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Collection and Culture of Alveolar Bone Marrow Multipotent Mesenchymal Stromal Cells From Older Individuals

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ABSTRACT

In this work, we examined the culture condition of alveolar bone marrow multipotent mesenchymal stromal cells (ABMMSCs), aiming to apply regenerative therapy to older periodontitis patients. To better understand the character of cultured cells from alveolar bone marrow, the expression profiles of well-known genes and their responses to the induction of osteogenic, chondrogenic, or adipogenic differentiation were examined. Using αMEM-based culture, ABMMSCs could be obtained from older individuals than in previous reports. Interestingly, ABMMSCs expressing Klf4 were able to differentiate into osteoblasts. The prediction of differentiation potential by Klf4 could be a useful guide for further improvement of the culture conditions required to culture ABMMSCs derived from older individuals. J. Cell. Biochem. 107: 1198–1204, 2009.

KEY WORDS: REGENERATIVE MEDICINE; ORAL; ALVEOLAR; BONE MARROW; STROMA; OLDER INDIVIDUALS

The choice of stem cell source is a matter of great importance because it directly influences the success of regenerative therapy. While various stem cell sources have been reported [Barrilleaux et al., 2006; Bjerknes and Cheng, 2006; Dominici et al., 2006; Burke et al., 2007; Yu et al., 2008], iliac bone marrow [Matsubara et al., 2005], alveolar bone marrow [Matsubara et al., 2005], dental pulp [Gronthos et al., 2000], and periodontal ligament [Seo et al., 2004] have been examined as stem cells for oral regenerative therapy. Alveolar bone marrow is a realistic candidate because tooth extraction is needed to obtain stem cells from dental pulp and the periodontal ligament. In a previous report, the success of the human alveolar bone marrow multipotent mesenchymal stromal cells (ABMMSCs) culture was relatively limited to young patients [Matsubara et al., 2005].

In this study, we examined the culture condition of ABMMSCs, aiming to apply regenerative therapy to older periodontitis patients. To better understand the character of ABMMSCs, the expression profiles of well-known genes associated with the maintenance of undifferentiated embryonic stem cells [Ulloa-Montoya et al., 2007; Jiang et al., 2008], and associated with the induction of pluripotent stem cells from somatic cells [Takahashi et al., 2007], osteogenesis [Song and Tuan, 2004; Delorme et al., 2008; Ogata, 2008], chondrogenesis [Song and Tuan, 2004; Delorme et al., 2008], and adipogenesis [Song and Tuan, 2004; Delorme et al., 2008] were

Abbreviations Used: ABMMSCs, alveolar bone marrow multipotent mesenchymal stromal cells; BSP, bone sialoprotein; ESCs, embryonic stem cells; FGF2, fibroblast growth factor 2; IBMMSCs, iliac bone marrow multipotent mesenchymal stromal cells; MAPK, mitogen-activated protein kinase; MSCs, mesenchymal stem cells; α-MEM, α-minimum essential medium; Runx2, runt homeodomain protein 2; SMC, smooth muscle cell.

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investigated. Their responses to the induction of osteogenic, chondrogenic and adipogenic differentiation were also examined.

METHODS

MATERIALS

Dulbecco's modified Eagle's medium (DMEM), Alpha minimum essential medium (αMEM), fetal calf serum (FCS), penicillin and streptomycin, and TrypLE™ Express were obtained from Invitrogen (Carlsbad, CA). Fibroblast growth factor 2 (FGF2) was obtained from Sigma–Aldrich (St. Louis, MO).

CELL PREPARATION

The use of all tissue was approved by the Committee on the Use of Human Subjects in Research at Nihon University School of Dentistry at Matsudo (EC03-046 and EC04-001). Human iliac bone marrow multipotent mesenchymal stromal cells (IBMMSCs) were purchased from Cambrex Bio Science (Cat. No.2M-125C) (Walkersville, MD). Cryopreserved samples of IBMMSCs were thawed rapidly in a 37°C water bath, suspended in DMEM or αMEM containing 10% FCS, and plated at a seeding density of approximately 4×10^4 cells/cm^2. Alveolar bone marrow aspirates were obtained from patients for dental implant treatment. Aspirates were suspended in αMEM and plated at a seeding density of approximately $3.7–6.2 \times 10^3$ white blood cells/cm^2 (details shown in Table II). Cells were left undisturbed for 3–9 days until they adhered to culture dishes, excluding the addition of 3 ng/ml FGF2 from day 6 of culture. Medium containing 3 ng/ml FGF2 was then replaced with fresh medium at 3-day intervals. Passages were performed when cells became subconfluent, and cell numbers and population doublings were measured throughout the culture period.

MTT ASSAY

IBMMSCs suspended in DMEM, DMEM supplemented with 40 μg/ml L-proline and 50 μg/ml ascorbic acid, or αMEM were plated in sextuplicate at a density of 9.6×10^4 cell/well in 24-well plate (BD Biosciences, San Jose, CA). On day 10, culture medium and non-adherent cells were washed off, MTT (1 mg/ml) was added to each well, and the 24-well plate was incubated for 4 h. The cells were treated with lysis solution (50% dimethylformamide, 20% SDS) and incubated for 2 h. The degree of MTT reduction in each sample was assessed by measuring absorption at 570 nm.

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR)

Total RNAs were extracted from IBMMSCs and ABMMSCs (passage 5–6) using guanidine thiocyanate as described previously [Ogata et al., 1997, 2000]. RT-PCR was carried out with a SuperScript III One-Step RT-PCR system with Platinum Taq High Fidelity (Invitrogen) in a 50 μl mixture containing 0.1 μg total RNA, 0.2 μM of the primer sets (Table I) and 2× Reaction Mix according to the manufacturer's instructions. The cycling condition was 1 cycle (cDNA synthesis at 50°C for 30 min, RTase inactivation and pre-denaturation at 94°C for 2 min), 35 cycles (denaturation at 94°C for 30 s, annealing at 55°C for 30 s and extension at 72°C for 30 s) and 1 cycle (final extension at 72°C for 10 min). PCR products were separated by 2% agarose gel and visualized under ultraviolet illumination after ethidium bromide staining.

REAL-TIME PCR

Total RNA (1 μg) was used as a template for cDNA synthesis. cDNA was prepared using an EXScript RT Reagent Kit. Quantitative real-time PCR was performed using human Runx2, Osterix, Klf4, and GAPDH primer sets (Table I) using the SYBR Premix EX Taq in a

TABLE I. Details for Surveyed Genes and the Primer Pairs for Their Amplification

Gene symbol	Entrez gene ID	Gene official full name	Primer pairs
GAPDH	2597	Glyceraldehyde-3-phosphate dehydrogenase	Fwd: GCACCGTCAAGGCTGAGAAC Rev: ATGGTGGTGAAGACGCCAGT
POU5F1	5460	POU class 5 homeobox 1	Fwd: GCAATTTGCCAAGCTCCTGAA Rev: AAGCTAAGCTGCAGAGCCTCAAAG
SOX2	6657	SRY (sex determining region Y)-box 2	Fwd: GTGAGCGCCCTGCAGTACAA Rev: GCTGCGAGTAGGACATGCTGTAG
NANOG	79923	Nanog homeobox	Fwd: CAACTGGCCGAAGAATAGCAATG Rev: TGGTTGCTCCAGGTTGAATTGTT
KLF4	9314	Kruppel-like factor 4 (gut)	Fwd: AAGAGTTCCCATCTCAAGGCACA Rev: GGGCGAATTTCCATCCACAG
MYC	4609	v-Myc myelocytomatosis viral oncogene homolog (avian)	Fwd: CGGATTCTCTGCTCTCCTCGAC Rev: CCTCCAGCAGAAGGTGATCCA
BSP	3381	Integrin-binding sialoprotein	Fwd: CTGGCACAGGGTATACAGGGTTAG Rev: ACTGGTGCCGTTTATGCCTTG
RUNX2	860	Runt-related transcription factor 2	Fwd: ATGTGTGTTTGTTTCAGCAGCA Rev: TCCCTAAAGTCACTCGGTATGTGTA
OSTERIX	121340	Sp7 transcription factor	Fwd: GCCATTCTGGGCTTGGGTATC Rev: GAAGCCGGAGTGCAGGTATCA
FGF4	2249	Fibroblast growth factor 4	Fwd: GAGCAGCAAGGGCAAGCTCTA Rev: ACCTTCATGGTGGGCGACA
PPARγ	5468	Peroxisome proliferator-activated receptor gamma	Fwd: TGGAATTAGATGACAGCGACTTGG Rev: CTGGAGCAGCTTGGCAAACA
SOX9	6662	SRY (sex determining region Y)-box 9	Fwd: TGTATCACTGAGTCATTTGCAGTGT Rev: AAGGTCTGTCAGTGGGCTGAT
GDF3	9573	Growth differentiation factor 3	Fwd: GTACTTCGCTTTCTCCCAGACCAA Rev: CCTTTCTTTGATGGCAGACAGGTT
TERT	7015	Telomerase reverse transcriptase	Fwd: TGTGCTACGGCGACATGGA Rev: GAGGTGAGGTGTCACCAACAAGAA

TP800 thermal cycler dice real-time system (Takara, Tokyo, Japan). Amplification was performed in 25 μl final volume containing 2× SYBR Premix EX Taq (12.5 μl), 0.2 μM forward and reverse primers (0.1 μl) and 25 ng cDNA (2.5 μl) for Klf4, Runx2 and 10 ng (1.0 μl) cDNA for GAPDH. To reduce variability between replicates, PCR premixes, which contained all reagents except for cDNA, were prepared and aliquoted into 0.2 ml Hi-8-tubes (Takara). The thermal cycling conditions were 1 cycle at 95°C for 10 s, 40 cycles at 95°C for 5 s, and 60°C for 30 s. Post-PCR melting curves confirmed the specificity of single-target amplification and fold expressions relative to GAPDH were determined in quadruplicate.

INDUCTION OF OSTEOGENESIS, CHONDROGENESIS, AND ADIPOGENESIS

Osteogenic, chondrogenic, or adipogenic differentiation was induced in cells at passage 5–6 with a Human Mesenchymal Stem Cell Functional Identification Kit (R&D Systems) according to the manufacturer's instructions. Cells were cultured for 21 days in induction medium (Osteogenic; αMEM containing 10% FCS, dexamethasone, ascorbate-phosphate and β-glycerolphosphate, Chondrogenic; αMEM containing insulin, transferrin, selenious acid, bovine serum albumin, linoleic acid, dexamethasone, ascorbate-phosphate, proline, pyruvate, and TGF-β3, Adipogenic; αMEM containing 10% FCS, hydrocortisone, isobutylmethylxanthine and indomethacin. R&D Systems did not reveal the concentration of drugs in the media). In this period, induction medium was replaced with fresh medium every 3 days. On the 21st day of induction, the cultures were washed with PBS, fixed for 10 min in 4% buffered paraformaldehyde at room temperature and stained with Alizarin Red for osteogenic cells or Oil Red-O for adipogenic cells. For chondrogenic cells, cultures were fixed with 100% ethanol for 10 min at room temperature and stained with Alcian Blue.

STATISTICAL ANALYSIS

MTT light absorption data were analyzed using one-way analysis of variance followed by Bonferroni's multiple *t* tests.

RESULTS

DMEM needed 36 days to expand 4.5×10^6 IBMMSCs to 2.0×10^7 cells. αMEM needed only 15 days to expand 1.0×10^7 IBMMSCs to 2.2×10^7 cells. IBMMSCs cultured with DMEM (I–D) were flatter and larger (Fig. 1a) than IBMMSCs cultured with αMEM (I-α), which were small and clear (Fig. 1b). IBMMSCs cultured with DMEM supplemented with L-proline and ascorbic acid showed a significantly higher MTT value than those cultured with DMEM ($P < 0.01$), and those cultured with αMEM had the highest MTT value ($P < 0.01$) (Fig. 1c).

Three alveolar bone marrow specimens collected from separate individuals were cultured. The details of initial data for each aspirated specimen are shown in Table II. Figure 2 shows population doubling per colony of ABMMSCs in each specimen, and doubling time in the logarithmic phase was approximately 36 h.

Figure 3 shows the results of RT-PCR using I–D, I-α, and ABMMSCs (A-1, A-2, and A-3) cultured with αMEM. Remarkably

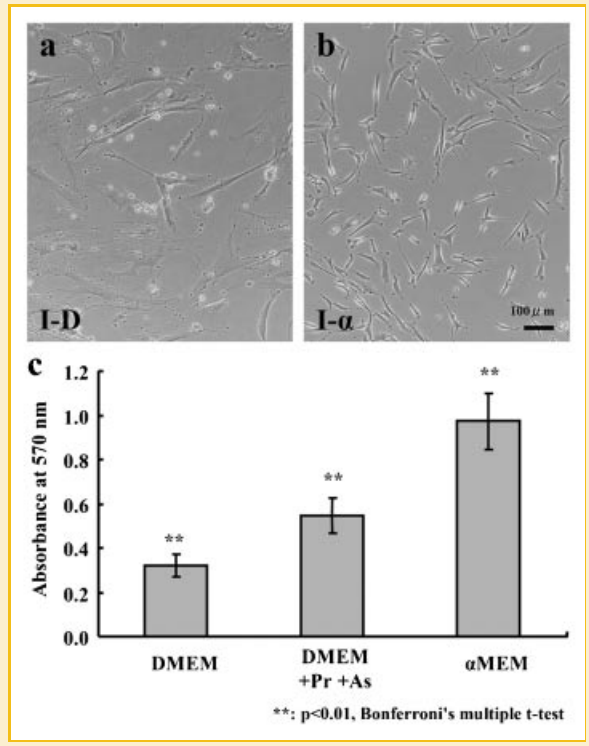

Fig. 1. Phase-contrast micrograph of iliac bone marrow multipotent mesenchymal stromal cells (IBMMSCs) cultured with (a) DMEM (I–D) and (b) αMEM (I–α). Two or more days were necessary for DMEM to produce equal numbers of cells compared with αMEM, and I–D was flatter and larger than I–α, which was small and clear. c: Difference in the number of primary cultured IBMMSCs among DMEM, DMEM supplemented with L-proline and ascorbic acid (DMEM + Pr + As), and αMEM. Significantly more IBMMSCs were cultured with DMEM + Pr + As than with DMEM ($P < 0.01$), and significantly more αMEM were cultured than the others ($P < 0.01$).

weak Nanog, c-Myc, bone sialoprotein (BSP) and Runx2, and lack of Klf4 gene expression in I-D. I-α and A-2 showed almost the same tendencies in all examined gene expressions except for Osterix. Weak c-Myc, BSP, Runx2, and Osterix gene expressions in A-1, and weak gene expression of Klf4, c-Myc, and BSP in A-3 were distinct. All specimens expressed POU5F1 and Sox2 at almost the same level, and did not express FGF4, PPARγ, Sox9, GDF3, and TERT. Quantitative real-time PCR was performed using human Runx2,

TABLE II. Detail Data for Primarily Cultured Alveolar Bone Marrow Aspirates

	Patient 1	Patient 2	Patient 3
Age	62	57	57
Sex	Female	Male	Female
Aspirated sample volume (μl)	250	400	30
Total number of red blood cells	1.2×10^8	9.8×10^7	9.8×10^7
Total number of white blood cells	1.2×10^5	1.7×10^5	9.1×10^4
WBC/RBC	1/1,000	1/578	1/1,081
Seeded sample volume (μl)	125	227	30
Seeded sample concentration (WBC/cm^2)	6.2×10^3	3.7×10^3	4.6×10^3
Number of colony (day 12)	5	10	35
Name of MSCs	ABMMSCs1	ABMMSCs2	ABMMSCs3

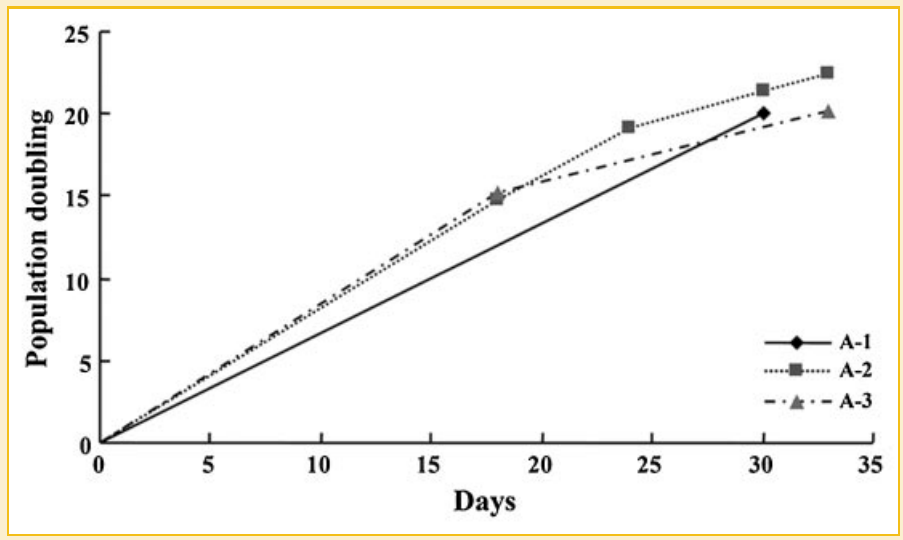

Fig. 2. Population doubling of alveolar bone marrow multipotent mesenchymal stromal cells cultured with αMEM (ABMMSCs-α) in each specimen. Vertical axis indicates base 2 logarithms of accumulated ABMMSCs-α numbers per colony, and horizontal axis indicates days of culture. All cases needed 1 month for ABMMSCs-α to expand up to 2^{20} (approximately 10^6) cells. ABMMSCs-α doubled every approximately 36 h. A-1, A-2, and A-3: ABMMSCs-α from patient 1, 2, and 3.

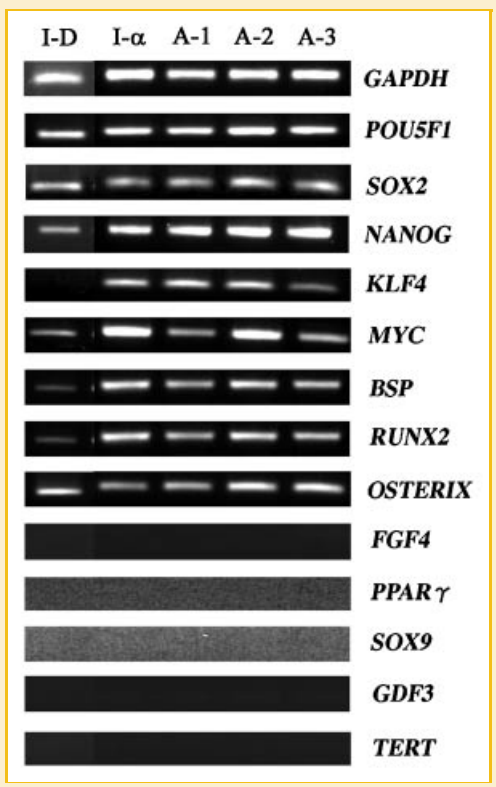

Fig. 3. Gene expression of IBMMSCs cultured with DMEM (I-D) and with αMEM (I-α), and ABMMSCs-α (A-1, A-2, A-3). Each gene was amplified with RT-PCR, and PCR products were separated by agarose gel electrophoresis and visualized under ultraviolet illumination after ethidium bromide staining. Lack of KLF4 gene expression in D-I, remarkably weak c-Myc, BSP, and Runx2 gene expression in A-1, weak gene expression of KLF4, c-Myc, and BSP in A-3.

Osterix, Klf4, and GAPDH primer sets to determine the exact levels of Klf4, Runx2, and Osterix gene expression in these cells. Results of real-time PCR were almost same as RT-PCR (Fig. 4).

Figure 5 shows the response of I-D, I-α, and ABMMSCs to the induction of osteogenic, chondrogenic and adipogenic differentiation. Osteogenic differentiation was strongly induced in I-α and A-1, moderately in A-2, slightly in A-3, and was not induced in I-D. Chondrogenic differentiations were induced in all specimens. Adipogenic differentiations were strongly induced in I-α and A-2, and slightly in A-1 and A-3.

DISCUSSION

Using an αMEM-based culture, ABMMSCs could be obtained from older individuals than in the previous report [Matsubara et al., 2005], and only the medium composition was different from the above report, in which DMEM had been used. In the IBMMSC culture, two or more days were necessary for DMEM to produce equal numbers of cells to αMEM, and moreover, IBMMSCs cultured with DMEM seemed to have the characteristic shape of extremely aged cells (Fig. 1a). DMEM could be a severe culture environment for cells from older individuals.

DMEM contains ferric nitrate and αMEM contains several kinds of amino acids, vitamins, lipoic acids and nucleic acids. The contribution of collagen to the adhesion of cells to the culture dish is expected because the addition of essential components (L-proline and ascorbic acid) for collagen production to DMEM significantly increased primary-cultured IBMMSCs (Fig. 1c). In addition, this was not due to radical-scavenger and reduction efficacy by ascorbic acid (data not shown). It has already been reported that integrin-mediated signaling prevented the anoikis of mesenchymal stem cells (MSCs) [Benoit et al., 2007] and also contributed to MSC survival

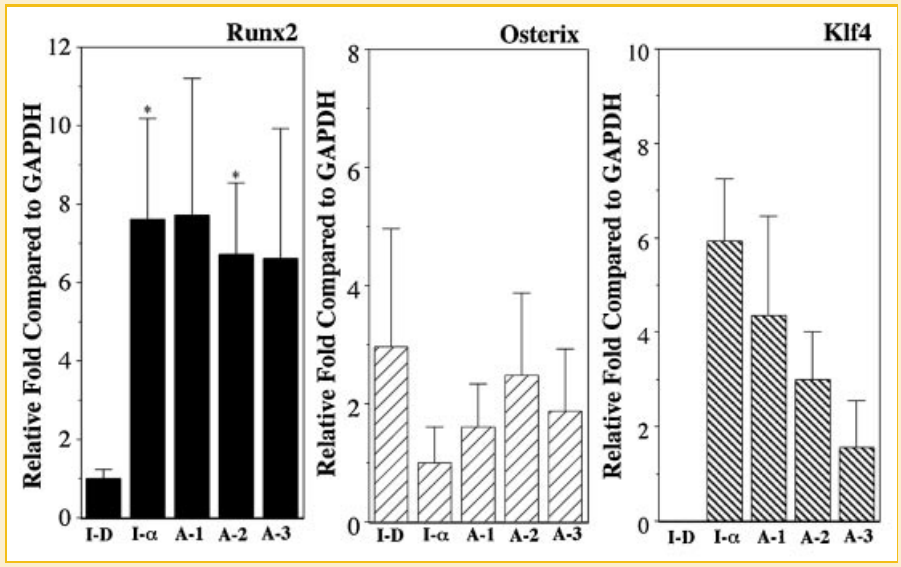

Fig. 4. The expressions of Runx2, Osterix, Klf4, and GAPDH mRNA in the cells were measured by real-time PCR. The relative amounts of mRNA of Runx2, Osterix and Klf4 to GAPDH were calculated. The experiments were performed in quadruplicate for each data point. Quantitative analyses of the quadruplicate data sets are shown with standard errors. Significant differences from control: $^{*}P < 0.1$.

[Song et al., 2007]. Such a mechanism might have functioned in our experiment. Furthermore, the efficacy remained unclear, because significantly more cells were cultured with αMEM than with DMEM. The supportive culture environment due to such efficacies might have enabled the culture of ABMMSCs from older individuals, but further study aiming at a more supportive culture condition is required because there is still room for improvement.

Some tendencies in the gene expression profile and in the response to the induction of differentiation are described as follows. The transcription factors associated with the maintenance of embryonic stem cells (ESCs) undifferentiated state (POU5F1, Sox2, and Nanog) were expressed similarly in all cells, while other ESC-associated genes (TERT and GDF3) were not expressed in all cells. The transcription factors crucial for osteogenesis (Runx2 and Osterix) were expressed in all cells, while adipogenesis-associated PPARγ and chondrogenesis-associated Sox9 were not expressed in all cells. The details are still unclear, although the above-mentioned gene expressions have been reported in MSCs cultured under various conditions, such as FGF2-containing medium [Song and Tuan, 2004; Alexanian, 2007; Battula et al., 2007; Isenmann et al., 2007; Roche et al., 2007; Ulloa-Montoya et al., 2007; Delorme et al., 2008]. In our culture condition, these genes did not seem to predict the differentiation potential of the cells. FGF4 is known as a soluble factor essential for trophoblast stem cell self-renewal [Niwa et al., 2000]. In adult tissues, FGF4 is locally and specifically expressed in the brain and testis [Yamamoto et al., 2000], regulates neural progenitor cell (NPC) proliferation and neuronal differentiation [Kosaka et al., 2006], and induces spermatogenesis and prevents adriamycin-induced testicular toxicity [Yamamoto et al., 2002]; however FGF4 was not expressed in all cells. c-Myc and BSP tend to be strongly expressed in IBMMSCs cultured with αMEM (I-α) and in ABMMSCs-2 cultured with αMEM (A-2) than in others. The expression of c-Myc promotes normal cell proliferation [Skaletz-Rorowski et al., 1999] and is found in undifferentiated MSCs [Mugrauer and Ekblom, 1991], although it is up-regulated as a symptom of transformation in senescent MSCs [Rubio et al., 2008]. BSP is non-collagenous protein that has multiple functions, including bone mineralization, and is a major component of mineralized connective tissues [Ogata, 2008]. Both c-Myc and BSP are activated by FGF2 stimulation via the mitogen-activated protein kinase (MAPK) pathway [Skaletz-Rorowski et al., 1999; Shimizu-Sasaki et al., 2001; Shimizu et al., 2006], and the observed similarity of these gene expression tendencies in each sample would be a reflection of the status of MAPK.

In recent years, Klf4 has been focused on as an essential transcription factor for the induction of pluripotent stem cells from somatic cells [Takahashi et al., 2007]. Klf4 binds to GT-rich or CACCC elements on the target gene, forms a complex with histone acetyltransferase or histone deacetylase, and regulates the target gene expression by modulating the chromatin structure in the neighborhood [Evans et al., 2007]. This gene stabilizes the ESC undifferentiated state cooperatively with Klf2 and Klf5, and contributes to the expression of ESC-specific genes [Jiang et al., 2008]. Klf4 also plays an important role in smooth muscle cell (SMC) dedifferentiation in injured blood vessels. c-fos and SMC-specific genes have CArG elements in their promoter region. Usually, the SMC phenotype is maintained by activation of the above genes due to serum response factor binding to the CArG element. The Klf4 binding site exists near the CArG element, and SMC dedifferentiation is induced by gene repression caused by Klf4 binding to CArG box after injury [McDonald et al., 2006; Kawai-Kowase and Owens, 2007]. Klf4 is closely related to the undifferentiated state, and dedifferentiation as described above functions as a tumor suppressor in somatic cells via $p21^{cip1/kip1}$ pathway-dependent cell-cycle arrest when its own DNA is damaged [Ghaleb et al., 2005; McConnell et al., 2007]. Moreover, an important transcription factor for the

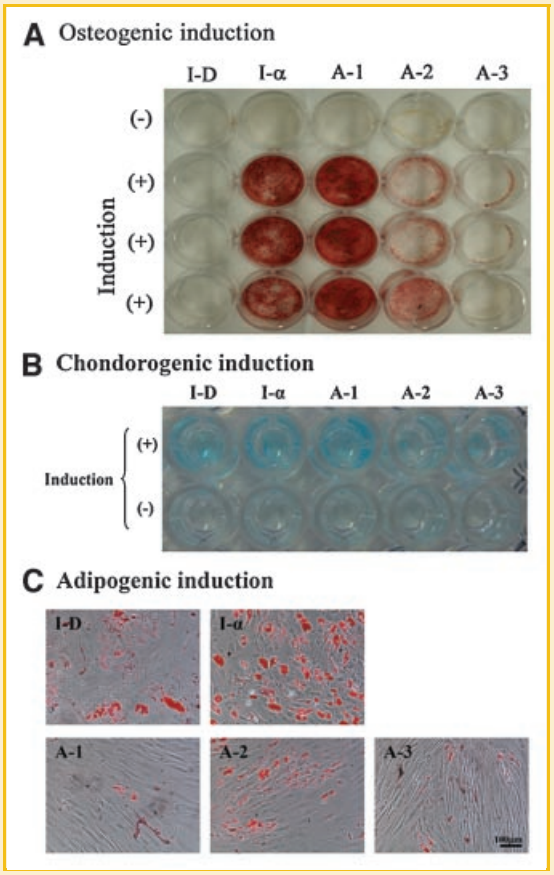

Fig. 5. Response of IBMMSCs cultured with DMEM (I-D) and with αMEM (I-α), and ABMMSCs-α (A-1, A-2, A-3) to induction of (A) osteogenic, (B) chondrogenic and (C) adipogenic differentiation. Osteogenic differentiation (triplicate) was strongly induced in I-α and A-1, moderately in A-2, slightly in A-3, and not in D-I. Chondrogenic differentiation was induced in all specimens. Adipogenic differentiation was strongly induced in I-α and A-2, and slightly in A-1 and A-3. Remarkably, IBMMSCs cultured with αMEM (I-α) and ABMMSCs-α expressing Klf4 (I-α, A-1, A-2, and A-3) were able to differentiate into osteogenic cells (see Fig. 3).

differentiation of osteoblasts, activator protein 1, is regulated by c-fos [Zayzafoon et al., 2005], which is repressed by Klf4 [McDonald et al., 2006; Kawai-Kowase and Owens, 2007]. We hypothesize that MSCs strongly expressing Klf4 were able to differentiate into osteoblasts and form bone nodules. In other words, there is a possibility that Klf4 predicts the osteogenic differentiation of MSCs.

In conclusion, αMEM is more advantageous than DMEM in the primary culture of ABMMSCs. The prediction of differentiation potential by Klf4 might become a useful guide for further improvement of the culture conditions required to culture ABMMSCs derived from older individuals.

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REFERENCES

Alexanian AR. 2007. Epigenetic modifiers promote efficient generation of neural-like cells from bone marrow-derived mesenchymal cells grown in neural environment. J Cell Biochem 100:362–371.

Barrilleaux B, Phinney DG, Prockop DJ, O'Connor KC. 2006. Ex vivo engineering of living tissues with adult stem cells. Tissue Eng 12:3007–3019.

Battula VL, Bareiss PM, Treml S, Conrad S, Albert I, Hojak S, Abele H, Schewe B, Just L, Skutella T, Buhring HJ. 2007. Human placenta and bone marrow derived MSC cultured in serum-free, b-FGF-containing medium express cell surface frizzled-9 and SSEA-4 and give rise to multilineage differentiation. Differentiation 75:279–291.

Benoit DS, Tripodi MC, Blanchette JO, Langer SJ, Leinwand LA, Anseth KS. 2007. Integrin-linked kinase production prevents anoikis in human mesenchymal stem cells. J Biomed Mater Res A 81:259–268.

Bjerknes M, Cheng H. 2006. Intestinal epithelial stem cells and progenitors. Methods Enzymol 419:337–383.

Burke ZD, Thowfeequ S, Peran M, Tosh D. 2007. Stem cells in the adult pancreas and liver. Biochem J 404:169–178.

Delorme B, Ringe J, Gallay N, Le Vern Y, Kerboeuf D, Jorgensen C, Rosset P, Sensebe L, Layrolle P, Haupl T, Charbord P. 2008. Specific plasma membrane protein phenotype of culture-amplified and native human bone marrow mesenchymal stem cells. Blood 111:2631–2635.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop Dj, Horwitz E. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315–317.

Evans PM, Zhang W, Chen X, Yang J, Bhakat KK, Liu C. 2007. Kruppel-like factor 4 is acetylated by p300 and regulates gene transcription via modulation of histone acetylation. J Biol Chem 282:33994–34002.

Ghaleb AM, Nandan MO, Chanchevalap S, Dalton WB, Hisamuddin IM, Yang VW. 2005. Kruppel-like factors 4 and 5: The yin and yang regulators of cellular proliferation. Cell Res 15:92–96.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. 2000. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci USA 97:13625–13630.

Isenmann S, Cakouros D, Zannettino A, Shi S, Gronthos S. 2007. hTERT transcription is repressed by Cbfa1 in human mesenchymal stem cell populations. J Bone Miner Res 22:897–906.

Jiang J, Chan YS, Loh YH, Cai J, Tong GQ, Lim CA, Robson P, Zhong S, Ng HH. 2008. A core Klf circuitry regulates self-renewal of embryonic stem cells. Nat Cell Biol 10:353–360.

Kawai-Kowase K, Owens GK. 2007. Multiple repressor pathways contribute to phenotypic switching of vascular smooth muscle cells. Am J Physiol Cell Physiol 292:C59–C69.

Kosaka N, Kodama M, Sasaki H, Yamamoto Y, Takeshita F, Takahama Y, Sakamoto H, Kato T, Terada M, Ochiya T. 2006. FGF-4 regulates neural progenitor cell proliferation and neuronal differentiation. FASEB J 20:1484–1485.

Matsubara T, Suardita K, Ishii M, Sugiyama M, Igarashi A, Oda R, Nishimura M, Saito M, Nakagawa K, Yamanaka K, Miyazaki K, Shimizu M, Bhawal UK, Tsuji K, Nakamura K, Kato Y. 2005. Alveolar bone marrow as a cell source for regenerative medicine: Differences between alveolar and iliac bone marrow stromal cells. J Bone Miner Res 20:399–409.

McConnell BB, Ghaleb AM, Nandan MO, Yang VW. 2007. The diverse functions of Kruppel-like factors 4 and 5 in epithelial biology and pathobiology. Bioassays 29:549–557.

McDonald OG, Wamhoff BR, Hoofnagle MH, Owens GK. 2006. Control of SRF binding to CArG box chromatin regulates smooth muscle gene expression in vivo. J Clin Invest 116:36–48.

Mugrauer G, Ekblom P. 1991. Contrasting expression patterns of three members of the myc family of protooncogenes in the developing and adult mouse kidney. J Cell Biol 112:13–25.

Niwa H, Miyazaki J, Smith AG. 2000. Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. Nat Genet 24:372–376.

Ogata Y. 2008. Bone sialoprotein and its transcriptional regulatory mechanism. J Periodontal Res 43:127–135.

Ogata Y, Niisato N, Furuyama S, Cheifetz S, Kim RH, Sugiya H, Sodek J. 1997. Transforming growth factor-beta 1 regulation of bone sialoprotein gene transcription: Identification of a TGF-beta activation element in the rat BSP gene promoter. J Cell Biochem 65:501–512.

Ogata Y, Nakao S, Kim RH, Li JJ, Furuyama S, Sugiya H, Sodek J. 2000. Parathyroid hormone regulation of bone sialoprotein (BSP) gene transcription is mediated through a pituitary-specific transcription factor-1 (Pit-1) motif in the rat BSP gene promoter. Matrix Biol 19:395–407.

Roche S, Richard MJ, Favrot MC. 2007. Oct-4, Rex-1, and Gata-4 expression in human MSC increase the differentiation efficiency but not hTERT expression. J Cell Biochem 101:271–280.

Rubio D, Garcia S, Paz MF, De la Cueva T, Lopez-Fernandez LA, Lloyd AC, Garcia-Castro J, Bernad A. 2008. Molecular characterization of spontaneous mesenchymal stem cell transformation. PLoS ONE 3:e1398.

Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. 2004. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364:149–155.

Shimizu E, Nakayama Y, Nakajima Y, Kato N, Takai H, Kim DS, Arai M, Saito R, Sodek J, Ogata Y. 2006. Fibroblast growth factor 2 and cyclic AMP synergistically regulate bone sialoprotein gene expression. Bone 39: 42–52.

Shimizu-Sasaki E, Yamazaki M, Furuyama S, Sugiya H, Sodek J, Ogata Y. 2001. Identification of a novel response element in the rat bone sialoprotein (BSP) gene promoter that mediates constitutive and fibroblast growth factor 2-induced expression of BSP. J Biol Chem 276:5459–5466.

Skaletz-Rorowski A, Waltenberger J, Muller JG, Pawlus E, Pinkernell K, Breithardt G. 1999. Protein kinase C mediates basic fibroblast growth factor-induced proliferation through mitogen-activated protein kinase in coronary smooth muscle cells. Arterioscler Thromb Vasc Biol 19:1608–1614.

Song L, Tuan RS. 2004. Transdifferentiation potential of human mesenchymal stem cells derived from bone marrow. FASEB J 18:980–982.

Song H, Chang W, Lim S, Seo HS, Shim CY, Park S, Yoo KJ, Kim BS, Min BH, Lee H, Jang Y, Chung N, Hwang KC. 2007. Tissue transglutaminase is essential for integrin-mediated survival of bone marrow-derived mesenchymal stem cells. Stem Cells 25:1431–1438.

Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872.

Ulloa-Montoya F, Kidder BL, Pauwelyn KA, Chase LG, Luttun A, Crabbe A, Geraerts M, Sharov AA, Piao Y, Ko MS, Hu WS, Verfaillie CM. 2007. Comparative transcriptome analysis of embryonic and adult stem cells with extended and limited differentiation capacity. Genome Biol 8:R163.

Yamamoto H, Ochiya T, Takahama Y, Ishii Y, Osumi N, Sakamoto H, Terada M. 2000. Detection of spatial localization of Hst-1/Fgf-4 gene expression in brain and testis from adult mice. Oncogene 19:3805–3810.

Yamamoto H, Ochiya T, Tamamushi S, Toriyama-Baba H, Takahama Y, Hirai K, Sasaki H, Sakamoto H, Saito I, Iwamoto T, Kakizoe T, Terada M. 2002. HST-1/FGF-4 gene activation induces spermatogenesis and prevents adriamycin-induced testicular toxicity. Oncogene 21:899–908.

Yu BD, Mukhopadhyay A, Wong C. 2008. Skin and hair: Models for exploring organ regeneration. Hum Mol Genet 17:R54–R59.

Zayzafoon M, Fulzele K, McDonald JM. 2005. Calmodulin and calmodulin-dependent kinase II alpha regulate osteoblast differentiation by controlling c-fos expression. J Biol Chem 280:7049–7059.