ORIGINAL RESEARCH

Icariin promotes the migration of bone marrow stromal cells via the SDF-1α/HIF-1α/CXCR4 pathway

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Purpose: In this study, a series of in vitro experiments were performed to investigate the molecular mechanisms underlying cell migration promoted by icariin (ICA) at low concentrations.

Materials and methods: Bone marrow stromal cells (BMSCs) were cultured with different concentrations of ICA to verify whether it can enhance the efficiency of BMSCs migration. Western blot was employed to measure the expression of hypoxia-inducible factor-1α (HIF-1α) and C-X-C chemokine receptor type 4 (CXCR4) at different time points in BMSCs treated with ICA. Subsequently, we evaluated the function of HIF-1α in the expression of CXCR4 and the migration of cells by transfecting plasmid HIF-1α small interfering RNA (siHIF-1α) into BMSCs model.

Results: Our data indicated that different concentrations of ICA (10, 1, and 0.1 µM) further enhanced the chemotactic capability of SDF-1α, and the most prominent cell migration stimulatory effect was observed with 1 µM ICA. Furthermore, ICA significantly enhanced the protein levels of CXCR4 and HIF-1α, and this effect was blocked by ICI 12,780 (estrogen receptor antagonis). Moreover, transfection of BMSCs with siHIF-1α reduced CXCR4 expression, suggesting that HIF-1α can regulate the migration of cells by influencing the expression of CXCR4.

Conclusion: ICA promoted BMSCs migration via the activation of HIF-1α and further regulated the expression of CXCR4, suggesting that ICA might have beneficial effects in stem cell therapy.

Keywords: cell migration, CXCR4, HIF-1α, SDF-1α, icariin

Introduction

Icariin (ICA) is the main active ingredient of Herba Epimedii, which is a traditional Chinese medicinal plant. It can increase mineral density, biomechanical strength, and thickness of the trabecular bone by inhibiting bone resorption and promoting bone formation.[1,2] Chinese herbal medicine has a long history of application in clinical practice due to rapid action, less side effects, and low price. In our previous study, we found that ICA can promote bone regeneration during expanded suture in a rat rapid maxillary expansion model. However, the specific mechanism of ICA-induced bone regeneration remains unknown.

Cell homing is a coordinated multistep process, involving several cell growth factors, chemical factors, and extracellular matrix, mainly through a series of chemokines, which affect the chemokine receptor shaft.[3,4] The stromal cell-derived factor-1α (SDF-1α)/C-X-C chemokine receptor type 4 (CXCR4) axis is widely studied in stem cell

homing and is sensitive to low levels of oxygen molecules. Under hypoxic condition, the expression of SDF-1α is increased, leading to stem cell homing to tissue damage, CXCR4-expressing mesenchymal stem cells (MSCs) accumulation, and tissue regeneration and repair.[3,5] The upregulation of CXCR4 level in bone marrow stromal cells (BMSCs) can engraft stem cell migration to accelerate the healing of injury. Additionally, the CXCR4 promoter includes four potential hypoxia-response elements (HRE), which are located within 2.6-kb upstream of the transcriptional start stream.[6] Therefore, increased expression of hypoxia-inducible factor-1α (HIF-1α) can increase the activity of the promoter CXCR4 luciferase. Furthermore, it can promote the migration of stem cells.

HIF-1α is regulated by various drugs,[7] radiation,[8] cytokines, and hormones.[9,10] Recent evidence has suggested that ICA can inhibit the activity of Von Hippel–Lindau tumor suppression protein (pVHL) and upregulate the mRNA and protein expression of HIF-1α in cartilage cells to promote cartilage restoration.[7] Therefore, we speculated whether ICA could regulate stem cell migration by regulating HIF-1α. Additionally, ICA is a type of phytoestrogen, which exerts biological activity through estrogen receptor (ER);[1] activated ERs can induce the expression of HIF-1α.[11] Therefore, in the present study, we hypothesized that ICA could evoke HIF-1α activity, in turn inducing CXCR4 expression.

The aim of this study was to investigate whether ICA is involved in the regulation of HIF-1α expression in BMSCs through ER, which regulates the expression of CXCR4.

Materials and methods
Materials
ICA (99% purity) was purchased from Biopurify Phytochemicals Ltd (Chengdu, China). It was dissolved in dimethyl sulfoxide to obtain 10 mM stock solution and stored at 4°C. Further dilution was performed with the cell culture medium, alpha-modified Eagle's medium (α-MEM; Thermo Fisher Scientific, Waltham, MA, USA), and FBS (Biological Industries, Cromwell, CT, USA). Trypsin-EDTA (0.25%) and antibiotic–antimycotic were procured from Thermo Fisher Scientific. Lactacystin and chloroquine were obtained from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). Antibodies against CXCR4 and HIF-1α were obtained from Abcam (Cambridge, UK). SDF-1α was purchased from R&D Systems, Inc. (Minneapolis, MN, USA).

BMSC isolation and culture
BMSCs were isolated by adherent culture of whole bone marrow. Three-to-four-week-old male Sprague Dawley rats weighing 60 g were purchased from the Animal Center, Shandong University, China. The rats were killed and the femur and tibia were cleared of connective tissue. The epiphyses were collected, and the marrow was harvested by inserting a 5-mL syringe into one end of the bone shaft and flushing the contents into a 60-mm culture dish containing α-MEM supplemented with 20% (v/v) screened FBS and 2% penicillin/streptomycin. After incubation of the cells at 37°C with 5% CO_2 for 4 days, the culture medium containing non-adherent cells was removed, and the adherent layer was washed once with fresh medium, and then cultured continuously. The cells were passaged at 1:3 dilution until they reached 80% confluence. The study was approved by the Institutional Animal Care and Use Committee of Shandong University and was carried out in accordance with the National Institutes of Health Guidelines for the Use of Laboratory Animals.

Cell counting kit-8 (CCK-8) assay
To examine the effect of ICA on the viability of BMSCs, the cells were treated with different concentrations of ICA and the viability was evaluated by the CCK-8 assay, according to the manufacturer's instruction (Dojindo Laboratories, Kumamoto, Japan). Briefly, BMSCs were seeded in 96-well plates at a density of 5,000 cells/well. After incubation for 24 hours, the cultured medium was changed to serum-free medium with serial concentrations of ICA (100, 10, 1, and 0.1 μM). After incubation for 24 hours, the medium was replaced with 100 μL of serum-free medium containing 10 μL of CCK-8 solution, and then the plates were incubated for 3–4 hours at 37°C. The absorbance of the solution was measured at 450 nm using a multi-well spectrophotometer (SPECTROstar Nano; BMG Labtech, Ortenberg, Germany). The viability index was calculated as the experimental OD value/control OD value. Five independent experiments were performed in quadruplicate.

Transwell migration assay
The effect of ICA on rat BMSCs migration was evaluated by a modified transwell-based migration assay (8.0-μm pore size; Corning, NY, USA). BMSCs at passage 2 were serum starved for 12 hours and resuspended in 200 μL of medium with 1% FBS and adjusted to a density of $4×10^4$ cells per well. The cells were then added into the upper chamber and 600 μL of serum-free α-MEM, with or without 200 ng/mL SDF-1α, and various concentrations of ICA (10, 1, and 0.1 μM) were added to the lower chamber. After incubation at 37°C in an atmosphere of 5% CO_2 and 95% air for 12 hours,

the filter was gently removed and non-migrating cells in the upper chamber were gently removed with a cotton swab. The cells that migrated to the lower chamber were fixed with 4% paraformaldehyde for 30 minutes, and then stained for 5 minutes with 0.1% crystal violet. The number of migrating cells was calculated by counting the cells in five randomly selected microscopic fields per filter under each condition; the average of three independent experiments is presented.

Western blot assay

BMSCs were cultured in the medium containing 1 µM ICA for 6, 12, 24, and 48 hours as previously described. The cells were then harvested and lysed in RIPA lysis buffer containing the protease inhibitor, phenylmethanesulfonyl fluoride (Solarbio Science & Technology Co., Ltd., Beijing, China), for 30 minutes at 4°C. Twenty micrograms of the sample was resolved by 10% SDS-PAGE and electro-transferred onto a PVDF membrane (Pall Corporation, Port Washington, NY, USA). The membranes were blocked and incubated with appropriate primary antibodies, including rabbit anti-rat CXCR4 and HIF-1α at dilutions of 1:400 and 1:1,000, respectively. To normalize protein loading, mouse anti-rat β-actin (Sigma-Aldrich Co., St Louis, MO, USA) antibody was used at a dilution of 1:5,000. After incubation with primary antibodies diluted in TBST with 5% nonfat milk at 4°C overnight, the membranes were exposed to the corresponding horseradish peroxidase-conjugated secondary antibodies and visualized by enhanced chemiluminescence. Protein band intensities on scanned films were compared with their respective controls using the ImageJ software (NIH, Bethesda, MD, USA). The bands were first rounded up using a volume Rect tool, and then target area intensity was calculated. The density of β-actin was used as control for the expression of CXCR4 and HIF-1α.

Plasmid construction and cell transfection

To investigate whether HIF-1α increases CXCR4 expression, synthetic HIF-1α small interfering RNA (siHIF-1α) and negative control siRNA (NC-siRNA) were transfected into BMSCs. The siRNA expression vector for HIF-1α was constructed (Gene Pharma, Shanghai, China). The specific siRNA sequence targeting HIF-1α was as follows: 5′-AGCAATTCTCCAAGCCCTCC-3′; 3′-TTCATCAGT GGTGGCAGTTG-5′.

The cells were grown to 70% confluence in each dish and were transfected for 24 hours with either siRNAs specific for HIF-1α (50 nmol/L) or non-targeting siRNAs as negative control (50 nmol/L, Dharmacon, Lafayette, CO, USA) using

Lipofectamine 2000 (Thermo Fisher Scientific), according to the manufacturer's instructions.

Statistical analyses

All experiments were repeated at least three times, and the experimental data are expressed as mean ± standard error of the mean. The data were analyzed using SPSS software (SPSS Inc., Chicago, IL, USA) with differences between groups assessed by one-way ANOVA. Statistical probability of $P < 0.05$ was considered significant.

Results

Isolation of BMSCs

BMSCs were isolated from rat bone marrow, cultured as in primary culture, and passaged 3–5 times. In the primary phase, BMSCs contained attached spindle-shaped cells with colonies and floating cells, reaching 90% confluence on day 7. BMSCs at passage 3 reached 90% confluence on day 4 and the floating cells were absent.

Effect of ICA concentration on BMSC viability

The CCK-8 assay was performed to evaluate the effects of ICA on BMSC viability. The effects of ICA at different concentrations on the proliferation of BMSCs were evaluated (Figure 1). A high concentration (100 µM) of ICA inhibited cell proliferation (control: 0.225±0.014; 100 µM: 0.144±0.027, $**P < 0.01$). All the other three concentrations of ICA (10 –0.1 µM) had positive effects on cell proliferation, of which 1 µM had the strongest effect (10 µM: 0.379±0.032;
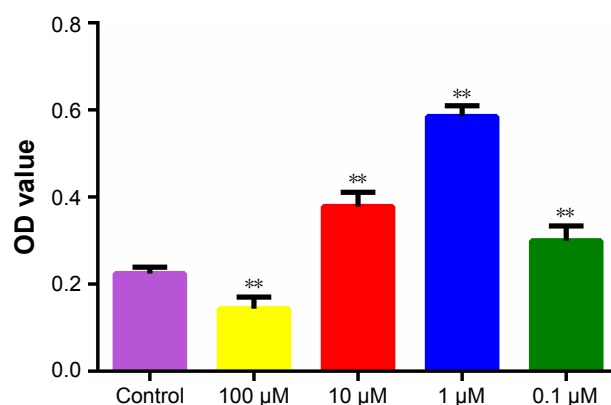

Figure 1 Effect of ICA on BMSC viability.
Notes: BMSCs were cultured in serum-free medium with or without various concentrations of ICA (100, 10, 1, and 0.1 µM) for 24 hours; then CCK-8 assay was performed to examine cell viability. Data are presented as mean ± SD (n=5). $**P < 0.01$ compared with the control group.
Abbreviations: BMSCs, bone marrow stromal cells; CCK-8, cell counting kit-8; ICA, icariin.

1 µM: 0.585±0.025; 0.1 µM: 0.299±0.034, **P<0.01). The data are presented as mean ± SD (n=5).

Transwell migration assay

As reported by several studies, we found that SDF-1α had a positive effect on the migration capacity of BMSCs. When treated with SDF-1α at a concentration of 200 ng/mL, the migration capacity of BMSCs significantly increased compared with that of the control group (P<0.05). More importantly, when treated with various concentrations of ICA (10, 1, 0.1 µM), the chemotactic capability of SDF-1α was further enhanced (Figure 2); the most prominent stimulatory effect on cell migration was observed with 1 µM ICA (control: 64.8±5.67; SDF-1α: 97±8.15; SDF-1α +10 µM ICA: 120.8±8.76; SDF-1α +1 µM ICA: 143.8±8.76; SDF-1α +0.1 µM ICA: 99±9.82). Therefore, this concentration was chosen for subsequent experiments. Additionally, we investigated the migratory ability of 1 µM ICA alone, and the results showed that the number of migrated cells in the ICA group was more than that in the control group (P<0.05), but relatively less compared with that in the SDF-1α group (P>0.05) (control: 41±10.42; ICA: 79±6.48; SDF-1α: 93.75±3.40; SDF-1α + ICA: 178.25±12.87) (Figure 3).

ICA enhances CXCR4 expression

To further explore the mechanism underlying the effect of ICA on BMSC migratory ability, we examined whether ICA preconditioning affected the expression of CXCR4. We analyzed CXCR4 expression level in BMSCs preconditioned with 1 µM ICA for 0, 6, 12, and 24 hours. The results showed that ICA significantly induced the expression of CXCR4, and this effect was increased with time from 6 to 24 hours (control: 0.180±0.005; 6 hours: 0.682±0.46; 12 hours: 0.796±0.04; 24 hours: 0.822±0.035). Subsequently, the pretreatment of BMSCs with ICI 182,780 for 30 minutes significantly decreased the upregulated expression of CXCR4 (control: 0.526±0.047; ICA: 0.719±0.062; ICA + ICI: 0.280±0.027; P<0.05) (Figure 4). Overall, ICA can enhance the expression of CXCR4 through the ER, while using ER antagonist ICI 182,780 verified this way.

ICA preconditioning increased HIF-1α protein expression in BMSCs

To verify whether HIF-1α specifically mediates the increase in CXCR4 expression during preconditioning with ICA, we examined the expression of HIF-1α protein by Western blotting. The level of HIF-1α increased significantly in
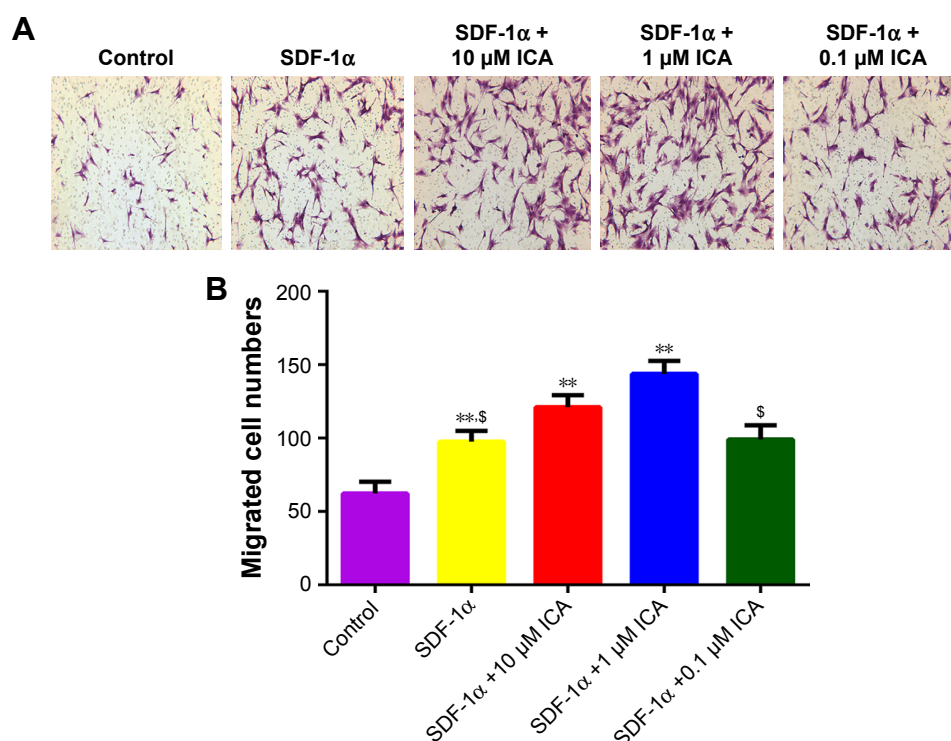

Figure 2 Effects of different concentrations of ICA and SDF-1α on stem cell migration.
Notes: BMSCs were treated with ICA at the indicated concentration for 12 hours. (**A**) Crystal violet staining showed cells that migrated to the undersurface of the membrane in the different groups. (**B**) The number of migrated cells was quantified by counting the cells in five random fields at 100× magnification. SDF-1α +10 µM ICA and SDF-1α +1 µM ICA groups were statistically significant compared with the SDF-1α group. **P<0.01. Additionally, there was no significant difference between SDF-1α +0.1 µM ICA and SDF-1α group. $P>0.05. Data are presented as mean ± SD. n=5.
Abbreviations: BMSCs, bone marrow stromal cells; ICA, icariin; SDF-1α, stromal cell-derived factor-1α.
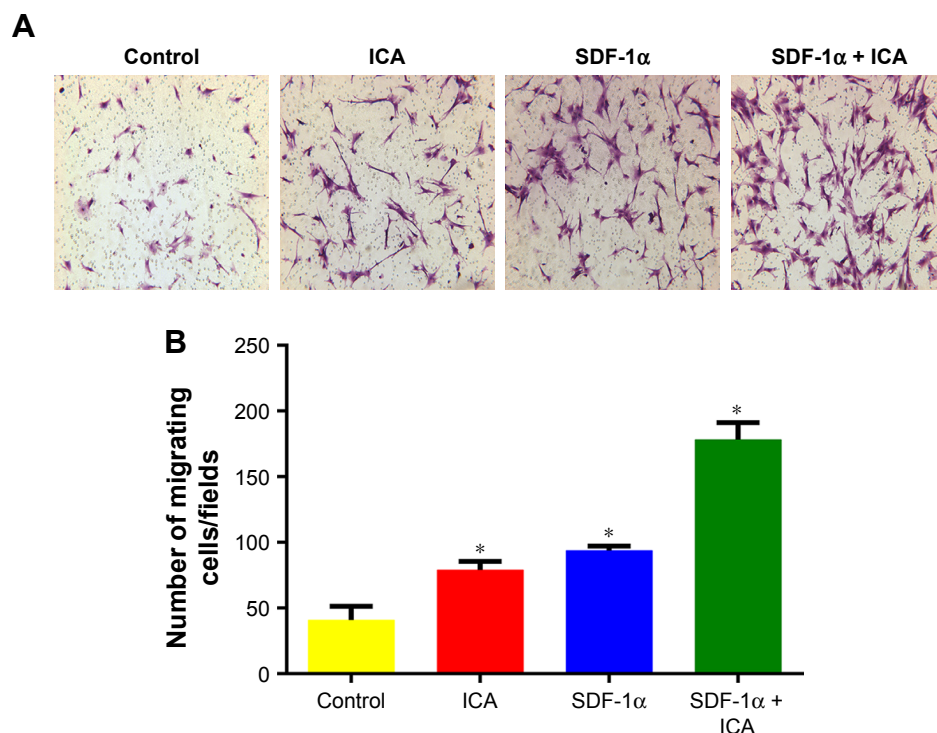
Figure 3 In vitro chemotactic capability of SDF-1α was enhanced when combined with ICA.

Notes: Transwell chemotaxis assay was performed on BMSCs with α-MEM (control), 1 μM ICA, 200 ng/mL SDF-1α, and 1 μM ICA +200 ng/mL SDF-1α in the lower chamber. After 12 hours of incubation, cells on the upper side of the membrane were removed with a cotton swab. (**A**) Crystal violet staining showed cells that migrated to the undersurface of the membrane in the different groups. (**B**) The number of migrated cells was counted in five random fields at 100× magnification. Cell migration in the SDF-1α +1 μM ICA group was significantly higher than that in the SDF-1α group. *P<0.05. Data are presented as mean ± SD.

Abbreviations: α-MEM, alpha-modified Eagle's medium; BMSCs, bone marrow stromal cells; ICA, icariin; SDF-1α, stromal cell-derived factor-1α.
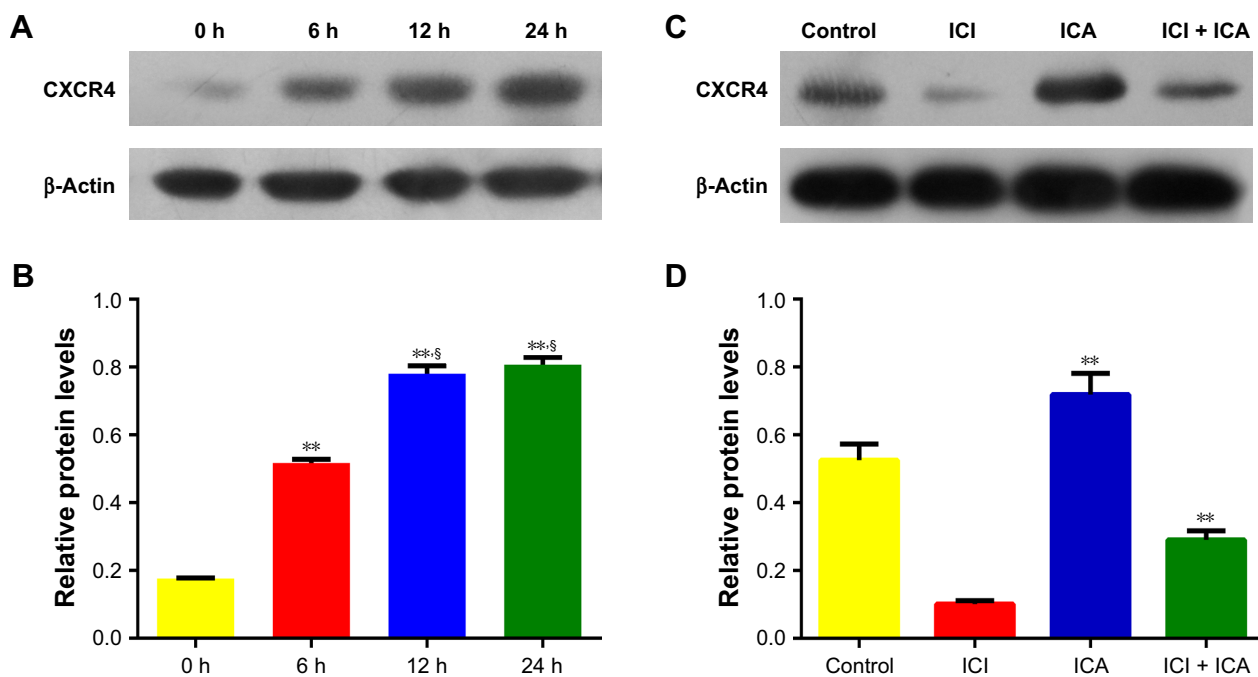

Figure 4 ICA induced the expression of CXCR4 in BMSCs.

Notes: (**A**) BMSCs were incubated in the presence of ICA (1 μM) for varying periods of time (0–24 hours) and with 10% serum (containing the same concentration of dimethyl sulfoxide) and then harvested. Total protein was extracted and blotted with the antibody against CXCR4. (**C**) Cells were pretreated with 1 μM ICI 182,780 (an ER antagonist) 30 minutes before ICA treatment, and then harvested for Western blot analysis. Each of the examples shown is representative of three independent experiments. (**B**, **D**) The relative expression of CXCR4 was quantified by ImageJ software. Values are mean ± SD of three independent experiments for each condition determined from densitometry relative to β-actin. **P<0.05 compared with the control group. §P>0.05 when comparing the 12-hour group with the 24-hour group.

Abbreviations: BMSCs, bone marrow stromal cells; CXCR4, C-X-C chemokine receptor type 4; ER, estrogen receptor; h, hours; ICA, icariin.
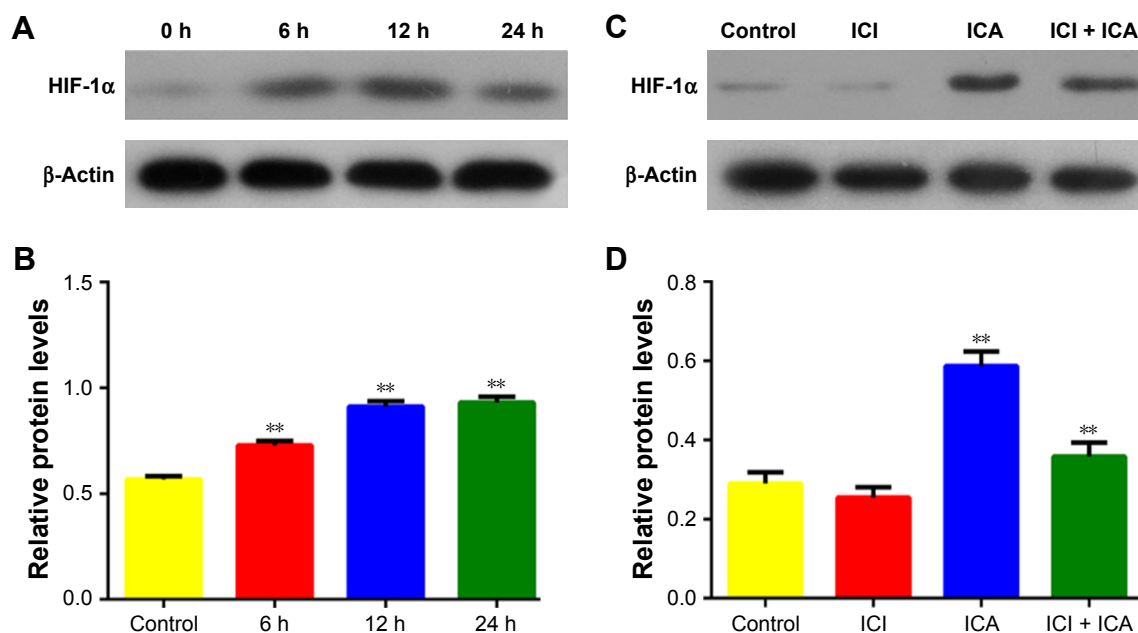
Figure 5 ICA induces HIF-1α expression.
Notes: (**A**) BMSCs were incubated with ICA (1 µM) for 24 hours and with culture medium and then harvested. Total protein was extracted and blotted with the antibody against HIF-1α. Expression of HIF-1α increased in a time-dependent manner from 6 to 24 hours. (**C**) BMSCs were treated with ICA (1 µM) in the presence of ICI 182,780 for 30 minutes, resulting in significant inhibition of ICA-induced HIF-1α expression. (**B**, **D**) The values were obtained by ImageJ software. Each of the examples shown is representative of three independent experiments. Values are mean ± SD of three independent experiments. **$P<0.05$ compared with the control.
Abbreviations: BMSCs, bone marrow stromal cells; h, hours; HIF-1α, hypoxia-inducible factor-1α; ICA, icariin.

response to ICA for 6 hours and progressively increased for up to 24 hours (control: 0.568±0.015; 6 hours: 0.728±0.021; 12 hours: 0.911±0.03; 24 hours: 0.930±0.03) (Figure 5). To examine the involvement of ERs in the ICA-induced increase in HIF-1α level, BMSCs were pretreated with ICI 182,780 before ICA treatment. As shown in Figure 5, Western blot analysis showed that the expression of HIF-1α was abolished by ICI 182,780 indicating that the expression of HIF-1α is regulated via the ER.

Subsequently, to further investigate whether HIF-1α was involved in the increase in CXCR4 expression, synthetic siHIF-1α and NC-siRNA were transfected into BMSCs before ICA treatment. Interestingly, ICA-induced CXCR4 and HIF-1α expressions were abolished when pre-transfected with siRNA that targets HIF-1α, suggesting that the expression of CXCR4 was modulated by HIF-1α (control + NC-siRNA: 0.111±0.010; ICA + NC-siRNA: 0.420±0.041; ICA + siHIF-1α: 0.275±0.028; **$P<0.05$) (Figure 6).

Effect of HIF-1α on BMSCs migration
To further explore the effect of HIF-1α on ICA-induced BMSCs migration, the cells were transfected with siHIF-1α for 12 hours, and then added into the upper chamber of a polycarbonate membrane insert, and co-cultured with ICA (1 µM) for 12 hours. As shown in Figure 7, we found

that the number of BMSCs that migrated in the SDF-1α + ICA + siHIF-1α group was significantly lower than that in the SDF-1α + ICA + NC-siRNA group, and the difference was statistically significant (control + NC-siRNA: 69.6±8.26; SDF-1α + ICA + NC-siRNA: 172.4±12.58; SDF-1α + ICA + siHIF-1α: 81.2±9.73; $P<0.05$).

Discussion
Bone injury repair is a complex process, including biological events at the tissue, cellular, and molecular level, in which the mechanism is mainly manifested as stem cells homing to the damaged site and the proliferation of stem cells.[12] Several studies have demonstrated that stem cell transplantation has a significant effect on promoting tissue healing and in shortening the healing period.[13,14] Guo et al reported that bone marrow mononuclear cell transplantation accelerated bone remolding by the intrusion of a large number of mesenchymal cells from the local stromal cell population.[14] Therefore, recruiting and homing endogenous stem cells to the injured tissue might be a more effective strategy to improve tissue repair. In the present study, our findings demonstrated that ICA can significantly promote the proliferation and migration of BMSCs and enhance the expression of CXCR4 through HIF-1α activation mediated by ER in vitro. This effect of promoting cell migration by ICA might play a key role in accelerating bone regeneration.
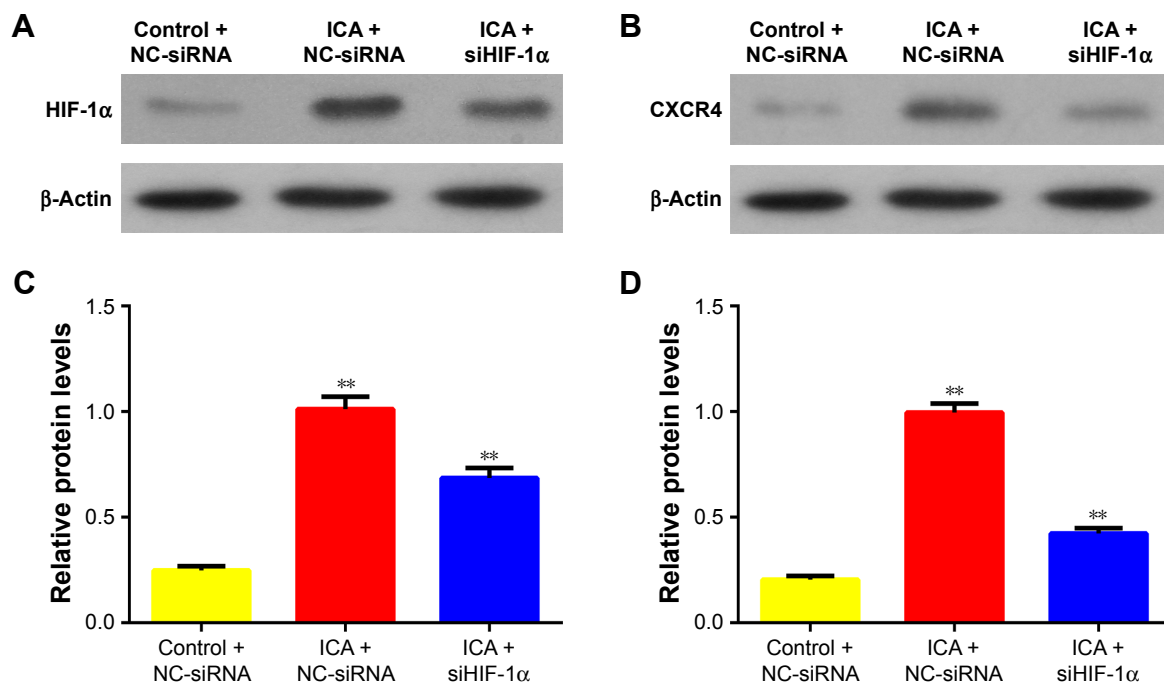
Figure 6 HIF-1α regulated ICA-induced CXCR4 expression.
Notes: BMSCs were transfected for 12 hours with either siRNA-HIF-1α (50 nmol/L) or non-targeting control siRNA (50 nmol/L) using Lipofectamine 2000 12 hours before ICA treatment, and (**A**) HIF-1α and (**B**) CXCR4 expression were then analyzed by using Western blotting. (**C, D**) The values were obtained by ImageJ software. Each of the examples shown is representative of three independent experiments. Values are mean ± SD of three independent experiments. **$P<0.05$ compared with the control + NC-siRNA.
Abbreviations: BMSCs, bone marrow stromal cells; CXCR4, C-X-C chemokine receptor type 4; HIF-1α, hypoxia-inducible factor-1α; ICA, icariin; NC-siRNA, negative control siRNA.
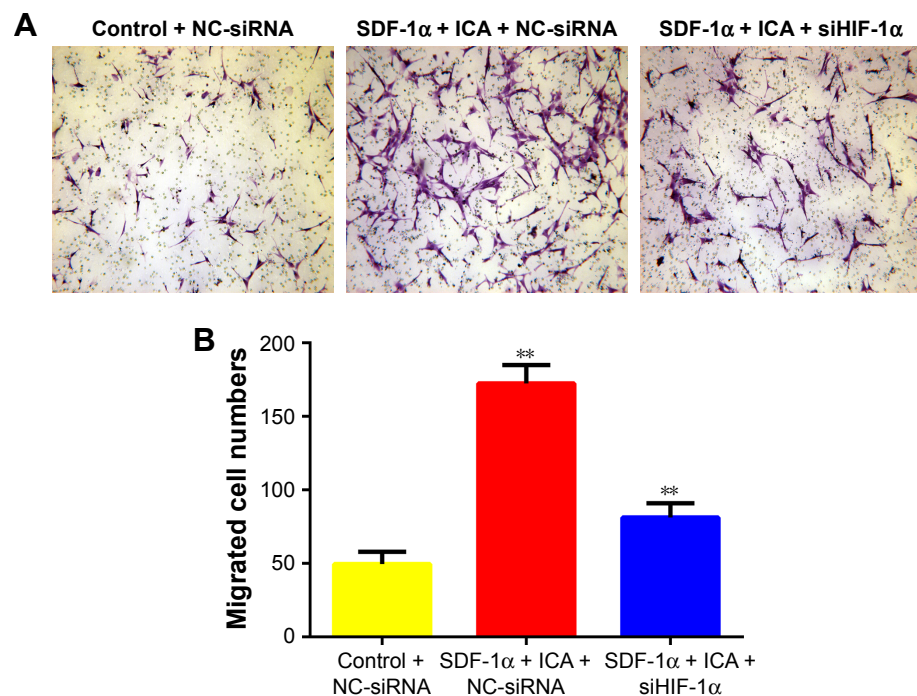

Figure 7 Effect of HIF-1α on BMSCs migration.
Notes: BMSCs were transfected for 12 hours with either siRNA-HIF-1α (50 nmol/L) or non-targeting control siRNA (50 nmol/L) using Lipofectamine 2000, and then resuspended in the upper chambers followed by treatment with SDF-1α or ICA. After incubation for 12 hours, BMSCs that migrated to the bottom surface of the membrane were stained with crystal violet, and the migrated cell number was calculated manually. (**A**) Crystal violet staining showed cells that migrated to the undersurface of the membrane in the different groups. (**B**) The numbers of migrated cells was counted in five random fields at 100× magnification. Data are presented as mean ± SD. **$P<0.05$ compared with the control + NC-siRNA group.
Abbreviations: BMSCs, bone marrow stromal cells; h, hours; HIF-1α, hypoxia-inducible factor-1α; ICA, icariin; NC-siRNA, negative control siRNA; SDF-1α, stromal cell-derived factor-1α.

The recruitment and incorporation of progenitor cells into ischemic or injured tissue require a coordinated multistep process, including adhesion to the endothelium, transendothelial migration, chemotaxis, matrix degradation and invasion, and in situ differentiation.[15] The SDF-1α/CXCR4 axis plays an important role in stem cell homing, and the expression of SDF-1α is upregulated at sites of injury, acting as a potent chemoattractant to recruit circulating or residing CXCR4-expressing MSCs, which are required for tissue repair or regeneration.[16] An increase in the expression of CXCR4 in BMSCs could engraft stem cells migration to accelerate the healing of injury. The transwell assay results revealed that ICA combined with SDF-1α can significantly enhance cell migration compared with that by SDF-1α alone. Furthermore, we performed Western blotting to examine the expression of CXCR4, and the results revealed that the expression was upregulated considerably. The result is similar to that reported by Tang et al, who stated that ICA can enhance the bone marrow-derived endothelial progenitor cells (BM-EPCs) chemotactic capacity.[17]

ICA is a type of phytoestrogen, which has structural similarities to that of estrogen in terms of conformation and binding capabilities to ERs, which can promote calcium absorption through the ER pathway within the intestinal cells[18] or might regulate bone remodeling via the same pathway within bone cells.[19] Therefore, we speculated that the expression of CXCR4 induced by ICA was regulated by the ER pathway. In the present study, preconditioning with ER-specific inhibitor ICI 182,780 attenuated the expression of CXCR4 induced by ICA.

We found that the expression of HIF-1α was upregulated when cultured with ICA. This expression reversed in the presence of an ER pure antagonist (ICI 182,780), indicating that it is a receptor-mediated effect. Furthermore, we used HIF-1α to explore the mechanism of regulating the expression of CXCR4 and found that CXCR4 expression is abolished in the siRNA HIF-1α group compared with that in the ICA group. In addition, we found that the number of stem cells migrated in the siRNA HIF-1α group was significantly less than that in the ICA group, indicating that HIF-1α regulates the migration of MSCs. It has been reported that HIF-1α plays a very important role in regulating the expression of CXCR4. The CXCR4 promoter includes four potential HREs located within 2.6-kb upstream of the transcriptional start site and one at 21.3-kb within the intron.[6] This result indicates that CXCR4 is a hypoxia-responsive gene. Therefore, increased expression of HIF-1α can enhance the activity of the promoter CXCR4

luciferase. Furthermore, it can promote the migration of stem cells, and this is supported by the results of the present study. Additionally, ICA significantly increases the level of hypoxia-responsive element luciferase reporter, which is accompanied by the increased accumulation and nuclear translocation of HIF-1α in murine chondrocytes.[7] This is consistent with the findings of the present study.

Currently, the exact mechanism of HIF-1α activation by ICA is not fully understood. Under normoxic condition, the oxygen-dependent domain of the HIF-1α subunit was recognized by pVHL via E3-ligase-mediated ubiquitination proteasomal degradation. Under hypoxic condition, the activity of prolyl hydroxylases (PHDs) is suppressed resulting in the accumulation of cytoplasmic HIF-1α and the subsequent translocation to the nucleus, where it dimerizes with the β-subunit and binds to the HREs in the promoter of target genes and enhances the transcription of those genes.[20,21] Peng W et al reported that ICA can inhibit the activity of PHD via the interaction between ICA and iron ions and upregulate the expression of HIF-1α mRNA; however, these effects have to be studied further.

Conclusion

Overall, our results indicate that ICA promotes the migration of BMSCs via the activation of HIF-1α, and further regulated the expression of CXCR4. This suggests that ICA might possess beneficial effects in stem cell therapy.

Acknowledgment

Disclosure

The authors report no conflicts of interest in this work.

References

1. Chen G, Wang C, Wang J, et al. Antiosteoporotic effect of icariin in ovariectomized rats is mediated via the Wnt/β-catenin pathway. *Exp Ther Med*. 2016;12(1):279–287.
2. Mok SK, Chen WF, Lai WP, et al. Icariin protects against bone loss induced by oestrogen deficiency and activates oestrogen receptor-dependent osteoblastic functions in UMR 106 cells. *Br J Pharmacol*. 2010;159(4):939–949.
3. Sun Y, Li QF, Yan J, Hu R, Jiang H. Isoflurane preconditioning promotes the survival and migration of bone marrow stromal cells. *Cell Physiol Biochem*. 2015;36(4):1331–1345.
4. Zhou W, Xu Y, Li X, Gao G, Jiang Z, Shao Z. Irradiation enhances expression of cxcr4 in murine glioma cells via HIF-1α-independent pathway. *J Int Med Res*. 2014;42(4):926–931.

5. Ge G, Zhang H, Li R, Liu H. The function of SDF-1-CXCR4 axis in SP cells-mediated protective role for renal ischemia/reperfusion injury by SHH/GLI1-ABCG2 pathway. *Shock*. 2017;47(2):251–259.

6. Guo M, Cai C, Zhao G, et al. Hypoxia promotes migration and induces CXCR4 expression via HIF-1α activation in human osteosarcoma. *PLoS One*. 2014;9(3):e90518.

7. Wang P, Zhang F, He Q, et al. Flavonoid compound icariin activates hypoxia inducible factor-1alpha in chondrocytes and promotes articular cartilage repair. *PLoS One*. 2016;11(2):e0148372.

8. Moeller BJ, Cao Y, Li CY, Dewhirst MW. Radiation activates HIF-1 to regulate vascular radiosensitivity in tumors: role of reoxygenation, free radicals, and stress granules. *Cancer Cell*. 2004;5(5):429–441.

9. Zhou J, Brune B. Cytokines and hormones in the regulation of hypoxia inducible factor-1alpha (HIF-1alpha). *Cardiovasc Hematol Agents Med Chem*. 2006;4(3):189–197.

10. Mirzamohammadi S, Aali E, Najafi R, et al. Effect of 17beta-estradiol on mediators involved in mesenchymal stromal cell trafficking in cell therapy of diabetes. *Cytotherapy*. 2015;17(1):46–57.

11. Estrogen receptor-α directly regulates the hypoxia-inducible factor 1 pathway associated with antiestrogen response in breast cancer. *Proc Natl Acad Sci U S A*. 2015;112(49):15172–7.

12. Ren G, Chen X, Dong F, et al. Concise review: mesenchymal stem cells and translational medicine: emerging issues. *Stem Cells Transl Med*. 2012;1(1):51–58.

13. Cao J, Wang L, Du ZJ, et al. Recruitment of exogenous mesenchymal stem cells in mandibular distraction osteogenesis by the stromal cell-derived factor-1/chemokine receptor-4 pathway in rats. *Br J Oral Maxillofac Surg*. 2013;51(8):937–941.

14. Guo J, Wang L, Xu H, Che X. Effect of heterologous bone marrow mononuclear cell transplantation on midpalatal expansion in rats. *ExpTher Med*. 2015;9(4):1235–1240.

15. Chavakis E, Urbich C, Dimmeler S. Homing and engraftment of progenitor cells: a prerequisite for cell therapy. *J Mol Cell Cardiol*. 2008;45(4):514–522.

16. Li Q, Zhang A, Tao C, Li X, Jin P. The role of SDF-1-CXCR4/CXCR7 axis in biological behaviors of adipose tissue-derived mesenchymal stem cells in vitro. *BiochemBiophys Res Commun*. 2013;441(3):675–680.

17. Tang Y, Jacobi A, Vater C, Zou L, Zou X, Stiehler M. Icariin promotes angiogenic differentiation and prevents oxidative stress-induced autophagy in endothelial progenitor cells. *Stem Cells*. 2015;33(6):1863–1877.

18. Chen Y, Wang J, Jia X, Tan X, Hu M. Role of intestinal hydrolase in the absorption of prenylated flavonoids present in Yinyanghuo. *Molecules*. 2011;16(2):1336–1348.

19. Wei Q, Zhang J, Hong G, et al. Icariin promotes osteogenic differentiation of rat bone marrow stromal cells by activating the ERalpha-Wnt/beta-catenin signaling pathway. *Biomed Pharmacother*. 2016;84:931–939.

20. Maxwell PH, Wiesener MS, Chang GW, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature*. 1999;399(6733):271–275.

21. Jaakkola P, Mole DR, Tian YM, et al. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science*. 2001;292(5516):468–472.