IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

Human Journals
Research Article
July 2019 Vol.:13, Issue:1

In Vitro Hepatocyte Differentiation of Human Adipose-Derived Stem Cells under Hypoxia and Photobiomodulation Irradiation

IJSRM
INTERNATIONAL JOURNAL OF SCIENCE AND RESEARCH METHODOLOGY
An Official Publication of Human Journals

In-Su Park[1]*

[1]Cell Therapy Center, Ajou University School of Medicine, Suwon, Korea

Submission: 22 June 2019
Accepted: 28 June 2019
Published: 30 July 2019

HUMAN JOURNALS
www.ijsrm.humanjournals.com

Keywords: Hepatocytes; photobiomodulation, low-level light, adipose-derived stem cells, hepatogenic differentiation

ABSTRACT

New research demonstrated adult stem cells could be harvested from a patient and used to repair own damaged liver. Additionally, stem cells may be manipulated in vitro to induce hepatic differentiation. We investigated effect of hypoxia and photobiomodulation therapy (PBMT) on the hepatogenic differentiation of human adipose-derived stem cells (hASCs). hASCs were exposed to different Carbon dioxide concentrations with photobiomodulation (PBM) using low-level light. Cell survival and secretion of hepatocyte growth factor of the hASCs were evaluated by immunostaining and Western blot analyses. Hepatic differentiation was assessed via immunocytochemical staining, FACS and western blot analysis for liver-specific genes and proteins, including albumin, cytokeratins 8/18, and alpha-fetoprotein. PBM therapy has been shown to enhance proliferation and cytokine secretion of a number of cells. In the hypoxia+PBMT group, the survival of hASCs was increased due to the decreased apoptosis of hASCs. The expression profiles of albumin, alpha-fetoprotein, and cytokeratin 8/18 demonstrated that when hepatocyte growth factor, hypoxia, or PBMT were treated individually, incomplete hepatocyte differentiation was achieved. On the other hand, quantitative analysis of albumin, cytokeratins 8/18, and alpha-fetoprotein showed that hepatocyte growth factor was enhanced significantly ($p<0.05$) by hypoxia+PBM treatment. The obtained cell populations contained progenitors that expressed both hepatic albumin and cytokeratin 8/18 markers, as well as alpha-fetoprotein. These data suggest that PBMT and hypoxia are an effective biostimulator of hASCs in hepatogenic differentiation, which enhances the survival of hASCs and stimulates the secretion of growth factors. These cells with hepatic function may become important tools for liver transplant procedures, liver development studies, and pharmacologic research.

INTRODUCTION

Because of the availability of suitable human livers for transplantation falls short of the number of potential patients, liver failure is a global medical problem [1, 2]. In addition, the availability of primary human hepatocytes for cell-therapy and drug development applications is significantly limited [3-7]. Thus, there is a compelling need for an abundant and accessible supply of cells that can safely and effectively improve liver function in these patients [1, 2].

The ability to implant progenitor cell populations that can be differentiated in vitro would be a major advance in current cell-based therapies [8]. In addition, and importantly for this application, the ability to utilize a non-hepatic progenitor cell to mimic hepatocytes in vitro would enable the scale-up production of cells for bioartifical liver assist devices, cell-therapy and drug discovery applications [9, 10].

Recent findings that mesenchymal stromal cell (MSC) can differentiate into a cell exhibiting a hepatocyte phenotype [10, 11]. Adult stem cell-based therapies may provide alternative or adjunct therapeutic approaches toward the treatment of liver diseases [4-7]. In vitro propagation of umbilical cord blood (UCB)-derived, bone marrow-derived mesenchymal stromal cells (BMSCs), human umbilical cord matrix cells, and human adipose-derived stromal/stem cells (hASCs) in media containing numerous growth factors and cytokines yield cell populations that express a number of hepatic characteristics [11]. Numerous cytokines and growth factors have been shown to have potent effects on hepatic growth and differentiation in vitro [12-14]. They include hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor (TGF), basic fibroblast growth factor (bFGF), insulin, insulin-like growth factor, and oncostatin M (OSM) [15] [16]. Moreover, the estimated fraction of MSCs in adipose tissue is markedly higher when compared to other common MSC sources such as bone marrow [17]. Unfortunately, limitations still remain regarding the clinical application of hASCs, predominately because of their low hepatic cell differentiation efficacy, and cultures over extended periods of time.

The photobiomodulation (PBM) has been implemented for various purposes for some time, such as to provide pain relief, to reduce inflammation, and to improve local circulation. Moreover, many studies have demonstrated that PBM has positive biostimulatory effects on stem cells [18]. It is well known that the proliferation and growth factor secretion (such as

HGF, FGF, VEGF) of hASCs was also enhanced by PBM irradiation [18, 19]; and PBM enhanced tissue healing by stimulating angiogenesis in various animal models of ischemia [20].

Many studies have indicated that hypoxia influences development of the embryo by regulating the differentiation and self-renewal (including the maintenance of stemness and proliferation) of stem cells. In some organs, such as the lung, nervous system and heart, hypoxia induces the differentiation of stem cells into mature cells [8]. Hypoxic preconditioning results have been reported in enhanced survival of human mesenchymal stem cells [13]. Also, hypoxia stimulates the production of growth factors, such as HGF, EGF, and bFGF that induce hepatocyte differentiate [8].

In this study was designed to further optimize conditions of differentiation in efforts to produce hASCs-derived hepatocytes that closely recapitulate the human hepatic phenotype.

MATERIALS AND METHODS

Culture of hASCs

hASCs supplied from CEFO (Seoul, Korea) were cultured in low-glucose Dulbecco's modified Eagle's medium F-12 (DMEM/F-12; Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (FBS, Welgene), 100 units/ml penicillin and 100 μg/ml streptomycin at 37.0°C in a 5% CO_2 incubator. hASCs between passage 5 and 8 were used for all experiments.

In vitro hepatic differentiation

hASCs were plated on collagen type I-coated dishes at a concentration of 4×10^4 cells/cm^2. When the cells reached confluency, hepatogenic induction was carried out over a period of 21 days. First, the cells were treated for 7 days with hepatogenic differentiation basal medium (HDBM) serum free supplemented with EGF and bFGF (PeproTech EC, London, UK). Afterwards, the cells were cultured for 14 days in a HDBM, containing HGF, FGF, Nicotinamide (Supplementary Table. 2).

Photobiomodulation irradiation

Light emitting diode (LED; WON Technology, Daejeon, Korea) was applied for 10 min daily. The light source used was LED (light emitting diode; WON Technology Co., Ltd., Korea) designed to fit over a standard multi-well plate (12.5 × 8.5 cm) for cell culture. The LED was had an emission wavelength peaked at 660 nm. The irradiance at the surface of the cell monolayer was measured by a power meter (Orion, Ophir Optronics Ltd., UT). To obtain the energy dose of 6 J/cm^2, exposure time for LED array was 10 min under power density of 10 mW/cm^2 (1 milliwatt × second = 0.001 joules).

Cells viability assay

The cell viability of spheroids was analyzed by using a live/dead viability cytotoxicity assay kit after 10 day in culture (Molecular probes, Carlsbad, CA). Briefly, 1 ml of HEPES-buffered saline solution (HBSS) containing 2 µl of green (SYTO 10 fluorescent nucleic acid stain solution) and 2 µl of red (ethidium homodimer-2) nucleic acid stain solution was added to plates and then incubated at 37°C in a 5% CO_2 incubator for 15 min. The negative control was prepared by freezing cells at -80°C for 30 min. Images were quantified by using ImageJ software (NIH, Bethesda, MD). The percentage of live/dead cells was scored by counting number of pixels per image.

Fluorescence-activated cell sorting (FACS)

Cells were washed with phosphate buffered saline (PBS) containing 0.5% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO). The cells were stained in PBS containing 1% BSA with either isotype controls or antigen specific antibodies for 60 min. The antibodies used were human CD34 (BD Biosciences, San Jose, CA), KDR (Beckman Coulter, Brea, CA), CD31 (Beckman Coulter), CD45 (Abcam, Cambridge, MA), CD90 (BD Biosciences), CD105 (Caltac Laboratories, Burlingham, CA) and CD29 (Millipore, Waltham, MA). The cells were washed thrice with PBS containing 0.5% BSA and resuspended in PBS for flow cytometry using an Accuri device (BD Biosciences). Isotype control IgG was used as a negative control.

Immunofluorescence staining

Indirect immunofluorescence staining was performed using a standard procedure. In brief, cells were fixed with 4% paraformaldehyde, blocked with 5% BSA/PBS (1 h, 24°C), washed twice with PBS, treated with 0.1% Triton X-100/PBS for 1 min and washed extensively in PBS. The sections were stained with specific primary antibodies and fluorescent-conjugated secondary antibodies (Supplementary Table 1) using a M.O.M. kit according to the manufacturer's instructions (Vector Laboratories, Burlingame, CA). The cells were counterstained with DAPI (4,6-diamino-2-phenylindole dihydrochloride; Vector Laboratories). Negative control-mouse IgG (Dako, Carpinteria, CA) and –rabbit IgG (Dako) antibody was used as a negative control. Stained sections were viewed with a model DXM1200F fluorescence microscope (Nikon, Tokyo, Japan). Processed images were analyzed for fluorescence intensity using ImageJ software (NIH).

Western blot

Samples were solubilized in lysis buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 1 mM phenylmethylsulfonyl fluoride,1µg/ml leupeptin, 2 µg/ml aprotinin) for 1 h at 4ºC. Lysates then were clarified by centrifugation at 15,000 g for 30 min at 4ºC, diluted in Laemmli sample buffer containing 2% SDS and 5% (v/v) 2-mercaptoethanol, and heated for 5 min at 90ºC. Proteins were separated by SDS polyacrylamide gel electrophoresis (PAGE) using 10% or 15% resolving gels followed by transfer to nitrocellulose membranes (Bio-Rad, Hercules, CA). The membranes were incubated with primary antibody for 1 h at room temperature. For detection, peroxidase-conjugated anti-mouse IgG or anti-rabbit IgG) and enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) were used as described by the manufacturer. Membranes were scanned to create chemiluminescent images and to quantify with an image analyzer (Kodak, Rochester, NY).

Statistical analyses

All the quantitative results were obtained from triplicate samples. Data were expressed as a mean ± SD. Statistical analysis was carried out using two-sample t test for comparing 2 groups of samples and One-way Analysis of Variance (ANOVA) for 3 groups. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Characterization of hASCs

Adherent cells obtained from human adipose tissue were expanded in vitro. The cells were positive for human MSC markers CD29 (□1 integrin), CD90 (Thy-1) and CD105 (endoglin). However, the cells were negative for human endothelial cell markers CD34, CD31, KDR (VEGF receptor) and hematopoietic cell marker CD45 on flow cytometry analyses (Fig. 1). These results indicated that the expanded cells included a large population of hASCs and were not contaminated with endothelial cells.

Table 1

Antibody	Host	Company	Catalogue number
anti-human CD29	mouse	Millipore	MAB2253Z
anti-human Flk-1	mouse	Santa Cruz	Sc-6251
anti-human CD34	mouse	Millipore	MAB4211
anti-human CD31	mouse	Dako	M0823
anti-human CD31	rabbit	abcam	ab76533
anti-human CD45	mouse	abcam	ab82595
anti-human CD90	mouse	BD biosciences	555595
anti-human CD105	mouse	Caltac Laboratories	MHCD10500
HIF-1 alpha	rabbit	Novus	NB100-134
anti-human FGF	rabbit	abcam	ab8880
anti-human VEGF	rabbit	abcam	ab52917
Alexa Fluor 488 anti-mouse IgG	goat	Invitrogen	A11001
Alexa Fluor 594 anti-rabbit IgG	goat	Invitrogen	A11012

Fig. 1

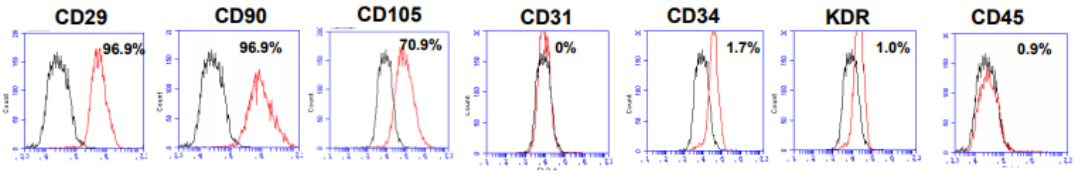


Figure 1. Immunofluorescence staining of hASCs. hASCs (passage 4) were stained with CD29, CD90 and CD105 for mesenchymal stem cell identification, with KDR, CD31 and CD34 for endothelial lineage cell identification, and SMA for smooth muscle cell identification. Scale bar: 200 μm

Inhibition of hASC apoptosis

To verify the cell viability of hASCs, a dead cell assay with of fluorescent dyes was carried out. Non-viable cells were stained red. Dead cells were significantly less ($p < 0.05$) in hypoxia and PBM treated- hASC as opposed to a hypoxia culture (Fig. 2A). Apoptosis induced by a lack of cell-matrix interaction (anoikis), was prevented in hASCs cultured as hypoxia and PBM. Furthermore, hASCs had reduced pro-apoptotic factors (PARP, Caspase-3, BAX) and enhanced anti-apoptotic factor (Bcl-2, pAKT) at the protein level (Fig. 2B). This indicated that hypoxia and PBM treated- hASC secrete higher levels of anti-apoptotic factors relative to hASCs in normoxia culture. Therefore, PBM-hypoxia cultured cells seemed to be more adaptable and more resistant to hypoxia compared to hASCs in normal cell culture condition.

Fig. 2

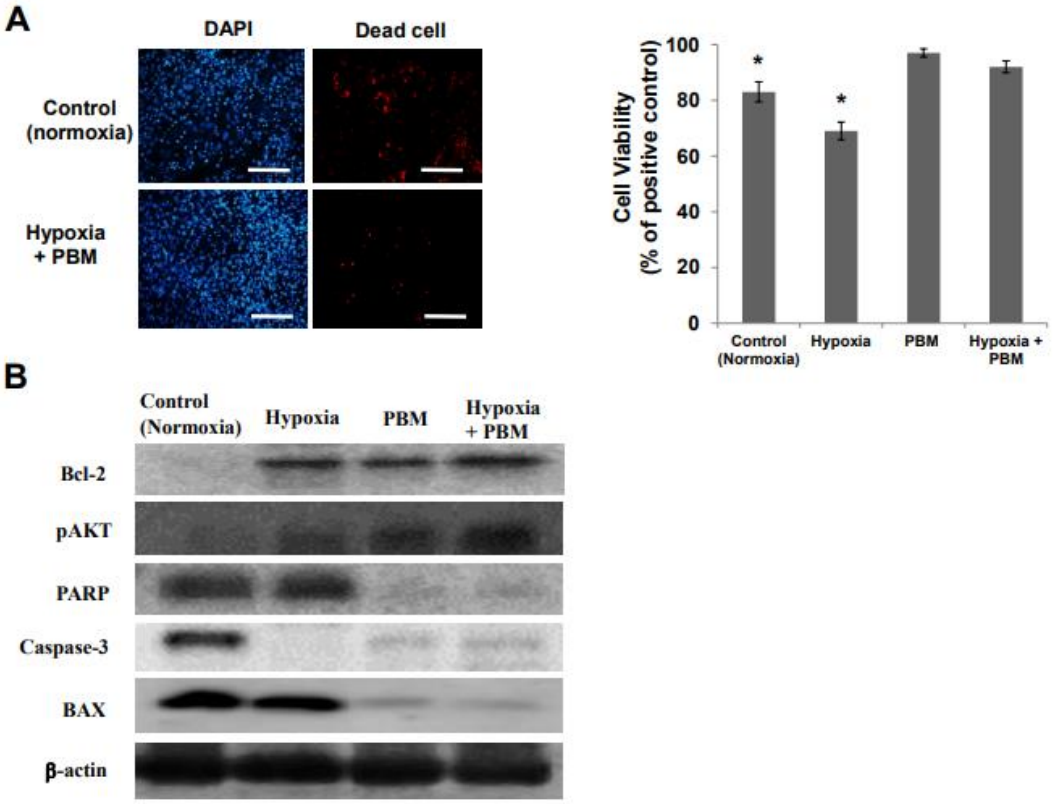


Figure 2. Inhibition of apoptosis in hASCs by PBM-hypoxia. hASCs were cultured for 21 days under normoxic (21% oxygen) or hypoxic (1% oxygen) conditions. (A) Fluorescence microscopic image of Dead stain on day 21. Dead cells were stained with ethidium homodimer (red). Scale bar: 500 μm. The ratio of live and dead cells in the hASCs. (B) hASCs had reduced pro-apoptotic factors (PARP, Caspase-3, BAX) and enhanced anti-apoptotic factor (Bcl-2, pAKT) at the protein level.

Table 2

Pretreatment Medium	
Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium(Cat. No. HUXMA-03101)	100 mL
EGF	20 μℓ
bFGF	10 μℓ

Hepatocyte Differentiation Medium	
Human Mesenchymal Stem Cell Hepatogenic Differentiation Basal Medium(Cat. No. HUXMA-03101)	100 mL
HGF	20 μℓ
bFGF	10 μℓ
Nicotinamide	100 μℓ

Production of growth factors by cells in PBM-hypoxia culture

Hypoxia condition was confirmed by the elevated expression of hypoxia-inducible factors 1α (HIF1α) under 5% O_2 condition (Fig. 3A). Additionally, PBM-hypoxia cultures showed a dramatic increase in the expression of hypoxia-induced survival factors, such as hypoxia-inducible factor (HIF)-1α, relative to cells in a normal cell culture condition (Fig. 3A). PBM is upregulate the expression of growth factors [19, 20], and PBM-hypoxia cultured cells showed considerable expression of hepatocyte growth factor (HGF), and fibroblast growth factor 2 (FGF2) (Fig. 3B). The expression of growth factors in PBM-hypoxia cultured cells was much greater than that of PBM treated cells or hypoxia treated cells (Fig. 3C).

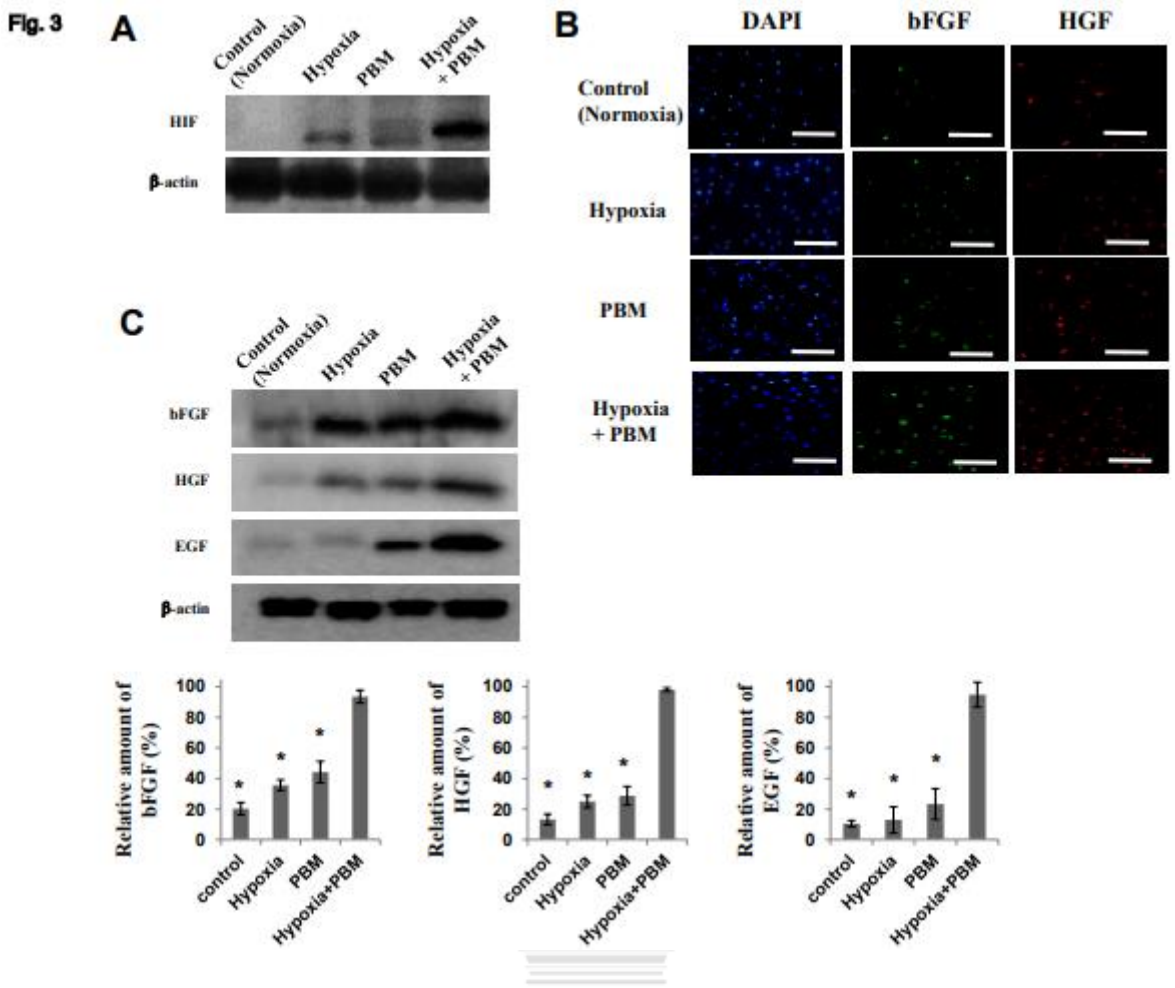


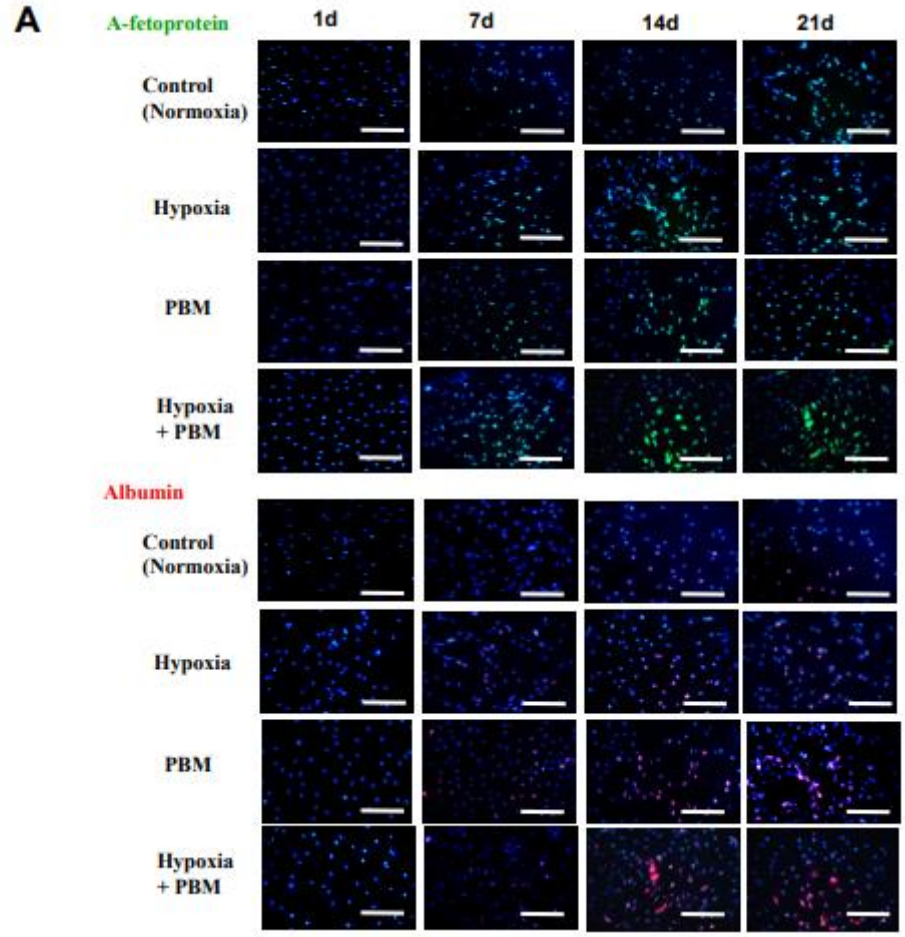
Figure 3. Production of growth factors by cells in PBM-hypoxia culture. (A) Western blot analysis and quantification of HIF-1 in hASCs treated with PBM and hypoxia. (B) Hepatocyte-related protein were monitored by immunofluorescent staining. hASC stained with anti-human FGF, HGF, and EGF antibodies. Scale bar: 100 μm. (C) Enhanced expression of PBM, hypoxia-induced survival factors and hepatic growth factors in hASC. hASCs in monolayer were cultured for 21 days. Relative amount of bFGF, HGF, and EGF.

Synerge of effect of PBM and hypoxia on the hepatic differentiation of the hASCs

To investigate the effect of PBM on the hepatic differentiation of the hASCs, we cultured the hASCs under PBM-hypoxia conditions for 21 days, respectively. Many more AFP (early liver specific markers)- or ALB (late liver specific markers)-positive cells were observed among cells exposed to hypoxia (5% O_2), compared with those subjected to normoxic conditions (21% O_2) by immunofluorescence staining (Fig. 4A). Also, the expression of alpha-fetoprotein (AFP) and albumin (ALB) was increased by PBM culture conditions on 21 days (Fig. 4A). These results confirm that PBM augments hepatic differentiation in MSC in

general. In particular, the expression of human AFP and ALB in PBM-hypoxia cultured cells was much greater than that of PBM treated cells or hypoxia treated cells (Fig. 4B). The protein level of AFP (early liver specific markers) was increased by PBM-hypoxia culture for 14 days. However, the protein level of ALB (late liver specific markers) was increased by PBM-hypoxia culture for 21 days (Fig. 4B). These data suggest that PBM-hypoxia induces upregulation of genes that are predominantly involved in hepatic differentiation, metabolism.

Fig. 4



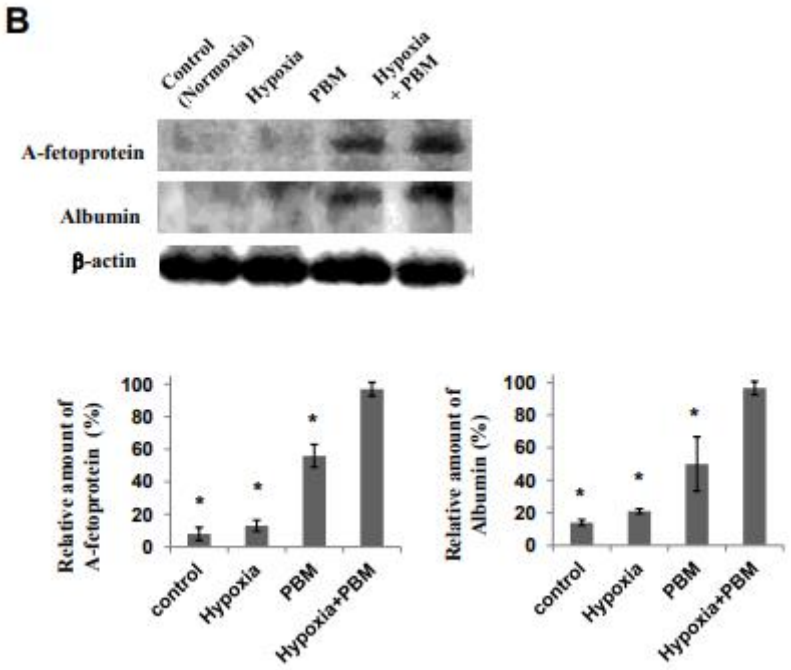


Figure 4. In vitro differentiation. Hepatogenic cell surface markers were monitored by immunofluorescent staining. Cells stained with anti-human ALB, and AFB antibodies. Scale bar: 100 μm. (B) Western blot analysis of liver specific markers with early (AFP) and late (ALB) in hASCs treated with PBM and hypoxia.

DISCUSSION

As multipotent stem cells residing in the stromal fraction of adipose tissue, hASCs are an abundant source of the cells required for transplantation [21, 22]. hASCs can be easily isolated from a small volume of adipose tissue and subsequently harvested by a simple, minimally invasive method [23]. In addition, their numbers can be expanded rapidly in vitro using standard cell culture techniques. Significant levels of cell-surface markers, including CD29, CD49d, CD44, CD144, CD54, and CD106 are expressed in hASCs [24]. Additionally, thyroxine-1 (CD90) and endoglin (CD105) are expressed in hASCs, allowing adherence to other cells or ECMs. hASCs can also secrete a wide spectrum of angiogenic and anti-apoptotic factors, including VEGF and HGF [25]. Most importantly, PBM increases the gene expression and release of several hepatic differention related growth factors, including FGF, EGF and HGF, from stem cells [19, 25]. In this case, the induction does not depend on the oxygen tension and involves the activation of a different regulatory mechanism possibly mediated by mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt signaling pathway [26].

Hypoxia is a critical microenvironmental factor for normal embryonic development [27]. Mammalian embryonic development occurs at low intrauterine O_2 levels ranging from 2% to 9% [28]. Also, Hypoxia is known to promote cell differentiation in many tissues [8]. However, the effect of hypoxia on the differentiation of stem cells varies among individual tissues [23]. Most importantly, the effect of hypoxia on the hepatic differentiation of hASCs was previously unclear. Our results revealed that hypoxia (5% O_2) induced the differentiation of mesenchymal cell progenitors into hepatic cells, as evidenced by increased expression of the hepatic cell marker ALB and an increased number of AFP- and CK8/18-positive cells.

Inefficient oxygen transport in hASCs may trigger the expression of hypoxia-induced survival factors (i.e., CXCL12 and HIF-1a), which in turn may precondition cells to hypoxia and nutrient deficiency [23]. Hypoxia induced upregulation of CXCL12 is ascribed to activation of a transcription factor, HIF-1a. HIF-1a binds directly to specific binding site in the CXCL12 promoter in hypoxic conditions thereby inducing CXCL12 expression [23].

Additionally, the metabolic/molecular biology studies performed on hASCs show that mitochondrial respiration can provide sufficient amount of energy under PBM-hypoxia culturing to permit cell proliferation [25]. Thus, we applied 5% O_2 treated with PBM to research the effect of PBM-hypoxia condition on the hASCs. We discovered that differentiation and secretion of EGF, HGF, and FGF were also enhanced by PBM. It has been reported that hypoxia promotes the differentiation and attenuates the stemness of hASCs through blocking of the Wnt/β-catenin pathway [29]. These data suggest that the PBM-hypoxia had effects on either hepatic differentiation or self-renewal of the hASCs.

In PBM-hypoxia cultures, if growth factors are secreted from stem cells after PBM and hypoxia, the factors might be stimulate the hepatic differentiation of stem cells in an autocrine or paracrine manner [23]. The physiologic concept is that PBM-hypoxia improves cellular metabolism from stem cells. The outcomes confirm and extend previous reports documenting the potential utility of ASC-derived hepatocytes for liver tissue repair and regeneration.

REFERENCES

[1] Gary S. Becker, Introducing Incentives in the Market for Live and Cadaveric Organ Donations, Journal of Economic Perspectives 21 (2007) 3-24.
[2] Rauchfuss F, Bärthel E, Scheuerlein H, Schüle S, Malessa C, Settmacher U, Living Donor Liver Transplantation - Past and Present Zentralbl Chir 141(5) (2016) 559-564.
[3] van de Kerkhove MP, Chamuleau RAFM, van Gulik TM, Clinical application of bioartificial liver support systems, Annals of surgery 240 (2004) 216.
[4] Takebe T, Kimura M, Yoshizawa E, Ayano S, Koido M, Funayama S, Massive and Reproducible Production of Liver Buds Entirely from Human Pluripotent Stem Cells Cell Rep 5(21) (2017) 2661-2670.
[5] Kopp JL, Sander M, Stem cells versus plasticity in liver and pancreas regeneration, Nat Cell Biol. 18(3) (2016) 238-245.
[6] Tsolaki E, Stem cell-based regenerative opportunities for the liver: State of the art and beyond World J Gastroenterol 21(43) (2015) 12334-12350.
[7] Goldman O, Human Pluripotent Stem Cells: Myths and Future Realities for Liver Cell Therapy Cell Stem Cell 18(6) (2016) 703-706.
[8] Park IS, Jung Y, Rhie JW, Kim SH, Endothelial differentiation and vasculogenesis induced by three-dimensional adipose-derived stem cells, Anat Rec (Hoboken) 296 (2013) 168-177.
[9] Dandri M, Torok E, Pollok JM, Iwanska A, Repopulation of mouse liver with human hepatocytes and in vivo infection with hepatitis B virus, Hepatology 33 (2001) 981-988.
[10] Bhogal RH, Bartlett DC, Weston CJ, Curbishley SM, Isolation of primary human hepatocytes from normal and diseased liver tissue: a one hundred liver experience, PLoS One 6 (2011) e18222.
[11] Aurich H, Kaltwasser P, Vetter M, Weise A, Liehr T, Brulport M, Hengstler JG, Dollinger MM, Fleig WE, Christ B, Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo, Gut. 58(4) (2009) 570-581.
[12] Park IS, Jung Y, Rhie JW, Kim SH, Endothelial differentiation and vasculogenesis induced by three-dimensional adipose-derived stem cells, Anat Rec (Hoboken) 1 (2013) 168-77.
[13] Bhang SH, La WG, Lee TJ, Yang HS, Sun AY, Baek SH, Rhie JW, Kim BS, Angiogenesis in ischemic tissue produced by spheroid grafting of human adipose-derived stromal cells, Biomaterials 32 (2011) 2734-2747.
[14] Valcarcel M, Jaureguibeitia A, Lopategi A, Martinez I, Mendoza L, Three-dimensional growth as multicellular spheroid activates the proangiogenic phenotype of colorectal carcinoma cells via LFA-1-dependent VEGF: implications on hepatic micrometastasis, J Transl Med. 6 (2008) 57.
[15] Pastar I, Yin NC, Ramirez H, Nusbaum AG, Sawaya A, Epithelialization in Wound Healing: A Comprehensive Review Adv Wound Care (New Rochelle) 3(7) (2014) 445-464.
[16] Li P, A review: therapeutic potential of adipose-derived stem cells in cutaneous wound healing and regeneration Stem Cell Res Ther 9(1) (2018) 302.
[17] Alessio Giai Via, Francesco Oliva, Biological properties of mesenchymal Stem Cells from different sources, Muscles Ligaments Tendons J 2(3) (2012) 154-162.
[18] Choi K, Kim H, Lee S, Bae S, Kweon OK, Kim WH., Low-level laser therapy promotes the osteogenic potential of adipose-derived mesenchymal stem cells seeded on an acellular dermal matrix., J Biomed Mater Res B Appl Biomater. 101(6) (2013) 919-928.
[19] Park IS, Ahn JC, Enhanced angiogenic effect of adipose-derived stromal cell spheroid with low-level light therapy in hind limb ischemia mice, Biomaterials 35(34) (2014) 9280-9289.
[20] de Sousa AP, Silveira NT, de Souza J, Cangussú MC, dos Santos JN, Pinheiro AL., Laser and LED phototherapies on angiogenesis, Lasers Med Sci 28(3) (2013) 981-987.
[21] Huang JI, Jones NF, Zhu M, Lorenz HP, Chondrogenic potential of multipotential cells from human adipose tissue, Plast. Reconstr. Surg. 113 (2004) 585–594.
[22] Gimble J, Adipose-derived adult stem cells: isolation, characterization, and differentiation potential, Cytotherapy 5 (2003) 362–369.
[23] Park IS, Kim SH, A novel three-dimensional adipose-derived stem cell cluster for vascular regeneration in ischemic tissue, Cytotherapy 13 (2013) 00681-6.
[24] Park IS, Rhie JW, Endothelial differentiation and vasculogenesis induced by three-dimensional adipose-

derived stem cells, Anat Rec (Hoboken) 1 (2013) 168-177.
[25] Park IS, Ahn JC, Adipose-derived stem cell spheroid treated with low-level light irradiation accelerates spontaneous angiogenesis in mouse model of hindlimb ischemia Cytotherapy 19(9) (2017) 1070-1078.
[26] Hu WP, Yu CL, Lan CC, Chen GS, Yu HS, Helium-neon laser irradiation stimulates cell proliferation through photostimulatory effects in mitochondria, J Invest Dermatol 127 (2007) 2048-2057.
[27] Imanirad P, Hypoxia and HIFs in regulating the development of the hematopoietic system Blood Cells Mol Dis. 51(4) (2013) 256-263.
[28] D. SL, The role of hypoxia in development of the Mammalian embryo Dev Cell. 17(6) (2009) 755-773.
[29] von Sengbusch A, Fisch KM, Enns A, Nicolson GL, Haier J, Focal adhesion kinase regulates metastatic adhesion of carcinoma cells within liver sinusoids, Am. J. Pathol 166 (2005) 585-596.