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Hypoxia-inducible factor-1α regulates matrix metalloproteinase-1 activity in human bone marrow-derived mesenchymal stem cells

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Abstract **We examined the mRNA levels of hypoxia-inducible factor-1α (HIF-1α) in bone marrow mesenchymal stem cells (bmMSCs) of eight osteoarthritis patients. BmMSC-1, expressing higher HIF-1α mRNA and protein than bmMSC-5, elicited higher matrix metalloproteinase-1 (MMP1) activity and stronger invasive capacity. In vitro invasion assays and quantitative PCR analyses showed that targeted inhibition of HIF-1α in bmMSC-1 decreased its invasion and expressions of MMP1 and MMP3, whereas overexpression of HIF-1α in bmMSC-5 increased its invasion and expressions of MMP1 and MMP3. Therefore, HIF-1α can regulate MMP1 and MMP3 expressions in human bmMSCs, which might suggest a pathophysiological role of bmMSC expressing high HIF-1α in bone diseases.**
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Keywords: Hypoxia-inducible factor-1α; Osteoarthritis; Bone marrow mesenchymal stem cells; Matrix metalloproteinase-1

1. Introduction

Hypoxia-inducible factor-1 (HIF-1) is an oxygen-labile heterodimeric transcription factor composed of HIF-1α and HIF-1β subunits. Cellular HIF-1α level is regulated by cellular oxygen concentrations, hence acting as the regulatory subunit of HIF-1 [1]. HIF-1 can transactivate a number of genes whose products participate in glucose uptake, erythropoietin synthesis, angiogenesis, etc. Thus, HIF-1 is the major factor maintaining oxygen homeostasis, which is crucial in embryonic development, healing of ischemic wounds, and even tumor progression [2,3]. These findings suggest that HIF-1 may play a physiological or pathophysiological role depending on its expression levels in different cell types.

In bone marrow, the mesenchymal stem cell (bmMSC) is a small population of cells capable of self-renewal proliferation and lineage differentiation into chondrocyte, osteoblast, adipocyte, myoblast, and fibroblast [4–7], and is believed to play a key role in bone regeneration and maintenance of adult bone mass. In addition, bmMSCs are also featured by their capability of targeted migration [8 and references therein], which has paved way for bmMSCs to serve as an ideal tool for therapeutic gene delivery in tissue repair. Other studies have shown that matrix metalloproteases (MMPs) produced by bmMSCs play an important role in their targeted migration [9]. However, how the expression of MMPs is regulated in bmMSCs is still not well addressed.

Previously, we and the other research group reported that HIF-1α can enhance invasive capacity of human cancer cells [10,11], indicating that HIF-1α may regulate the cellular proteolytic activities. In the present study, we examined the expression of HIF-1α in bmMSCs isolated from old donors, and studied the link between HIF-1α and expression of MMPs.

2. Materials and methods

2.1. Cell culture and treatment

Human bone marrow samples were collected from osteoarthritis patients (69–77 years old). Informed consent was obtained from each donor. The use of human bmMSCs in this study was approved by both the Institutional Review Board at National Health Research Institutes and hospital. To isolate bmMSCs, marrow aspirate was diluted with equal volume of Dulbecco's phosphate-buffered saline (DPBS), and then transferred on top of 10 ml of Ficoll (Amersham Biosciences) in a 50-ml conical tube. Cell separation was accomplished by centrifugation at $1100 \times g$ for 30 min at 20 °C. The nucleated cells were collected from the interface, diluted with two volumes of DPBS, and collected by centrifugation at $900 \times g$. The cells were plated in a 10-cm culture plate, and cultured in Dulbecco's modified Eagle's medium (DMEM) (low glucose) (GIBCO-BRL) containing 10% fetal bovine serum (FBS) (Hyclone), glutamine (GIBCO-BRL), penicillin and streptomycin (GIBCO-BRL), and maintained in a humidified atmosphere containing 5% CO_2 at 37 °C. Cells were passaged at 70–80% confluence. Cells of the fourth passage were subjected to flow cytometric analysis, and those that were CD31- and CD45-negative, but CD90- and CD105-positive were defined as bmMSCs. For hypoxic treatment, cells (4×10^5) plated in 10-cm culture dishes were incubated in 6 ml medium as mentioned above, and maintained in a humidified atmosphere containing 2% O_2, 2% CO_2, and 96% N_2 at 37 °C for 4 h. For overexpression or inhibition of HIF-1α, pCEP4/HIF-1α (American Type Culture Collection, ATCC) or $siRNA_{HIF\text{-}1\alpha}$ was transfected into bmMSCs, respectively, by electroporation using MicroPorator system (NanoEnTek Inc., South Korea) following the protocol provided by manufacturer. The sequences of siRNAs were as follows: $siRNA_{HIF\text{-}1\alpha}$ (sense), AGAGGUGGAUAUGUGUGGGdTdT; $siRNA_{HIF\text{-}1\alpha}$ (antisense), CCCACACAUAUCCACCUCUdTdT; $siRNA_{GFP}$ (sense), GGCUACGUCCAGGAGCGCACC; and $siRNA_{GFP}$ (antisense), UGCGCUCCUGGACGUAGCCUU.

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Abbreviations: HIF-1α, hypoxia-inducible factor-1α; siRNA, small interfering RNA; GFP, green fluorescence protein; MMP, matrix metalloproteinase; APMA, *p*-aminophenylmercuric acetate

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2.2. Western blot analysis

Cells (1×10^6) were washed with PBS and collected by centrifugation. Cell pellet was suspended into 150 μl cold RIPA buffer plus protease inhibitors for 30 min, and then subjected to centrifugation to collect supernatant. Aliquots (40 μg) of whole-cell lysates were separated on 10% SDS–polyacrylamide gels, and electrotransferred onto polyvinylidene membranes. The membranes were incubated with anti-HIF-1α (BD Biosciences, catalog no. 610959) and anti-α-tubulin (Neo markers, catalog no. MS-581-P1) antibodies, and the signals were obtained by enhanced chemiluminescence (PIERCE).

2.3. In vitro invasion and migration assays

Quantitative Cell Migration Assay Collagen I system (Chemicon International) was used to examine invasion and migration of bmMSCs. Briefly, cells (1.6×10^4) were suspended in 0.5 ml medium and added to the upper chamber which was lodged into the lower chamber containing 0.75 ml medium. After incubating at 37 °C for the time as indicated, the cells in the upper chamber were removed with cotton swabs. The membranes were then soaked in the fixation solution for 10 min and then stained with Liu stain. The stained cells were counted in three fields under a microscope.

2.4. RNA isolation and RT-PCR

Total RNA was isolated from bmMSCs and subjected to RT-PCRs for detection of HIF-1α expression as described previously [11]. The 5′ and 3′ primers used in PCRs were as follows: HIF-1α (479 bp), AGAAAAAGATAAGTTCTGAACGTC and GAGAAAAAAGCT-TCGCTGTGTG; and GAPDH (597 bp), CCACCCATGGCA-AATTCCATGGCA and TCTAGACGGCAGGTCAGGTCCACC. The PCR products were resolved in 2% agarose gels and then stained with ethidium bromide. Signals were visualized with an AlphaImager (Alpha Innotech).

2.5. Quantitative real-time PCR

Real-time qPCR was performed using the Power SYBR Green PCR Master Mix (Applied Biosystems, CA). The 5′ and 3′ primers used were as follows: MMP1 (154 bp), GATGGACCTGGAGGAAATC and GTCCAAGAGAATGGCCGA; MMP2 (400 bp), CACTGAGG-GCCGCACGGAT and CTTGATGTCATGCTGGGACA; MMP3 (152 bp), CGCCTGTCTCAAGATGATATAAAT and CTGACA-GCATCAAAGGACAA; MMP14 (186 bp), GGTGTTTGAT-GAGGCGT and GGGTACTCGCTATCCACT; and β-actin (71 bp), CCCTGGCACCCAGCAC and GCCGATCCACACGGAG-TAC. Primers for HIF-1α were as described above. All real-time qPCRs were performed in triplicate on an ABI PRISM 7000 Sequence Detector System as follows: 50 °C for 2 min, 95 °C for 10 min and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. The relative mRNA levels were calculated using the $2^{-\Delta\Delta CT}$ method, with β-actin mRNA as a normalizer.

2.6. Conditioned medium preparation

Cells (1.5×10^5) were seeded into 6-cm tissue culture plates and maintained in the original medium for 24 h. Then cells were washed with PBS and cultured in 1.5 ml serum-free medium for 24 h. Then the medium was collected and stored at −80 °C.

2.7. Matrix metalloproteinase-1 activity assay

The levels of endogenous free active MMP1 and total free MMP1 in the conditioned media were determined using a MMP1 Biotrak Activity Assay System (Amersham Biosciences) following the protocol provided by the manufacturer. Briefly, 100 μl of each standard (proMMP1 provided by the manufacturer) and conditioned media were incubated at 4 °C for 16 h in microplate wells precoated with anti-MMP1 antibody which binds pro and active MMP1. The wells were washed four times with wash buffer and aspirated. Fifty microliters of *p*-aminophenylmercuric acetate (APMA) solution plus 50 μl detection reagent was added into the wells containing standards or conditioned medium to measure the total (pro and active) MMP1 activity. On the other hand, 50 μl of assay buffer (without APMA) plus 50 μl detection reagent was added into the wells containing conditioned medium to measure the endogenous active MMP1. The plates were read at 405 nm immediately and 3 h after incubating at 37 °C. The endogenous active MMP1 as well as total MMP1 contents were determined by interpolation from the standard curve. The resulting data were used to calculate the relative level of MMP1 in media.

2.8. Statistical analysis

Data shown were the means ± S.D. Statistical difference between two groups was determined by paired *t*-test. A value of $P < 0.05$ was considered to denote statistical significance.

3. Results

We performed RT-PCR analyses to examine HIF-1α mRNA levels in bmMSCs obtained from old donors. Data showed that cellular HIF-1α mRNA levels varied among bmMSC cells (Fig. 1A). We chose bmMSC-1 and bmMSC-5 for subsequent studies because of the dramatic difference of the HIF-1α mRNA levels between these two cultures. Quantitative PCR showed that HIF-1α mRNA level of bmMSC-1 was approximately 3-folds of that of bmMSC-5 (Fig. 1B). Western blot analyses showed that under normoxic condition HIF-1α protein level of bmMSC-1 was approximately 2.5-folds of that of bmMSC-5 (Fig. 1C). Hypoxia increased HIF-1α protein in both bmMSCs. Next, we performed in vitro invasion assays to examine the invasive capability of bmMSC-1 and bmMSC-5. Data showed that the invasive activity of bmMSC-1, as measured using collagen I-coated filter membranes, was 2-folds of that of bmMSC-5, whereas the migratory activity of bmMSC-1, as measured using BSA-coated filter membranes, was similar to that of bmMSC-5 (Fig. 1D). These data showed that bmMSC-1 expressed more HIF-1α and was more invasive than bmMSC-5. Accordingly, we studied the role of HIF-1α in the invasiveness of bmMSCs. As shown, overexpression of HIF-1α (63% increase) caused approximately 60% increase in the invasiveness of bmMSC-5 (Fig. 2A). On the other hand, inhibition of HIF-1α expression (57% decrease) using $siRNA_{HIF\text{-}1\alpha}$ caused approximately 35% decrease in the invasiveness of bmMSC-1 (Fig. 2B). These data showed a functional link between HIF-1α and invasion of bmMSCs.

That bmMSC-1 was more efficiently in migrating through collagen I-coated filter membranes than bmMSC-5 implied that bmMSC-1 might produce more MMP1 than bmMSC-5. Thus, RNA isolated from bmMSC-5 and bmMSC-1 was subjected to real-time qPCR analyses. Results showed that the MMP1 mRNA level of bmMSC-1 was approximately 5.7-folds of that of bmMSC-5 (Fig. 3A). Moreover, ELISA assays showed that the levels of total free MMP1 and endogenous active MMP1 in bmMSC-1-derived conditioned media were approximately 5-folds and 2-folds of those of bmMSC-5 derived media, respectively (Fig. 3B and C), indicating that bmMSC-1 produced more MMP1 and elicited higher MMP1 activity than bmMSC-5.

Thus, we studied if HIF-1α regulated MMP1 expression in bmMSCs. Complementary DNAs prepared from the various bmMSC-1 and bmMSC-5 cultures used in the experiments of Fig. 2 were subjected to real-time qPCRs to examine the mRNA levels of HIF-1α and several MMPs. As shown, the mRNA levels of HIF-1α, MMP1, and MMP3 (a MMP1 activator) in pCEP4/HIF-1α-transfected bmMSC-5 were approximately 2.6-, 1.6-, and 1.9-folds of those of untreated bmMSC-5 (Fig. 4A). The levels of these mRNAs in $siRNA_{HIF\text{-}1\alpha}$-transfected bmMSC-1 were approximately 66%, 69%, and 61% of those of untreated bmMSC-1 (Fig. 4B). In comparison, expres-

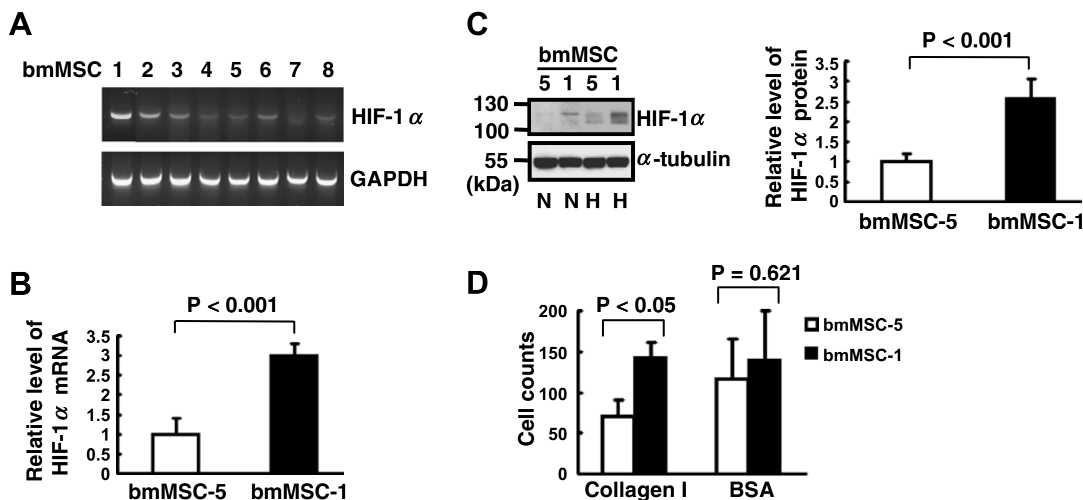

Fig. 1. HIF-1α expression and invasive activity of human bmMSCs. (A) Two micrograms of RNA prepared from each bmMSC culture was subjected to RT-PCR analyses to show the levels of HIF-1α mRNA of these cultures. GAPDH served as a control. (B) Equal amount of cDNA (0.5 μg) prepared from bmMSC-1 and bmMSC-5 were subjected to real-time qPCR assays to detect HIF-1α mRNA and β-actin mRNA (as a normalizer). The ΔCT value of bmMSC-1 was calibrated against that of bmMSC-5 to obtain the ΔΔCT value. The level of HIF-1α mRNA of bmMSC-1 was presented relatively to that of bmMSC-5. Data represent the means ± S.D. from three experiments. (C) Whole-cell lysates prepared from normoxic (N) bmMSC-1 and bmMSC-5 cultures were subjected to Western blot analyses to measure the relative abundance of HIF-1α protein. Cell lysates from hypoxic (H) cultures served as positive controls. Signals were quantitated, and the HIF-1α/α-tubulin values were calculated. The level of HIF-1α protein of bmMSC-1 was presented relatively to that of bmMSC-5. Data represent the means ± S.D. from three experiments. (D) BmMSC-1 and bmMSC-5 (1.6×10^4 each) were seeded on collagen I-coated, and BSA-coated filter membranes. These cells were incubated for 24 h at 37 °C. Cells migrated through the filter membranes were counted under a 40-fold high power field. Cell counts of bmMSC-5 and bmMSC-1 that migrated through collagen I- and BSA-coated membranes were 71 ± 19, 142 ± 66, 117 ± 49, and 140 ± 60 cells, respectively. Data represent the means ± S.D. from three assays.

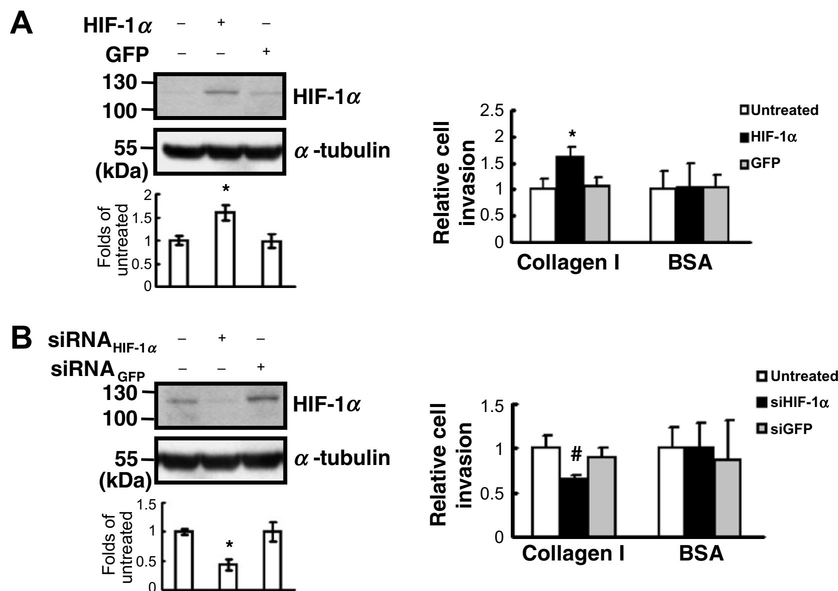

Fig. 2. HIF-1α regulated invasive activity of bmMSCs. BmMSC-5 (1.2×10^6) were transfected with 2 μg of pCEP4/HIF-1α (HIF-1α) or pGFP (GFP) (A), whereas bmMSC-1 (1.2×10^6) were transfected with 100 nM of $siRNA_{HIF\text{-}1\alpha}$ (siHIF-1α) or $siRNA_{GFP}$ (siGFP) (B). A part of cells were harvested 40 h after transfection for Western blot analyses (left panels), and for real-time qPCRs (see Fig. 4). Meanwhile, a part of cells (1.6×10^4 each) were seeded on collagen I-coated and BSA-coated filter membranes 16 h after transfection. Subsequent invasion and migration assays were performed as described in Fig. 1D. Cell counts of the plasmid- or siRNA-transfected cells were compared with those of untreated cells to obtain the relative invasion and migration activities. Data represent the means ± S.D. from three experiments. $*P < 0.05$ and $\#P < 0.01$ versus control.

sions of MMP2 and MMP14 mRNA in bmMSC-5 and bmMSC-1 were not affected by the manipulation of HIF-1α expression. Taken together, our data indicated that HIF-1α could up-regulate the expression of MMP1, and possibly its activity by inducing MMP3 expression in bmMSCs.

4. Discussion

Our investigation revealed that the basal expression of HIF-1α in bmMSCs of old human subjects varies among subjects, and that the elevated HIF-1α can up-regulate at least the

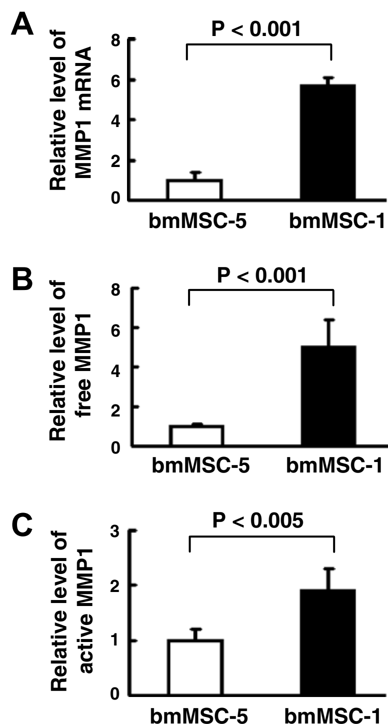

Fig. 3. Measurement of MMP1 expression and activity. (A) Equal amount of cDNA (0.5 μg) prepared from bmMSC-1 and bmMSC-5 were subjected to real-time qPCR assays to detect MMP1 mRNA and β-actin mRNA (as a normalizer). The △CT value of bmMSC-1 was calibrated against that of bmMSC-5 to obtain the ΔΔCT value. The level of MMP1 mRNA of bmMSC-1 was presented relatively to that of bmMSC-5. Data represent the means ± S.D. from three experiments. In addition, bmMSC-1 and bmMSC-5 (1.5×10^5 each) were cultured in 1.5 ml serum-free medium to generate conditioned media. Two batches of media were prepared. These media were subjected to ELISA analyses for the levels of total free MMP1 (B) and endogenous active MMP1 (C). The concentrations of total free and endogenous active MMP1 of bmMSC-1 were 2.9 ± 0.14 and 0.43 ± 0.08 ng/ml, and those of bmMSC-5 were, 0.58 ± 0.07 and 0.23 ± 0.04 ng/ml, respectively. The levels of total free and endogenous active MMP1 of bmMSC-1 were compared with those of bmMSC-5 to obtain relative MMP1 levels. Data represent the means ± S.D. from four triplicate analyses.

expressions of MMP1 and MMP3. Although the role of elevated HIF-1α expression in the biological properties of bmMSCs has not been defined, it is reasonable to assume that bmMSCs with high HIF-1α activity may survive hypoxic/ischemic stress more successfully because HIF-1α can mediate cellular adaptive response to hypoxia [3]. It was reported that the vascular function and limb blood flow are reduced with age, which lowers oxygen level in tissues and represents a risk factor of age-related diseases [12]. So, it is reasonable to speculate that bmMSCs with high HIF-1α level may be less affected by low oxygen level associated with aging. Whether high HIF-1α/MMP1 production may affect the migratory activity of bmMSCs in the collagen I-rich bone marrow microenvironments during the recruitment of bmMSC is not clear. However, based on our data (Fig. 2), it is likely to occur. Since hypoxia increases HIF-1α expression, our data suggest that HIF-1α-induced MMP1 and MMP3 may participate in hypoxia-promoted bmMSC migration. A somewhat different result was reported that hypoxia promotes migration of MSCs mainly through inducing MMP14 [13]. For the signaling mediating bmMSC migration per se, it has been reported that Wnt/β-catenin signaling plays an important role in the migratory capacity of human bmMSCs [8]. Our data reveal that the complicated mechanisms underlying bmMSC migration may also include HIF-1α-related signaling.

Elevated HIF-1α up-regulates MMP1 and MMP3 expression in bmMSCs. So then, could it play a role in the pathogenesis and/or progression of osteoarthritis? Our study design does not provide data directly addressing this question partly because the bone marrow donors are all aged osteoarthritis patients. However, it has been shown that HIF-1 may initiate hypoxia-induced apoptosis by increasing the expression of BNIP3 and/or by stabilizing p53 [14]. Moreover, Komatsu et al. reported that partial HIF-1α deficiency enhances bone regeneration in vivo by decreasing the apoptosis of osteoblast and chondrocyte [15]. These findings suggest that in spite of being a cell survival signal, higher level of HIF-1α may cause pathological effects, especially when certain target genes are excessively induced. Using MMP1 as an example, while low level of MMP1 may participate in healthy tissue remodeling, its level is elevated in arthritic tissues. Based on current theory, osteoarthritis is the consequence of long term mechanical stress on the articular cartilage. In response, the cartilage chondrocytes produce inflammatory cytokines and MMPs including MMP1, which eventually causes destruction of articular cartilage [16,17]. So, under pathological conditions, excess production of MMP1 by chondrocytes may facilitate the destruction of cartilage and development of osteoarthritis. Given that bmMSC can differentiate into chondrocyte and osteoblast, it is

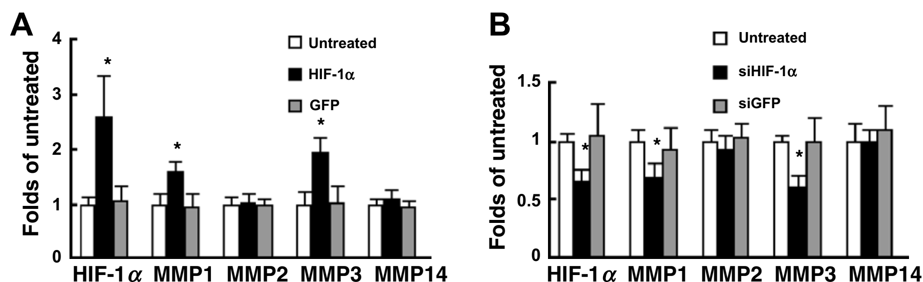

Fig. 4. HIF-1α regulated MMP1 and MMP3 expression in bmMSCs. A part of bmMSC-1 and bmMSC-5 cultures (8×10^5 each) used in the experiments of Fig. 2 were used to prepare cDNA. Equal amount of cDNA (0.5 μg) from various bmMSC-5 cultures (A) and bmMSC-1 cultures (B) were subjected to real-time qPCR analyses to measure the levels of HIF-1α, MMP1, MMP2, MMP3, and MMP14 mRNAs with β-actin mRNA as a normalizer. The ΔCT values of the plasmid- or siRNA-transfected cells were calibrated against that of untreated cells to obtain the ΔΔCT values. The levels of HIF-1α, MMP1, MMP2, MMP3, and MMP14 mRNAs of the plasmid- or siRNA-transfected cells were presented relatively to those of untreated cells. Data represent the means ± S.D. from three analyses. $^*P < 0.05$ versus control.

reasonable to assume that a subclass of bmMSCs expressing higher HIF-1α/MMP1/MMP3 is more likely to play a critical role in the progression of osteoarthritis and other bone diseases. Further characterization of the impact of high HIF-1α expression to the lineage differentiation of bmMSCs and to the proteolytic performance of bmMSC-derived progeny will shed light on the question regarding the putative pathophysiological role of bmMSCs expressing high HIF-1α in bone diseases.

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References

[1] Semenza, G.L. (2000) Hypoxia, clonal selection, and the role of HIF-1 in tumor progression. Crit. Rev. Biochem. Mol. Biol. 35, 71–103.
[2] Crew, S.T. and Fan, C.-M. (1999) Remembrance of things PAS: regulation of development by bHLH-PAS proteins. Curr. Opin. Genet. Dev. 9, 580–587.
[3] Semenza, G.L. (2000) HIF-1: mediator of physiological and pathophysiological responses to hypoxia. J. Appl. Physiol. 59, 47–53.
[4] Owen, M. and Friendenstein, A.J. (1988) Stromal stem cells: marrow-derived osteogenic precursors. Ciba Found. Symp. 136, 42–60.
[5] Bruder, S.P., Fink, D.J. and Caplan, A.I. (1994) Mesenchymal stem cells in bone development, bone repair, and skeletal regeneration therapy. J. Cell Biochem. 56, 283–294.
[6] Prockop, D.J. (1997) Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276, 71–74.
[7] Halleux, C., Sottile, V., Gasser, J.A. and Seuwen, K. (2001) Multi-lineage potential of human mesenchymal stem cells following clonal expansion. J. Musculoskel. Neuron Interact. 2, 71–76.
[8] Neth, P., Ciccarella, M., Egea, V., Hoelters, J., Jochum, M. and Ries, C. (2006) Wnt signaling regulates the invasion capacity of human mesenchymal stem cells. Stem Cells 24, 1892–1903.
[9] Ries, C., Egea, V., Karow, M., Kolb, H., Jochum, M. and Neth, P. (2007) MMP-2, MT1-MMP, and TIMP-2 are essential for the invasive capacity of human mesenchymal stem cells: differential regulation by inflammatory cytokines. Blood 109, 4055–4063.
[10] Krishnamachary, B., Berg-Dixon, S., Kelly, B., Agani, F., Feldser, D., Ferreira, G., Iyer, N., LaRusch, J., Pak, B., Taghavi, P. and Semenza, G.L. (2003) Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. Cancer Res. 63, 1138–1143.
[11] Shyu, K.-G., Hsu, F.-L., Wang, M.J., Wang, B.-W. and Lin, S. (2007) Hypoxia-inducible factor 1α regulates lung adenocarcinoma cell invasion. Exp. Cell Res. 313, 1181–1191.
[12] Dinenno, F.A., Jones, P.P., Seals, D.R. and Tanaka, H. (1999) Limb blood flow and vascular conductance are reduced with age in healthy humans. Circulation 100, 164–170.
[13] Annabi, B., Lee, Y.T., Turcotte, S., Naud, E., Desrosiers, R.R., Champagne, M., Eliopoulos, N., Galipeau, J. and Béliveau, R. (2003) Hypoxia promotes murine bone-marrow-derived stromal cell migration and tube formation. Stem Cells 21, 337–347.
[14] Greijer, A.E. and Wall, E.V.D. (2004) The role of hypoxia inducible factor 1 in hypoxia induced apoptosis. J. Clin. Pathol. 57, 1009–1014.
[15] Komatsu, D.E., Bosch-Marce, M., Semenza, G.L. and Hadjiargyrou, M. (2007) Enhanced bone regeneration associated with decreased apoptosis in mice with partial HIF-1alpha deficiency. J. Bone Miner. Res. 22, 366–374.
[16] Shlopov, B.V., Lie, W.R., Mainardi, C.L., Cole, A.A., Chubinskaya, S. and Hasty, K.A. (1997) Osteoarthritic lesions: involvement of three different collagenases. Arthritis Rheum. 40, 2065–2074.
[17] Mitchell, P.G., Magna, H.A., Reeves, L.M., Lopresti-Morrow, L.L., Yocum, S.A., Rosner, P.J., Geoghegan, K.F. and Hambor, J.E. (1996) Cloning, expression, and type II collagenolytic activity of matrix metalloproteinase-13 from human osteoarthritic cartilage. J. Clin. Invest. 97, 761–768.