Role and mechanism of the lncRNA SNHG1/miR-450b-5p/IGF1 axis in the regulation of myocardial ischemia reperfusion injury

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Abstract. The increasing rates of morbidity and mortality caused by ischemic heart disease pose a serious threat to human health. Long non-coding (lnc)RNA small nucleolar RNA host gene 1 (SNHG1) has a protective effect on the myocardium. In the present study, the role of lncRNA SNHG1 in myocardial ischemia reperfusion injury (MIRI) and the underlying mechanisms were investigated. After hypoxia/reoxygenation (H/R) induction, the expression levels of lncRNA SNHG1 were detected using reverse transcription-quantitative PCR. After lncRNA SNHG1 overexpression via cell transfection, cell viability was detected using an MTT assay, apoptotic rates were detected using TUNEL staining, apoptosis-related protein expression levels were detected using western blotting and respective kits were used to measure the oxidative stress levels. The Encyclopedia of RNA Interactomes database predicted the presence of binding sites between lncRNA SNHG1 and microRNA (miR)-450b-5p, and between miR-450b-5p and insulin-like growth factor 1 (IGF1). These interactions were then verified using luciferase reporter assays. Subsequently, the regulatory mechanism underlying the lncRNA SNHG1/miR-450b-5p/IGF1 axis in MIRI was investigated by overexpressing miR-450b-5p and knocking down IGF1 expression in H/R-induced cells. Finally, the expression of PI3K/Akt signaling pathway-related proteins was detected using western blotting. lncRNA SNHG1 expression was significantly downregulated in H/R-induced AC16 cells. lncRNA SNHG1 overexpression significantly inhibited apoptosis and decreased oxidative stress levels in H/R-induced AC16 cells, which was mediated via regulation of the miR-450b-5p/IGF1 axis and activation of the PI3K/Akt signaling pathway. Therefore, the present study suggested that activation of the PI3K/Akt signaling pathway via the lncRNA SNHG1/miR-450b-5p/IGF1 axis inhibited the apoptosis and oxidative stress levels of H/R-induced AC16 cells.

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Introduction

The increasing rates of morbidity and mortality in patients with ischemic heart disease (IHD) pose a serious threat to human health (1,2). The timely and effective restoration of coronary blood flow is an effective strategy for the treatment of IHD. However, the process of restoration of blood flow inevitably induces an additional type of damage, namely myocardial ischemia-reperfusion injury (MIRI) (3). Thus, the treatment of IHD can result in sequelae, and it is of significance to further study the relevant mechanisms underlying MIRI to identify effective targets and strategies for alleviating or preventing MIRI.

Long non-coding RNAs (lncRNAs) are non-coding RNAs that are >200 nucleotides in length (4). Recent studies have shown that lncRNA small nucleolar RNA host gene 1 (SNHG1) is involved in the occurrence and development of a variety of diseases (5,6). lncRNA SNHG1 can enhance cell proliferation, migration and invasion in cervical cancer (7). lncRNA SNHG1 also suppresses gastric cancer cell proliferation and promotes apoptosis via the Notch1 signaling pathway (8). Moreover, lncRNA SNHG1 displays a protective effect on the myocardium. For example, lncRNA SNHG1 can serve a protective role in cardiomyocyte hypertrophy by targeting the microRNA (miRNA/miR)-15a-5p/high mobility group AT-hook 1 axis (6). lncRNA SNHG1 also protects AC16 cells against adriamycin-induced toxicity by regulating the miR-195/Bcl-2 axis (9). Moreover, Liang *et al* (10) demonstrated that upregulated lncRNA SNHG1 expression promoted the proliferation, migration and angiogenesis of vascular endothelial cells, and reduced the injury to these cells after hypoxia/reoxygenation (H/R) induction. However, to the best of our knowledge, no relevant studies on lncRNA SNHG1 in MIRI have been reported.

lncRNAs can function as molecular sponges to adsorb miRNAs, competitively binding with miRNAs internally, thus exerting their biological effects via downregulated inhibition of the target genes of these miRNAs, effectively

increasing the activity of the target gene, which is termed the lncRNA/miRNA/mRNA pathway (11). A previous study has shown that inhibition of miR-450b-5p significantly alleviates H/R injury and improves liver function in mice by targeting crystallin αB (CRYAB) (12). However, whether miR-450b-5p serves a role in MIRI is not completely understood. IGF1 serves an important role in MIRI. For example, it has been shown that IGF1 serves a protective role in MIRI model rats by activating the PI3K/Akt signaling pathway (13). Overexpression of suppressor of cytokine signaling 2 inhibited IGF1 expression via the Janus kinase 1/STAT signaling pathway, thereby exacerbating MIRI in type 2 diabetes (14). Moreover, lncRNA SNHG1 was involved in sorafenib resistance by activating the Akt signaling pathway (15). lncRNA SNHG1 can inhibit neuronal apoptosis in rats with cerebral infarction via the PI3K/Akt signaling pathway (16). However, to the best of our knowledge, the specific role of the lncRNA SNHG1/miR-450b-5p/IGF1 axis in H/R-induced MIRI has not been previously reported.

In the present study, the effects of the lncRNA SNHG1/miR-450b-5p/IGF1 axis on H/R-induced myocardial apoptosis and oxidative stress levels were investigated and the underlying mechanisms were also assessed. The results of the present study may provide a theoretical basis for future clinical studies and highlight potential targets for the treatment of MIRI.

Materials and methods

Cell culture and establishment of the H/R model. AC16 cells (American Type Culture Collection) were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) in a humidified incubator with 5% CO_2 at 37˚C. For establishment of the H/R model, the cell culture atmosphere was N_2 (94%), O_2 (1%) and CO_2 (5%) to simulate hypoxia for 6 h, followed by 12-h reoxygenation under normal atmospheric culture conditions as previously described (17).

Database. ENCORI database (http://starbase.sysu.edu.cn/index.php) was used to predict the interactions among lncRNA SNHG1, miR-450b-5p and IGF1 (Figs. S1 and S2).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RNA was reverse transcribed into cDNA using a TIANScript II RT Kit (Tiangen Biotech Co., Ltd.) according to the manufacturer's protocol. Subsequently, qPCR was performed using a RealMastcrMix (SYBR-Green) kit (Tiangen Biotech Co., Ltd.). The following thermocycling conditions were used for qPCR: 95˚C for 2 min; 40 cycles at 95˚C for 20 sec, 60˚C for 15 sec and 72˚C for 30 sec. The sequences of the primers (obtained from Sangon Biotech) used for qPCR were as follows: lncRNA SNHG1 forward, 5'-AGGCTGAAGTTACAGGT-3' and reverse, 5'-TTGGCTCCCAGTGTCTT-3'; miR-450b-5p forward, 5'-GCTTTTGCAATATGTTCCG-3' and reverse, 5'-CAGTGCGTGTCGTGGAGT-3'; IGF1 forward, 5'-CCCAGAAGGAAGTACATTTG-3' and reverse, 5'-GTTTAACAGGTAACTCGTGC-3'; U6 forward, 5'-AGAGAAGATTAGCATGGCCCCTG-3' and reverse, 5'-ATCCAGTGCAGGGTCCGAGG-3'; and GAPDH forward, 5'-ACCACAGTCCATGCCATCAC-3' and reverse, 5'-GTGAGGGAGATGCTCAGTGT-3'. miRNA and mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (18) and normalized to the internal reference genes U6 and GAPDH, respectively.

Cell transfection. A SNHG1 overexpression plasmid (Oe-SNHG1; pBluescript vector) and the empty vector [Oe-negative control (NC)], miR-450b-5p mimic (forward, 5'-UUUUGCAGUAUGUUCCUGAAUA-3' and reverse 5'-UUCAGGAACAUACUGCAAAAUU-3'; lentiviral vector pLVTHM), mimic NC (forward, 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse, 5'-ACGUGACACGUUCGGAGAATT-3'; lentiviral vector pLVTHM), IGF1 short hairpin (sh)RNA lentiviral particles (shRNA-IGF1; shRNA-IGF1#1, 5'-GAAGAATTGTGAAAGTTTA-3' and shRNA-IGF2#1, 5'-GCTAGAGTGTCATAATAAA-3') and non-targeting NC shRNA lentiviral particles (shRNA-NC; 5'-TTCTCCGAACGTGTCACGT-3') were all purchased from Genecopoeia, Inc. Cells ($1x10^5$ cells/well) were transfected with 20 nM overexpression plasmid, miRNA mimic, shRNA or the corresponding NC using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 48 h according to the manufacturer's protocol. At 48 h post-transfection, RT-qPCR and western blotting were used to detect transfection efficiency.

MTT. AC16 cells were seeded ($8x10^3$ cells/well) into 96-well plates. At 48 h post-transfection, the medium was removed and 10 μl MTT (Sigma-Aldrich; Merck KGaA) was added to each well for 4 h. Subsequently, DMSO was added. The optical density was measured at a wavelength of 570 nm using a microplate analyzer.

TUNEL assay. Apoptosis was measured using a TUNEL staining kit (Beyotime Institute of Biotechnology). Transfected cells were fixed using 4% paraformaldehyde for 1 h at 4˚C and then 0.1% Triton X-100 was added at room temperature for 5 min. Subsequently, cells were washed with PBS and incubated with TUNEL reagent for 1 h at 37˚C. Subsequently, 50 μl DAB color development was performed for 10 min at 15˚C according to the manufacturer's instructions. The cells were stained with DAPI for 10 min at room temperature in the dark. Stained cells were observed under a glass coverslip with PBS using a fluorescent microscope (magnification, x200; Carl Zeiss AG). ImageJ software (version 1.8.0; National Institutes of Health) was used to count the total cells and the $TUNEL^+$ cells. The number of apoptotic cells was calculated to be the average number of positive cells out of the total number of cells in six fields of view per slide.

Western blotting. Total protein was isolated from cells using RIPA lysis buffer (Thermo Fisher Scientific, Inc.) and then quantified using a BCA kit (Bio-Rad Laboratories, Inc.). Proteins (30 μg) were separated via 12% SDS-PAGE and transferred to PVDF membranes (Bio-Rad Laboratories, Inc.). After blocking with 5% skimmed milk at room temperature for 1.5 h, the membranes were incubated at 4˚C overnight with primary antibodies targeted against: Bcl-2 (1:1,000; cat. no. ab32124; Abcam), Bax (1:1,000; cat. no. ab182733; Abcam), cytochrome *c* (1:1,000; cat. no. ab133504; Abcam), cleaved caspase 3 (1:1,000; cat. no. ab32042; Abcam), IGF1 (1:1,000; cat. no. ab134140; Abcam), phosphorylated (p)-PI3K (1:1,000; cat. no. ab278545; Abcam), p-Akt (1:1,000; cat. no. ab8805; Abcam), PI3K (1:1,000;

cat. no. ab191606; Abcam), Akt (1:1,000; cat. no. ab8805; Abcam) and GAPDH (1:1,000; cat. no. ab8245; Abcam). Subsequently, the membranes were probed with horseradish peroxidase-conjugated secondary antibodies (1:5,000; cat. no. ab150113 or ab9482; Abcam) for 2 h at room temperature. Signals were visualized using Super Signal ECL (Thermo Fisher Scientific, Inc.) and semi-quantified using ImageJ software (version 1.46; National Institutes of Health).

Detection of malondialdehyde (MDA), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px). The level of SOD was detected using the SOD Assay Kit (cat. no. A001-3-2; Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocol. MDA concentrations were determined using an ELISA kit (cat. no. A003-1-2; Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's protocol. All above were used in accordance with the manufacturer's protocol. GSH-Px enzyme activity was measured using a GSH-Px Assay Kit (cat. no. A005-1-2, Nanjing Jiancheng Bioengineering Institute) according to a manufacturer's protocol.

Luciferase reporter assay. The amplified PCR products of the 3' untranslated regions of SNHG1 and IGF1 were ligated into the miRNA target expression vector pmirGLO-dual-luciferase (Promega Corporation). A QuickChange II XL site-directed mutagenesis kit (Agilent Technologies, Inc.) was used to generate the SNHG1 (SNHG1 MUT) and IGF (IGF1 MUT) mutants. Cells were co-transfected with miR-450b-5p mimic or miR-NC and SNHG1 MUT, SNHG1 wild-type (WT), IGF1 WT or IGF1 MUT using Lipofectamine 2000 reagent, all at a final concentration of 100 nmol/l. At 48 h post-transfection, the luciferase activities were assayed using a dual-luciferase reporter gene assay system (Promega Corporation) according to the manufacturer's protocol. *Renilla* luciferase activity was used for normalization and results were recorded using a GloMax 96 Microplate Luminometer (Promega Corporation).

Statistical analysis. Data are presented as the mean ± standard deviation. Comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. All statistical analyses were performed using SPSS software (version 22.0; IBM Corp.). $P<0.05$ was considered to indicate a statistically significant difference. Each experiment was repeated at least three times.

Results

lncRNA SNHG1 overexpression inhibits H/R-induced apoptosis and oxidative stress in AC16 cells. After AC16 cells were induced by H/R, the expression levels of lncRNA SNHG1 were detected using RT-qPCR. The results showed that lncRNA SNHG1 expression in the H/R group was significantly decreased compared with that in the control group (Fig. 1A). Subsequently, lncRNA SNHG1 was overexpressed in cells (Fig. 1B). The MTT assay showed that cell viability was significantly decreased following H/R induction compared with that in the control group. SNHG1 overexpression significantly increased cell viability compared with that in the H/R + Oe-NC group (Fig. 1C). Cell apoptosis was detected using a TUNEL assay and western blotting. Compared with the control group, apoptosis was significantly increased following H/R induction, which was accompanied by significantly increased expression levels of Bax, cytochrome *c* and cleaved caspase 3 and significantly decreased expression levels of Bcl-2. Compared with the H/R + Oe-NC group, apoptosis was significantly decreased in the H/R + Oe-SNHG1 group, which was accompanied by significantly decreased expression levels of Bax, cytochrome *c* and cleaved caspase 3 and significantly increased expression levels of Bcl-2 (Fig. 1D and E). The levels of MDA, SOD and GSH-Px were detected using ELISAs. The results demonstrated that the level of MDA was significantly increased, whereas the levels of SOD and GSH-Px were significantly decreased in the H/R group compared with those in the control group. However, SNHG1 overexpression significantly inhibited the effects of H/R induction on the levels of oxidative stress-related indicators (Fig. 2A-C). The aforementioned results indicated that lncRNA SNHG1 overexpression inhibited H/R-induced apoptosis and oxidative stress levels in AC16 cells.

lncRNA SNHG1 overexpression increases the expression of IGF1 by sponging miR-450b-5p. The ENCORI database predicted the presence of binding sites between lncRNA SNHG1 and miR-450b-5p, as well as between miR-450b-5p and IGF1 (Fig. 3A and B). In addition, compared with that in the control group, the expression of miR-450b-5p was significantly increased in H/R-induced cells, whereas the expression of IGF1 was significantly decreased (Fig. 3C and D). Cell transfection was used to overexpress miR-450b-5p, which was confirmed via RT-qPCR (Fig. 3E). Subsequently, the luciferase reporter gene assay demonstrated that in SNHG1 WT, the luciferase activity significantly decreased when transfected with the miR-450b-5p mimic compared with the mimic NC group. However, no significant change in luciferase activity was exhibited in the SNHG1 MUT group. In the IGF1 WT group, luciferase activity was significantly decreased following transfection with the miR-450b-5p mimic compared with the mimic NC group. However, no significant change in luciferase activity was exhibited following miR-450b-5p mimic transfection in the IGF1 MUT group. These results verified the targeted binding between lncRNA SNHG1 and miR-450b-5p and between miR-450b-5p and IGF1 (Fig. 3F). In addition, following lncRNA SNHG1 overexpression, the expression of miR-450b-5p in H/R-induced cells was significantly decreased, whereas the expression of IGF1 was significantly increased. The expression of IGF1 in the H/R + miR-450b-5p mimic group was significantly increased compared with that in the H/R + mimic NC group (Fig. 3G-I). These results indicated that lncRNA SNHG1 overexpression upregulated IGF1 expression by sponging miR-450b-5p.

lncRNA SNHG1/miR-450b-5p/IGF1 axis regulates PI3K/Akt signaling and affects apoptosis and oxidative stress levels of H/R-induced AC16 cells. IGF1 expression was knocked down using shRNAs and successful knockdown was confirmed via RT-qPCR (Fig. 4A). shRNA-IGF1#2 was selected for subsequent experiments as it displayed the optimum knockdown efficiency. Cells were divided into the following groups: i) Control; ii) H/R; iii) H/R + Oe-SNHG1; iv) H/R + Oe-SNHG1 + mimic NC; v) H/R + Oe-SNHG1 + miR-450b-5p mimic; vi) H/R + Oe-SNHG1 + shRNA-NC; and vii) H/R + Oe-SNHG1 + shRNA-IGF1. The MTT and TUNEL assay results demonstrated that cell viability was significantly decreased and apoptosis was significantly

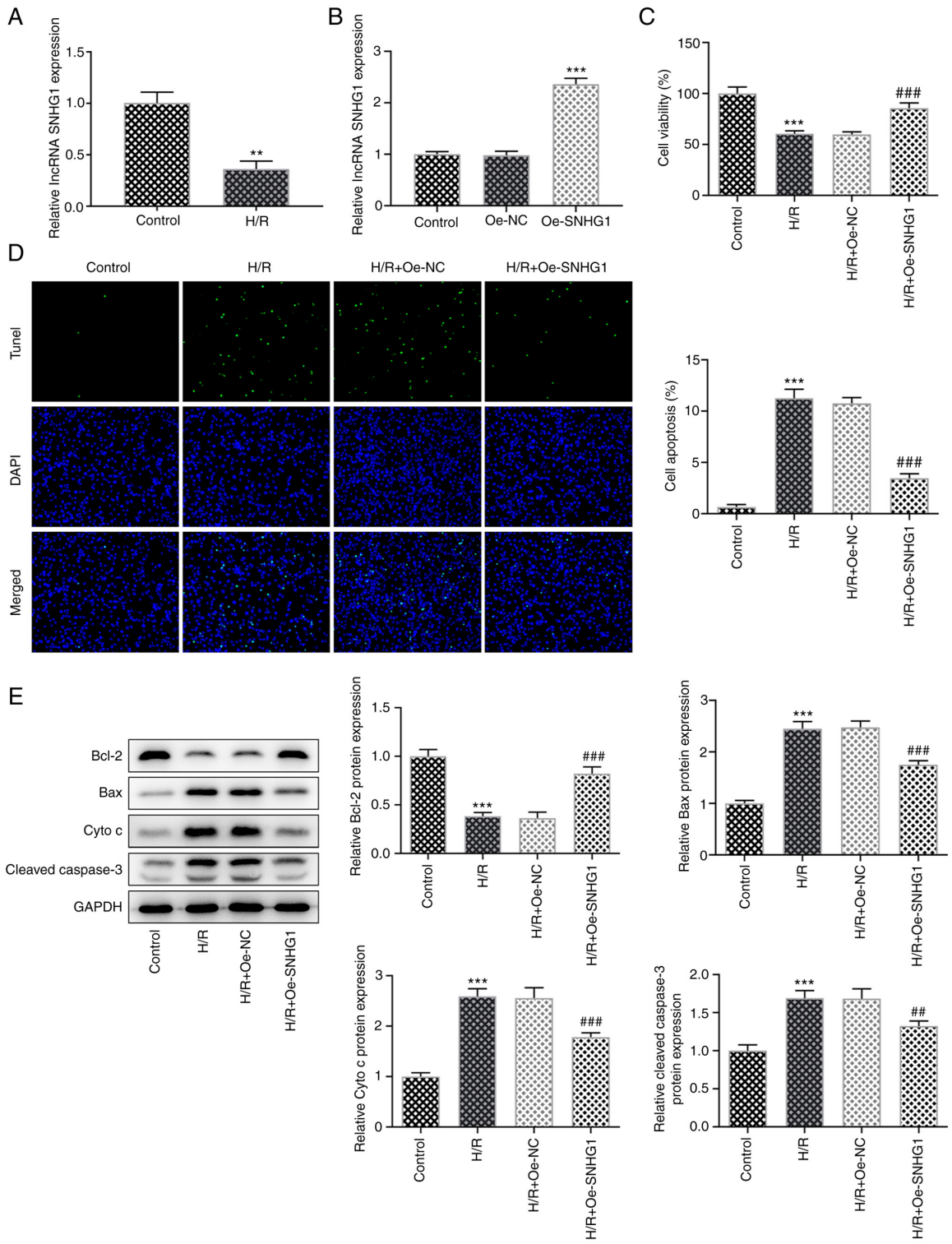

Figure 1. lncRNA SNHG1 overexpression inhibits H/R-induced apoptosis in AC16 cells. Reverse transcription-quantitative PCR was performed to detect the expression levels of lncRNA SNHG1 in (A) AC16 cells (**P<0.01 vs. control) and (B) transfected AC16 cells (***P<0.001 vs. Oe-NC). (C) MTT assays were performed to detect cell viability. (D) TUNEL assays were performed to detect cell apoptosis. (E) Western blotting was performed to measure the expression levels of apoptosis-related proteins. ***P<0.001 vs. control; $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. H/R + Oe-NC. lncRNA, long non-coding RNA; SNHG1, small nucleolar RNA host gene 1; H/R, hypoxia/reoxygenation; Oe, overexpression; NC, negative control; Cyto c, cytochrome *c*.

increased in the H/R + Oe-SNHG1 + shRNA-IGF1 group compared with that in the H/R + Oe-SNHG1 + mimic NC group. This was accompanied by a significant increase in the expression levels of the apoptosis-related proteins Bax, cytochrome *c* and cleaved caspase 3, whereas Bcl-2 expression was significantly decreased. Compared with that in the H/R + Oe-SNHG1 + shRNA-NC group, cell viability was significantly decreased and apoptosis was significantly increased in the H/R + Oe-SNHG1 + shRNA-IGF1 group, which was accompanied by increased Bax, cytochrome *c* and cleaved caspase 3 expression levels and decreased Bcl-2 expression levels (Fig. 4A-D). These results indicated that miR-450b-5p overexpression and IGF1 knockdown

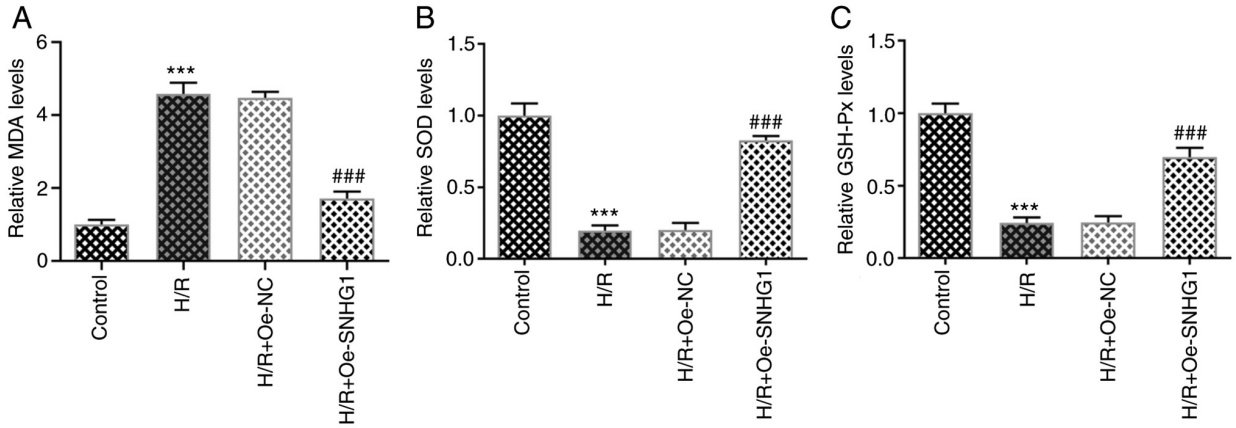

Figure 2. Long non-coding RNA SNHG1 overexpression inhibits H/R-induced oxidative stress in AC16 cells. (A) MDA, (B) SOD and (C) GSH-Px were detected using commercial kits. ***P<0.001 vs. H/R; ###P<0.001 vs. H/R + Oe-NC. SNHG1, small nucleolar RNA host gene 1; H/R, hypoxia/reoxygenation; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; Oe, overexpression; NC, negative control.

could reverse the effects of SNHG1 overexpression on cell viability and apoptosis. Subsequently, the levels of oxidative stress-related indicators were detected. Compared with those in the H/R + Oe-SNHG1 + mimic NC group, SOD and GSH-Px levels were significantly decreased and MDA levels were significantly increased in the H/R + Oe-SNHG1 + miR-450b-5p mimic group. Compared with those in the H/R + Oe-SNHG1 + shRNA-NC group, the levels of SOD and GSH-Px were significantly decreased and the levels of MDA were significantly increased in the H/R + Oe-SNHG1 + shRNA-IGF1 group (Fig. 5A-C).

Additionally, the expression levels of PI3K/Akt signaling pathway-related proteins were dysregulated. Compared with that in the control group, the expression of p-PI3K and p-AKT was significantly decreased following H/R induction, whereas SNHG1 overexpression resulted in significant upregulation of p-PI3K and p-Akt expression levels. miR-450b-5p overexpression or IGF1 knockdown significantly inhibited the effects of SNHG1 overexpression on the PI3K/Akt signaling pathway (Fig. 6).

Discussion

In the present study, the role of the lncRNA SNHG1/miR-450b-5p/IGF1 axis in H/R injury was assessed. Oxidative stress serves a significant disruptive role in the state of ischemia and reperfusion, which lead to cell apoptosis and ultimately result in MIRI (19). In the present study, the results demonstrated that the lncRNA SNHG1/miR-450b-5p/IGF1 axis regulated the PI3K/Akt signaling pathway to affect H/R-induced apoptosis and oxidative stress levels in AC16 cells, thus suggesting a regulatory role in MIRI.

lncRNA SNHG1 downregulation reduced apoptosis, oxidative stress and inflammation in a model of Parkinson's disease via inhibition of the miR-125b-5p/MAPK1 axis (20). In H/R-induced endothelial cells, the expression of lncRNA SNHG1 was significantly decreased, and as an endogenous competitive RNA, lncRNA SNHG1 can alleviate H/R-induced injury of vascular endothelial cells via the hypoxia-inducible factor-1α/VEGF signaling pathway (10). In the present study, lncRNA SNHG1 expression levels were significantly decreased in H/R-induced AC16 cells. In addition, lncRNA SNHG1 has been shown to serve a protective effect on the myocardium. Zhang *et al* (21) demonstrated that hydrogen peroxide treatment could significantly inhibit the activity and promote the apoptosis of cardiomyocytes, whereas SNHG1 overexpression could significantly inhibit hydrogen peroxide-induced damage to cardiomyocytes. Another study reported that SNHG1 overexpression could significantly reduce the myocardial hypertrophy induced by deoxyadrenaline (6). The results of the present study demonstrated that lncRNA SNHG1 overexpression significantly inhibited apoptosis and decreased oxidative stress levels in H/R-induced AC16 cells, which may serve a therapeutic role in the treatment of MIRI.

In the present study, the ENCORI database predicted that lncRNA SNHG1 could bind to miR-450b-5p and miR-450b-5p could bind to IGF1. These interactions were verified using luciferase reporter assays. miR-450b-5p was reported to be a latent biomarker in transient ischemic attack and liver self-healing plasmodium malaria (22,23). Additionally, it has been shown that the expression of miR-450b-5p decreased in mice with hepatic ischemia/reperfusion injury, and miR-450b-5p inhibition significantly alleviates hepatic ischemia/reperfusion injury and improves liver function in mice by targeting CRYAB (12). In the present study, miR-450b-5p expression levels were significantly increased in H/R-induced AC16 cells. Following myocardial ischemia reperfusion, miR-29a and lethal-7 can affect cell apoptosis by regulating IGF1 (24). miR-320 downregulation inhibited myocardial apoptosis and protected against myocardial ischemia-reperfusion injury by targeting IGF1 (25). Degradation of IGF1 by mouse mast cell protease 4 promoted cell death and adverse cardiac remodeling after myocardial infarction (26). In the present study, IGF1 expression levels were significantly decreased in H/R-induced AC16 cells. lncRNA SNHG1 overexpression significantly downregulated the expression of miR-450b-5p, resulting in upregulated IGF1 expression to inhibit apoptosis and decrease the levels of oxidative stress in H/R-induced AC16 cells.

PI3K/Akt is a classical signaling pathway that serves an important role in MIRI (27,28). In the present study, the expression levels of p-PI3K and p-Akt were downregulated

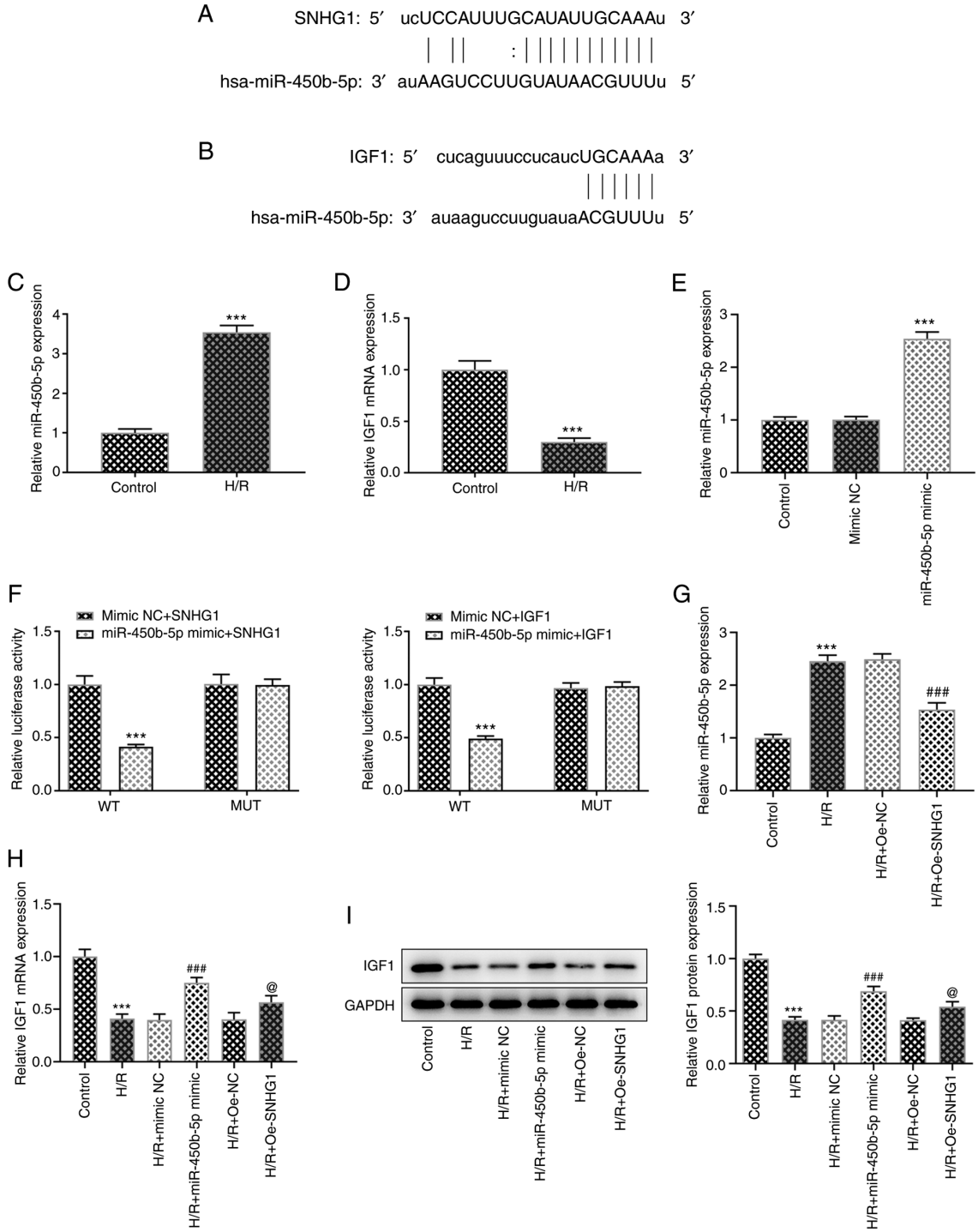

Figure 3. lncRNA SNHG1 overexpression upregulates IGF1 expression by sponge adsorption of miR-450b-5p. Binding sides of (A) lncRNA SNHG1 and miR-450b-5p, and (B) miR-450b-5p and IGF1. (C) RT-qPCR was performed to detect the expression levels of (C) miR-450b-5p and (D) IGF1. ***P<0.001 vs. control. (E) RT-qPCR was performed to assess the transfection efficiency of miR-450b-5p mimic. ***P<0.001 vs. mimic NC. (F) Luciferase reporter assays confirmed the binding sides between lncRNA SNHG1 and miR-450b-5p, and between miR-450b-5p and IGF1. ***P<0.001 vs. mimic NC + IGF1 or mimic NC + SNHG1. (G) RT-qPCR was performed to assess the effect of lncRNA SNHG1 overexpression on miR-450b-5p expression. ***P<0.001 vs. control; ###P<0.001 vs. H/R + Oe-NC. (H) RT-qPCR and (I) western blotting were performed to assess the effect of lncRNA SNHG1 and miR-450b-5p overexpression on IGF1 expression. ***P<0.001 vs. control; ###P<0.001 vs. H/R + mimic NC; @P<0.05 vs. H/R + Oe-NC. lncRNA, long non-coding RNA; SNHG1, small nucleolar RNA host gene 1; IGF1, insulin-like growth factor 1; miR, microRNA; RT-qPCR, reverse transcription-quantitative PCR; NC, negative control; H/R, hypoxia/reoxygenation; Oe, overexpression; WT, wild-type; MUT, mutant.

after H/R induction. In addition, IGF1 has been reported to serve a protective role on MIRI model rats by activating the PI3K/Akt signaling pathway (13). These results indicated that IGF1 regulated the PI3K/Akt signaling pathway. In the present study, the results demonstrated that the lncRNA SNHG1/miR-450b-5p/IGF1 axis regulated the PI3K/Akt signaling pathway and affected H/R-induced apoptosis and oxidative stress levels in AC16 cells. The results of the present study should be verified with the use of PI3K/Akt signaling pathway inhibitors or activators in future studies.

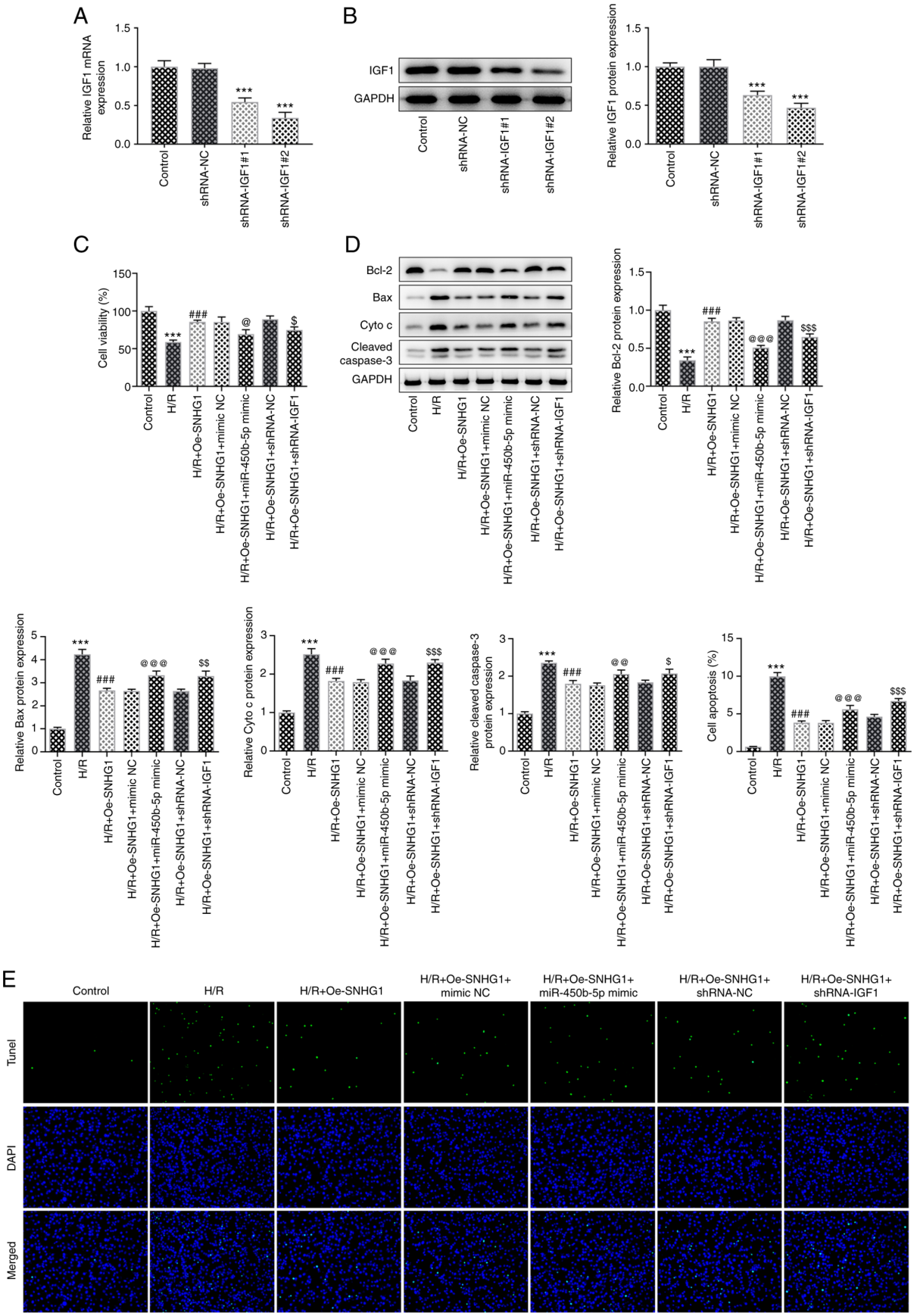

Figure 4. Long non-coding RNA SNHG1/miR-450b-5p/IGF1 axis affects the apoptosis of H/R-induced AC16 cells. (A) Reverse transcription-quantitative PCR and (B) western blotting were performed to assess the transfection efficiency of shRNA-IGF1#1 and shRNA-IGF1#2. ***P<0.001 vs. shRNA-NC. (C) MTT assays were performed to detect cell viability. (D) Western blotting was performed to detect the expression of apoptosis-related proteins. (E) TUNEL assays were performed to detect apoptosis. ***P<0.001 vs. control; $^{###}$P<0.001 vs. H/R; $^{@}$P<0.05, $^{@@}$P<0.01 and $^{@@@}$P<0.001 vs. H/R + Oe-SNHG1 + mimic NC; $^{\$}$P<0.05 and $^{\$\$\$}$P<0.001 vs. H/R + Oe-SNHG1 + shRNA-NC. SNHG1, small nucleolar RNA host gene 1; miR, microRNA; IGF1, insulin-like growth factor 1; H/R, hypoxia/reoxygenation; shRNA, short hairpin RNA; NC, negative control; Oe, overexpression; Cyto c, cytochrome *c*.

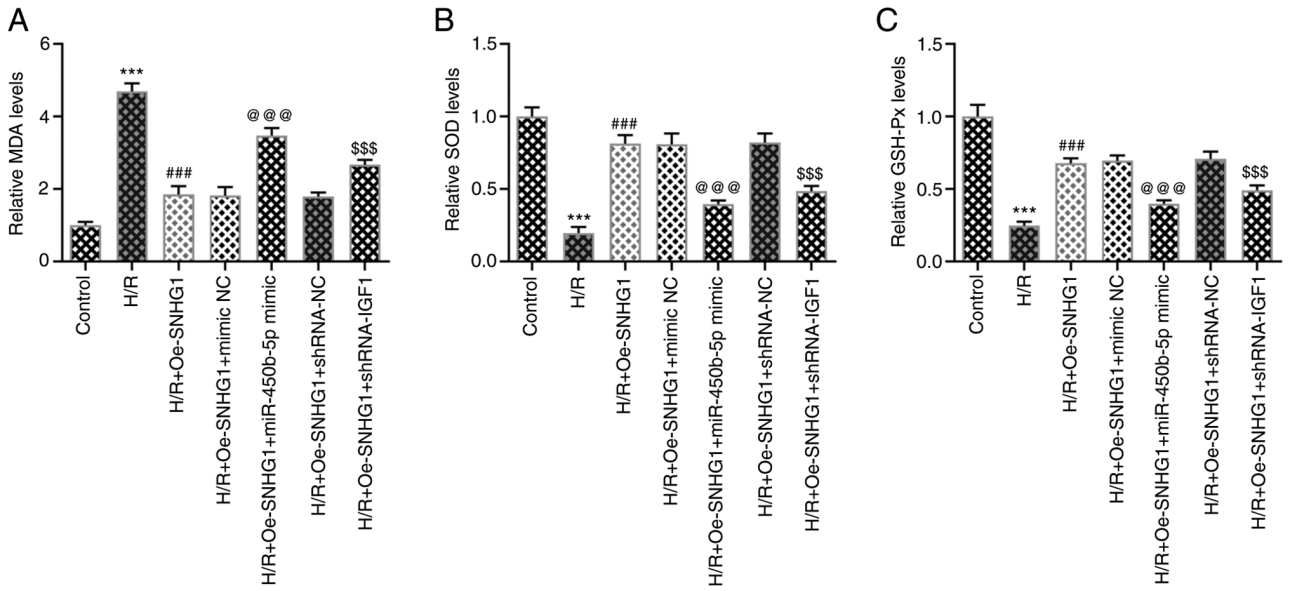

Figure 5. Long non-coding RNA SNHG1/miR-450b-5p/IGF1 axis affects oxidative stress levels of H/R-induced AC16 cells. (A) MDA, (B) SOD and (C) GSH-Px were detected using commercial kits. ***P<0.001 vs. control; ###P<0.001 vs. H/R; @@@P<0.001 vs. H/R + Oe-SNHG1 + mimic NC; $$$P<0.001 vs. H/R + Oe-SNHG1 + shRNA-NC. SNHG1, small nucleolar RNA host gene 1; miR, microRNA; IGF1, insulin-like growth factor 1; H/R, hypoxia/reoxygenation; MDA, malondialdehyde; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; Oe, overexpression; NC, negative control; shRNA, short hairpin RNA.

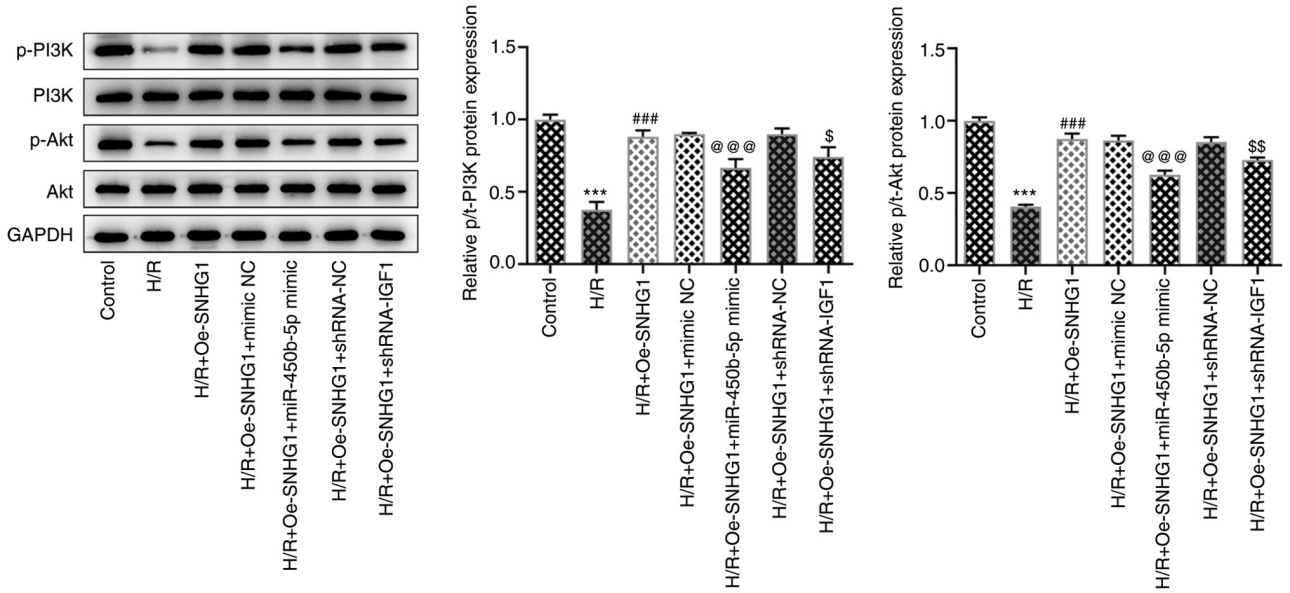

Figure 6. Long non-coding RNA SNHG1/miR-450b-5p/IGF1 axis regulates the PI3K/Akt signaling pathway, and affects apoptosis and oxidative stress levels in H/R-induced AC16 cells. Western blotting was performed to detect the expression of PI3K/Akt pathway-related proteins. ***P<0.001 vs. control; ###P<0.001 vs. H/R; @@@P<0.001 vs. H/R + Oe-SNHG1 + mimic NC; $P<0.05 and $$P<0.01 vs. H/R + Oe-SNHG1 + shRNA-NC. SNHG1, small nucleolar RNA host gene 1; miR, microRNA; IGF1, insulin-like growth factor 1; H/R, hypoxia/reoxygenation; Oe, overexpression; NC, negative control; shRNA, short hairpin RNA; p, phosphorylated.

The present study had a number of limitations. Firstly, the study only used one cell line (AC16), which is not reliable enough to explain the mechanism. Future experiments should investigate the results of the present study in additional cell lines. Moreover, the results were not verified by performing animal experiments, thus further investigations are required to verify the conclusions of the present study.

In conclusion, the present study demonstrated that the activation of the PI3K/Akt signaling pathway via the lncRNA SNHG1/miR-450b-5p/IGF1 axis inhibited the apoptosis and oxidative stress levels of H/R-induced AC16 cells. These results provided a theoretical basis for the mechanistic study of MIRI.

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Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LH and JZ contributed to the conception and design of the present study, analyzed and interpreted the data, and critically revised the manuscript for important intellectual content. QY and PZ contributed to designing the study, analyzed the data, and drafted and revised the manuscript. All authors read and approved the final manuscript. LH and JZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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