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HIF-1α Overexpression Induces Angiogenesis in Mesenchymal Stem Cells

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Abstract

Stem cell therapy continues to be an innovative and promising strategy for heart failure. Stem cell injection alone, however, is hampered by poor cell survival and differentiation. This study was aimed to explore the possibility of improving stem cell therapy through genetic modification of stem cells, in order for them to promote angiogenesis in an auto- and paracrine manner under hypoxic conditions. Hypoxia inducible factor-1α was overexpressed in bone marrow-derived mesenchymal stem cells (MSCs) by stable transduction using a lentiviral vector. Under hypoxic and normoxic conditions, the vascular endothelial growth factor (VEGF) concentration in the cells' supernatant was measured by an enzyme-linked immunosorbent assay. Migration was assayed by wound healing and c-Met expression by flow cytometry. Tube formation was evaluated on a Matrigel basement membrane. The concentration of VEGF was significantly increased in the supernatant of HIF-1α–overexpressing MSCs; this medium was significantly more effective in inducing endothelial cell migration compared to untransduced MSCs. Transduced cells showed increased levels of c-Met expression and were more efficient at tube formation. However, no indication of differentiation toward an endothelial phenotype was observed. This study indicated that genetic modification of MSCs by HIF-1α overexpression has the potential to improve components of the angiogenesis process under a hypoxic condition by paracrine and autocrine mechanisms.

Key words: angiogenesis; hypoxia; HIF-1α; mesenchymal stem cells

Introduction

Despite the progress achieved in prevention and conventional treatments, ischemia, as the main cause of cardiac injury, and postischemic heart failure have now become a worldwide problem.[1] In this setting, the possibility to develop innovative therapeutic approaches using stem cells has engendered a significant interest, which is fully supported by preclinical findings in small and large animals of cardiac ischemia.[2] Despite the enormous potential of stem cell therapy, however, a reason for growing concern is that the behavior of donor cells, once implanted *in vivo*, might be very different from that shown in cell culture, and that this might essentially limit their therapeutic potential.[2–5] One way to circumvent this problem, and thus optimize cell-based therapy for organ repair, could be via the genetic modification of donor stem cells, in order for them to maintain their potential and promote their proliferation and survival *in vivo*. Alternatively, stem cell genetic modification might also be exploited to ameliorate the microenvironment by inducing neovascularization of the target tissue.[6–10]

In recent two decades, identification of cardiac dysfunction molecular mechanisms and dissection of the molecular and cellular mechanisms effective in tissue regeneration have evolved rapidly. As a consequence, a growing body of knowledge is now available on the possible genes that might be exploited, either alone or in the context of genetic modification of stem cells.[11–13]

As a platform for stem cell and gene therapy, mesenchymal stem cells (MSCs) have attracted significant attention for the repair of damaged heart tissue. This attention is partly due to their *ex vivo* expansion potential while maintaining their plasticity, and partly due to their immunosuppressive properties, their trophic effects resulting from paracrine secretions, and their capacity to promote neovascularization.[14–21]

In particular, it has been reported that bone marrow MSCs protect cardiomyocytes by upregulation of vascular endothelial growth factor-A (VEGF) under hypoxic culture conditions

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through the activation of hypoxia inducible factor (HIF)-1α.[22] This might be of particular interest, especially when considering that MSCs after transplantation *in vivo* into the infarcted myocardium, find themselves inside an ischemic microenvironment, characterized by reduced oxygen (O_2) tension and nutrient deprivation that may jeopardize their viability.[23] Indeed, HIF-1α is a master transcription factor, which is stabilized by hypoxia and regulates the expression of several genes that are effective in angiogenesis, preventing apoptosis, and inducing migration and homing of cells into the site of ischemia.[2,4,24,25] *In vitro* culture of MSCs under low oxygen tension close to the *in vivo* situation (1%–7%) influences their paracrine secretion pattern, differentiation, proliferation, migration, and survival.[4,22,26–28] However, MSCs resist hypoxia for only a few days, which is in contrast to the requirement to be met upon *in vivo* implantation, where they need to withstand hypoxia significantly longer. Of interest, hypoxic preconditioning, a condition known to induce HIF-1α, has frequently been reported to act as a very powerful cytoprotective stimulus, which attenuates apoptosis and improves survival and differentiation of stem cells.[4,24]

For all the above reasons, inducing prolonged expression of HIF-1α in MSCs appears as an appealing possibility on one hand to promote survival of MSCs themselves in hypoxia, whereas, on the other, to promote production of a cocktail of growth factors that might improve endothelial functional angiogenesis *in vivo*.

This study attempted to elucidate the effect of long-term expression of HIF-1α in human bone marrow-derived MSCs, upon gene delivery using a lentiviral vector, on MSCs' angiogenesis potential.

Material and Methods—Experimental

Culture and characterization of MSCs

MSCs were isolated similarly to a previously described protocol.[29] Human MSCs were obtained from iliac crest aspirates of healthy donors ranging in age from 19 to 32 years at the Bone Marrow Transplantation Center, Shariati Hospital, Tehran, Iran. Samples were collected after obtaining informed consent from individuals and being approved by the Institutional Review Board protocol, according to guidelines of the Medical Ethics Committee, Ministry of Health, Iran. The mononuclear cell fraction was separated by centrifugation over a Ficoll-Paque gradient and suspended in a basal medium [Dulbecco's modified Eagle's medium (DMEM) high glucose, supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine (all from Gibco), 100 U/mL of penicillin, and 100 μg of streptomycin (Sigma-Aldrich Co.)].

After 3 days, the nonadherent cell fraction was removed by washing with PBS. Monolayer adherent cells were cultured in the same medium until they reached 70%–90% confluence. Cells were passaged four times to ensure removal of hematopoietic cells. Human bone marrow mesenchymal stem cell (hBMSC) cultures were kept under normoxia at 37°C in a humidified atmosphere containing 95% air and 5% CO_2 and under a hypoxic condition at 37°C in a humidified atmosphere containing 93% nitrogen, 2% oxygen, and 5% CO_2.

To characterize expression of surface markers, cultures were harvested at 80% confluency by 0.025% trypsin containing 0.02% EDTA (Gibco), and labeled with PE-conjugated mouse anti-human CD31, FITC-conjugated mouse anti-human CD34, FITC conjugated mouse anti-human CD105, and FITC-conjugated mouse anti-human CD73 for flow cytometry (Beckman coulter; Fullerton). FITC-conjugated mouse IgG2 and PE-conjugated mouse IgG1 were used as isotype controls. Data were analyzed using WinMDI2.8 (PUCL).

The phenotype of MSCs is defined in part by the multipotency of these cells in culture, and so they were induced to differentiate into osteocytes and adipocytes to identify their MSC properties. About 1×10^4 hMSCs were seeded in six-well culture plates under osteogenesis and adipogenesis media. In addition to the basal medium, the osteogenesis medium contained dexamethazone (10 μM), ascorbic acid (50 μg/mL) and beta-glycerol phosphate (10 μM), whereas the adipogenesis medium (all from Sigma-Aldrich Co.) contained dexamethazone (1 μM), indomethacin (200 μM), insulin (1.7 μM), and isobutylmethylxanthine (500 μM). After 14 days of differentiation (with replacing the medium every 3 days), the cells were fixed using a 4% paraformaldehyde solution and stained by Alizarin red staining and Oil red O staining for calcium deposition in osteocytes and fat droplet in adipocytes, respectively.

hMSCs were seeded in a six-well plate at the density of 1×10^5cells per well for infection by recombinant lentiviruses.

Cloning of HIF-1α cDNA, recombinant lentivirus production, and transduction of MSCs

HIF-1α cDNA was subcloned from puc57 in to the iG2 transfer vector by digesting both plasmids with *Bam*H1 (Fermentas Co.), and ligate cDNA into iG2 by a ligation reaction kit (Fermentas Co.) containing T4 ligase. Fidelity of cloning was proved by restriction mapping by *NCO*1 and sequencing.

iG2 contains a retroviral enhancer/promoter of spleen focus-forming virus and an encephalomyocarditis virus internal ribosome entry site in front of the enhanced green fluorescent protein (eGFP), which is preceded by unique cloning sites. This construct facilitates simultaneous and high expression of the transgene, in addition to eGFP, in a broad range of cell types. iG2, mD2G, encoding VSV-G envelope to facilitate infection of a variety of cell types, and psPAX2 plasmids were cotransfected, using the calcium–phosphate method, into the HEK-293. T monolayer cell cultures to produce the lentiviral particles expressing HIF-1α. Lentiviral particles were collected in 24, 48, and 72 h after transfection from 293-T cells' supernatant, concentrated using Amicon Ultra Centrifugal Filter Devices (Millipore), and stored at −80°C.

The vector titer was evaluated by plating 5×10^5 HEK-293. T cells in five wells of a six-well plate and infecting with serial dilutions from collected viruses to four wells. One well remained as a negative control. To determine transducing units per milliliter, the 293. T cells were trypsinized after 72 h, and GFP-expressing cells were quantified using flow cytometry.

One round of MSC transduction was performed at several consecutive multiplicity of infection (MOI) (5–50) in the presence of polybrene (8 mg/mL final concentration). After 8 h, the medium was replaced, and after 3 days, cultures were visualized using an invert fluorescent microscope (Nikon), and finally the percent of GFP-positive cells was determined using flow cytometry.

Reverse transcriptase-polymerase chain reaction and ELISA for expression of HIF-induced genes

Total RNA was extracted using an RNeasy plus microkit (Qiagene Co.) and overexpression of HIF-1α and GAPDH was investigated by reverse transcriptase (RT)-polymerase chain reaction (PCR), using a power cDNA synthesis kit and a maxim PCR premix kit (Intron Co., South Korea) by designing the following primers: HIF-1α: f–cttctggatgct ggtgatttgg, r–gtgtccagttagttcaaactgag; GAPHD: f–agaagg ctggggctcatttgc, r–tgcaggaggcattgctgatg. Expression of the HIF-1α mRNA was analyzed in both normoxic and hypoxic conditions by semiquantitative RT-PCR. In these assays, amplification was carried out for 25 PCR cycles to maintain the reaction in the linear range; primer pairs were able to amplify both the endogenous HIF-1α mRNA and the lentiviral transgene. Expression of endothelial marker genes in MSCs was investigated by RT-PCR for vWF, CD34, and Tie2 using the following primers: vWF, f–ccgccaggtccaacagag, r–gcaaatcc taacaaatccagagc; CD34, f–accccagagttacctacccag, r–tgtcgtttct gtgatgtttgttg; Tie2, f–gttccttcatccattcagtgc, r–cacttctgggcttcac atctc. The VEGF concentration was quantified in the medium of MSCs and transduced MSCs (HIF-MSCs) after 24-h incubation under hypoxic conditions using an ELISA kit (hVEGF ELISA kit; Invitrogen Co.).

Tube formation assay

The tube formation assay was used as a model for assessment of angiogenesis. Matrigel (BD Matrigel™; BD Biosciences Co.) was thawed overnight at 4°C and administered by cold tips in 50 μL per well in cold 96-well cell culture plates. Matrigel made a thin gel layer after incubation at 37°C for 1 h. About 1.0×10^4 of infected and noninfected MSCs were seeded on each Matrigel-coated well and after 24-h hypoxia. Network formation was evaluated under the invert fluorescent microscope.

Wound-healing assay

Wound-healing assay on endothelial cells, human umbilical vein endothelial cell (HUVECs), was used to study the paracrine effect of MSCs on endothelial cell migration potential. After 24-h culture of MSCs and HIF-MSCs under low oxygen tension, the supernatants from hypoxic cells were collected. The wound-healing assay was employed by scratching the monolayer HUVEC confluent cell cultures using sterile 200-μL sterile tips. After 8 h FBS starvation (0.5% FBS) for HUVECs, wound healing initiated by administering the collected supernatants from MSCs and HIF-MSCs on HUVEC cultures in different wells of a 24-well plate. The assay was accomplished by recording the time required for each wound to be closed completely by migrating HUVECs from borders of the wounds in to the scratched zone.

Expression level of c-MET

The expression level of c-Met receptor was quantified by flow cytometry (anti-hHGF R/c-Met fluorescein-conjugated mouse IgG1 antibody; R&D Systems) on the surface of both MSCs and HIF-MSCs after 24-h hypoxia.

Results

Characterization of MSCs

Figure 1A represents a picture of spindle-shaped MSCs at passage 3 that were obtained from bone marrow of normal donors. To characterize these cells, flow cytometry results showed (Fig. 1B) that MSCs expressed CD105 (92%) and CD73 (80%) while they scored negative for hematopoietic and endothelial cell markers (CD31: 1% and CD34: 0.5%).

Differentiation potential of MSCs toward adipogenic and osteogenic lineages was investigated, and upon staining with Oil Red and Alizarin red, presence of oil-containing vacuoles showed differentiation into adipocytes (Fig. 2A), while

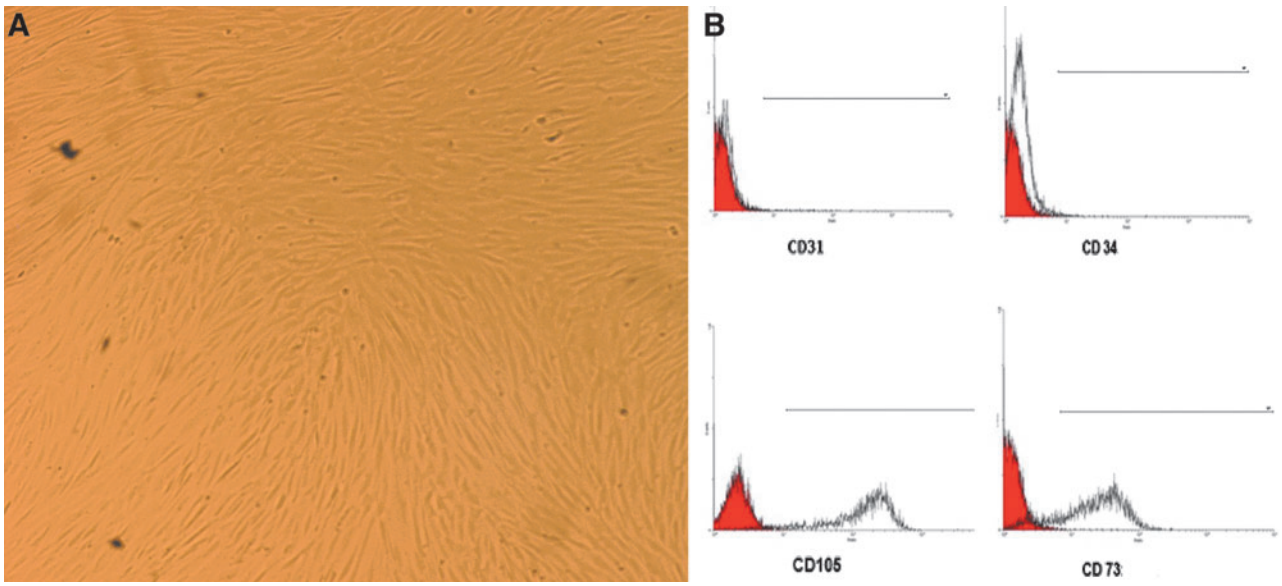

FIG. 1. Morphology of mesenchymal stem cells (MSCs) during culture **(A)** and their expression level of CD31 (1%), CD34 (0.5%), CD105 (92%), and CD73 (80%) **(B).**

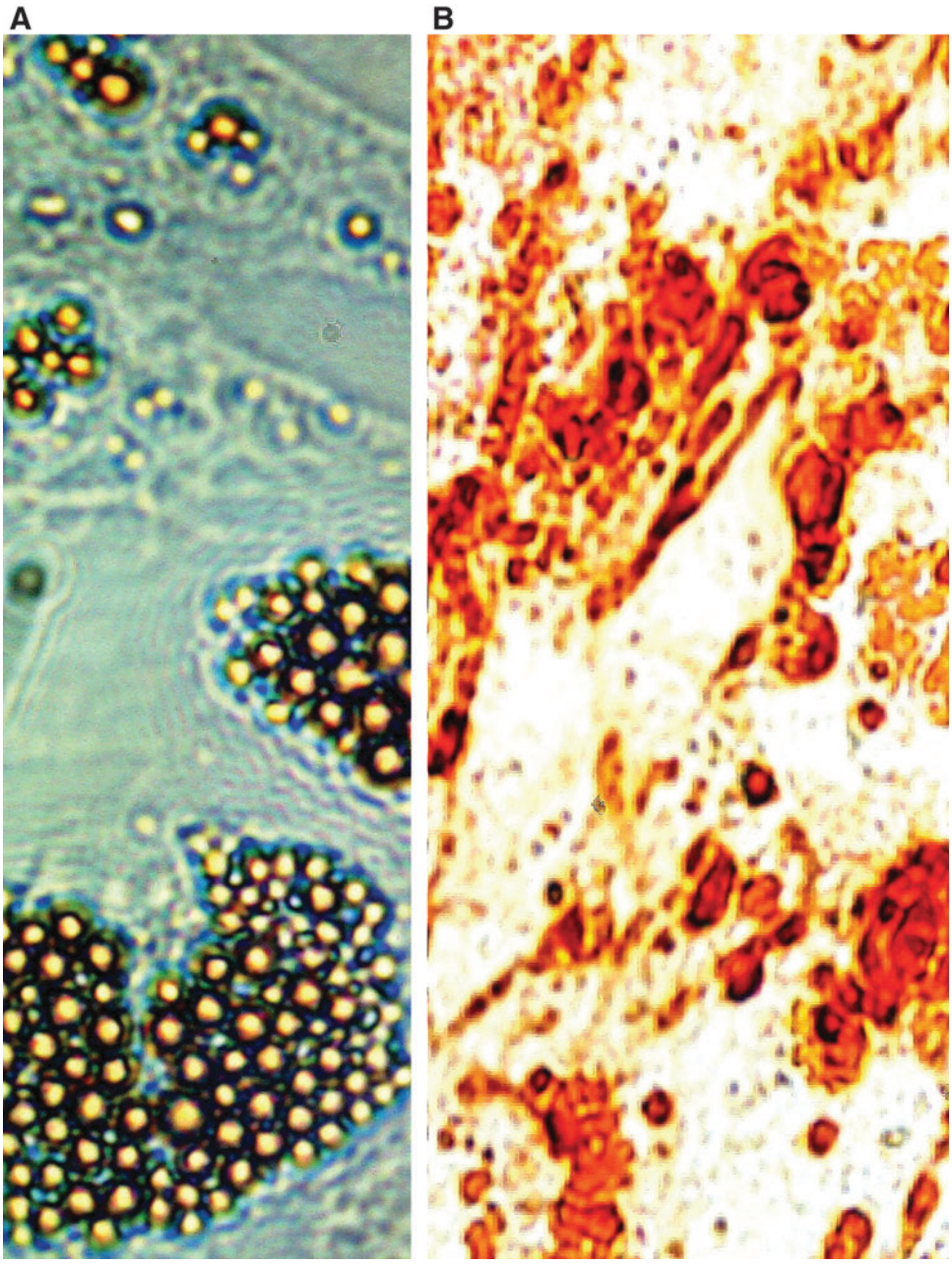

FIG. 2. Adipogenesis differentiation **(A)** and osteogenesis differentiation **(B)** of MSCs; note the vacuoles containing fat and calcium deposition in Oil Red and Alizarin red staining, respectively.

calcium deposition was indicative of differentiation into osteocytes (Fig. 2B). MSCs maintained in a standard medium scored negative for both staining procedures (data not shown).

Taken together, the flow cytometry and histochemical results are consistent with the conclusion that MSCs retain undifferentiated potential while they show a pluripotent differentiation capacity upon proper stimulation.

Transduction of MSCs with lentiviral vectors

MSCs were transduced with lentiviral vectors, delivering a bicistronic construct expressing GFP and the HIF-1α transcription factor. As evaluated by analysis of GFP fluorescence at 96 h after infection, the efficiency of transduction at MOI of 35 ranged from 80% to 90% in the different experiments (see Fig. 3 for a representative experiment). As shown in Figure 4, the HIF-1α mRNA overexpressed in HIF-MSCs compared to the endogenous transcript of MSCs in both normoxic and hypoxic conditions.

MSCs expressing HIF-1α have increased angiogenic potential

We next explored the angiogenic potential of transduced (HIF-MSCs) and nontransduced (MSCs) cells in hypoxia. Vascular tube formation was assessed microscopically after 24-h culture on Matrigel-coated plates. As shown in Figure 5A, nontransduced MSCs formed aggregates with short-protruding sprouts. As a control, HUVECs on Matrigel were capable to form an interconnected tubular network (Fig. 5B). In contrast to uninfected MSCs, cells transduced with the

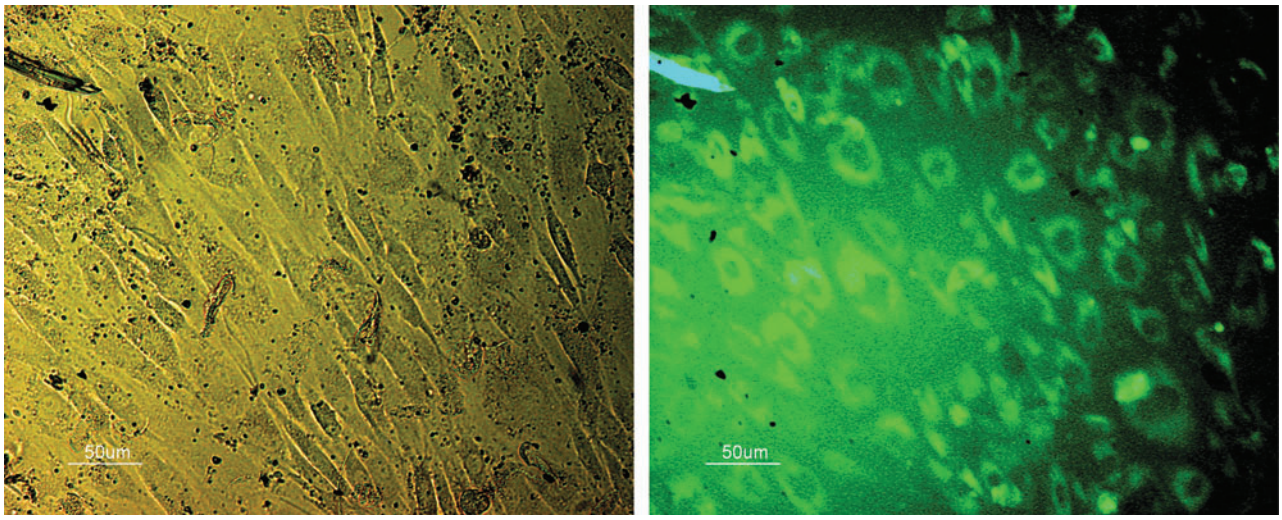

FIG. 3. Transducted MSCs; note the GFP expression in infected cells. GFP, green fluorescent protein.

HIF-1α lentiviral vector were efficient at forming tubes in an organized pattern (shown in Fig. 5C). Of interest, the vast majority of tube-forming cells also expressed the GFP marker, indicative of transduction with the lentiviral vector (Fig. 5D).

Under hypoxia, the levels of secreted VEGF significantly increased in the untransduced cells ($p < 0.05$). Of note, this increase was even higher in the cells transduced with HIF-1α ($p < 0.05$ between transduced and nontransduced cells in hypoxic conditions; Figure 6).

To assess the possibility of mesenchymal-to-endothelial differentiation, we analyzed, by semi-quantitative PCR, the expression level of various endothelial cell markers, including CD34, Tie2, and vWF, after 24 h of hypoxia. As shown in Figure 7, however, none of these factors was found to be expressed in HIF-MSCs, while they were clearly detected in total mRNA from HUVECs.

HIF-MSCs express c-Met in hypoxic conditions

c-Met, as one of the genes regulated by HIF-1α, expression analysis by flow cytometry indicated that in hypoxic conditions, 1.3% of untransduced MSCs expressed c-Met, while this percentage quantified as 25.7% (>20-fold increase) when the HIF-MSC cells were analyzed (Fig. 8).

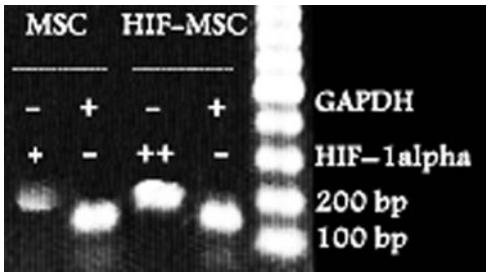

FIG. 4. HIF-1α expression level; lane 1: size marker; lane 2: GAPDH in HIF-MSCs; lane 3: HIF-1α in HIF-MSCs; lane 4: GAPDH in MSCs; lane 5: HIF-1α in MSCs. HIF, hypoxia inducible factor.

HIF-MSC cell supernatant promotes endothelial cell migration

Although HIF-1α overexpression in MSCs did not lead to endothelial differentiation, it resulted in increased migration potential of endothelial cells through a paracrine manner. As shown in Figure 9, the supernatant of hypoxic HIF-MSCs led to the complete closure of the wound after 20 h, while this process was still largely incomplete upon incubation with the supernatant of untransduced MSCs.

Discussion

In this study, we explored the effects of HIF-1α overexpression on the angiogenic potential of human bone marrow-derived MSCs. We report that expression of this factor results in increased secretion of VEGF, improved motogenic activity of the cells toward tube formation and c-Met expression, and increased secretion of factors that induce endothelial cells' migration. In no case, however, we could detect direct differentiation of MSCs to endothelial cells.

Increased expression of VEGF and other angiogenic factors upon HIF-1α expression is consistent with the established binding of the HIF-1α to the promoter regions of these genes.[30]

The results presented above indicate that HIF-MSCs, when cultured in hypoxia, form a capillary-like tubular structure resembling angiogenic structures, while apparently, they did not directly differentiate into endothelial cells. We therefore tried to understand by which molecular mechanisms they were able to spread into and form tube-like structures. Since this process depended on the expression of HIF-1α, we reasoned that it might be due to activation of one of the HIF-1α downstream genes with a defined role in angiogenesis, cell motility, and spreading. One of the HIF-1-regulated factors possessing mitogenic and motogenic properties is the hepatocyte growth/scatter factor receptor, c-Met. Expression of c-Met might explain, to some extent, the organized tube formation in the Matrigel assay.

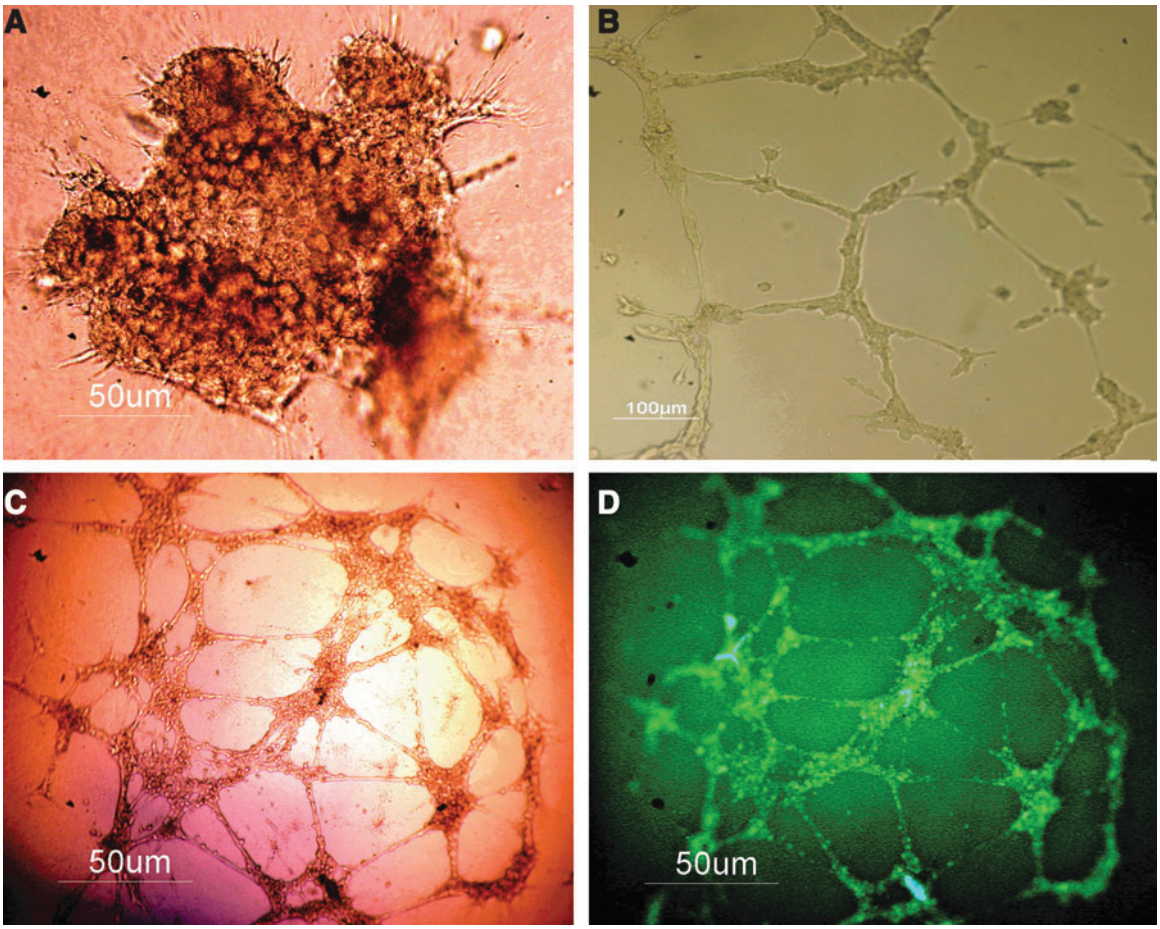

FIG. 5. Tube-formation assay on Matrigel after 24 h hypoxia; **(A)**: MSCs formed aggregations with some sprouting tubes; **(B)**: human umbilical vein endothelial cell (HUVEC) tube formation as endothelial cells; **(C)**: HIF-MSCs, note the organized and interconnected tube formations; **(D)**: most HIF-MSCs expressing GFP contributed in tube formation.

As far as c-Met is concerned, other studies have reported that ischemic or traumatic rat brain extracts induce production of HGF in hMSCs.[31,32] Expression of this factor in hMSCs is fully consistent with its involvement in cell migration, wound healing, and tissue regeneration.[31–34] Elevated expression of HGF and c-Met, by increasing the levels and stabilizing their mRNAs in response to hypoxia, has been shown to be directly dependent on HIF-1α.[35–37]

Hepatocyte growth factor (HGF) or scatter factor is a mesenchyme-derived pleiotropic growth factor and a powerful stimulator of angiogenesis and tissue regeneration, which acts in initial steps of angiogenesis, including cell migration and proliferation, via binding to its tyrosine kinase receptor, c-Met. It is well known that cells expressing c-Met are more responsive to migration through HGF ligand binding *in vivo* and *in vitro*.[38–43] Stimulation of c-Met can lead to scattering, angiogenesis, proliferation, enhanced cell motility, and tubulogenesis.[44,45]

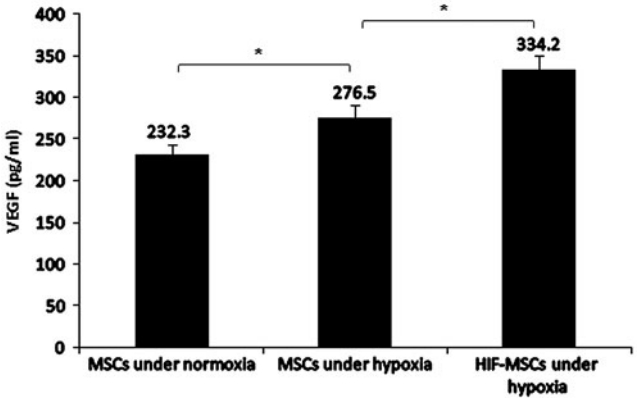

FIG. 6. Vascular endothelial growth factor concentration in MSC and HIF-MSC media after 24 h hypoxia. Note the significant differences between groups indicated by $*p < 0.05$.

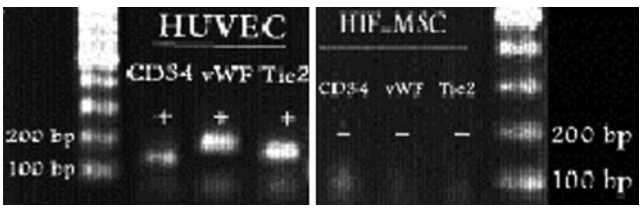

FIG. 7. Reverse transcriptase-polymerase chain reaction for endothelial markers. (*Left*) CD34 (152 bp), vWF (196 bp), and Tie2 (171 bp) in HUVECs; (*right*) HIF-MSCs under hypoxic conditions.

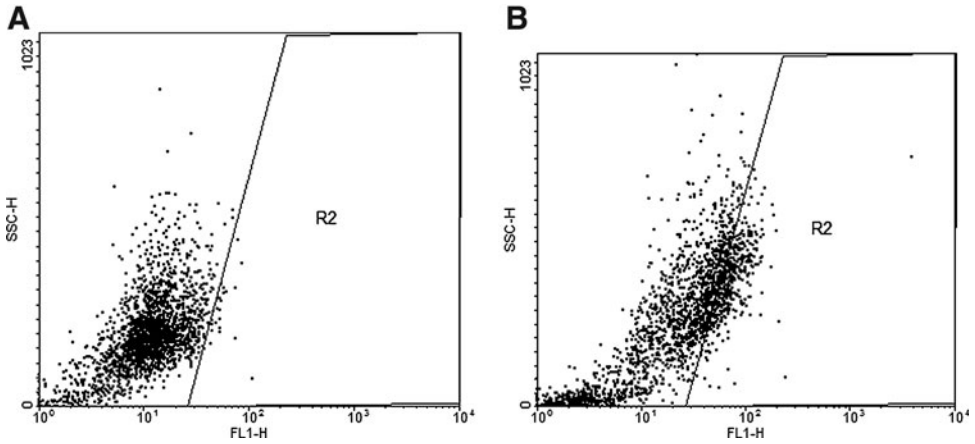

FIG. 8. Flow cytometry results for c-Met expression; **(A)**: MSCs (1.3%); **(B)**: HIF-MSCs (25.7%).

In our study, we observed that HIF-1α–induced c-Met overexpression (~20-fold increase over control) was concomitant with a loss in MSC aggregation, as observed in hypoxic conditions in the untransduced cells. This is well consistent with the established role of this receptor in inducing tumor cell migration.[46] In a similar manner, c-Met expression might also contribute to spreading of the cells on Matrigel and cellular network formation.

Of potential interest, the high expression level of c-Met was noted in the peripheral region of myocardial infarction and in the surrounding myocardial cells of blood vessels.[34] In addition, HGF preferentially reaches the ischemic region and has local and direct effects on the myocardium in patients with myocardial infarction.[34] Increased presence of c-Met as a chemoattractant receptor might indicate a high migratory capability toward gradient of HGF. Based on this consideration, we wish to suggest that HIF-MSCs expressing c-Met might be very effective at inducing recruitment and angiogenesis after *in vivo* transplantation. In concomitant with the HGF-cMET axis, it has been reported that pretreatment of MSCs with hypoxia upregulates expression of SDF-1α and its receptor CXCR4. The SDF-1-CXCR4 axis is regulated by HIF-1α and required for MSC chemotaxis and organ-specific homing in ischemic tissue.[27] In addition, HIF-1α regulates expression of matrix metalloproteinases (MMPs), since matrix degradation plays a pivotal role in network formation, and *in vivo*, in targeted migration of BM-MSCs, this could represent and additional advantage of MSC transduction with HIF-1α.[47,48] Contribution of HGF-cMET, SDF-1-CXCR4 axes, and MMPs in migration of MSCs has been previously shown.[49]

In light of the capacity of HIF-MSCs to form tubes in the Matrigel cultures and to express high levels of VEGF in response to hypoxia, we wondered whether these cells might acquire an endothelial phenotype. Also, we hypothesized that elevated VEGF secretion in an HIF-MSC–conditioned

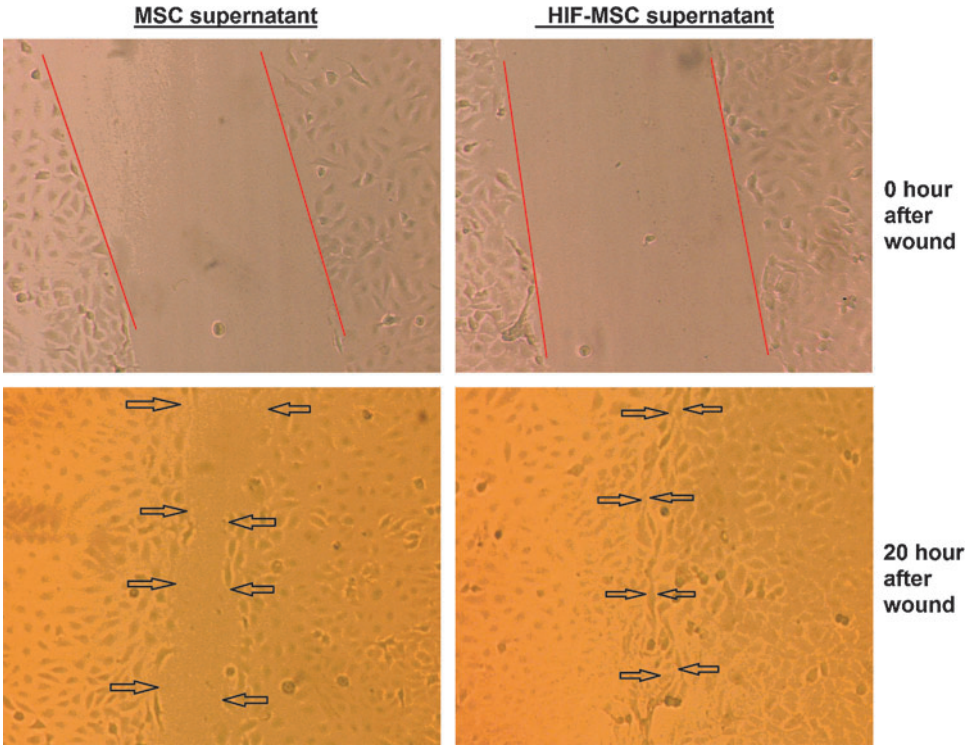

FIG. 9. Wound- (region indicated between two red lines, *upper panels*) healing assay by applying a 24-h conditioned medium of hypoxic MSCs and HIF-MSCs (*lower panels*) on HUVECs. Note the black arrows showing wound closure only in HIF-MSCs after 20 h, which failed to be closed in MSCs during the same period of culture.

medium after 24 h of hypoxia and higher expression of c-Met protein might enable MSCs to differentiate into endothelial cells. However, the RT-PCR results indicated that the transduced MSCs did not express any endothelial markers, including CD34, vWF, and Tie2. This finding is in agreement with previous studies, which reported the elevated neovascularization in rat and mouse by MSCs and bone marrow mononuclear cells, however, not through the direct incorporation of these cells into the newly formed vasculature.[50,51] In some researches, however, contribution of transplanted MSCs in capillaries formed in rat hind limb ischemia models has been reported.[52]

As shown in Figure 5, HUVECs, as endothelial cells, could form endothelial tubes on a Matrigel-coated surface. In this study, we showed that overexpression of HIF-1α in MSCs (HIF-MSCs) enabled these cells to form tube-like structures, as well. HIF-MSCs showed overexpression of c-MET, which may interpret to increase the motility and spreading of the cells.

In the same manner, HIF-1α overexpression speculated to influence the paracrine migration potential of MSCs on endothelial cells. Wound-healing assay showed the elevated cell motility of HUVECs by administering HIF-MSC supernatant. This result clearly indicated that HIF-1α activation in MSCs led to the secretion of factors increasing the migratory potential of endothelial cells.

It seemed that the pattern and/or amount of paracrine factors secreted by MSCs was altered in a way that promoted migration in endothelial cells. VEGF effect on endothelial cell migration has been reviewed and reported previously.[53] Correspondence of VEGF quantification results with higher motility-inducing ability of transduced cell supernatants during wound-healing assay could suggest VEGF as one of the factors that effectively changes the paracrine pattern of HIF-MSCs toward inducing migration of endothelial cells. Although it needs more research, but cautiously, it could be hypothesized that overexpression of HIF-1α in MSCs (HIF-MSCs) may promote angiogenesis by tissue-resident endothelial cells at the site of ischemia by inducing migration pathway of angiogenesis.

In this respect, MSCs should thus be better considered as supporting and paracrine-activating cells for endothelial cells during the various steps of angiogenesis rather than directly contributing to vessel formation through a vasculogenic process. It is notable that in some experiments, stabilization of HIF-1α in MSCs led to the expression of endothelial markers, including Tie2 and vWF in an HIF-1α–dependent fashion. It has been done by overexpression of ANG-1 and Akt and endothelial markers evaluated after a different time period compared to our study.[54] Similar results have been also reported in a study that evaluated the human MSC differentiation and angiogenic potential, by a Matrigel-based tube-formation assay, in a conditioned medium from glioblastoma multiform. Immunofluorescence staining indicated that hMSCs expressed CD151, VE-cadherin, desmin, and alpha-smooth muscle actin, whereas an expression analysis for von-Willebrand factor (vWF) and smooth myosin was negative.[55]

The paracrine effect of genetically modified MSCs on endothelial cells is supported by the strong migratory stimulus provided to HUVECs by the conditioned media from these cells. Despite the lack of direct transdifferentiation of MSCs into endothelial cells, the increased auto- and paracrine migratory activity and the capacity of MSCs tube formation by HIF-1α overexpression can provide fields for future investigations to explore the importance and supporting role of this genetic modification on improving MSC potential for vascularization in regenerating tissues.

In conclusion, this study indicated the potential of HIF-1α overexpression in MSCs as a tool to improve some angiogenesis pathways under hypoxic conditions through elevated VEGF secretion and c-MET protein expression, as well as paracrine effects on endothelial cells. Further studies will be necessary to further explore these findings *in vivo*.

Author Disclosure Statement

No competing financial interests exist.

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