Article

Discovery Medicine 2024; 36(189): 2071–2078
https://doi.org/10.24976/Discov.Med.202436189.191

Sevoflurane Activates PI3K/AKT Signaling Pathway by Upregulating *GDF11* Expression to Attenuate Ischemia/Reperfusion Injury in Cardiomyocytes

Rong-sheng Zhou[1], Xiao-hong Xue[2], Yang Bi[1], Ting-ting Yang[1], Zhan-qin Zhang[1], Hao-yang Zhu[1], Qiang Wang[1,*]

[1]Department of Anesthesiology, The First Affiliated Hospital of Xi'an Jiaotong University, 710061 Xi'an, Shaanxi, China
[2]Department of Hemodialysis, The First Affiliated Hospital of Xi'an Jiaotong University, 710061 Xi'an, Shaanxi, China
*Correspondence: drwangqiang1@163.com (Qiang Wang)
Published: 20 October 2024

Background: Myocardial ischemia/reperfusion (I/R) injury stands as a primary contributor to ischemic heart disease. Sevoflurane (SEVO), a commonly used inhalation anesthetic, has been shown to exert a direct protective effect on ischemic heart injury. However, the specific mechanism by which it exerts the protective effect remains unclear. This study was designed to investigate the role of SEVO in myocardial I/R injury and its potential molecular mechanisms.

Methods: Blood samples were collected from patients with acute myocardial infarction (AMI) (n = 20) and healthy volunteers (n = 20). The human cardiomyocytes AC16 models of I/R injury were induced by hypoxia/reoxygenation. The mRNA expression levels of growth differentiation factor 11 (*GDF11*) in the cells and blood were determined by reverse transcription quantitative real-time PCR (RT-qPCR). The cell proliferation was detected by Cell Counting Kit-8 (CCK-8). Enzyme-Linked Immunosorbent Assay (ELISA) was utilized to detect the levels of inflammatory factors interleukin (IL)-8, IL-1β and IL-6 in the cells. And biochemical assay kits were applied for the measurement of the activity of lactate dehydrogenase (LDH) and superoxide dismutase (SOD) as well as the malondialdehyde (MDA) level in the cells. Moreover, western blot was employed to evaluate the levels of the p-serine-threonine protein kinase (AKT), AKT, and phosphatidylinositol 3-kinase (PI3K), protein expression in the cells.

Results: The *GDF11* expression was decreased in the blood of AMI patients and cardiomyocytes induced by I/R ($p < 0.01$). Besides, 1% SEVO was presented to promote cardiomyocyte proliferation, inhibit apoptosis, oxidative stress and inflammation, and activate the PI3K/AKT signaling pathway through up-regulation of *GDF11* expression ($p < 0.01$).

Conclusion: SEVO promotes proliferation and inhibits inflammatory response, apoptosis, and oxidative stress of I/R-treated cardiomyocytes by elevating *GDF11* expression, thereby reducing myocardial I/R injury. Notably, the mechanism underlying the alleviation of the I/R injury may involve the activation of PI3K/AKT signaling pathway.

Keywords: sevoflurane; PI3K/AKT signaling pathway; myocardial ischemia/reperfusion; *GDF11*

Introduction

Cardiovascular disease has high mortality and morbidity worldwide [1]. Acute myocardial infarction (AMI) results from myocardial necrosis triggered by acute sustained hypoxia and ischemia in the coronary arteries. It stands as leading cause of cardiovascular-related death globally [2]. Myocardial ischemia/reperfusion (I/R) is currently an effective treatment for myocardial ischemia. However, it can lead to several adverse effects on myocardial cardiac function, energy metabolism, and electrophysiology. In some cases, it may even result in death due to the promotion of severe arrhythmias. In summary, I/R injury is a primary risk factor in the pathological process of cardiovascular disease [3]. Therefore, alleviating I/R injury is crucial for treating AMI.

Anesthetics is used for pretreatment in an expanding number of therapeutic procedures to minimize the size of myocardial infarction and mitigate I/R injury [4]. Sevoflurane (SEVO), a common anesthetic agent, is widely used in clinical settings for inducing and maintaining general anesthesia. Its popularity stems from its ability to easy diffuse into cells and cell membranes. Additionally, sevoflurane has been shown to reduce perioperative mortality and morbidity [5,6]. Several studies have reported that pretreatment with SEVO during myocardial ischemia can significantly reduce I/R injury [7,8]. This suggested that SEVO could be a potential therapeutic approach to alleviate myocardial injury and protect myocardial function. However, uncertainty still surrounds the specific mechanism by which sevoflurane exerts its protective effect. Growth differentiation factor 11 (*GDF11*), also recognized as bone morphogenetic protein 11, is a member of the transform-

Copyright: © 2024 The Author(s). Published by Discovery Medicine. This is an open access article under the CC BY 4.0 license.
Note: *Discovery Medicine* stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

ing growth factor-β superfamily [9]. An early study has demonstrated that *GDF11* plays an essential role primarily in the axial skeletal patterning as well as the formation and development of the appendicular skeleton in animals [10]. Subsequent studies have revealed that *GDF11* concentration has been linked to a lower risk of cardiovascular events and mortality [11,12], implying that *GDF11* may serves as a protective agent against cardiovascular events. However, there have been no reports on the interaction between *GDF11*, I/R injury and SEVO. Phosphatidylinositol 3-kinase (PI3K)/serine-threonine protein kinase (AKT) signaling pathway stands as one of the critical pathways in reperfusion injury and is an essential pathway for cell membrane receptors to transduce signals from external stimuli into cells. As an initiation factor of this signaling pathway, PI3K can be triggered by a range of factors such as ischemia, hypoxia and stress, and energy metabolism disorders [13].

Therefore, this study was designed to explore the mechanism underlying SEVO in reducing I/R injury through *in vitro* experiments and to reveal the interaction between SEVO, *GDF11*, PI3K/AKT signaling pathway and I/R injury. This study aims to establish a theoretical foundation for the clinical use of SEVO in treating I/R injury and for considering *GDF11* as a therapeutic target.

Materials and Methods

Blood Samples

Blood samples were acquired from 20 patients with AMI underwent treatment at The First Affiliated Hospital of Xi'an Jiaotong University between March 2022 and August 2022, as well as from 20 healthy volunteers during the same period. The study was approval by the Ethics Committee of The First Affiliated Hospital of Xi'an Jiaotong University and was conducted in accordance with the approved guidelines (Grant no.: 2021-W6). This study was conducted in accordance with the principles outlined in the Declaration of Helsinki and informed consent form was obtained from all participants prior to their involvement in the study.

Cell Culture

Human cardiomyocytes cell line AC16 (BFN60808678, Bluefbio, Shanghai, China) was cultured in DMEM medium (SH30022.01, Cytiva, South Logan, UT, USA) in a 37 °C and 5% CO_2 cell culture incubator. After reaching the logarithmic growth phase, the cells were digested using 0.25% trypsin (T1350, Solarbio, Beijing, China) for passaging. Both mycoplasma testing and short tandem repeats (STR) certification have been performed.

The shRNA interference plasmid was designed and synthesized by GenScript (Nanjing, China). Cells were seeded at 5×10^5 cells per well into 6-well plates and cultured. Subsequently, the medium was replaced with Opti-MEM serum-free medium. The shRNA was mixed with Lipofectamine 2000 at a ratio of 1 µg/µL in Opti-MEM serum-free medium and incubated at room temperature for 20 min. The mixture was then added to the attached cells. After culturing for 6 hours, the medium was replaced with complete medium. The sequences of shRNA negative control (NC) and sh-*GDF11* (the final concentration was 20 nM) were as follows:

(1) sh-NC-sense: GATCCCAACAAGATGAAGAG CACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTT TTTG.

(2) Anti-sense: AATTCAAAAACAACAAGATGA AGAGCACCAACTCGAGTTGGTGCTCTTCATCTTG TTGG.

(3) sh-*GDF11*-sense: GATCCCCCTTCTGACATCT CCTACATCTCGAGATGTAGGAGATGTCAGAAGGG TTTTTG.

(4) Anti-sense: AATTCAAAAACCCTTCTGACAT CTCCTACATCTCGAGATGTAGGAGATGTCAGAAG GGG.

Cell Grouping and Treatment

I/R injury was induced in AC16 cells by hypoxia/reoxygenation (H/R) according to the literature [14]. Briefly, AC16 cells at logarithmic growth phase were exposed to a hypoxic environment (95% N_2, 5% CO_2). Six hours later, the cells were reoxygenated (5% CO_2) for 6 h to generate AC16 cell models of I/R injury (I/R group).

SEVO was purchased from Aladdin (28523-86-6, Shanghai, China). AC16 cells were treated as follows: control group, AC16 cells received no treatment; I/R group, AC16 cells were induced by H/R; I/R + SEVO group, AC16 cells induced by H/R were treated with 1% SEVO [14]; I/R + SEVO + sh-NC group: AC16 cells transfected with negative control vector (sh-NC) received 1 h of 1% SEVO and 12 h of H/R induction treatment; I/R + SEVO + sh-*GDF11* group: AC16 cells transfected with shRNA that used to silenced *GDF11* (sh-*GDF11*) received 1 h of 1% SEVO and 12 h of H/R induction treatment.

Reverse Transcription Quantitative Real-Time PCR (RT-qPCR)

The Total RNA Extraction Kit (R1200, Solarbio, Beijing, China) was utilized for the extraction of the total RNA from the cells or blood sample. The total RNA samples were stored at –80 °C. Following that, cDNA synthesis was conducted through reverse transcription, adhering to the provided instructions accompanying with the FastKing RT Kit (With gDNase) (KR116-01, TIANGEN, Beijing, China). A Nanodrop instrument (13-400-518, Thermo Fisher Scientific, Wilmington, DE, USA) was utilized for the measurement of the purity and concentration of the synthesized cDNA. Subsequently, the relative expression level of *GDF11* in the samples was determined by following the procedure outlined in the RealUniversal Color Pre-

Table 1. RT-qPCR primer sequences.

Genes	Primer sequences
GDF11	F: 5′-CTGGAGGAGGACGAGTACCA-3′
	R: 5′-GAACATCACCTTGGGGCTGA-3′
GAPDH	F: 5′-GAGTCAACGGAT TTGGTCGT-3′
	R: 5′-GACAAGCTTCCCGTTCTCAG-3′

RT-qPCR, reverse transcription quantitative real-time PCR; *GDF11*, growth differentiation factor 11; *GAPDH*, Glyceraldehyde-3-Phosphate Dehydrogenase.

Mix kit (SYBR Green) (FP201-02, TIANGEN, Beijing, China), with Glyceraldehyde-3-Phosphate Dehydrogenase (*GAPDH*) serving as the internal control gene. Table 1 displays the primer sequences used. A $2^{-\Delta\Delta Ct}$ method was adopted for data analysis.

Cell Counting Kit-8 (CCK-8) Assay

AC16 cells, at the logarithmic growth phase, whether transfected or untransfected, were diluted to 4×10^4 cells/mL. Subsequently, 96-well plates were selected for cell inoculation with each well receiving 100 µL of cell suspension. Each experiment group was established with 6 duplicated wells. The cells were subjected to the SEVO and H/R treatment in accordance with the grouping and treatment after cell adherence. And cell proliferation was measured at 0 h and 24 h after the treatment using the CCK-8 kit (C0038, Beyotime, Shanghai, China). Briefly, the wells were supplemented with a mixture of 90 µL fresh complete culture and 10 µL CCK-8 solution, and incubated at 37 °C for 2 h. An enzyme marker was applied to examine the absorbance at 450 nm.

Flow Cytometry

Transfected or untransfected AC16 cells (1×10^5 cells/well) were inoculated in 6-well plates. Likewise, after the cell adherence, the cells received SEVO and H/R treatment. Later, after rinsing with pre-chilled phosphate buffered saline (PBS), the cells were centrifuged at 1000 rpm, 4 °C for 5 min. Following the removal of the supernatant, pre-chilled PBS was employed to wash the cells again, followed by centrifugation. Upon resuspension in 1 × binding buffer, the concentration of the cells was set to 1×10^6/mL. Subsequently, we transferred the 100 µL of the cell suspension to a 5 mL flow tube. After that, 5 µL of Propidium Iodide (PI) and AnnexinV-FITC (CA1020, Solarbio, Beijing, China) was added, mixed, and incubated at ambient temperature in the dark for 5 min. Finally, flow cytometry (656385, BD Biosciences, LSRFortessa, San Jose, CA, USA) was applied to determine the apoptosis rate within 1 h.

Enzyme-Linked Immunosorbent Assay (ELISA)

AC16 cells were planted in 24-well plates and subjected to SEVO and H/R treatment. Next, the cells were conducted for centrifugation at 1000 ×g for 10 min after 24 h of the treatment. Then, upon the collection of the supernatant, we measured the levels of interleukin (IL)-1β, IL-8 and IL-6 in the cell culture supernatant using the respective ELISA kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) following the steps of the corresponding. Each indicator underwent three repeated detections to ensure the accuracy and reliability of the results.

Biochemical Assay

A 24-well plate was picked for the inoculation of AC16 cells. Next, the cells were exposed to SEVO and H/R. Upon the 24 h treatment, the cells were centrifuged, and the supernatant was discarded. The extraction buffer was added, and the cells were broken up by ultrasound, followed by centrifugation at 8000 ×g at 4 °C for 10 min. After centrifugation, the supernatant was collected and placed on ice for measurement. We assessed the activity of malondialdehyde (MDA) and superoxide dismutase (SOD), as well as lactate dehydrogenase (LDH) levels in the cells using the corresponding MDA (A003-1), SOD (A001-3), LDH (A020-2-2) kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) in accordance with the kit procedures. All indicators were tested in triplicate to ensure the reliability and accuracy of the results.

Western Blot

A protein lysis solution (P0013, Beyotime, Shanghai, China) was conducted to lyse the cells on ice for 30 min. After centrifugation, we transferred the supernatant to a centrifuge tube on ice. After diluting the BSA protein standard (P0006, Beyotime, Shanghai, China), 20 µL of protein standard solution was supplemented. A BCA working solution was added upon diluting the sample to 20 µL with 1× PBS solution. The total protein concentration was calculated and the absorbance value was determined. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel was generated by adding concentrated gel and separation gel in the glass gel mold, followed by electrophoresis to separate 30 µg of total protein samples, with the voltage set to 80 V. Once the protein marker had completely separated and reached the interface of the separation gel, the voltage was set to 120 V. The transfer of the protein on the gel after electrophoresis was conducted to a polyvinylidene fluoride (PVDF) membrane (IPVH00010, Millipore Sigma, Burlington, MA, USA) at a continuous current of 200 mA for 2 h in an electric tank. After blocking in 5% skimmed milk powder for 2 h, the membrane was subjected to overnight incubation with primary antibodies in a shaker at 4 °C. Upon washing, secondary antibodies were conducted to incubate the membrane at ambient temperature for 1 h. Following the addition of Enhanced chemiluminescent (ECL) solution (P0018S, Beyotime, Shanghai, China), the protein membrane was placed on a gel imaging system. ImageJ software (version 1.8.0, NIH, Bethesda, MD, USA)

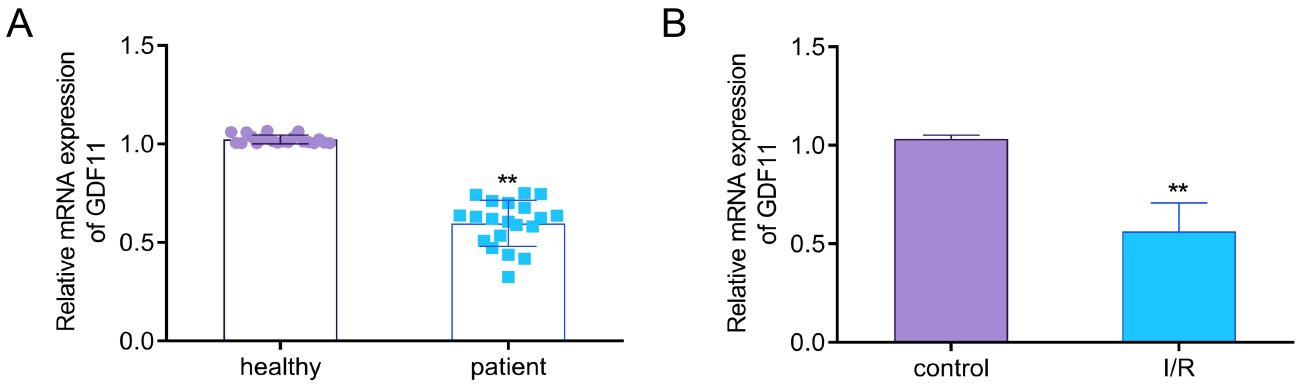

Fig. 1. *GDF11* expression is downregulated in acute myocardial infarction patients and the I/R induced cell line. (A,B) The mRNA expression levels of *GDF11* in clinical blood samples (n = 20) (A) and AC16 cells (n = 3) (B) were detected by RT-qPCR, $^{**}p < 0.01$. I/R, ischemia/reperfusion.

was utilized for the quantification of protein bands, with *GAPDH* serving as an internal control to assess the relative expression of the target proteins. The antibodies information used were as follows: anti-phosphatidylinositol 3-kinase (PI3K) antibody (1:1000 dilution, ab86714, Abcam, Cambridge, UK); anti-p-PI3K (1:1000 dilution; ab138364, Abcam, Cambridge, UK); anti-AKT antibody (1:1000 dilution; ab59380, Abcam, Cambridge, UK), anti-p-AKT antibody (1:5000 dilution; ab8933, Abcam, Cambridge, UK), anti-*GAPDH* antibody (1:5000 dilution; ab8245, Abcam, Cambridge, UK), Goat anti-Mouse IgG H&L (HRP) (1:2000 dilution, ab205719, Abcam, Cambridge, UK).

Statistical Analysis

The experimental data were presented as mean ± standard deviation and analyzed using Graphpad prism 9.0 software (GraphPad Software, San Diego, CA, USA). Statistical analyses, including the *t*-test and one-way analysis were performed using SPSS 21.0 software (IBM Corp, Armonk, NY, USA). $p < 0.05$ represented the statistical significance threshold.

Results

GDF11 is Downregulated in Acute Myocardial Infarction

Since *GDF11* was previously reported to be a possible protective factor against cardiovascular events, we examined *GDF11* expression levels in I/R-induced AC16 cells and serum from patients with AMI. The results exhibited significantly lower *GDF11* expression in serum from AMI patients and AC16 cells induced by I/R compared to the corresponding serum from healthy volunteers and AC16 cells in the control group ($p < 0.01$) (Fig. 1A,B). These findings suggest a potential protective role for *GDF11* against cardiovascular events, as its reduced expression was correlated with the pathological state of AMI and the cellular stress condition induced by I/R.

The Impact of SEVO on Apoptosis and Proliferation in I/R-Induced AC16 Cells and Its Modulation via GDF11

We treated these cells with 1% SEVO and sh-*GDF11* to explore the role of SEVO in cell proliferation and apoptosis in I/R-induced AC16 cells, as well as its relationship with the elevation of *GDF11*. Subsequently, we examined the effects on cell apoptosis and proliferation. The results depicted in Fig. 2. AC16 cells subjected to I/R exhibited a notable decrease in cell activity and *GDF11* expression alongside a significant increase in apoptosis. However, following treatment with 1% SEVO, there was a clear upregulation of *GDF11* expression and cell viability in I/R-induced AC16 cells, accompanied by a suppression of apoptosis ($p < 0.01$). Moreover, compared to the I/R + SEVO + sh-NC group, the I/R + SEVO + sh-*GDF11* group displayed a marked reduction in *GDF11* expression and cell viability, alongside an increase in apoptosis rate ($p < 0.01$). In summary, SEVO appears to inhibit apoptosis and promote proliferation in I/R-induced AC16 cells through the upregulation of *GDF11* expression.

Sevoflurane Modulates Inflammatory and Oxidative Responses in I/R-Induced AC16 Cells via GDF11 Expression

We then further explored the effect of SEVO on oxidative stress and inflammatory response in I/R-induced AC16 cells as well as its relationship with *GDF11* expression. Fig. 3A–F presented the results. Compared with the control group, the I/R group exhibited an upward trend in the levels of oxidative stress and inflammatory factors IL-6, IL-8, IL-1β and MDA, as well as LDH activity in AC16 cells, and a downward trend in SOD activity. It was evident that the oxidative stress and inflammatory response in AC16 cells were induced by I/R. After SEVO treatment, the levels of IL-1β, IL-8, IL-6, MDA and LDH activity were significantly reduced ($p < 0.01$) and SOD activity was significantly increased ($p < 0.01$) in I/R-induced AC16

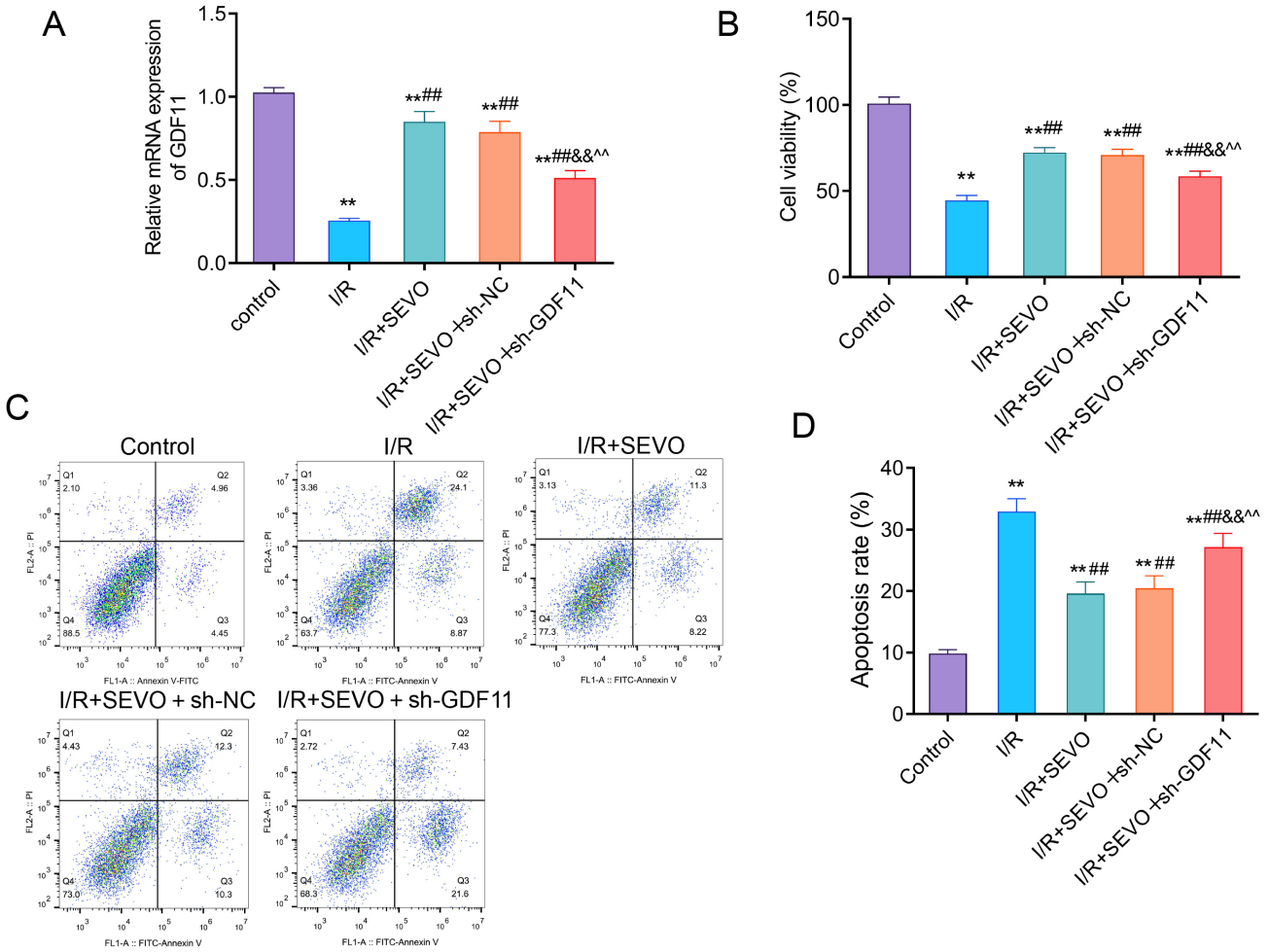

Fig. 2. Effect of SEVO on apoptosis and proliferation in I/R-induced AC16 cells and its regulation via *GDF11*. (A–D) *GDF11* mRNA expression levels (A), cell viability (B) and apoptosis (C,D) in AC16 cells of control group, I/R group, I/R + SEVO group, I/R + SEVO + sh-NC group and I/R + SEVO + sh-*GDF11* group by RT-qPCR, CCK-8 assay and flow cytometry, respectively. One-way analysis of variance, $^{**}p < 0.01$ *vs.* control group; $^{\#\#}p < 0.01$ *vs.* I/R group; $^{\&\&}p < 0.01$ *vs.* I/R + SEVO group; $^{\wedge\wedge}p < 0.01$ *vs.* I/R + SEVO + sh-NC group. SEVO, Sevoflurane; NC, negative control; CCK-8, Cell Counting Kit-8.

cells. Furthermore, compared to the I/R + SEVO + sh-NC group, the I/R + SEVO + sh-*GDF11* group significantly increased levels of MDA, IL-6, IL-1β, IL-8, and LDH activity, while decreasing SOD activity ($p < 0.01$). All these results suggest that SEVO inhibits oxidative stress and inflammatory responses in AC16 cells caused by I/R through the up-regulation of *GDF11* expression.

Sevoflurane's Role in Modulating PI3K/AKT Pathway via GDF11 in I/R Cardiomyocytes

SEVO was discovered to suppress the inflammatory responses and oxidative stress induced by I/R through up-regulation of *GDF11* expression. However, further discussion was required on whether it has been implicated in activating the PI3K/AKT signaling pathway. Therefore, we examined the protein expression levels of PI3K/AKT signaling pathway-related factors p-PI3K, PI3K, p-AKT, and AKT. We found that the ratios of both p-AKT/AKT and p-PI3K/PI3K in AC16 cells in the I/R group were significantly lower than those in the control group. The levels of p-AKT/AKT and p-PI3K/PI3K in cells after SEVO pretreatment were notably increased compared to the I/R group ($p < 0.01$). After silencing *GDF11* expression, relative to the I/R + SEVO + sh-NC group, the levels of p-AKT/AKT and p-PI3K/PI3K in cells in the I/R + SEVO + sh-*GDF11* group were markedly declined ($p < 0.01$), demonstrating that PI3K/AKT signaling was effectively inhibited in this case (Fig. 4). In summary, these results pointed out that SEVO activates the PI3K/AKT signaling pathway through the up-regulation of *GDF11* expression.

Discussion

Previous studies have documented the protective effects of SEVO in rats with myocardial I/R injury [15,16]. Consistent with these findings, our study reveals that SEVO

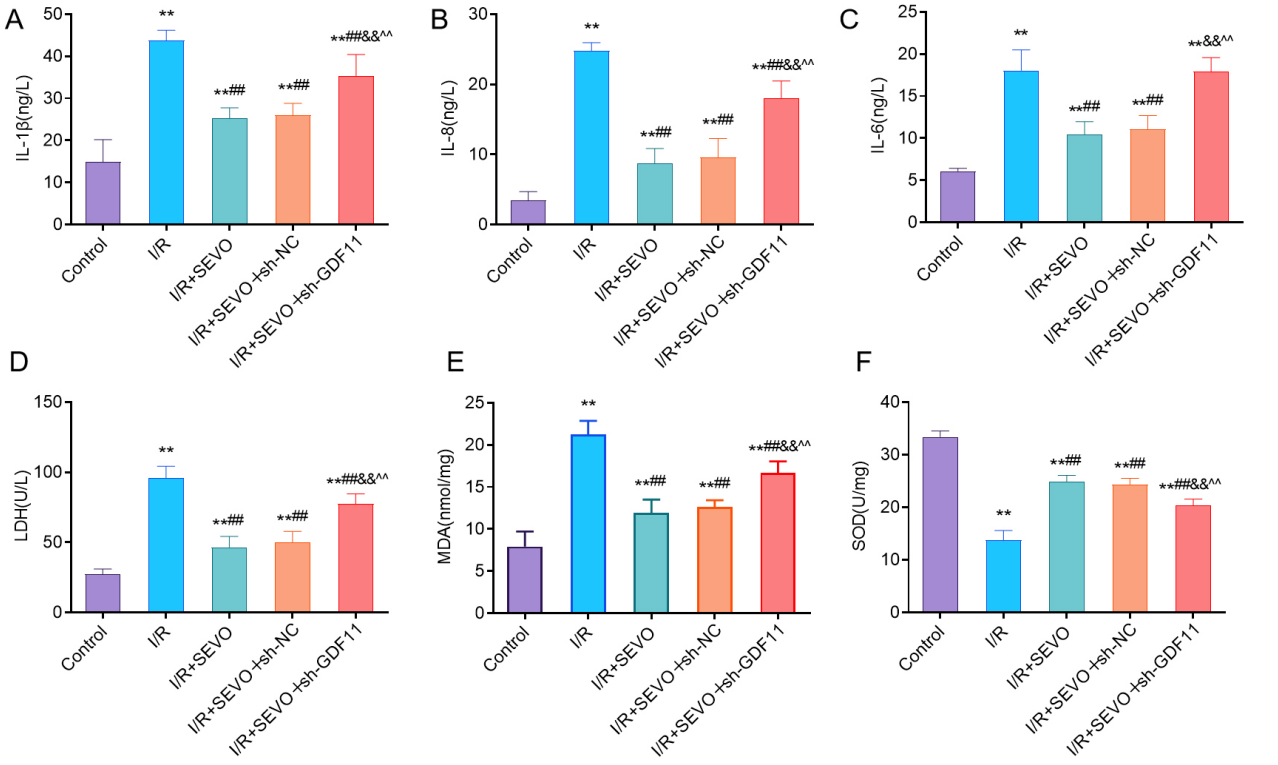

Fig. 3. Sevoflurane modulates inflammatory and oxidative responses in AC16 cells induced by I/R via *GDF11* expression. (A–C) ELISA detecting the levels of IL-1β (A), IL-8 (B) and IL-6 (C) in AC16 cells of the control group, I/R group, I/R + SEVO group, I/R + SEVO + sh-NC group and I/R + SEVO+sh-*GDF11* group. (D–F) Biochemical kits measuring the levels or activities of LDH (D), MDA (E) and SOD (F) in the control group, I/R group, I/R + SEVO group, I/R + SEVO + sh-NC group and I/R + SEVO + sh-*GDF11* group. One-way analysis of variance. $^{**}p < 0.01$ *vs.* control group; $^{\#\#}p < 0.01$ *vs.* I/R group; $^{\&\&}p < 0.01$ *vs.* I/R + SEVO group; $^{\wedge\wedge}p < 0.01$ *vs.* I/R + SEVO + sh-NC group. IL, interleukin; LDH, lactate dehydrogenase; MDA, malondialdehyde; SOD, superoxide dismutase; ELISA, Enzyme-Linked Immunosorbent Assay.

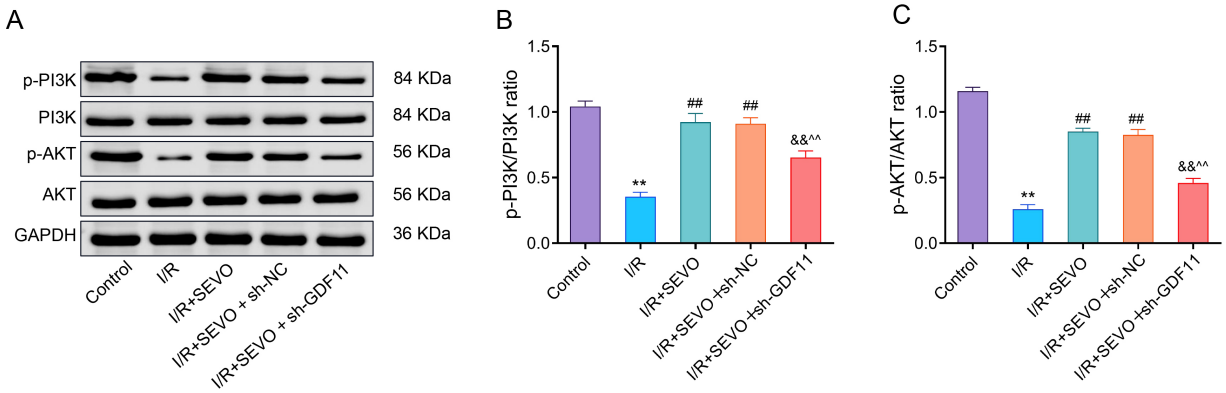

Fig. 4. Sevoflurane's role in modulating PI3K/AKT pathway and via *GDF11* in I/R cardiomyocytes. (A–C) The detection for expression levels of PI3K/AKT signaling pathway-related proteins PI3K, p-PI3K, AKT, p-AKT in AC16 cells of the control group, I/R group, I/R + SEVO group, I/R + SEVO + sh-NC group and I/R + SEVO + sh-*GDF11* group using western blot. One-way analysis of variance, $^{**}p < 0.01$ *vs.* control group; $^{\#\#}p < 0.01$ *vs.* I/R group; $^{\&\&}p < 0.01$ *vs.* I/R + SEVO group; $^{\wedge\wedge}p < 0.01$ *vs.* I/R + SEVO + sh-NC group. AKT, serine-threonine protein kinase; PI3K, phosphatidylinositol 3-kinase.

suppresses apoptosis, inflammatory response and oxidative stress in AC16 cells exposed to I/R, while promoting cell proliferation. These results further support the notion that SEVO has a preventive effect against I/R injury. Importantly, a significant increase in *GDF11* expression level in I/R-induced cardiomyocytes was observed follow-

ing SEVO treatment. Conversely, *GDF11* expression was reduced in cardiomyocytes subjected to I/R and in the blood of AMI patients. This suggests that *GDF11* may act as a protective factor against myocardial injury, and the protective effect exerted by SEVO may be mediated through the up-regulation of *GDF11* expression.

A close association between I/R injury and inflammatory response has been documented. When I/R injury occurs, the levels of inflammatory factors including tumor necrosis factor-alpha (TNF-α), IL-6, IL-8, and IL-1β increase, which in turn aggravate the I/R injury [17]. In this study, AC16 cells subjected to I/R had considerably lower levels of IL-6, IL-8, and IL-1 following SEVO pretreatment, indicating that SEVO helps prevent inflammatory response-related myocardial injury. To further confirm whether SEVO exerts anti-inflammatory effects in I/R-treated cells through up-regulation of *GDF11* expression, we transfected sh-*GDF11* in I/R-induced cells with SEVO treatment. A remarkable increase in the levels of inflammatory factors was observed in the cells, and this result suggests that SEVO inhibits the I/R-induced cellular inflammatory response via up-regulating *GDF11* expression. Additionally, I/R injury is closely linked to oxidative stress. As demonstrated in a study, I/R injury induces overproduction of reactive oxygen species (ROS), disrupts redox homeostasis, and finally leads to oxidative stress [18]. ROS can induce lipid peroxidation and degradation, thereby producing MDA. Additionally, ROS can alter cell permeability and result in massive intracellular LDH leakage [19]. In this scenario, cell membrane damage occurs and cardiomyocytes begin to deteriorate following I/R. To maintain the redox state in balance, excess ROS are usually eliminated by various enzymes, such as SOD and Glutathione peroxidase (GSH-Px) [20]. In this study, LDH activity and MDA level in AC16 cells subjected to I/R were noticeably decreased and SOD activity was significantly increased following SEVO pretreatment, alleviating the myocardial injury caused by oxidative stress. In contrast, the inhibitory effect of SEVO on oxidative stress was attenuated after transfection with sh-*GDF11* in I/R-induced AC16 cells treated with SEVO. This result implies that SEVO inhibits I/R-induced cellular oxidative stress response through up-regulation of *GDF11* expression.

The role of the PI3K/AKT/mTOR signaling pathway in cancer has been discussed in detail in the literature, highlighting the importance of this pathway in cell survival, proliferation, and cycle progression, as well as its frequent abnormalities in a variety of cancers [21]. After activation, the PI3K/AKT signaling pathway exerts protective effects in cardiomyocytes [22]. In this study, the levels of p-PI3K/PI3K and p-AKT/AKT were presented to considerably elevate in I/R-induced AC16 cells after pretreatment by SEVO, indicating that SEVO could activate the PI3K/AKT signaling pathway. In contrast, PI3K/AKT signaling pathway was inhibited after transfection of sh-*GDF11* in I/R-induced AC16 cells. This suggests that SEVO may exert protective effects through activating the PI3K/AKT signaling pathway through up-regulation of *GDF11* expression.

This study still has some limitations. For instance, this study mainly investigated the effect of SEVO on I/R injury and the possible mechanism of action through i*n vitro* cellular experiments. Relevant *in vivo* animal experiments are required to delve deeper into the role of SEVO in alleviating I/R injury and to elucidate its mechanism of action.

Conclusion

In summary, SEVO facilitates cell proliferation and suppresses oxidative stress, apoptosis, and inflammatory response in cardiomyocytes induced by I/R by up-regulating *GDF11* expression, the mechanism underlying the alleviation of the I/R injury may involve the activation of the PI3K/AKT signaling pathway.

Availability of Data and Materials

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

RZ and QW conceptualized and designed the study, drafted the initial manuscript. XX, YB, TY, ZZ and HZ collected the data and carried out the initial analyses. QW critically reviewed the manuscript for important intellectual content. All authors contributed significantly to editorial changes of important content. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of The First Affiliated Hospital of Xi'an Jiaotong University and was conducted in accordance with the approved guidelines (Grant no.: 2021-W6). This study was conducted in accordance with the principles outlined in the Declaration of Helsinki, and informed consent was obtained from all participants prior to their involvement in the study.

Acknowledgment

Not applicable.

Funding

This work was supported by the Science and Technology Development of Shanxi Province (No.2019SF-154).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Lu D, Thum T. RNA-based diagnostic and therapeutic strategies for cardiovascular disease. Nature Reviews. Cardiology. 2019; 16: 661–674.

[2] Benjamin EJ, Virani SS, Callaway CW, Chamberlain AM, Chang AR, Cheng S, *et al*. Heart Disease and Stroke Statistics-2018 Update: A Report from the American Heart Association. Circulation. 2018; 137: e67–e492.

[3] Jabbar AY, Baydoun H, Janbain M, Ferdinand KC. Current concepts in the management of stable ischemic heart disease and acute coronary syndrome in patients with hemophilia. Annals of Translational Medicine. 2018; 6: 299.

[4] Cai S, Liu Y, Cheng Y, Yuan J, Fang J. Dexmedetomidine protects cardiomyocytes against hypoxia/reoxygenation injury via multiple mechanisms. Journal of Clinical Laboratory Analysis. 2022; 36: e24119.

[5] Ohsumi A, Marseu K, Slinger P, McRae K, Kim H, Guan Z, *et al*. Sevoflurane Attenuates Ischemia-Reperfusion Injury in a Rat Lung Transplantation Model. The Annals of Thoracic Surgery. 2017; 103: 1578–1586.

[6] Zeng B, Liu Y, Xu J, Niu L, Wu Y, Zhang D, *et al*. Future Directions in Optimizing Anesthesia to Reduce Perioperative Acute Kidney Injury. American Journal of Nephrology. 2023; 54: 434–450.

[7] Hou T, Ma H, Wang H, Chen C, Ye J, Ahmed AM, *et al*. Sevoflurane preconditioning attenuates hypoxia/reoxygenation injury of H9c2 cardiomyocytes by activation of the HIF-1/PDK-1 pathway. PeerJ. 2020; 8: e10603.

[8] Wang J, Zheng H, Chen CL, Lu W, Zhang YQ. Sevoflurane at 1 MAC provides optimal myocardial protection during off-pump CABG. Scandinavian Cardiovascular Journal. 2013; 47: 175–184.

[9] Hannan NRF, Jamshidi P, Pera MF, Wolvetang EJ. BMP-11 and myostatin support undifferentiated growth of human embryonic stem cells in feeder-free cultures. Cloning and Stem Cells. 2009; 11: 427–435.

[10] Suh J, Eom JH, Kim NK, Woo KM, Baek JH, Ryoo HM, *et al*. Growth differentiation factor 11 locally controls anterior-posterior patterning of the axial skeleton. Journal of Cellular Physiology. 2019; 234: 23360–23368.

[11] Jamaiyar A, Wan W, Janota DM, Enrick MK, Chilian WM, Yin L. The versatility and paradox of GDF 11. Pharmacology & Therapeutics. 2017; 175: 28–34.

[12] Harper SC, Johnson J, Borghetti G, Zhao H, Wang T, Wallner M, *et al*. GDF11 Decreases Pressure Overload-Induced Hypertrophy, but Can Cause Severe Cachexia and Premature Death. Circulation Research. 2018; 123: 1220–1231.

[13] Pulido R. PTEN Inhibition in Human Disease Therapy. Molecules. 2018; 23: 285.

[14] Yu Z, Ren Q, Yu S, Gao X. Sevoflurane protects cardiomyocytes against hypoxia/reperfusion injury via LINC01133/miR-30a-5p axis. Bioscience Reports. 2020; 40: BSR20200713.

[15] Wu Q, Wang H, He F, Zheng J, Zhang H, Cheng C, *et al*. Depletion of microRNA-92a Enhances the Role of Sevoflurane Treatment in Reducing Myocardial Ischemia-Reperfusion Injury by Upregulating KLF4. Cardiovascular Drugs and Therapy. 2023; 37: 1053–1064.

[16] Ma Y, Cao Y, Gao H, Tong R, Yi J, Zhang Z, *et al*. Sevoflurane Improves Ventricular Conduction by Exosomes Derived from Rat Cardiac Fibroblasts After Hypothermic Global Ischemia-Reperfusion Injury. Drug Design, Development and Therapy. 2023; 17: 1719–1732.

[17] Wu J, Yang Y, Xun N, Zeng L, Li Z, Yang W, *et al*. Osthole attenuates myocardial ischemia/reperfusion injury in rats by inhibiting apoptosis and inflammation. American Journal of Translational Research. 2018; 10: 1109–1116.

[18] Kalogeris T, Baines CP, Krenz M, Korthuis RJ. Ischemia/Reperfusion. Comprehensive Physiology. 2016; 7: 113–170.

[19] Cao J, Qin G, Shi R, Bai F, Yang G, Zhang M, *et al*. Overproduction of reactive oxygen species and activation of MAPKs are involved in apoptosis induced by PM2.5 in rat cardiac H9c2 cells. Journal of Applied Toxicology. 2016; 36: 609–617.

[20] He L, He T, Farrar S, Ji L, Liu T, Ma X. Antioxidants Maintain Cellular Redox Homeostasis by Elimination of Reactive Oxygen Species. Cellular Physiology and Biochemistry. 2017; 44: 532–553.

[21] Ersahin T, Tuncbag N, Cetin-Atalay R. The PI3K/AKT/mTOR interactive pathway. Molecular BioSystems. 2015; 11: 1946–1954.

[22] Deng RM, Zhou J. The role of PI3K/AKT signaling pathway in myocardial ischemia-reperfusion injury. International Immunopharmacology. 2023; 123: 110714.