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SHORT COMMUNICATION

Regulation of high mobility group box 1 and hypoxia in the migration of mesenchymal stem cells

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Abstract

Mesenchymal stem cells (MSCs) have been increasingly offered for tissue regeneration with the premise that they can survive and thrive amidst the microenvironment of injured or degenerate tissues. The role of high mobility group box 1 (HMGB1) and hypoxia in the proliferation and migration of rat bone marrow MSCs (rBM-MSCs) has been investigated. First, the effect of HMGB1 on the proliferation of rBM-MSCs was determined. Second, to evaluate the regulation of hypoxia and HMGB1 in the migration of rBM-MSCs, cells in the wound healing model were exposed to four conditions: normoxia (20% O_2) and complete medium, normoxia and HMGB1, hypoxia (1% O_2) and complete medium, hypoxia and HMGB1. RT-PCR and Western blotting were used to measure the expression of migration-related genes and proteins. HMGB1 inhibited the proliferation of rBM-MSCs; HMGB1 alone or together with hypoxia and promoted the migration of MSCs and upregulated the expression of HIF-1α and SDF-1. These results demonstrated that HMGB1 arrested the proliferation of rBM-MSCs, but enhanced the migration of rBM-MSCs which could be further improved by hypoxia. This study strengthens current understanding of the interaction between MSCs and the microenvironment of damaged tissues.

Keywords: BM-MSCs; HMGB1; hypoxia; migration; SDF-1/CXCR4

Introduction

Mesenchymal stem cells (MSCs) may be the most promising graft cells for tissue regeneration, having been successfully isolated from bone marrow, adipose tissue, peripheral blood, placenta, amniotic fluid and other tissues (Pittenger et al., 1999; Kern et al., 2006; Tsai et al., 2007; Parolini et al., 2008; Huang et al., 2009). To repair damaged tissue, endogenous MSCs have to leave the native niches, circulate following the blood stream and relocate to initiate the regenerative process; exogenous MSCs must anchor and integrate with the host cells and tissues. Both approaches depend on the migration of MSCs. In animals with myocardial infraction, most of MSCs die post-implantation and few congregate in the injured zone (Toma et al., 2002; Gonzales and Pedrazzini, 2009; Toma et al., 2009). A large proportion of cells were trapped either in the lung or microvasculature (Hara et al., 2008). How tissue microenvironment regulates the migration of MSCs is critical for understanding the regenerative process.

In damaged tissues, endogenous cells can release several kinds of cytokines after apoptosis or necrosis (Voll et al., 1997; Uccelli et al., 2008). These cytokines affect the biological behaviour of implanted MSCs (Abarbanell et al., 2009). High mobility group box 1 (HMGB1) exists in the nuclei and cytoplasm of nearly all cell types, is secreted

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Abbreviations: HMGB1, high mobility group box 1; FBS, foetal calf serum; L-DMEM, low glucose Dulbecco's modified Eagle's medium; rBM-MSCs, rat bone marrow-derived mesenchymal stem cells; CFU-F, colony forming unit fibroblasts; SDF-1, stromal- derived factor 1; CXCR4, C-X-C chemokine receptor type 4; HIF-1α, hypoxia inducible factor-1α

into the extracellular milieu, and acts as a pro-inflammatory cytokine under hypoxia, apoptosis and necrosis (Palumbo et al., 2007 Paola Scaffidi, 2002). HMGB1 could mobilise bone marrow cells to target the engrafted skin in vivo (Tamai et al., 2011). Nevertheless, whether the resident stem cells in bone marrow contribute to this regeneration is not clearly elucidated. Hence, the first aim was to test how HMGB1 regulates the proliferation and migration of BM-MSCs. As one of the major components in the damaged microenvironment, hypoxia affects MSCs dramatically, especially the migration ability (Raheja et al., 2011). Therefore, the other aim was to explore the combined regulation of hypoxia and HMGB1 in BM-MSCs migration.

Materials and methods

Isolation of rat BM-MSCs

Fifteen Sprague–Dawley rats were obtained from the West China Medical Center Laboratorial Animal Center of Sichuan University. Experimental procedures were approved by Laboratory Animal Management Committee of Sichuan Province (Permit No.: SYXK-2008-119). BM-MSCs from third passage were harvested and identified per our previous description (Huang et al., 2012).

Proliferation assay

Rat BM-MSCs were seeded in 96-well plates at an initial density of 3,000 cells/cm^2, incubated with complete medium at 37°C with 5% CO_2 for 24 h, and treated with HMGB1 at three different concentrations (i.e. 10, 50 and 100 ng/mL) (Prospec, Israel) for 72 h ($n = 6$, triplicate); cells cultured in the complete medium without HMGB1 were the control group. At each time-point, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide) assay was used and absorbance measured in an ELISA plate reader (Patents, USA) at 490 nm. For colony-forming unit (CFU) assay, cells were plated at 80 cells/cm^2 into 10-cm culture dishes and incubated in complete medium with concentrations of HMGB1 as described above for 5 days. Upon harvesting, cells were fixed with methanol and stained with 0.1% crystal violet for 30 min ($n = 3$, triplicate). The number of clones was counted using Image-Pro Plus 6.0 software (Media Cybernetics, Crofton, MD)

Migration assay

MSCs were seeded at 1×10^4 cells/cm^2 in the 12-well plate. After 80–90% confluence, a cell-free gap was scratched using a sterile pipette tip as the wound model. The cells were treated with HMGB1 (10–100 ng/mL). The gaps were observed after 24 h incubation using an inverted microscope (Olympus IX71, Japan). Wound closure was analysed by DP2-BSW microscope digital camera software (Olympus, Japan) and the healing was calculated as the percentage of the remaining cell-free area at 24 h in comparison to the area of the initial wound at 0 h ($n = 3$, triplicate). Transwell migration was assayed using cell culture inserts (Corning, USA) with 8 μm pore filters. HMGB1 in complete medium (1–100 ng/mL) were added into the lower chamber and 3×10^5 serum-starved MSCs were seeded in the upper one. After 24 h incubation, non-migrating cells were removed with cotton swabs and the wells fixed with 4% paraformaldehyde, and stain with Haematoxylin and Eosin (H&E). The cells were counted under a light microscope in 5 random fields ($n = 3$, triplicate).

Experimental hypoxic condition

The hypoxic system was generated in hypoxia incubator chambers (Thermo Fisher Scientific, USA) in which O_2 was adjusted to 1%. Pre-cultured in normoxia (20% O_2) with complete medium for 24 h, the wound model was established and the cells were treated in the following conditions: (1) NC group: cells cultured in complete medium and normoxia; (2) NH group: cells cultured in 50 ng/mL HMGB1 within complete medium and normoxia; (3) HC group: cells cultured in complete medium and 1% O_2; (4) HH group: cells cultured in 50 ng/mL HMGB1 within complete medium and 1% O_2. Healing was calculated as above ($n = 3$, triplicate).

Real-time polymerase chain reaction (RT-PCR)

After 24 h treatment, total RNA of each groups were extracted and 1 μg RNA of each sample was used for cDNA synthesis using isoPLUS reagent (TAKARA, Japan). Gene expression of each group was analysed by RT-PCR. Briefly, cycling conditions included 95°C for 30 s, followed by 40 cycles at 95°C for 5 s and 56°C for 30 s and the primers of each gene are listed in the Table S1. Target gene expressions = target gene (i.e. SDF-1, CXCR4 or HIF-1α)/GAPDH ratios. Experiments were repeated 3 times in triplicate.

Western blotting

After 24 h treatment, total protein of each groups were extracted and detected by Western blotting. Whole-cell lysates were isolated; 20 μg protein was electrophoresed through 12% PAGE gel (Novex; Invitrogen, USA) and electrotransfered to nitrocellulose membrane (Amersham Pharmacia Biotech, USA). After blocking with TBST containing 5% non-fat milk, the membrane was incubated overnight at 4°C with the following primary antibodies: CXCR4 (Abcam, UK) (1:1,000), SDF-1 (Ebioscience, USA)

(1:1,000), GAPDH (Abcam, UK) (1:1,000) and HIF-1α (Abcam, UK) (1:400). The primary antibodies were diluted in TBST containing 5% non-fat milk. After being washed of the non-combined primary antibodies, the membrane was incubated with HRP-conjugated secondary antibody (1:6,000) and the immune complexes were visualised using chemiluminescence reagent kit (Thermo, USA). Autoradiographs were scanned by an imaging densitometer (HP, USA).

Statistical analysis

Statistical analysis used the software SPSS 16.0. The results were expressed as the mean ± SD of three independent experiments. One-way ANOVA was used to compare data from proliferation, CFU formation and migration assay; the difference between groups was further evaluated by LSD (least significant difference) test. Significance was accepted at $P < 0.05$.

Results and discussion

The concentration of HMGB1 ranging from 10 to 100 ng/mL was referenced to that of serum HMGB1 in septic patients (Wang, 1999). Inhibited proliferation of rBM-MSCs was observed after HMGB1 treatment for 3 days. Compared to the control group (0.73 ± 0.06), all three HMGB1 treatment groups (10 ng/mL, 0.64 ± 0.05; 50 ng/mL, 0.60 ± 0.01; 100 ng/mL, 0.57 ± 0.01) obviously suppressed the proliferation of rBM-MSCs after 72 h treatment ($P < 0.01$) (Figure 1A). Similarly, this inhibition was found in the CFU formation analysis; in HMGB1 treatment groups, the number of CFU decreased sharply (10 ng/mL, 554.3 ± 18.8; 50 ng/mL, 548 ± 2.16; 100 ng/mL, 530.33 ± 8.38) ($P < 0.01$) (Figures 1B–1F) compared to the control group (592.3 ± 8.7) after 5-day stimuli. Hence, HMGB1 significantly suppressed the proliferation and CFU formation ability of rBM-MSCs.

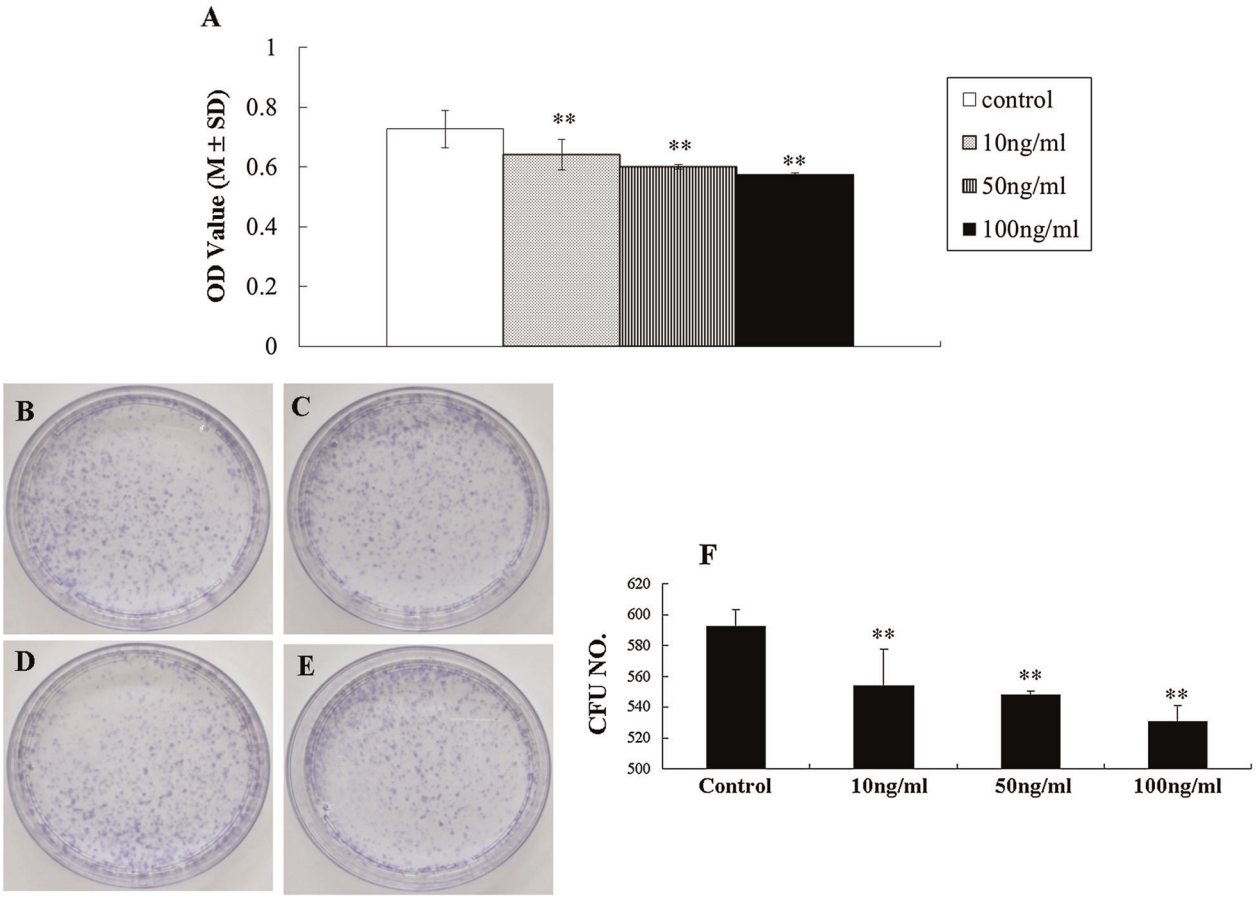

Figure 1 Effect of HMGB1 on the proliferation and CFU-formation potential of rBM-MSCs. (A) BM-MSCs were treated by complete medium with different concentrations of HMGB1 for 3 days; cell proliferation was determined by MTT assay. (B–E) 0.1% crystal violet staining of rBM-MSCs cultured in medium with the control (B), 10 ng/mL (C), 50 ng/mL (D) and 100 ng/mL HMGB1 (E) for 5 days. (F) Statistical analysis of CFU-F numbers of rBM-MSCs under HMGB1 treatment. Values were presented as mean ± SD of three independent experiments. $^{**}P < 0.01$ compared to the control group.

Transwell analysis showed that HMGB1 treatment for 24 h significantly increased the migration of rBM-MSCs ($P < 0.05$) (Figures 2A–2E). Similarly, the percentage of cell-free area in the wound healing assay decreased in HMGB1-treatment groups (Figures 2F and 2G). RT-PCR showed expression of migration-related genes encoding SDF-1 and HIF-1α were strongly upregulated in the 50 ng/mL group compared with the group without HMGB1 in normoxia ($P < 0.01$) (Figures 3D and 3E) (SDF-1, 1.38-fold; HIF-1α, 1.75- fold); additionally, Western blotting confirmed the increased expression of SDF-1, CXCR4 and HIF-1α in HMGB1 treatment groups in normoxia (Figure 3F) (SDF-1, 1.18-fold; CXCR4, 1.31-fold; HIF-1α, 1.81-fold). These indicated the vital role of SDF-1-CXCR4 axis in regulating migration of rBM-MSCs. MSCs migration in vitro and in vivo recruitment to injured sites was partly mediated by SDF-1-CXCR4 axis (Kitaori et al., 2009; Yuan et al., 2013). On the basis of our data, HMGB1 may promote the migration of BM-MSCs through SDF-1/CXCR4 axis.

Stimulation of HMGB1 enhanced rBM-MSCs migration induced by hypoxia (Figure 3A); the percentage of cell-free area in NH, HC and HH groups were less obvious than NC group ($P < 0.01$) and the gap disappeared in groups HC and HH (Figures 3A and 3B). Hypoxia significantly improved the expression of SDF-1 and HIF-1α genes expression in the presence of HMGB1 ($P < 0.01$) (SDF-1, 1.47-fold; HIF-1α, 1.46-fold), while comparative expression of CXCR4 occurred (Figures 3C–3E). At the protein expression level, Western blotting showed that hypoxia enhanced the expression of SDF-1 by 1.37-fold in the HMGB1 containing group (Figure 3F). Hence, hypoxia promoted the migration of rBM-MSCs induced by 50 ng/mL HMGB1 in the wound healing assay. Increased expression of SDF-1 at both gene and protein level occurred while the increased transcription of HIF-1α in HH group did not increase protein level. This inconsistent phenomenon may due to post-transcriptional control of HIF-1α gene expression. Thus, HMGB1 has an apparent promoting effect on HIF-1α expression. These findings indicated the possible enhancement of HMGB1 on HIF-1α expression in normoxia.

Migration of BM-MSCs was increased by hypoxia partly via the SDF-1-CXCR4 pathway (Liu et al., 2012). Based on the results of RT-PCR and Western blotting, the expression of SDF-1 and HIF-1α in HH group was much greater than those in the NH group, from which we infer that hypoxia preconditioning might facilitate migration of rBM-MSCs

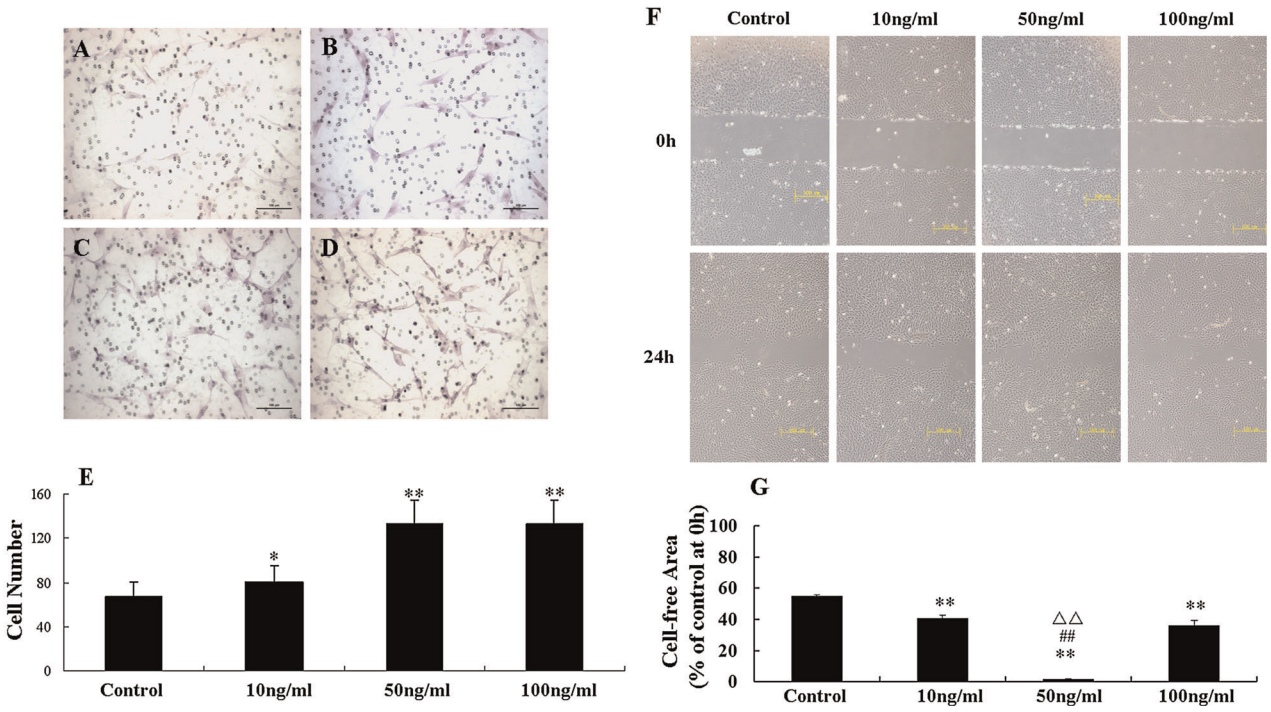

Figure 2 HMGB1 increased the migration of rBM-MSCs in wound healing assay and transwell analysis. (A–D) In transwell assay, the transmembrane cells were stained by H&E after 24 h treatment. (C–F) Cells treated with complete medium, 10, 50 and 100 ng/mL HMGB1 in complete medium, scale bar = 100 μm. (E) Cells stained by H&E were counted after treated by different concentration HMGB1 for 24 h. (F) Phase contrast images of the closure gap after treated by 0, 10, 50 and 100 ng/mL HMGB1 at 24 h, scale bar = 500 μm. (G) Percentage of cell-free area at 24 h compared to that at 0 h was determined. Values were presented as mean ± SD of three independent experiments. $^{*}P < 0.05$ compared to the control group; $^{**}P < 0.01$ compared to the control group; $^{\#\#}P < 0.01$ compared to the 10 ng/mL group; $^{\triangle\triangle}P < 0.01$ compared to the 100 ng/mL group.

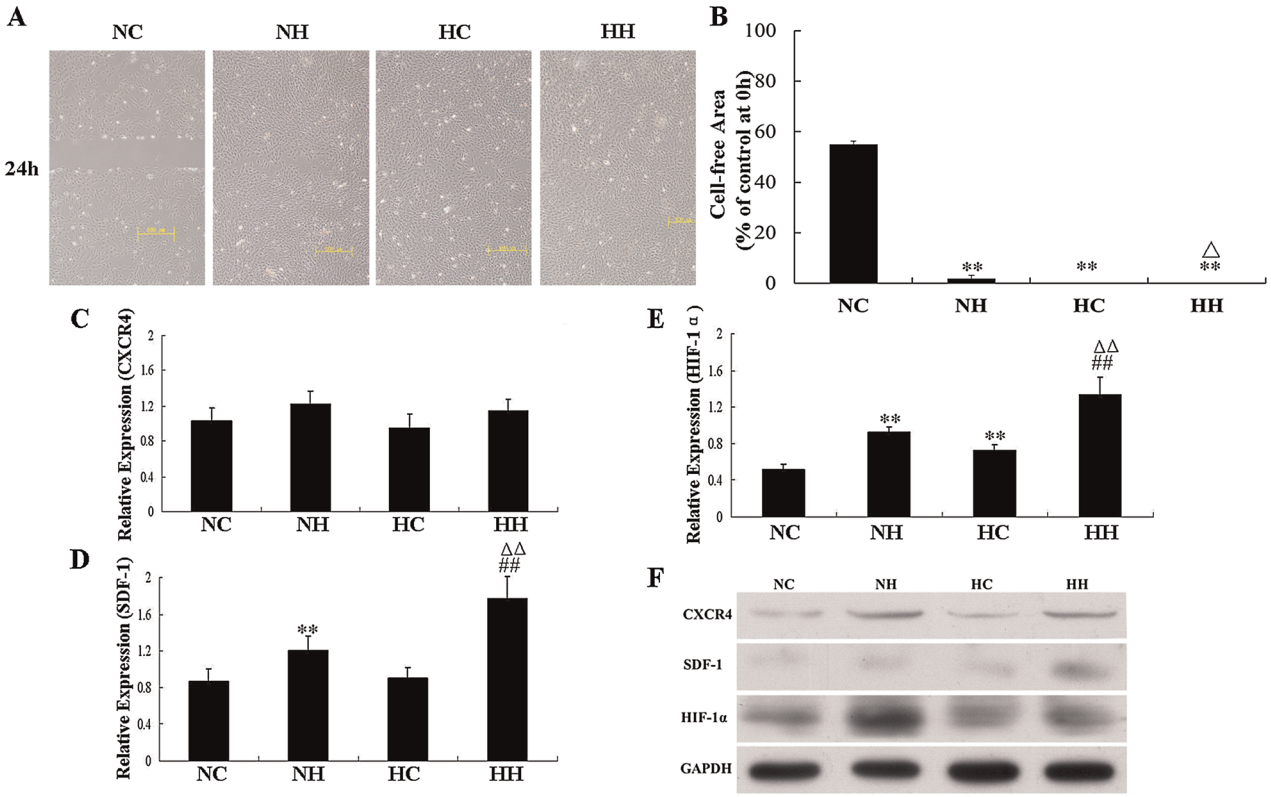

Figure 3 Hypoxia enhanced rBM-MSCs migration induced by HMGB1 treatment and related mechanisms. (A) Phase contrast images of the closure gap under the four treatments at 24 h, scale bar = 500 μm. (B) Percentage of cell-free area at 24 h compared to that at 0 h was determined; values were presented as mean ± SD of three independent experiments. (C–E) Expression of CXCR4 (C), SDF-1 (D), HIF-1α (E) mRNA were analysed by RT-PCR in the four groups; expression levels were relative to GAPDH. (F) Expression of CXCR4 (~44 kDa), SDF-1 (~15 kDa), HIF-1α (~110 kDa) protein on MSCs after four treatments for 24 h were analysed by Western blotting; values were presented as mean ± SD of three independent experiments. $^{**}P < 0.01$ compared to the group NC (cells cultured in complete medium and normoxia); $^{\#\#}P < 0.01$ compared to the group NH (cells cultured in 50 ng/mL HMGB1 within complete medium and normoxia); $^{\triangle}P < 0.05$ compared to the group NH (cells cultured in 50 ng/mL HMGB1 within complete medium and normoxia), $^{\triangle\triangle}P < 0.01$.

induced by HMGB1 via HIF-1α-SDF-1 pathway. That is because HIF-1α-SDF-1 pathway regulates the migration of progenitor cells in hypoxia (Ceradini et al., 2004). However, hypoxia also induces the release of HMGB1 in hepatocellular carcinomacells (Yan et al., 2012); but whether this phenomenon can be replicated by BM-MSCs remains unclear. We acknowledge that the absence of the precise mechanism of this enhancement is the major limitation of this study. This work shows the influence of hypoxia and HMGB1 on MSCs migration, which extends the knowledge of the interaction between implanted MSCs and the microenvironment of the damaged tissues.

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References

Abarbanell AM, Coffey AC, Fehrenbacher JW, Beckman DJ, Herrmann JL, Weil B, Meldrum DR (2009) Proinflammatory cytokine effects on mesenchymal stem cell therapy for the ischemic heart. Ann Thorac Surg 88: 1036–43.

Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, Capla JM, Galiano RD, Levine JP, Gurtner GC (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 10: 858–64.

Gonzales C, Pedrazzini T (2009) Progenitor cell therapy for heart disease. Exp Cell Res 315: 3077–85.

Hara M, Murakami T, Kobayashi E (2008) In vivo bioimaging using photogenic rats: fate of injected bone marrow-derived mesenchymal stromal cells. J Autoimmun 30: 163–71.

Huang YC, Yang ZM, Chen XH, Tan MY, Wang J, Li XQ, Xie HQ, Deng L (2009) Isolation of mesenchymal stem cells from human placental decidua basalis and resistance to hypoxia and serum deprivation. Stem Cell Rev 5: 247–55.

Huang YC, Zhu HM, Cai JQ, Huang YZ, Xu J, Zhou Y, Chen XH, Li XQ, Yang ZM, Deng L (2012) Hypoxia inhibits the spontaneous calcification of bone marrow-derived mesenchymal stem cells. J Cell Biochem 113: 1407–15.

Kern S, Eichler H, Stoeve J, Kluter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 24: 1294–301.

Kitaori T, Ito H, Schwarz EM, Tsutsumi R, Yoshitomi H, Oishi S, Nakano M, Fujii N, Nagasawa T, Nakamura T (2009) Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheum 60: 813–23.

Liu H, Liu S, Li Y, Wang X, Xue W, Ge G, Luo X (2012) The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. PLoS One 7: e34608.

Palumbo R, Galvez BG, Pusterla T, De Marchis F, Cossu G, Marcu KB, Bianchi ME (2007) Cells migrating to sites of tissue damage in response to the danger signal HMGB1 require NF-kappaB activation. J Cell Biol 179: 33–40.

Paola Scaffidi TMMEB (2002) Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. Nature 418: 191–5.

Parolini O, Alviano F, Bagnara GP, Bilic G, Buhring HJ, Evangelista M, Hennerbichler S, Liu B, Magatti M, Mao N, Miki T, Marongiu F, Nakajima H, Nikaido T, Portmann-Lanz CB, Sankar V, Soncini M, Stadler G, Surbek D, Takahashi TA, Redl H, Sakuragawa N, Wolbank S, Zeisberger S, Zisch A, Strom SC (2008) Concise review: isolation and characterization of cells from human term placenta: outcome of the first international Workshop on Placenta Derived Stem Cells. Stem Cells 26: 300–11.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284: 143–7.

Raheja LF, Genetos DC, Wong A, Yellowley CE (2011) Hypoxic regulation of mesenchymal stem cell migration: the role of RhoA and HIF-1alpha. Cell Biol Int 35: 981–9.

Tamai K, Yamazaki T, Chino T, Ishii M, Otsuru S, Kikuchi Y, Iinuma S, Saga K, Nimura K, Shimbo T, Umegaki N, Katayama I, Miyazaki J, Takeda J, McGrath JA, Uitto J, Kaneda Y (2011) PDGFRalpha-positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia. Proc Natl Acad Sci U S A 108: 6609–14.

Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD (2002) Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. Circulation 105: 93–8.

Toma C, Wagner WR, Bowry S, Schwartz A, Villanueva F (2009) Fate of culture-expanded mesenchymal stem cells in the microvasculature: in vivo observations of cell kinetics. Circ Res 104: 398–402.

Tsai MS, Hwang SM, Chen KD, Lee YS, Hsu LW, Chang YJ, Wang CN, Peng HH, Chang YL, Chao AS, Chang SD, Lee KD, Wang TH, Wang HS, Soong YK (2007) Functional network analysis of the transcriptomes of mesenchymal stem cells derived from amniotic fluid, amniotic membrane, cord blood, and bone marrow. Stem Cells 25: 2511–23.

Uccelli A, Moretta L, Pistoia V (2008) Mesenchymal stem cells in health and disease. Nat Rev Immunol 8: 726–36.

Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I (1997) Immunosuppressive effects of apoptotic cells. Nature 390: 350–1.

Wang H (1999) HMG-1 as a late mediator of endotoxin lethality in mice. Science 285: 248–51.

Yan W, Chang Y, Liang X, Cardinal JS, Huang H, Thorne SH, Monga SP, Geller DA, Lotze MT, Tsung A (2012) High-mobility group box 1 activates caspase-1 and promotes hepatocellular carcinoma invasiveness and metastases. Hepatology 55: 1863–75.

Yuan L, Sakamoto N, Song G, Sato M (2013) Low-level shear stress induces human mesenchymal stem cell migration through the SDF-1/CXCR4 axis via MAPK signaling pathways. Stem Cells Dev 22: 2384–93.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1. Specific primers used for real-time polymerase chain reaction.