverse transcriptasefor 1 hr at 42℃ followed by inactivation at 99℃ for 5 min and cooling to 4℃. The cDNA was amplified by 35 cycles of PCR using Taq polymerase (Promega). The amplified cDNAs were separated on 1 % gels, and the bands were visualized by ethidium bromide staining.

RESULTS

1. Induction of Neural Cells from AT-MSCs

AT-MSCs that had been grown to near confluence were treated with EGF and bFGF overnight in the preinduction media and then in the N2 medium plus BHA and DMSO, according to the neural-inducing protocol described by Woodbury et al (Woodbury et al., 2000). This treatment induced AT-MSCs to undergo profound changes in morphology (Fig. 1), with some cells developing multiple neurite extensions from cell body (Fig. 1C). Immunocytochemical staining for neural markers was performed to confirm the neural cells. The immunoreactive cells for β-tubulin Ⅲ, a neuron-specific marker, GFAP, an astrocyte marker, or Gal-C, an oligodendrocyte marker, were found (Fig. 2). RT-PCR analysis confirmed that AT-MSCs differentiated into neural cells. Nestin expression is restricted to

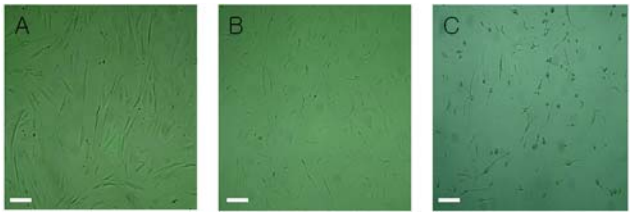

Fig. 1. Changes in cellular morphology of AT-MSCs during neural differentiation. A, AT-MSCs before addition of differentiation medium. Most cells were fibroblast like. B, the cells were exposed to 20 ng/mL bFGF and EGF preinduction medium for 24 hr. Cell morphology became thin. C, the cells were exposed to neural induction medium containing DMSO and BHA for 1.5 days. Extensive outgrowth of cellular processes was noted. bar=100 μm.

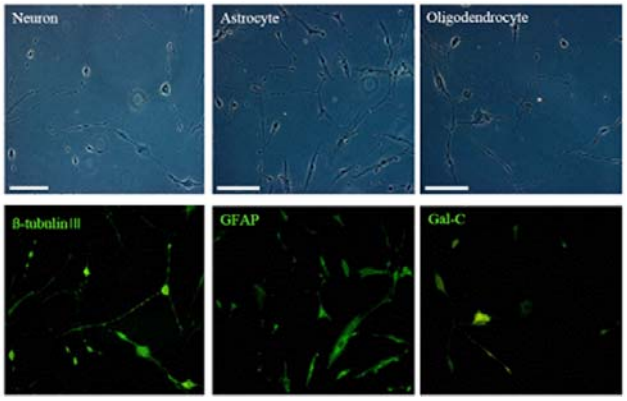

Fig. 2. Cell type-specific immunofluorescence staining of AT-MSCs cultured inneural induction medium. After culture in neural induction medium for 1.5 days, immunohistochemistry was done with antibodies for β-tubulin Ⅲ, a neuronal marker, GFAP, an astrocyte marker and Gal-C, an oligodendrocyte maker. Scale bar represents 100 μm.

neuroepithelial precursor cells in the neural tube and thus has been used as a cell type-specific marker for neural stem and precursor cells. While the stem cell markers such as SCF, C-kit, and Stat-3 were not expressed after preinduction medium culture, expression of β-tubulin Ⅲ and GFAP were induced during neural differentiation (Fig. 3).

2. Dopaminergic and Cholinergic Neuron Differentiation

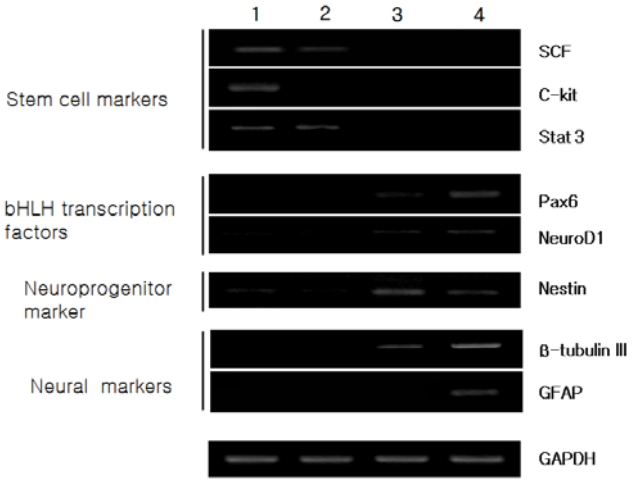

Fig. 3. Expression of various specific maker genes in AT-MSCs during nueral differentiation. RT-PCR analyses showed that SCF, C-kit and Stat-3 (stem cell markers) were only expressed by plain medium, and an increasing tendency in levels of Nestin (neural progenitor marker) after culture in preinduction medium. Expressions of Pax-6 (ectoderm markers), β-tubulin Ⅲ and NeuroD1 (neuronal markers) and GFAP (astrocyte marker) were induced by neural induction medium. Reaction condition and cycle number for PCR was optimized for each gene. Lane 1, plain media; lane 2, dedifferentiation media; lane 3, preinduction media; lane 4, cells grown in neural differentiation media.

We further studied immunocytochemistry to identify dopaminergic or cholinergic cells respectively after dopaminergic or cholinergic neuronal induction. Fig. 4 shows dopaminergic differentiation of AT-MSCs. Dopaminergic phenotype was confirmed by *in vitro* morphological examination including fluorescence microscopic visualization of β-tubulin Ⅲ and TH-positive cell. Fig. 5 shows cholinergic differentiation of AT-MSCs. Cholinergic phenotype was confirmed by *in vitro* morphological examination including fluorescence microscopic visualization of β-tubulin Ⅲ and ChAT-positive cell.

DISCUSSION

Adipose tissue has been identified as an alternative

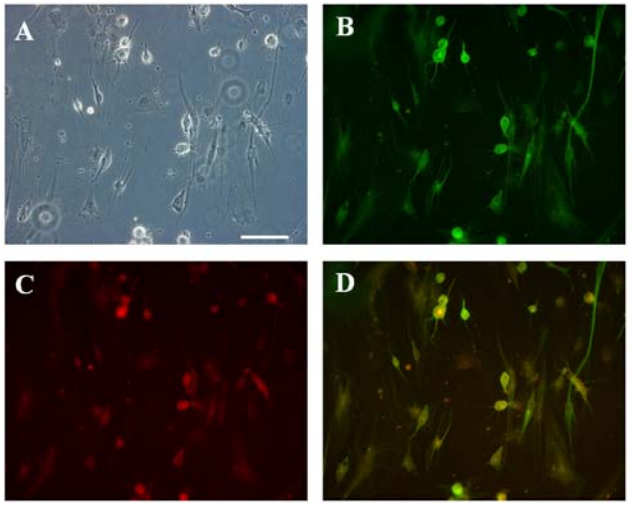

Fig. 4. Dopaminergic differentiation of AT-MSCs. Cell-type specific immunostaining for dopaminergic neurons were performed after dopaminergic induction. A, Phase contrast micrographs of differentiated cells. B, Green fluoresce indicates β-tubulin Ⅲ-immunoreactivity. C, Red fluoresce indicates TH-immunoreactivity. D representsthe merged images of the B and C figures. Scale bar represents 100 μm.

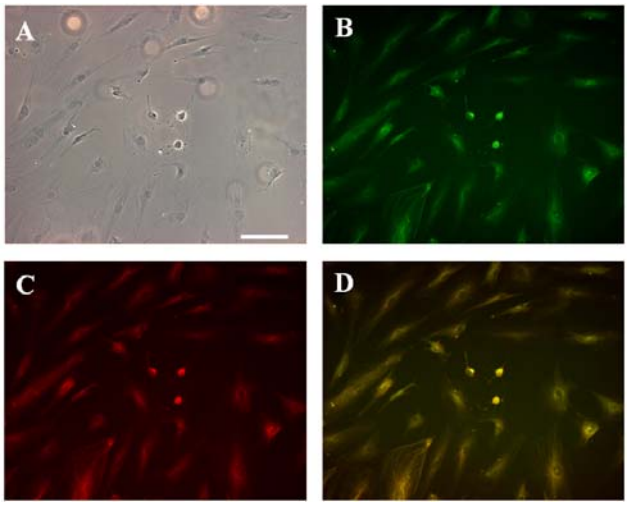

Fig. 5. Cholinergic differentiation of AT-MSCs. Cell-type specific immunostaining for Cholinergic neurons were performed after Cholinergic induction. A, Phase contrast micrographs of differentiated cells. B, Green fluoresce indicates β-tubulin Ⅲ-immunoreactivity. C, Red fluoresce indicates ChAT-immunoreactivity. D represents the merged images of the B and C figures. Scale bar represents 100 μm.

source of stromal cells capable of differentiating into mesodermal lineages. Previous studies have demonstrated the osteogenic, chondrogenic, adipogenic, myogenic, and cardiomyogenic, potential of stromal cells isolated from adipose tissue (Harvorsen et al., 2001; Sen et al., 2001; Erickson et al., 2002; Charrière et al., 2003; Rangappa et al., 2003; Winter et al., 2003 Park et al., 2007). However, recent reports have documented the differentiation of MSCs to neural-like cells (Sanchez et al., 2000; Woodbury et al., 2000), suggesting that adult mesenchymal stem cells may not be as restricted in original lineagesas previously thought.

Here we suggest thatAT-MSCs retain the capacity to differentiate into nonmesenchymal derivatives, specifically neural cells. AT-MSCs showed a response to antioxidantssuch as DMSO and BHA. This antioxidant-dependent neural differentiation occurred with a remarkable speed so that we could observe major changes in cellular and molecular levels within 24 hr (Jeong et al., 2004). Cells with neuronal and glial phenotypes were induced, and several specific markers for neurons and glial cells were identified in these cells by RT-PCR and immunocytochemistry. DMSO- and BHA-treated cells expressed mRNA transcripts for β-tubulin Ⅲ, a neuron-specific marker, GFAP, an astrocyte marker or nestin, a neural progenitor marker, determined by RT-PCR. DMSO-and BHA-treated cells also showed a neural morphology and β-tubulin Ⅲ-, GFAP- or Gal-C-immunoreactivity, as demonstrated by immunocytochemistry.

A recent work on MSCs undergoing early neurogenic differentiation reported the expression of nestin, an intermediate filament protein thought to be expressed at high levels in neural stem cells (Sanchez et al., 2000). Consistent with this, nestin expression was also detected in AT-MSCs cultured in neurogenic media conditions.

Basic Helix-Loop-Helix (bHLH) transcription factors are known to regulate neuronal differentiation of embryonic stem cell and neural stem cells and appear in early stages of neural development (Bertrand et al., 2002). The bHLH

transcription factors included Pax6 and NeuroD1. Pax6, transcription factor crucial during the early phase of neurogenesis were not detected in control medium but expression was induced byneural induction medium. Also NeuroD1, which is reported to be expressed in subsets of CNS neurons during terminal differentiation (Lee et al., 1995), was expressed after cultivation in induction medium, indicating that NeuroD1 closely participates closely in transdifferentiation of AT-MSCs into neurons. Therefore, it might be possible to induce neuronal and glial cell types from AT-MSCs through controlling expression of neurogenic bHLH factor genes.

In a further study, We examed the possibility of dopaminergic differentiation of AT-MSCs. The development of midbrain dopaminergic (DA) neurons requires a complex combination of transcriptional regulators and diffusible signals to control both the acquisition and maintenance of a neurotransmitter specific phenotype (Maxwell et al., 2005). Their initial specification requires the patterning information that is provided by sonic hedgehog (SHH) and fibroblast growth factor 8 (FGF8) (Yan Y et al., 2005). Early exposure to FGF8 during the process of neuroepithelial specification directs the precursors preferentially to a midbrain fate and subsequent differentiation to midbrain DA neurons. Exposure of FGF2-expanded neuroepithelial cells to FGF8 and SHH promotes differentiation of DA neurons (Perrier et al., 2004). So We induced AT-MSCs to differentiate into dopaminergic neurons *in vitro* using a FGF2, SHH and FGF8. Up to 40% of all of the neurons express TH, the dopaminergic phenotype is confirmed by morphological *in vitro* examination including fluorescence microscopic visualization of TH-positive immunocytochemistry.

Also we tried to differentiate AT-MSCs into further specific cholinergic neuron. The cholinergic neurons in the human basal forebrain play a key role in neurodegenerative disorders such as Alzheimer's disease. In the present study, the AT-MSCs were differentiated into cholinergic neurons in N2 medium containing Shh, RA, bFGF, BDNF and AA combinatorially. Some cells of the differentiated AT-MSCs were shown to express ChAT, a cholinergic neuronal marker by immunocytochemistry. RA and Shh are well-known molecules involved in cholinergic neuron differentiation (Barberi et al., 2003). When mouse ES cells were induced to neural fate and exposed to Shh and RA, the differentiating population was preferentially directed to cholinergic neuron fate (Wichterle et al., 2002; Wu et al., 2002). In addition, bFGF alone induced long-term cultured fetal human neural stem cells to form cholinergic neurons (Weiset et al., 2001), suggesting that bFGF may also be an effective growth factor for *in vitro* differentiation to human cholinergic neurons. A recent study reported that combined treatment with Shh, RA, BDNF and AA had additive and synergistic effects on cholinergic neuron differentiation from human embryonic stem cells (Lee et al., 2007). We found that the combined treatment with such factors could differentiate AT-MSCs to cholinergic neuron phenotypes very efficiently.

In conclusion, AT-MSCs retain potential mesenchyme-related multipotency and to differentiate into cells that express neuronal and glial to differentiate into cells that express neuronal and glial markers *in vitro*. Also AT-MSCs differentiated into dopaminergic and cholinergic neuron *in vitro*. The findings here implicate that AT-derived MSCs may be alternative cell source of treatment for neurodegenerative diseases.

ACKNOWLEDGEMENTS

This work was supported by the 2006 Inje University research grant.

REFERENCES

Arendt T, Bigl V, Arendt A, Tennstedt A (1983) Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's Disease. Acta Neuropathol 61:101-108.

Barberi T, Klivenyi P, Calingasan NY, Lee H, Kawamata H, Loonam K, Perrier AL, BrusesJ, Rubio ME, Topf N, Tabar V, Harrison NL, Beal MF, Moore MA, Studer L (2003) Neural subtype specification of fertilization and nuclear transfer embryonic stem cells and application in parkinsonian mice. Nat Biotechnol 21:1200-1207.

Bertrand N, Castro DS, Guillemot F (2002) Proneural genes and the specification of neural cell types. Nat Rev Neurosci 3:517-530.

Charrière G, Cousin B, Arnaud E, André M, Bacou F, Penicaud L, Casteilla L (2003) Preadipocyte conversion to macrophage. Evidence of plasticity. J Biol Chem 278: 9850-9855.

Dazzi F, Horwood NJ (2007) Potential of mesenchymal stem cell therapy. Curr Opin Oncol 19:650-655.

Dubois B, Ruberg M, Javoy-Agid F, Ploska A, Agid Y (1983) A subcortico-cortical cholinergic system is affected in Parkinson's disease. Brain Res 288:213-218.

Erickson GR, Gimble JM, Franklin DM, Rice HE, Awad H, Guilak F (2002) Chondrogenic potential of adipose tissue-derived stromal cells *in vitro* and *in vivo*. Biochem Biophys Res Commun 290:763-769.

Guo L, Yin F, Meng HQ, Ling L, Hu-He TN, Li P, Zhang CX, Yu S, Duan DS, Fan HX (2005) Differentiation of mesenchymal stem cells into dopaminergic neuron-like cells *in vitro*. Biomed Environ Sci 18:36-42.

Halvorsen YD, Franklin D, Bond AL, Hitt DC, Auchter C, Boskey AL, Paschalis EP, Wilkison WO, Gimble JM (2001) Extracellular matrix mineralization and osteoblast gene expression by human adipose tissue-derived cells. Tissue Eng 7:729-741.

Jeong JA, Gang EJ, Hong SH, Hwang SH, Kim SW, Yang IH, Ahn C, Han H, Kim H (2004) Rapid neural differentiation of human cord blood-derived mesenchymal stem cells. Neuroreport 15:1731-1734.

Kabos P, Ehtesham M, Kabosova A, Black KL, Yu JS (2002) Generation of neural progenitor cells from whole adult bone marrow. Exp Neurol 178:288-293.

Lee H, Shamy GA, Elkabetz Y, Schofield CM, Harrsion NL, Panagiotakos G, Socci ND, Tabar V, Studer L (2007) Directed differentiation and transplantation of human embryonic stem cell-derived motoneurons. Stem Cells 25:1931-1939.

Lee JE, Hollenberg SM, Snider L, Turner DL, Lipnick N, Weintraub H (1995) Conversion of Xenopus ectoderm into neurons by NeuroD, a basic helix-loop-helix protein. Science 268:836-844.

Li XJ, Du ZW, Zarnowska ED, Pankratz M, Hansen LO, Pearce RA, Zhang SC (2005) Specification of motoneurons from human embryonic stem cells. Nat Biotechnol 23:215-221.

Lindvall O, Kokaia Z, Martinez-Serrano A (2004) Stem cell therapy for human neurodegenerative disorders-how to make it work. Nat Med 10 Suppl S:42-50.

Maxwell SL, Li M (2005) Midbrain dopaminergic development *in vivo* and *in vitro* from embryonic stem cells. Anat. 207:209-18.

Mays RW, van't Hof W, Ting AE, Perry R, Deans R (2007) Development of adult pluripotent stem cell therapies for ischemic injury and disease. Expert Opin Biol Ther 7:173-184.

Park S, Kang HM, Kim ES, Kim JY, Kim HK (2007) Characterization analysis for cardiogenic potential of three human adult stem cell. Dev Reprod 11:167-177.

Perrier AL, Tabar V, Barberi T, Rubio ME, Bruses J, Topf N, Harrison NL, Studer L (2004) Derivation of midbrain dopamine neurons from human embryonic stem cells. Proc Natl Acad Sci USA 101:12543-12548.

Rangappa S, Fen C, Lee EH, Bongso A, Sim EK (2003) Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. Ann Thorac Surg 75.

Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, Freeman TB, Saporta S, Janssen W, Patel N, Cooper DR, Sanberg PR (2000) Adult bone marrow stromal cells differentiate into neural cells *in vitro*. Exp Neurol 164:247-56.

Sanchez-Ramos JR, Song S, Kamath SG, Zigova T, Will-

ing A, Cardozo-Pelaez F, Stedeford T, Chopp M, Sanberg PR (2001) Expression of neural markers in human umbilical cord blood. Exp Neurol 171:109-115.

Sen A, Lea-Currie YR, Sujkowska D, Franklin DM, Wilkison WO, Halvorsen YD, Gimble JM (2001) Adipogenic potential of human adipose derived stromal cells from multiple donors is heterogeneous. J Cell Biochem 81:312-319.

Shin S, Dalton S, Stice SL (2005) Human motor neuron differentiation from human embryonic stem cells. Stem Cells Dev 14:266-269.

Tuan RS, Boland G, Tuli R (2003) Adult mesenchymal stem cells and cell-based tissue engineering. Arthritis Res Ther 5:32-45.

Weis C, Marksteiner J, Humpel C (2001) Nerve growth factor and glialcell line-derived neurotrophic factor restore the cholinergic neuronal phenotype in organotypic brain slices of the basal nucleus of Meynert. Neuroscience 102:129-138.

Wichterle H, Lieberam I, Porter JA, Jessell TM (2002) Directed differentiation of embryonic stem cells into motor neurons. Cell 110:385-397.

Winter A, Breit S, Parsch D, Benz K, Steck E, Hauner H, Weber RM, Ewerbeck V, Richter W (2003) Cartilage-like gene expression in differentiated human stem cell spheroids: a comparison of bone marrow-derived and adipose tissue- derived stromal cells. Arthritis Rheum 48: 418-429.

Woodbury D, Schwarz EJ, Prockop DJ, Black IB (2000) Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 61:364-370.

Wu P, Tarasenko YI, Gu Y, Huang LY, Coggeshall RE, Yu Y (2002) Region-specific generation of cholinergic neurons from fetal human neural stem cells grafted in adult rat. Nat Neurosci 5:1271-1278.

Yan Y, Yang D, Zarnowska ED, Du Z, Werbel B, Valliere C, Pearce RA, Thomson JA, Zhang SC (2005) Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. Stem Cells 23:781-790.

Zhao LR, Duan WM, Reyes M, Keene CD, Verfaillie CM, Low WC (2002) Human bone marrow stem cells exhibit neural phenotypes and ameliorate neurological deficits after grafting into the ischemic brain of rat. Exp neurol 174:11-20.

Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279-4295.