Hypoxia Promotes Proliferation and Osteogenic Differentiation Potentials of Human Mesenchymal Stem Cells

Shun-Pei Hung,[1] Jennifer H. Ho,[2,3] Yu-Ru V. Shih,[1] Ting Lo,[1] Oscar K. Lee[1,4,5]

[1]Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan, [2]Graduate Institute of Clinical Medicine, Taipei Medical University, Taipei, Taiwan, [3]Department of Ophthalmology, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan, [4]Stem Cell Research Center, National Yang-Ming University, Taipei, Taiwan, [5]Department of Orthopaedics and Traumatology, Taipei Veterans General Hospital, 201 Shi-Pai Rd., Sec. 2, Taipei 11221, Taiwan

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ABSTRACT: Mesenchymal stem cells (MSCs), which can be isolated from bone marrow and other somatic tissues, are residing in an environment with relative low oxygen tension. The purpose of this study is to investigate the effects of hypoxia on MSCs, and we hypothesize that oxygen concentration regulates the intricate balance between cellular proliferation and commitment towards differentiation. In this study, human bone marrow-derived MSCs were cultured under hypoxia with 1% O_2. The proliferation ability of MSCs was increased after a 7-day hypoxic culture period. Migration assay showed that hypoxia enhanced the migration capabilities of MSCs. Moreover, expression of stemness genes Oct4, Nanog, Sall4 and Klf4 was increased under hypoxia. Furthermore, the differentiation ability of MSCs under hypoxia favored osteogenesis while adipogenesis was inhibited during a 4-week induction period. Cytokine antibody array analysis showed that a number of growth factors were up-regulated after a 7-day hypoxic incubation and the differential expression of growth factors may account for the increased proliferation and osteogenic potentials of MSCs under hypoxic condition. Taken together, hypoxia provides a favorable culture condition to promote proliferation as well as osteogenesis of MSCs through differential growth factor production. © 2011 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 30:260–266, 2012

Keywords: mesenchymal stem cells; hypoxia; proliferation; differentiation; migration

In mammalians, oxygen plays an important role in energy homeostasis in which oxidative phosphorylation is the most important reaction for energy production. The physiologic oxygen partial pressure in the human body is much lower than the atmosphere, ranging from 1% in cartilage and 1–7% in bone marrow to 10–13% in arteries, lungs, and liver.[1,2] However, in the ex vivo culture system, the oxygen concentration is almost 21% which is much higher than the physiologic oxygen partial pressure in the body. It has been reported that low oxygen pressure in tissue cultures demonstrated positive effects on cell growth[3] and, connective tissue cultured in high oxygen conditions promoted bone formation while low oxygen conditions favored cartilage differentiation.[4] It has also been reported that reduced oxygen tension enhances proliferation of some cell types. For example, cell proliferation increased for bovine pericytes[5] and rat calvarial osteoblasts[6] cultured at low oxygen tension. A recent study has reported that hypoxia (1–5% O_2) also enhanced the self-renewal of murine neural stem cells[7] and embryonic stem (ES) cells[8] while some experiments showed that hypoxia induced differentiation.[9,10]

Mesenchymal stem cells (MSCs) can be isolated from a variety of somatic tissues.[11] These cells have self-renewal capacity and can differentiate into mesodermal lineages. However, the exact influences of hypoxia on differentiation of MSCs still remain inconclusive as there are contradictory reports in the literature.[27–10] The purpose of this study was to investigate the effects of hypoxia on human MSCs. We hypothesized that oxygen participates in the intricate balance between cellular proliferation and commitment towards differentiation. In this study, the effects of hypoxia on the proliferation ability, migration ability, cell cycle regulation, stemness gene expression, differentiation potentials, as well as cytokine production of MSCs were examined.

METHODS

MSC Isolation, Culture Expansion, In Vitro Differentiation, and Hypoxia Treatment

Human bone marrow-derived MSCs were harvested as previously reported ($n = 15$).[11,12] An approval from the Institutional Review Board as well as the informed consents of the bone marrow donors were obtained prior to the commencement of the study. Single cell-derived, clonally expanded MSCs were tested for their surface immune-phenotype and tri-lineage differentiation potentials as previously described before these cells were used for subsequent experiments.[11,12] Expansion medium consisted of Iscove's modified Dulbecco's medium (IMDM; Gibco, Grand Island, NY) and 10% fetal bovine serum (FBS; Hyclone, Logan, UT) supplemented with 10 ng/ml FGF2, 100 U penicillin, 1,000 U streptomycin, and 2 mM L-glutamine (Gibco). Cells of passage 11–15 were used for the following experiments. Hypoxia (1% O_2) was simulated in a water-saturated gas mixture of 1% O_2, 5% CO_2, and 94% N_2 at 37°C. In additional experiments, cells were exposed to hypoxia as described above for different time courses (0, 1, 3, 5, 7 days) and the media were changed twice per week.

Cell Proliferation Assay

Direct Cell Counting

In order to examine the difference of proliferative ability of MSCs between normoxia and hypoxia, cells from passage 9–12 were seeded at 1.5×10^5 or 1.5×10^6 cells/dish density in 100 mm culture dishes with growth medium. Cells were exposed to hypoxia or under normoxia, then harvested and counted at different time courses (1, 3, 5, 7 days).

Additional supporting information may be found in the online version of this article.

Correspondence to: Oscar K. Lee (T: +886-2-2875-7391; F: +886-2-2875-7824; E-mail: kslee@vghtpe.gov.tw).

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Bromodeoxyuridine (BrdU) Incorporation Assay

Cell proliferation was estimated by flow cytometry based on BrdU incorporation during the S-phase of cycling cells. MSCs were cultured under normoxia or hypoxia for 7 days. Cultures of MSCs were treated with fluorescein-labeled antibody against BrdU (Cat. No. 559619; BD Biosciences, San Diego, CA) in a biologically effective concentration (10 mM) at day 6. After 24 h, cells were harvested, fixed, and DNA was denaturized. The immune complexes were detected and read by FACScan flow cytometer (BD Biosciences). Data were analyzed by CELL Quest software for determination of the number of $BrdU^+$ cells.

Western Blot Analysis, RNA Isolation, and Quantitative Real-Time PCR

These procedures were performed as previously reported[12,13] and detailed experimental methods were described in the supplementary information.

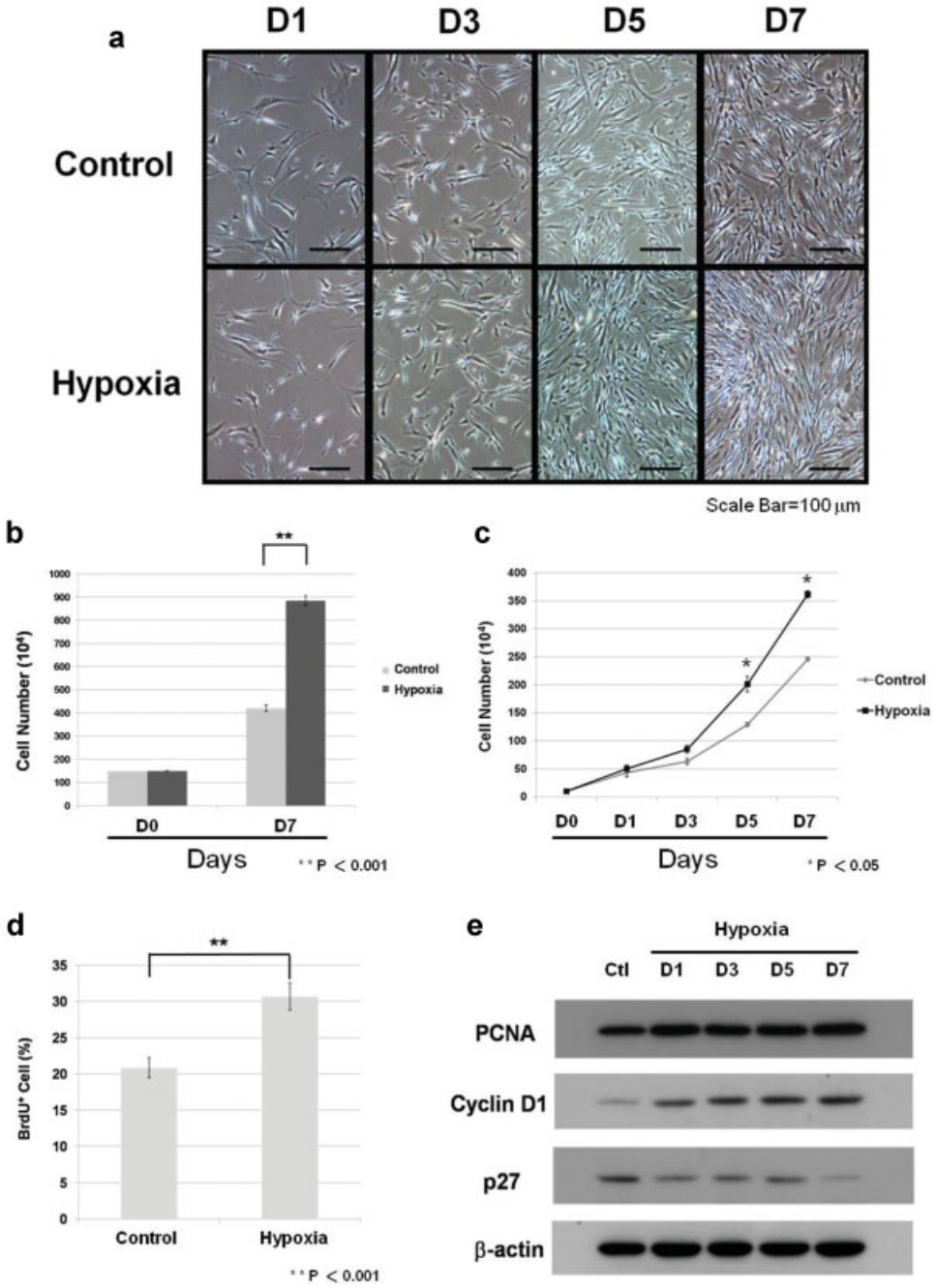


Figure 1. Hypoxic condition increases the proliferation ability of MSCs. (a) Morphology of the cells under hypoxic culture. (b) Cell number of MSCs under hypoxia condition was significantly higher on day 7. (c) Growth curves of MSCs were determined by the level of cumulative cell number at different incubation times (0, 1, 3, 5, 7 days) under normoxia or hypoxia. (d) The number of proliferating cells was also significantly higher after hypoxic culture for 7 days by BrdU incorporation assay. (e) Western blot analysis showed that expression of PCNA and cyclin D1 were increased with time and Cdk inhibitor p27 gradually decreased during the course when MSCs cultured under hypoxia. Ctl: Control, MSCs cultured under normoxia for 7 days; Hy: MSCs cultured under hypoxia at different time courses. Scale bar represents 100 μm. Data were shown as mean ± SD ($n = 3$) $^{*}p < 0.05$; $^{**}p < 0.001$.

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Cell Migration Assay and Cytokine Array Analysis

Migration assays were performed using the Culture-Insert method (ibidi GmbH, Martinsried, Germany). Human cytokine and growth factor assay was performed using Human Cytokine Antibody Array 1 (Cat #AAH-GF-1, RayBiotech, Norcross, GA) that following the manufacturer's instructions. The detail experimental methods were described in supplementary information.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Science-10 software (SPSS, Inc., Chicago, IL). The data were presented as mean ± SD of the results from three or four independent experiments. Results of cell number as well as stemness gene expressions were analyzed by ANOVA tests with Tukey's Post-Hoc tests. Results of osteogenic, chondrogenic and adipogenic marker gene expressions were analyzed by two-tail, non-paired t tests. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Hypoxia Increased the Proliferation Ability and Cyclin Protein Expression in MSCs

Compared with normoxic controls, there was no significant change in morphology after hypoxic culture (Fig. 1a). The cell number was higher after hypoxic culture for 7 days by direct cell counting (Fig. 1b). From days 0–7, the slop of growth curve in hypoxic group was steeper. That demonstrated the proliferation ability of MSCs in hypoxic group was increased (Fig. 1c). Cell proliferation assay was determined by BrdU incorporation analysis. Under hypoxia, the percentage of BrdU-positive MSCs was higher than normoxia (Fig. 1d). To investigate whether hypoxia affects the expression of cell cycle regulating proteins, MSCs were incubated in hypoxia for different time points (0, 1, 3, 5, 7 days). Results show hypoxia increased the expression of the cell proliferation marker

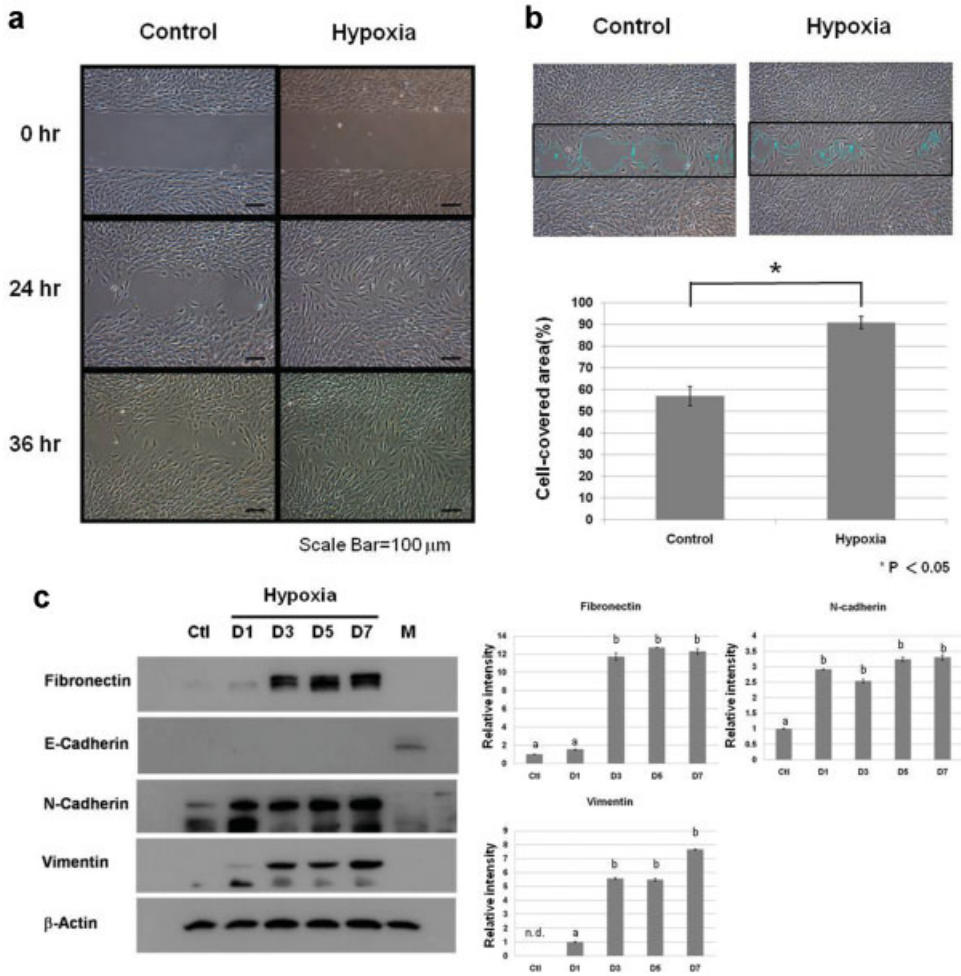


Figure 2. Hypoxia enhances the migration ability of MSCs. (a) MSCs migration assay was performed and photographed after culture insert removal for 24 and 36 h. Hypoxic culture obviously increased the migration capabilities of MSCs as there were more cells migrating towards the scratched space compared to the control group. (b) Comparison of cell-covered area of MSCs cultured under normoxia and hypoxia after culture-insert removal for 24 h. (c) Western blot analysis of fibronectin, N-cadherin and vimentin were up-regulated by hypoxia, which accounted for increased migration capabilities. Ctl: Control, MSCs cultured under normoxia for 7 days; Hy: MSCs cultured under hypoxia at different time courses; M: cell lysates of MCF-7 cells, which served as positive control of E-cadherin. Scale bar represents 100 μm. Data were shown as mean ± SD ($n = 3$). n.d., non-detectable. Different alphabets represent different levels of significance. $^{*}p < 0.05$.

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proliferating cell nuclear antigen (PCNA). In addition, cyclin D1 expression was gradually increased with time, and the expression of cyclin-dependent kinases (Cdk) inhibitor p27 was decreased gradually with time (Fig. 1e).

Hypoxia Enhanced the Migration Ability of MSCs

Hypoxia enhanced the migration ability of MSCs as shown in the photomicrographs taken 24 and 36 h after insert removal in which more cells migrated into the scratched area in the hypoxic group (Fig. 2a). Twenty-four hours post removal of the culture insert, the cell-covered area was almost completely full (90% $\pm$ 2.89) in the hypoxic group more than normoxic group (56% $\pm$ 4.3) (Fig. 2b). Moreover, hypoxia treatment up-regulated the protein level of vimentin as well as fibronectin and N-cadherin as shown by Western blot (Fig. 2c).

Hypoxia Up-Regulated Stemness Gene Expression in MSCs

Real-time RT-PCR analysis showed that hypoxia enhances the important stemness genes such as Oct4, Nanog, Sall4, and Klf4 expression (Fig. 3a–d, respectively). It was also found that hypoxia increases expression of RNA component (TERC) of telomerase in MSCs (Fig. 3e).

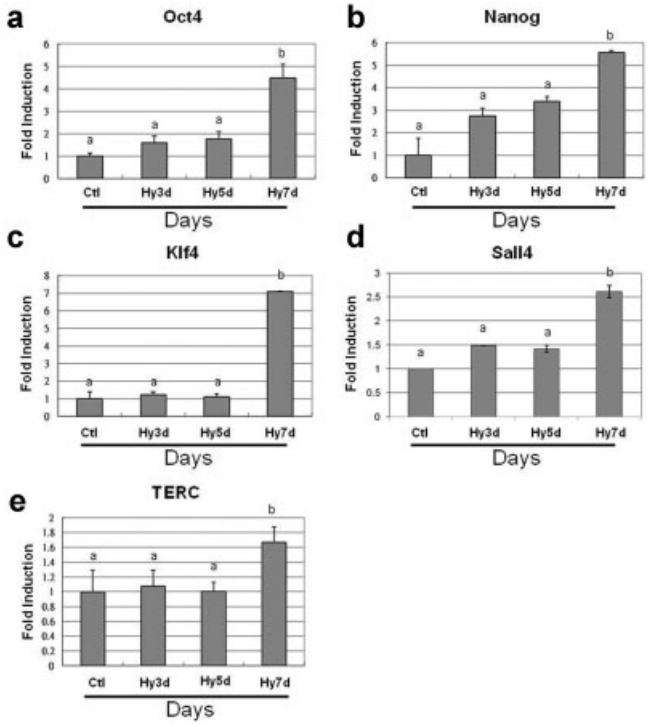


Figure 3. Hypoxia up-regulates stemness genes and telomerase RNA component expression. Expression of (a) Oct4, (b) Nanog, (c) Klfl4, (d) Sall4, and (e) TERC in MSCs after hypoxia treatment. It was found that hypoxia significantly up-regulated these genes. Ctl: Control, MSCs cultured under normoxia for 7 days; Hy: MSCs cultured under hypoxia at different time courses. Data were shown as mean $\pm$ SD ($n = 3$). Different alphabets represent different levels of significance, $p < 0.05$.

Hypoxia Enhanced Osteogenic, Inhibited Adipogenic, and Chondrogenic Potentials of MSCs

During 4 weeks of osteogenic induction, MSCs cultured in hypoxia expressed higher Alk-p (Fig. 4a, upper panel) and mineralization as shown by Von Kossa stain (Fig. 4a, lower panel). MSCs under hypoxia expressed higher levels of osteocalcin and osteopontin than normoxia (Fig. 4b). After 4 weeks of chondrogenic differentiation, the size of pellets cultured under normoxia was larger than those under hypoxia (Fig. 4c, upper panel) and the histological section showed mature chondrocyte morphology in normoxic group (Fig. 4c, lower panel). Under hypoxia, MSCs expressed reduced level of type II collagen (COL2A1), cartilage oligometric protein (COMP) and aggrecan (Fig. 4d) compared to normoxia. Light microscopy revealed increased numbers of lipid vacuoles in cells during the 4-week period of adipogenic induction under normoxic condition. In addition, the number of Oil Red O positive lipid vacuoles was decreased under hypoxia (Fig. 4e). MSCs under hypoxia possessed lower expression levels of adipsin, fatty acid synthase (FASN) and fatty acid binding protein 4 (FABP4) than normoxic controls (Fig. 4f). From the above mentioned findings, MSCs cultured in hypoxia had enhanced osteogenic, but inhibited chondrogenic and adipogenic differentiation.

Hypoxia Induced the Differential Production of Growth Factors in MSCs

The above-mentioned experimental results indicate that hypoxia promotes growth and increases the expression of several cell cycle regulating proteins in MSCs (Fig. 1). In order to explore the mechanisms, cytokine array analysis was performed. MSCs were cultured at normoxia or hypoxia for 7 days. The cultured media were harvested and incubated with cytokine array membranes. Results indicate that hypoxic condition regulates the production of a variety of growth factors on MSCs (Fig. 5).

DISCUSSION

In this study, we demonstrated that hypoxia increased proliferation potentials and promoted MSCs entry into the cell cycle (Fig. 1). The effects of low oxygen environment on cell growth had been examined by many investigators in different cell models.[8,14,15] However, the detailed mechanisms of how hypoxia induced proliferation are still unclear. Serum and mitogenic signals are known to activate the cyclin-dependent kinases that are the key regulators of cell cycle progression.[16] In view of this proliferation effect, hypoxia regulated the expression of a number of growth factors elucidated from our cytokine array data (Fig. 5). In our model system, it was possible that hypoxia switched on the program of cell cycle through autocrine or paracrine effects. Various mitogens, for example, insulin-like growth factor (IGFs) and VEGF are important for cell proliferation, survival, and maintenance of normal biological motility. It has been

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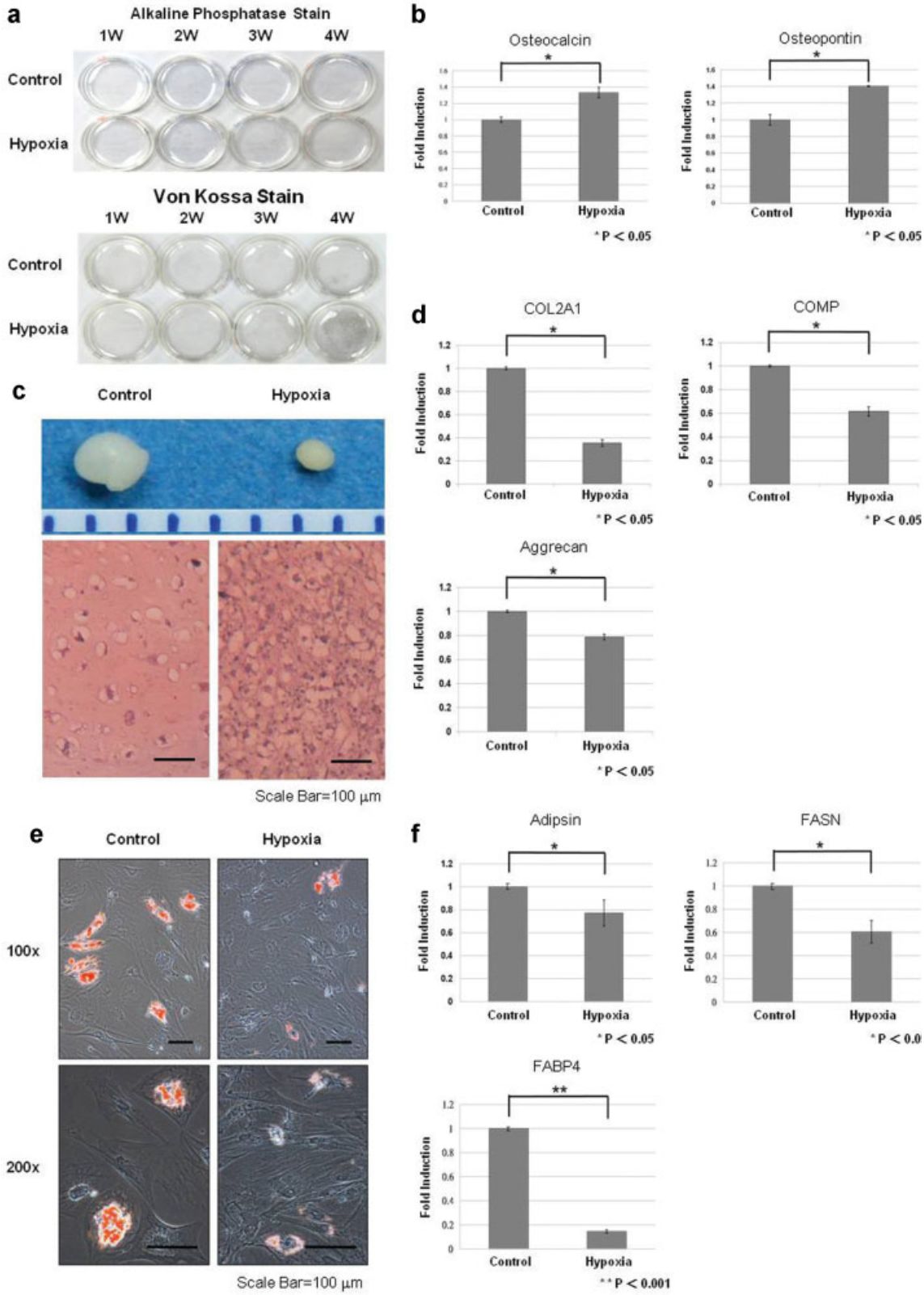
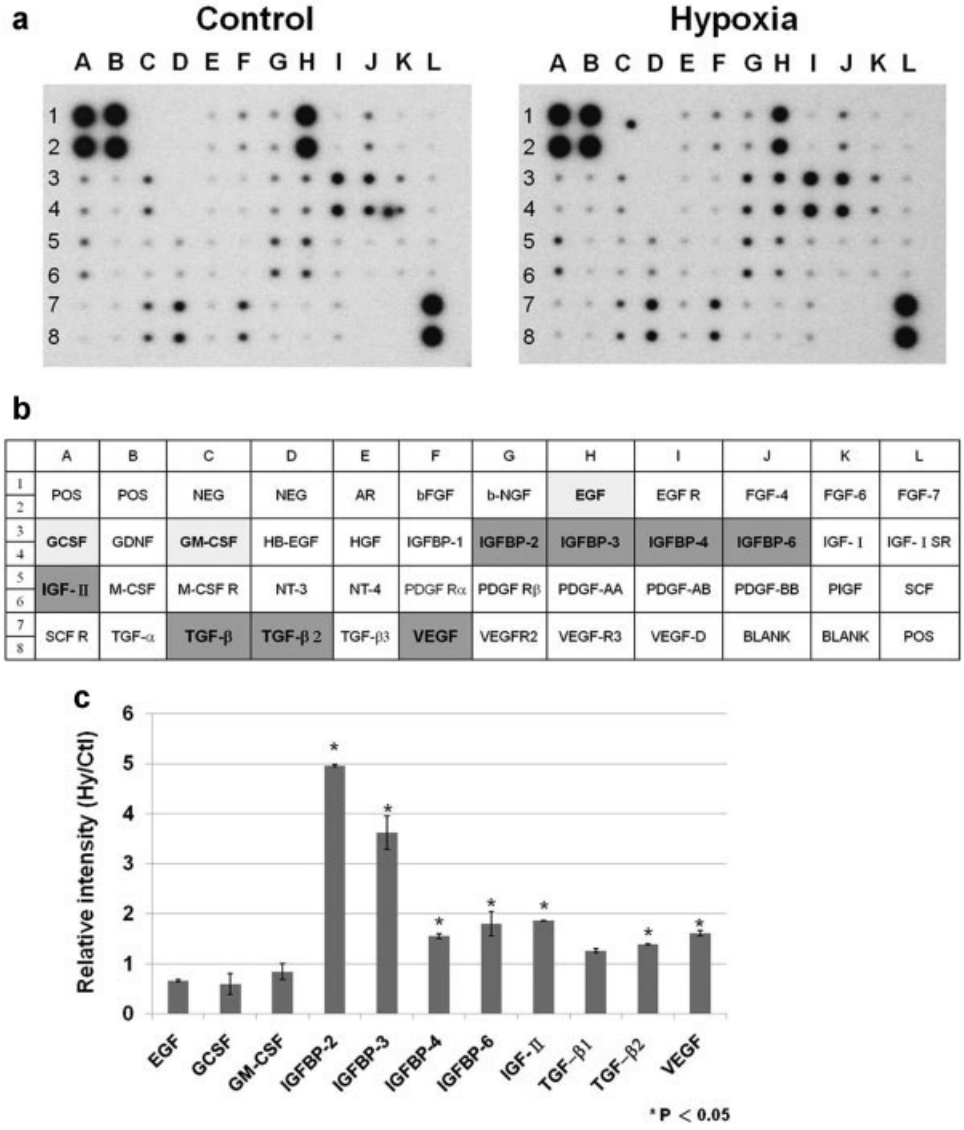


Figure 4. Hypoxia enhanced osteogenesis, inhibited chondrogenesis and adipogenesis of MSCs. (a) Alkaline phosphatase staining and Von Kossa staining of MSCs after osteogenic differentiation revealed that hypoxia enhanced osteogenic differentiation of MSCs. (b) Osteogenic marker genes including osteocalcin and osteopontin were significantly up-regulated. (c) Gross picture of pellets cultured for 4 weeks (upper panel) and histological sections for HE (haematoxylin and eosin) stain. (d) Chondrogenic marker genes including type II collagen (COL2A1), cartilage oligometric protein (COMP) and aggrecan expression were decreased by hypoxia. (e) Oil Red O staining of MSCs after adipogenic differentiation for 4 weeks revealed that hypoxia inhibited adipogenesis as fat droplet formation was reduced in hypoxic culture. (f) Adipogenic lineage marker genes including adipsin, fatty acid synthase (FASN), and fatty acid binding protein-4 (FABP4) were down regulated by hypoxia. Scale bar represents 100 μm. Data were shown as mean ± SD ($n = 3$). $^{*}p < 0.05$; $^{**}p < 0.001$.

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	A	B	C	D	E	F	G	H	I	J	K	L
1 2	POS	POS	NEG	NEG	AR	bFGF	b-NGF	EGF	EGF R	FGF-4	FGF-6	FGF-7
3 4	GCSF	GDNF	GM-CSF	HB-EGF	HGF	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-6	IGF- I	IGF- I SR
5 6	IGF- II	M-CSF	M-CSF R	NT-3	NT-4	PDGF Rα	PDGF Rβ	PDGF-AA	PDGF-AB	PDGF-BB	PIGF	SCF
7 8	SCF R	TGF-α	TGF-β	TGF-β 2	TGF-β3	VEGF	VEGFR2	VEGF-R3	VEGF-D	BLANK	BLANK	POS

Figure 5. Cytokine profiles of MSCs under normoxia and hypoxia treatment. Supernatants harvested from normoxia or hypoxia cultured MSCs were compared by cytokine array on day 7. (a) The cytokine array analyzed revealed differential expression of MSCs. (b) The map of cytokine array. (c) Quantization of cytokine expression levels from cytokine array membranes. Data were normalized with an internal positive control on the same membrane and presented as the mean ± SD ($n = 3$). $^{*}p < 0.05$.

reported that VEGF and its receptor play an autocrine or paracrine mitogenic role in MSCs.[17] Together, we infer that hypoxia regulated growth factor production, that resulted in mitogenic effects, and thereby induced MSC proliferation.

We also observed that oxygen concentration influenced the physiological motility on MSCs in which hypoxia increased the migration ability and induced mesenchymal gene expression such as vimentin, fibronectin, and N-cadherin (Fig. 2). During embryogenesis, oxygen was a key regulator of ontogeny and regulates the embryonic stem or progenitor cells. In the 1970s, Morriss demonstrated that successful development of the neural fold in vitro was dependent on the creation of culture conditions with low oxygen condition.[18] Hypoxia can promote migration of vascular smooth muscle cells through an autocrine pathway.[19] Based on our experimental data, hypoxia induced many growth factor and cytokine secretion (Fig. 5) and promoted migration ability of MSCs. These growth factors or cytokines may play crucial roles to regulate cell motility under low oxygen condition.

We also found that hypoxia affects the differentiation potentials of MSCs (Fig. 4). In our experiments, hypoxia promoted differentiation of osteogenesis, but inhibited chondrogenesis and adipogenesis. Rat-derived MSCs cultivated in hypoxic conditions produced more bone than those cultured in normoxic condition when harvested and loaded into porous ceramic cubes and implanted into syngeneic host animals.[9] However, contradictory findings were reported by D'Ippolito et al.[2] showing that low oxygen tension inhibited

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osteogenic differentiation of human bone marrow stromal cells. Taken together, the effects of hypoxia on MSCs are cell source- and species-dependent. Furthermore, different procurement and isolation protocols may account for differences in experimental results. In this study, we used single cell-derived, clonally expanded MSCs harvested from human bone marrow aspirates, which have been devoid of committed progenitors. Hypoxia enhanced osteogenesis and reciprocally inhibited adipogenesis in this clearly defined MSC population. In conclusion, we have shown here that hypoxia can influence the balance between proliferation and differentiation of MSCs. Under hypoxia, MSCs proliferate and retain stem cell properties, and may be used to yield great cell numbers for osteoblastic progenies. Hypoxic culture may therefore serve as a useful strategy in the tissue engineering of bones.

ACKNOWLEDGMENTS

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REFERENCES

1. Kofoed H, Sjontoft E, Siemssen SO, et al. 1985. Bone marrow circulation after osteotomy. Blood flow, pO2, pCO2, and pressure studied in dogs. Acta Orthop Scand 56:400–403.
2. D'Ippolito G, Diabira S, Howard GA, et al. 2006. Low oxygen tension inhibits osteogenic differentiation and enhances stemness of human MIAMI cells. Bone 39:513–522.
3. Cooper PD, Burt AM, Wilson JN. 1958. Critical effect of oxygen tension on rate of growth of animal cells in continuous suspended culture. Nature 182:1508–1509.
4. Markway BD, Tan GK, Brooke G, et al. 2010. Enhanced chondrogenic differentiation of human bone marrow-derived mesenchymal stem cells in low oxygen environment micropellet cultures. Cell Transplant 19:29–42.
5. Brighton CT, Lorich DG, Kupcha R, et al. 1992. The pericyte as a possible osteoblast progenitor cell. Clin Orthop Relat Res 275:287–299.
6. Tuncay OC, Ho D, Barker MK. 1994. Oxygen tension regulates osteoblast function. Am J Orthod Dentofacial Orthop 105:457–463.
7. Horie N, So K, Moriya T, et al. 2008. Effects of oxygen concentration on the proliferation and differentiation of mouse neural stem cells in vitro. Cell Mol Neurobiol 28:833–845.
8. Ezashi T, Das P, Roberts RM. 2005. Low O2 tensions and the prevention of differentiation of hES cells. Proc Natl Acad Sci USA 102:4783–4788.
9. Lennon DP, Edmison JM, Caplan AI. 2001. Cultivation of rat marrow-derived mesenchymal stem cells in reduced oxygen tension: Effects on in vitro and in vivo osteochondrogenesis. J Cell Physiol 187:345–355.
10. Chen HL, Pistollato F, Hoeppner DJ, et al. 2007. Oxygen tension regulates survival and fate of mouse central nervous system precursors at multiple levels. Stem Cells 25:2291–2301.
11. Lee KD, Kuo TK, Whang-Peng J, et al. 2004. In vitro hepatic differentiation of human mesenchymal stem cells. Hepatology 40:1275–1284.
12. Kuo TK, Hung SP, Chuang CH, et al. 2008. Stem cell therapy for liver disease: Parameters governing the success of using bone marrow mesenchymal stem cells. Gastroenterology 134:2111–2121.
13. Chen CT, Shih YR, Kuo TK, et al. 2008. Coordinated changes of mitochondrial biogenesis and antioxidant enzymes during osteogenic differentiation of human mesenchymal stem cells. Stem Cells 26:960–968.
14. Cipolleschi MG, Dello Sbarba P, Olivotto M. 1993. The role of hypoxia in the maintenance of hematopoietic stem cells. Blood 82:2031–2037.
15. Grayson WL, Zhao F, Bunnell B, et al. 2007. Hypoxia enhances proliferation and tissue formation of human mesenchymal stem cells. Biochem Biophys Res Commun 358:948–953.
16. Takahashi K, Murakami M, Yamanaka S. 2005. Role of the phosphoinositide 3-kinase pathway in mouse embryonic stem (ES) cells. Biochem Soc Trans 33:1522–1525.
17. Mayer H, Bertram H, Lindenmaier W, et al. 2005. Vascular endothelial growth factor (VEGF-A) expression in human mesenchymal stem cells: Autocrine and paracrine role on osteoblastic and endothelial differentiation. J Cell Biochem 95:827–839.
18. Morriss GM, New DA. 1979. Effect of oxygen concentration on morphogenesis of cranial neural folds and neural crest in cultured rat embryos. J Embryol Exp Morphol 54:17–35.
19. Osada-Oka M, Ikeda T, Akiba S, et al. 2008. Hypoxia stimulates the autocrine regulation of migration of vascular smooth muscle cells via HIF-1alpha-dependent expression of thrombospondin-1. J Cell Biochem 104:1918–1926.

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