Long-Term Sevoflurane Exposure Inhibits The Proliferation, Differentiation, and Homing Potential of Bone Marrow Mesenchymal Stem Cells

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Research Article

Keywords: BMSCs, Sevoflurane; Proliferation, Differentiation, Homing

Posted Date: March 9th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-218648/v1

Long-term sevoflurane exposure inhibits the proliferation, differentiation, and homing potential of bone marrow mesenchymal stem cells

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Abstract

Background: Bone marrow mesenchymal stem cells (BMSCs) are widely used in many fields such as wound repair, gene delivery, and microenvironment improvement. In some cases, BMSC transplantation requires long-term anesthesia. However, the effects of anesthetics on the characteristics of BMSCs are poorly understood.

Methods: In this study, we examined the effect of sevoflurane, a gas anesthetic drug most commonly used in children, on the proliferation, differentiation, and homing potential of BMSCs.

Results: Short-term (6 h) sevoflurane exposure had almost no effect on the proliferation, differentiation, and homing of BMSCs. However, long-term (24 h) sevoflurane exposure inhibited the proliferation of BMSCs, accelerated their differentiation into nerve cells, and inhibited their homing potential to damaged vascular endothelial cells and intact glioma cells.

Conclusion: Short-term anesthesia with sevoflurane as the main inducer is safe and harmless to BMSCs, but long-term sevoflurane exposure may reduce their repair potential. Therefore, because of the high proportion of BMSCs in children, the application of long-term anesthesia with sevoflurane should be cautious, or more suitable anesthetic drugs are needed.

Keywords: BMSCs; Sevoflurane; Proliferation; Differentiation; Homing

Introduction

Bone marrow mesenchymal stem cells (BMSCs) are a population of cells in mammalian bone marrow stroma, which have the potential to differentiate into bone, cartilage, fat, and nerve cells as well as myoblasts [1]. They not only mechanically support hematopoietic stem cells in bone marrow, but can also be amplified and genetically modified *in vitro*. Moreover, after re-introduction into the body, BMSCs migrate to damaged tissues (homing), differentiate into specific cells, secrete growth factors to improve the microenvironment, and repair damage [2, 3].

BMSC transplantation often occurs following surgery such as tumor resection and removal of necrotic tissue [4]. Some patients who undergo surgery are inevitably exposed to prolonged anesthesia. However, there are few studies on the effects of anesthetics on the characteristics of BMSCs.

Sevoflurane was discovered by Ross Terrell, synthesized by Regan in 1968, completed phase III clinical trials in 1986, and approved for clinical use by the Japanese Drug Administration in 1990 [5]. In recent years, sevoflurane has been considered by many anesthesiologists as a landmark drug for inhalation anesthesia because it has significant advantages in the induction and maintenance of general anesthesia in children [6]. Many hospitals have reported successful experiences in their extensive use of general anesthesia for children [6].

However, in recent years, some studies have found that sevoflurane has various effects on the characteristics of BMSCs. Zhou et al. [7] found that exposure to sevoflurane for 4 hours causes a concentration-dependent decrease in the cell viability of BMSCs. Low-dose (1.7%) sevoflurane has no effect on cell viability, but higher concentrations of sevoflurane (>2.3%) have obvious cytotoxicity in BMSCs. Moreover, after combined exposure to sevoflurane and propofol, BMSCs become raritas with wizened cytoplasm and have fewer connections to each other. However, propofol alone up to 20 μg/ml does not harm BMSCs. These studies indicate that it is necessary to choose an appropriate anesthesia for BMSC transplantation. However, Sun et al. [8] suggested that sevoflurane preconditioning has protective effects on the survival and migration of BMSCs against hypoxia and serum deprivation, which improve their therapeutic potential. Cheng et al. [9] found that soluble factors secreted by BMSCs attenuate sevoflurane-induced oxidative stress and apoptosis of neuronal cells by preserving their mitochondrial functions. Thus, the conclusions of these studies are inconsistent or even contradictory.

For a more comprehensive and in-depth understanding of the effects of anesthetic drug exposure on the characteristics of BMSCs, we treated BMSCs with sevoflurane because there is a high proportion and large number of BMSCs in children, which may have a more profound effect on their health.

Materials and methods

Isolation of rat BMSCs. 3 Sprague-Dawley pregnant rats were purchased from Sibeifu Biotechnology Co., Ltd. (Beijing, China). After pregnant rats delivery, 7-day-old rats (n=5) were decapitated and immersed in 70% alcohol for 10 minutes for disinfection. On a sterile operating table, rat skin and muscles were carefully peel off to fully expose the femurs. Both ends of the femurs were cut off and then a 1-mL syringe was used to flush the femur cavity with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) (both from Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The cells were then incubated at 37°C with 5% CO_2 for 3 days. Then, half of the culture medium was replaced and the first complete medium change was performed on day 7. On day 15, adherent cells were passaged with 0.25% trypsin (Invitrogen Life Technologies, Carlsbad, CA, USA) at a ratio of 1:3. The expression of surface antigens CD34, CD45, CD71, and CD105 (Abcam Trading Co. Ltd., Shanghai, China) was detected by flow cytometry at passage 4.

Cell culture and isoflurane exposure. Passage 5 cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Prior to isoflurane exposure, the BMSCs were cultured in 60-mm dishes at 1×10^6 cells per dish for 12 h. The cells were then placed in a 1.5-L airtight chamber with continuous flow of 2% isoflurane (Abbott Laboratories Ltd, Maidenhead, UK), 21% oxygen, 5% carbon dioxide, and 72% nitrogen for 24 h, followed by a 48-h period of drug withdrawal. The control group did not receive isoflurane treatment.

Cell proliferation assay. The proliferation of BMSCs was measured using a Cell Counting Kit-8 (CCK-8; Yeasen, Shanghai, China). BMSCs were cultured in 96-well plates (3000/well) for 24 h, followed by exposure to 2% isoflurane 24 h. The culture medium was then replaced with fresh medium, followed by an additional 5 days of culture. Cells viability was determined each day. To determine the proliferation rate of the cells, CCK-8 (5 mg/ml) was added to each well and the cells were incubated for 1 h, followed by measuring the absorbance of each well at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). The absorbance values were used to derive the relative cell number from a standard curve.

Cell cycle analysis. Cells were digested with 0.25% trypsin and subjected to repeated mechanical pipetting to harvest a single cell suspension. After fixation in 70% ethanol at 4°C overnight, the cells were incubated with 100 mL RNase A (1 mg/mL) for 30 min at 37°C. Then, 400 mL of 10 mg/mL PI was added and the solution, followed by incubation in the dark for 15 min. The cell cycle was analyzed by flow cytometry and CellQuest software. The cell proliferation index was calculated by the formula PIx = (S+G2M)/(G0G1 + S+G2M).

Immunofluorescence. BMSCs were cultured on coverslips coated with polylysine for 24 h, followed by 24 h of exposure to 2% isoflurane. Then, the isoflurane was

removed and the BMSCs were cultured in 3% oxygen for 48 h to induce differentiation into neural cells. The initial stage of BMSC differentiation into nerve cells is marked by Nestin and the final stages to glial cells is marked by glial fibrillary acidic protein (GFAP) or neurons marked by βIII-Tubulin. BMSCs were fixed in 4% paraformaldehyde for 15 min at room temperature (RT), permeabilized in 0.5% Triton X-100 at RT for 20 min, and then incubated with anti-Nestin (ab134017; 1:10,000; Abcam, Cambridge, MA, USA), anti-GFAP (ab7260; 1:5,000; Abcam), and anti-βIII-Tubulin (ab247375; 1:5000; Abcam) antibodies at 4°C overnight. Then, the cells were incubated with Alexa Fluor 488-conjugated anti-rabbit IgG H&L (ab150077, Abcam; 1:500) to detect Nestin and Alexa Fluor 594-conjugated anti-rabbit IgG H&L (ab150080, Abcam; 1:500) to detect GFAP and βIII-Tubulin at RT for 1 h in the dark. Specimens were mounted on glass slides in VECTASHIELD mounting medium (Vector Laboratories) containing 3 μg/ml 4′,6-diamidino-2-phenylindole (DAPI) (D1306; Invitrogen). Images were captured under an LSM800 confocal laser scanning microscope (Zeiss).

Lactate dehydrogenase (LDH) detection. BMSCs were cultured in 96-well plates for 24 h, followed by 24 h of exposure to 2% isoflurane. The cells were then collected and centrifuged at 12,000 rpm for 10 min. The LDH content in the conditioned medium was measured by an enzyme-linked immunosorbent assay-based LDH Activity Assay Kit (Yuanmu Biotechnology Co., Ltd., Shanghai, China) in accordance with the manufacturer's instructions.

Apoptosis analysis. BMSCs were cultured for 24 h, followed by 24 h of exposure to 2% isoflurane. The cells were resuspended in 100 μl binding buffer and then 5 μl FITC-Annexin V and 5 μl PI were added to the solution, followed by incubation in the dark box at room temperature for 15 min. Flow cytometric analysis of apoptosis was performed in accordance with the Annexin V-FITC/PI apoptosis detection kit (Cat: 40302, Shanghai Yisheng Biological Technology Co., Ltd., Shanghai, China) instructions.

Transwell assay. Because BMSCs have the potential to home to injured cells and tumor cells, scratched vascular endothelial cell line HUVEC and glioma cell line C6 (both from ATCC, Maryland, USA) were used to assess the homing potential of BMSCs. HUVECs were cultured in the lower chambers of Transwells. After 24 h, the surface of the cells was lightly scratched using a 200-μl pipette tip to create a wound model. C6 cells were cultured in lower chambers of Transwell for 24 h. BMSCs were seeded in the upper chambers of Transwells. Then, the Transwell chamber was transferred into an airtight chamber and exposed to 2% isoflurane for 24 h. Then, the membrane of the upper chamber was stained with crystal violet for 10 min. The number of invading cells was counted under an inverted microscope (cellSens Entry 1.16; Olympus, Japan).

Western blot analysis. BMSCs were cultured for 24 h, followed by 24 h of exposure to 2% isoflurane. Then, total protein was extracted and the expression levels of CXC chemokine receptor 4 (CXCR4) and vascular endothelial growth factor receptor 2 (VEGFR2) were detected by western blotting. Briefly, the proteins were separated on 4%–12% SDS-PAGE gels, transferred onto polyvinylidene fluoride membranes (EMD Millipore, Inc.), blocked, and then incubated with antibodies against CXCR4 (1:100, ab155072, Abcam) or VEGFR2 (1:1,000, ab39638, Abcam), followed by incubation with an HRP-conjugated secondary antibody (1:500, ab7090, Abcam). The proteins were detected using a SuperSignal protein detection kit (Pierce). The density of each band was quantified by image analysis software ImageJ version 1.48 (National Institutes of Health, Bethesda, MD, USA). The membrane was stripped and re-probed with a primary antibody against β-actin (1:1,000, Santa Cruz Biotechnology).

Statistical analysis. All experiments were repeated three times. Data are presented as means ± SD and were analyzed using GraphPad Prism 6 software (San Diego, CA). Comparisons between two groups were conducted by the Student's *t*-test. $P<0.05$ was considered statistically significant.

Results

BMSC phenotypes of isolated cells. On the second day after the cells were harvested, a small number of cells were attached to the bottom of the culture dish (Fig. 1A). With the extension of culture time, these small clusters of cells gradually expanded into sheets (Fig. 1A). On day 14, the cells underwent the first passage. As the number of passages increased, the cells became more uniform (Fig. 1A). These cells strongly expressed MSC marker antigens CD71 and CD105, but hardly expressed hematopoietic stem cell marker antigens CD34 and CD45 (Fig. 1B). These results confirmed that the cultured cells were highly pure BMSCs.

-Fig. 1-

Figure 1 Isolation of BMSCs. (A) Morphologies of BMSCs at passage 0 (P0) and passage 4 (P4) were observed under an inverted microscope (scale bar, 20 μm). (B) Expression of BMSC markers CD71 and CD105, and hematopoietic stem cell markers CD34 and CD45 was detected by flow cytometry.

Long-term isoflurane exposure inhibits the proliferation of BMSCs. Next, we examined the effect of long-term exposure to isoflurane on the proliferation of BMSCs. Observation of cell morphology showed that BMSCs that had been subjected to isoflurane exposure for 24 h followed by withdrawal for 48 h lost their typical spindle-shaped morphology and shrank into a cord shape (Fig. 2A). CCK-8 assays also showed that the proliferation rate of BMSCs subjected to isoflurane exposure for 24 h and withdrawal for 5 days was significantly lower than that of the control group

(Fig. 2B). Flow cytometry showed that a greater proportion of BMSCs that were exposed to sevoflurane for 24 h and withdrawal for 48 h were arrested in G2/M phase, while the proportion of cells in S phase was decreased significantly (Fig. 2C and 2D). These results indicated that 24 h of isoflurane exposure reduced the proliferative potential of BMSCs.

-Fig. 2-

Figure 2 Long-term exposure to sevoflurane inhibits the proliferation of BMSCs. (A) After long-term sevoflurane exposure, BMSC morphology was observed under a microscope (scale bar, 20 μm). (B) CCK-8 assays were used to assess the effect of long-term exposure to sevoflurane on BMSC proliferation. (C) Flow cytometry was used to examine the effect of long-term exposure to sevoflurane on the cell cycle of BMSCs. (D) Cell cycle phases of BMSCs. Data are shown as means ± SD (n = 3); $*P < 0.05$ and $**P < 0.01$ compared with the control group (Student's t-test).

Long-term isoflurane exposure inhibits the differentiation of BMSCs into nerve cells. The multilineage differentiation potential of BMSCs is needed for tissue regeneration and repair. Therefore, we next investigated the effect of long-term exposure to sevoflurane on the differentiation of BMSCs into neural cells. BMSCs in the control group differentiated into glial cells (marked by GFAP) and neurons (marked by βIII-Tubulin) under specific induction conditions. However, the proportion of BMSCs subjected to long-term sevoflurane exposure, which differentiated into glial cells and neurons was increased significantly (Fig. 3). The results indicated that long-term exposure to sevoflurane promoted the differentiation of BMSCs into nerve cells.

-Fig. 3-

Figure 3 Long-term exposure to sevoflurane inhibits the differentiation of BMSCs. Immunofluorescence was used to analyze the effect of sevoflurane exposure on the differentiation of BMSCs into neural precursor cells (marked by Nestin; green), glial cells (marked by GFAP; red), and neurons (marked by βIII-Tubulin; red). DAPI was used to stain cell nuclei (scale bar, 20 μm). GFAP, glial fibrillary acidic protein.

Long-term sevoflurane exposure damages BMSCs. Next, we examined whether long-term exposure to sevoflurane damaged BMSCs. The level of LDH is often used to measure the degree of cell damage. The results showed that exposure to sevoflurane for 24 h increased the level of LDH in BMSC culture medium (Fig. 4A). Additionally, flow cytometry showed that 24 h of exposure to sevoflurane caused a higher proportion of BMSCs to undergo apoptosis (Fig. 4B and 4C). These results confirmed that long-term exposure to sevoflurane damaged BMSCs.

-Fig. 4-

Figure 4 Long-term sevoflurane exposure damages BMSCs. (A) ELISA was used to measure the protein level of LDH. (B and C) Flow cytometry was used to detect apoptosis. Data are shown as means ± SD (n = 3). $*P < 0.05$ and $**P < 0.01$ compared with the control group (Student's t-test).

Long-term sevoflurane exposure inhibits the migration of BMSCs. BMSCs migrate and home to damaged tissues or tumors, which is considered to be an important

mechanism for BMSCs to perform tissue repair through blood transport or structural medium migration. Therefore, we assessed the effect of long-term exposure to sevoflurane on the migration potential of BMSCs. First, we scratched cultured HUVECs to establish cell damage models. Transwell assays showed that the number of BMSCs that migrated to injured HUVECs was decreased by more than three times (Fig. 5A and 5B). Next, we assessed the effect of long-term exposure to sevoflurane on the migration potential of BMSCs toward glioma cells. As a result, long-term exposure to sevoflurane also reduced the migration ability of BMSCs toward C6 glioma cells (Fig. 5A and 5B).

Two cytokine/receptor pairs, SDF-1/CXCR4 and VEGF/VEGFR, play important roles in mediating recruitment of BMSCs to damaged tissues or tumors [10, 11]. Therefore, we measured the protein expression levels of CXCR4 and VEGFR in BMSCs after exposure to sevoflurane. The results showed that exposure to sevoflurane reduced the protein expression levels of CXCR4 and VEGFR in BMSCs (Fig. 5C, S1 and 5D).

-Fig. 5-

Figure 5 Long-term sevoflurane exposure inhibits the migration of BMSCs. (A and B) Transwell assays were used to assess the migration potential of BMSCs toward damaged HUVECs and C6 glioma cells (scale bar, 30 μm). (C and D) Protein expression levels of CXCR4 and VEGFR in BMSCs were detected by western blotting. Data are shown as means ± SD (n = 3). $^{**}P < 0.01$ compared with the control group (Student's t-test).

Discussion

MSCs can be extracted, cultured, and amplified from bone marrow, fat, dental pulp, and other tissues in adults and then induced to differentiate into multilineage cells under specific conditions for tissue repair [1, 12-15]. BMSCs are an important cell source for regenerative medicine because of convenient tissue sampling, easy *in vitro* amplification and genetic modification, and avoidance of ethical concerns [16-18]. However, in clinical practice, the process of implanting BMSCs into specific areas of the body is often accompanied by surgical anesthesia, especially some complicated operations that require prolonged exposure to anesthetic drugs.

Sevoflurane is currently one of the most commonly used gas anesthetics for pediatric anesthesia [19, 20]. However, because the proportion of BMSCs in children is significantly higher than that in adults [21, 22], the effect of sevoflurane on the characteristics and functions of BMSCs may be a problem. To the best of knowledge, there are few convincing studies on the effect of sevoflurane on BMSCs. Sun et al. [8] found that short-term sevoflurane preconditioning has protective effects on survival and migration of BMSCs against H/SD and improves the therapeutic potential of BMSCs. These beneficial effects might be mediated at least in part by upregulating HIF-1α, HIF-2α, VEGF, and p-Akt/Akt. Ti et al. [23] showed that sevoflurane preconditioning protected BMSCs against hypoxia by activating miR-210 expression

and promoted their paracrine functions and effects on resident cardiac stem cells. These results appear to support the conclusion that sevoflurane exposure is beneficial to BMSCs. However, Sun et al. only examined the effect of 2-h sevoflurane exposure on BMSC, and lacked observations of the biological characteristics of BMSCs after long-term sevoflurane exposure. Ti et al. focused on the protective effect of sevoflurane-pretreated BMSCs on hypoxic cardiomyocytes rather than the effects of sevoflurane on BMSCs. We speculate that short-term exposure to sevoflurane may promote the release of growth factors and anti-inflammatory factors from BMSCs to exert protective effects on target cells or organs, but this benefit may be obtained by consuming BMSC energy reserves.

Because these studies are not sufficient to fully understand the effect of sevoflurane exposure on BMSCs, we applied long-term sevoflurane exposure (24 h) to BMSCs derived from young rats to examine its effect on the biological characteristics of BMSCs. We evaluated the effects of long-term exposure to sevoflurane on the proliferation, differentiation, health, and homing potential of BMSCs. Our results showed that long-term exposure to sevoflurane inhibited the proliferation of BMSCs, which was characterized by a decrease in cell proliferation rate and cell cycle arrest in G2/M phase. Further experiments showed that long-term exposure to sevoflurane promoted the differentiation of BMSCs into nerve cells. Additionally, long-term sevoflurane exposure increase the LDH content in the medium and apoptosis of BMSCs, which suggested that long-term sevoflurane treatment damaged BMSCs.

The characteristic migration of BMSCs toward injured cells and tumors is considered to be an important mechanism of BMSCs to endogenously mobilize and repair distantly damaged tissues (4). In this study, we first scratched HUVECs to establish a damaged vascular endothelial cell model and then Transwell assays were used to assess the migration potential of BMSCs toward damaged HUVECs. The results showed that long-term exposure to sevoflurane inhibit the migration ability of BMSCs toward injured HUVECs. Further experiments showed that long-term exposure to sevoflurane also inhibited the migration of BMSCs toward glioma cells.

Two important cytokine/receptor pairs, SDF-1/CXCR4 and VEGF/VEGFR, are involved in regulating the migration of BMSCs toward damaged tissues and tumors (10,11). Therefore, we also measured the protein expression of CXCR4 and VEGFR in BMSCs. The results showed that long-term exposure to sevoflurane reduced the protein expression levels of CXCR4 and VEGFR in BMSCs. Taken together, these results support that long-term exposure to sevoflurane reduced the homing potential of BMSCs toward damaged tissues and tumors.

In this study, we found that long-term exposure to sevoflurane reduced the potential of BMSCs to participate in tissue repair. This result is inconsistent with previous studies indicating that short-term sevoflurane exposure promotes BMSCs to participate in damage repair, but short-term sevoflurane exposure mainly triggers the

acute reaction of BMSCs. Oxidative stress reactions exert anti-inflammatory and anti-oxidant effects and therefore elicit a protective effect on damaged tissues in a short time. However, long-term exposure to sevoflurane will consume BMSCs excessively, causing them to fatigue and even damage, which manifests as decreases in their proliferation, differentiation, and homing potential. These results promote our understanding of the effect of long-term sevoflurane exposure on the biological characteristics of BMSCs and serve as a guide for future clinical practice.

Acknowledgment

We thank Mitchell Arico from Liwen Bianji, Edanz Group China (www.liwenbianji.cn/ac), for editing the English text of a draft of this manuscript.

Funding

This study was supported by a grant from National Natural Science Foundation of China (Grant No. 81602363), Tianjin Natural Science Foundation of China (Grant No. 18JCQNJC12800 and 19JCZDJC35200), Tianjin Special Project of New Generation Artificial Intelligence Technology (Grant No. 18ZXZNSY00260), and Binhai Health and Family Planning Commission Science and Technology Projects (Grant No. 2012BWKZ004, 2018BWKZ001, and 2019BWKQ030).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The current study did not involve human participation, human data or human tissue. The study was carried out in compliance with the ARRIVE guidelines 2.0. Ethical Approval was obtained from the Animal Ethics Committee of The Fifth Central Hospital of Tianjin (Tianjin, China; Approval no. TJWZX2020014) for this study. The experiments were performed in accordance with the relevant regulations and guidelines.

Authors' contributions

DY Zhang and YW Bai designed the experiments. YX Bai, P Wang, LL Li, and CY Zhang performed the experiments and collected data. WC Shi and XZ Liu analyzed and interpreted the data. XF Ma, DY Zhang, and YW Bai drafted the manuscript. DY Zhang and YW Bai agreed to be accountable for all aspects of the work to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and gave final approval of this version to be published.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interests.

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Figures

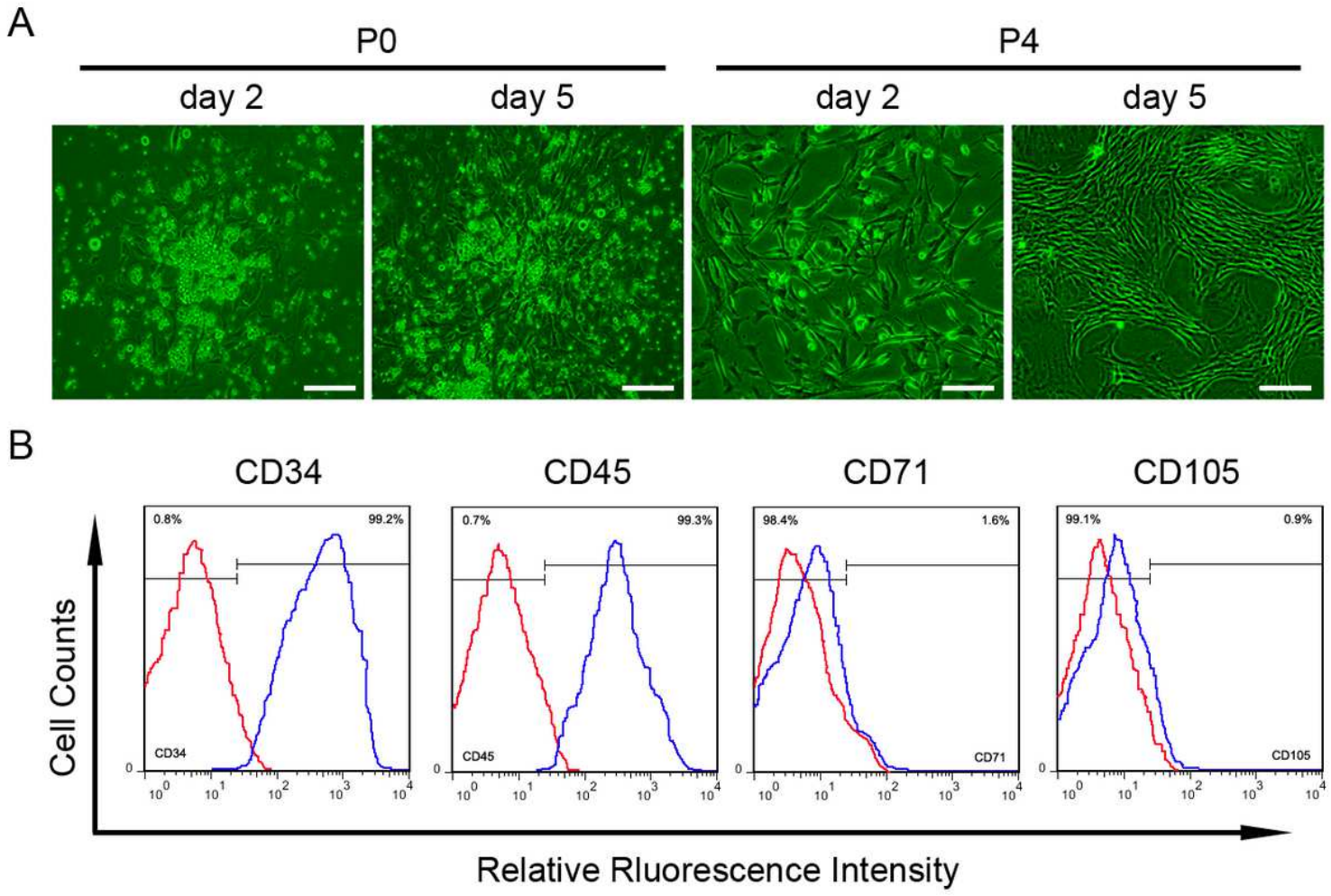

Figure 1

Isolation of BMSCs. (A) Morphologies of BMSCs at passage 0 (P0) and passage 4 (P4) were observed under an inverted microscope (scale bar, 20 μm). (B) Expression of BMSC markers CD71 and CD105, and hematopoietic stem cell markers CD34 and CD45 was detected by flow cytometry.

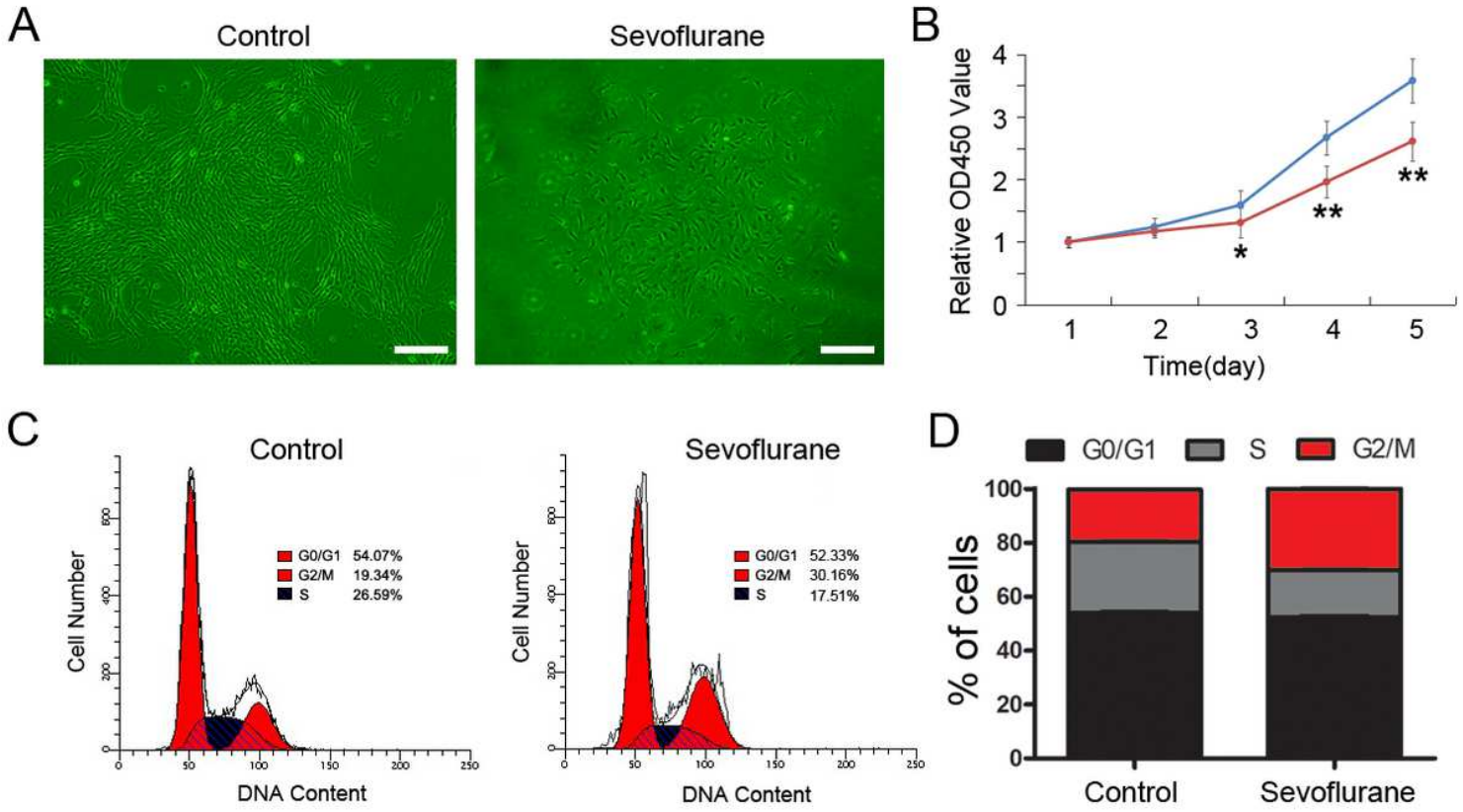

Figure 2

Long-term exposure to sevoflurane inhibits the proliferation of BMSCs. (A) After long-term sevoflurane exposure, BMSC morphology was observed under a microscope (scale bar, 20 μm). (B) CCK-8 assays were used to assess the effect of long-term exposure to sevoflurane on BMSC proliferation. (C) Flow cytometry was used to examine the effect of long-term exposure to sevoflurane on the cell cycle of BMSCs. (D) Cell cycle phases of BMSCs. Data are shown as means ± SD (n = 3); *P < 0.05 and **P < 0.01 compared with the control group (Student's t-test).

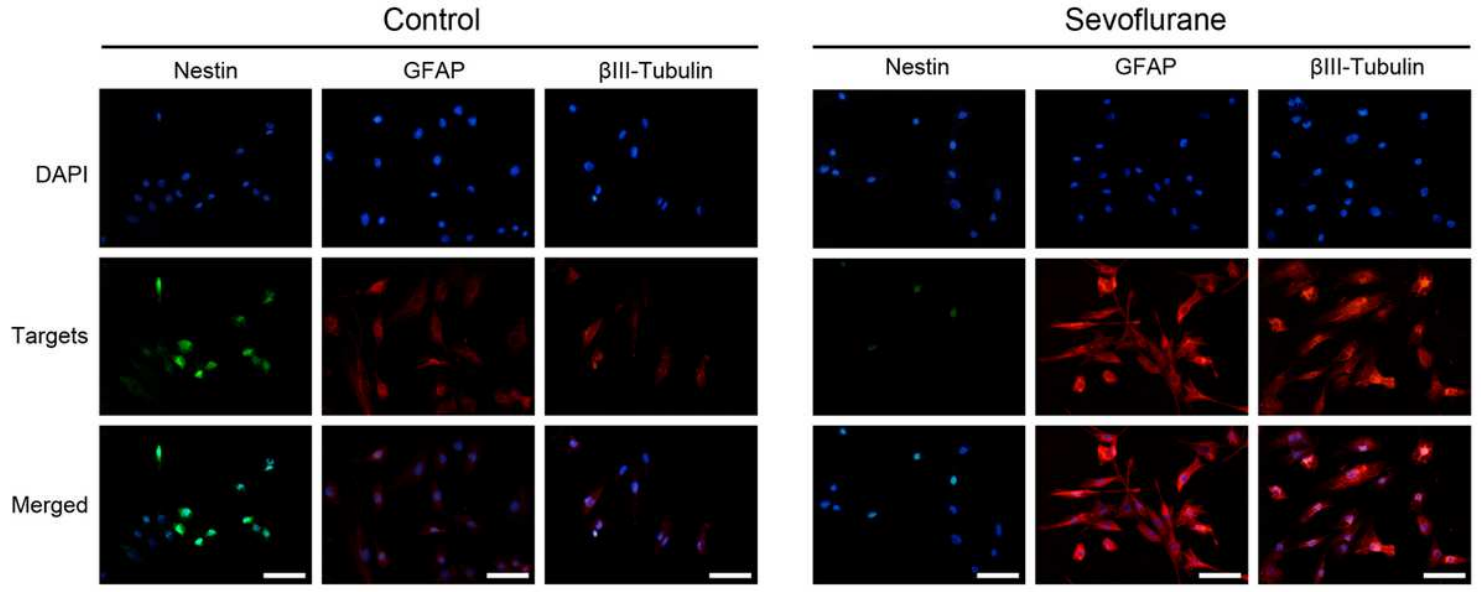

Figure 3

Long-term exposure to sevoflurane inhibits the differentiation of BMSCs. Immunofluorescence was used to analyze the effect of sevoflurane exposure on the differentiation of BMSCs into neural precursor cells (marked by Nestin; green), glial cells (marked by GFAP; red), and neurons (marked by βIII-Tubulin; red). DAPI was used to stain cell nuclei (scale bar, 20 μm). GFAP, glial fibrillary acidic protein.

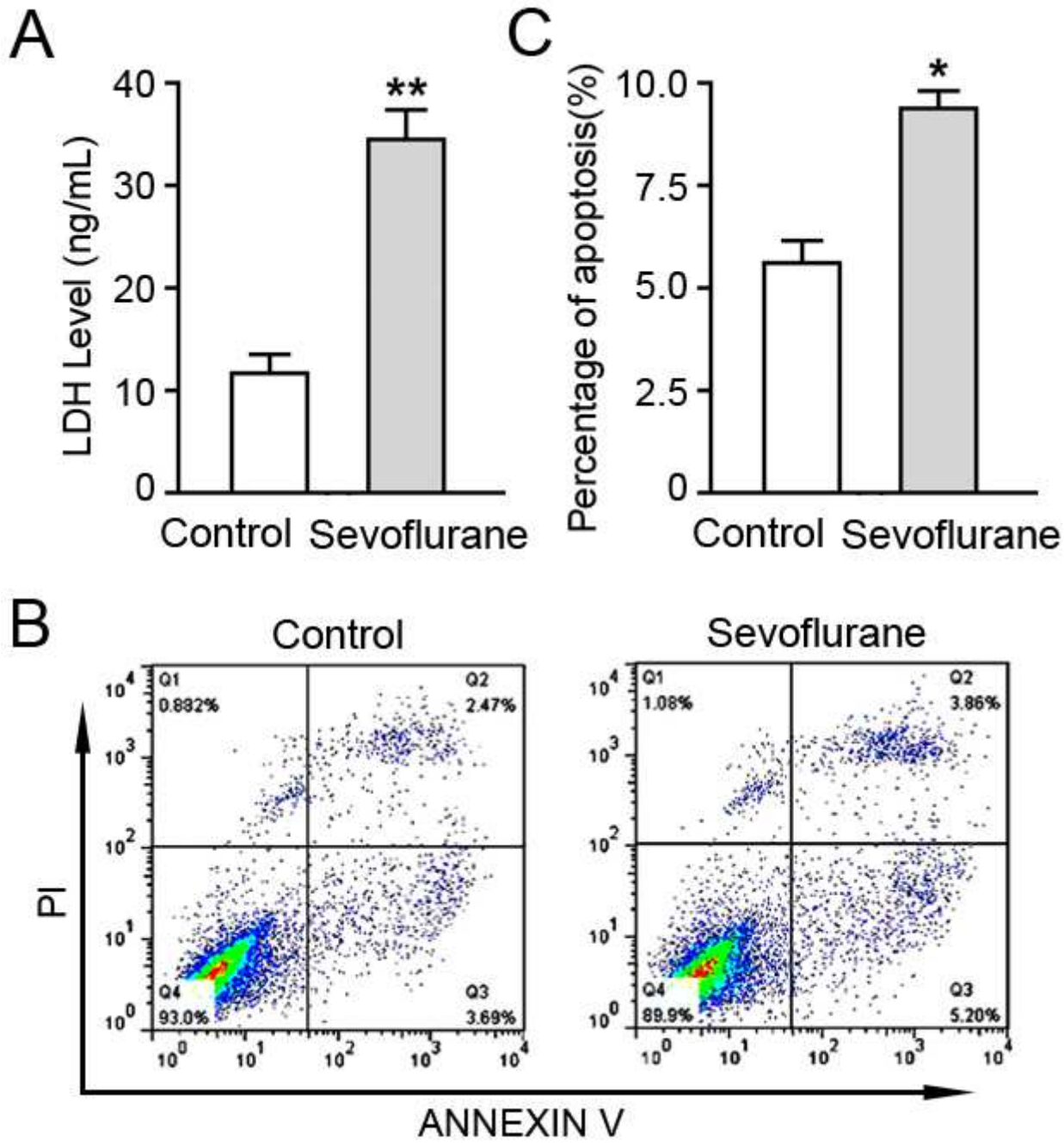

Figure 4

Long-term sevoflurane exposure damages BMSCs. (A) ELISA was used to measure the protein level of LDH. (B and C) Flow cytometry was used to detect apoptosis. Data are shown as means ± SD (n = 3). *P < 0.05 and **P < 0.01 compared with the control group (Student's t-test).

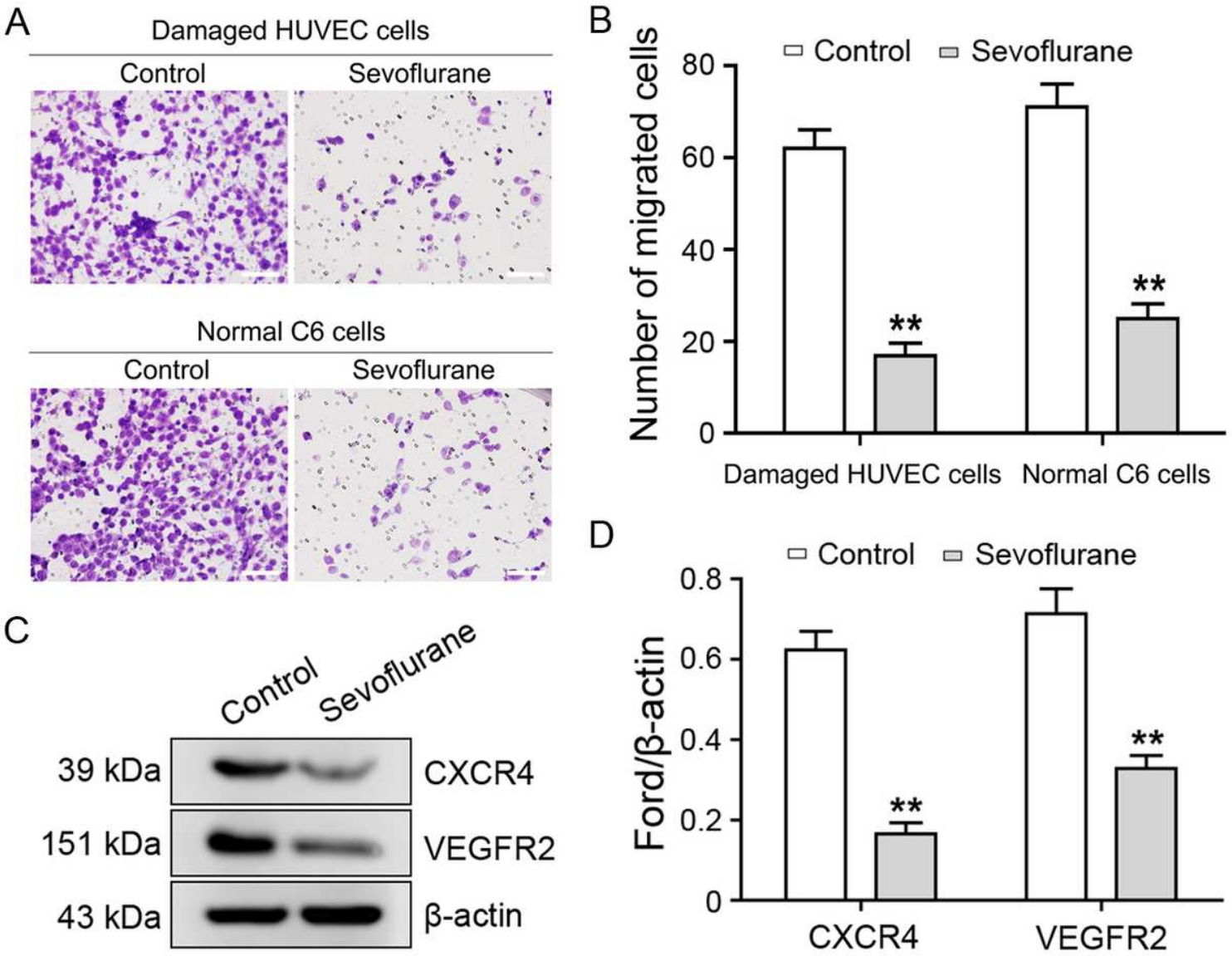

Figure 5

Long-term sevoflurane exposure inhibits the migration of BMSCs. (A and B) Transwell assays were used to assess the migration potential of BMSCs toward damaged HUVECs and C6 glioma cells (scale bar, 30 μm). (C and D) Protein expression levels of CXCR4 and VEGFR in BMSCs were detected by western blotting. Data are shown as means ± SD (n = 3). **P < 0.01 compared with the control group (Student's t-test).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Fig.S1.UncroppedwesternblotsrelatedtoFigure5.docx