Overexpression of Long noncoding RNA Oprm1 Attenuates Myocardial Ischemia/Reperfusion Injury by Increasing Endogenous Hydrogen Sulfide Production via Oprm1/miR-30b-5p/CSE axis

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Research

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Abstract

Background: Ischemia/Reperfusion (I/R) Injury largely limits the efficacy of revascularization in acute myocardial infarction. Long noncoding RNA (lncRNA) Oprm1 is protective in cerebral I/R injury. However, the effect of lncRNA Oprm1 on myocardial I/R injury and its mechanism remains unknown.

Methods: We ligated and then released the left anterior descending coronary artery of adult male rats to build the I/R model in vivo, while an H9c2 cardiomyocytes hypoxia-reoxygenation (H/R) model was also used. Myocardial infarction area, cardiac function, histology, Tunnel staining, cell viability, and vital protein expression was conducted and compared.

Results: lncRNA Oprm1 was significantly down-regulated in the I/R injury model. When administered with the AAV9-Oprm1 vector, the myocardial injury and cardiac function were mitigated and preserved, with apoptosis reduced. The cystathionine-γ-lyase (CSE) expression and hydrogen sulfide (H_2S) expression were increased. The dual-luciferase reporter gene revealed the targeted relationship between lncRNA Oprm1 and miR-30b-5p. In H9c2 cardiomyocytes models, the miR-30b-5p blocked the protective effect of lncRNA Oprm1 on H/R injury, when Bcl-2, Bcl-xl was down-regulated, and HIF-1α, Bnip-3, Caspase-3, and Caspase-9 up-regulated.

Conclusions: lncRNA Oprm1can competitively combines with miR-30b-5p, which down-regulates the expression of CSE. When administered with lncRNA Oprm1, increased endogenous H_2S can reduce apoptosis and protect the myocardium from I/R injury via activating PI3K/Akt pathway and inhibiting HIF1-α activity.

1. Background

Recent years have witnessed the substantial improvement of the prognosis of patients with acute myocardial infarction (AMI), the most threatening disease worldwide, due to the promotion of revascularization therapy, including thrombolytic therapy, percutaneous coronary intervention, and coronary artery bypass graft (CABG) surgery[1,2]. However, the revascularization efficacy is substantially limited by following complications involving malignant arrhythmia, increasing infarct area, and persistent systolic dysfunction[3], which are associated with myocardial ischemia/reperfusion injury (MIRI)[4]. MIRI is the primary factor affecting the prognosis of AMI[5]. Hence, mitigation and inhibition of MIRI is the key to protecting the myocardium, which necessitates novel therapeutic targets and medicine.

Long non-coding RNA (LncRNA) refers to RNA without coding function and size over 200 nt[6]. LncRNAs are now emerging as an essential target involving various diseases, including cancer and cardiovascular diseases[7]. LncRNA Oprm1 is demonstrated to be abundant in heart tissue, and overexpression of Oprm1 significantly reduces cerebral ischemia/reperfusion injury[8]. However, the effect of lncRNA Oprm1 on MIRI and its underlying mechanism remains elusive.

Recently, hydrogen sulfide (H_2S) has been recognized as another endogenous critical gas signaling molecule, except for nitric oxide (NO) and carbon monoxide (CO)[9]. As a signaling molecule, H_2S participates in multiple physiological and pathological reactions, including cancer[10], burns[11], inflammation[12], as well as cardiovascular diseases[13]. Endogenous H_2S is produced by the cystathionine-γ-lyase (CSE), which can be inhibited by microRNA(miR)-30 family[14]. Apoptosis, as a programmed cell death process, has been demonstrated to be initiated and enhanced throughout the MIRI, directly affecting the myocardial vitality[15]. Previous studies have shown that the reactive oxygen species (ROS) was produced excessively and overcome the protection of the antioxidant enzyme system, resulting in mitochondrial dysfunction and apoptosis[15].

In this study, we tried to use the animal model in vivo and cell model in vitro to explain the protecting role and mechanism of lncRNA Oprm1 in MIRI.

2. Materials And Methods

2.1 Animals

Male SPF grade Sprague-Dwaly (SD) rats (weighing 250-300g) were purchased from the Department of Animal Resources of Tianjin Medical University. The experimental animals were kept under standard room conditions with a temperature of 25±1°C, the humidity of 60%, and natural light from 6 a.m. to 6 p.m. Standard rodent chow and water were given routinely. The Committee on Ethics of Biomedicine of Tianjin Third Central Hospital approved this study, which also conformed to the revised Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH) Publication Nos. 85-23 (1996).

A total of 60 rats were randomly divided into three groups, including the Sham group, the I/R group, and the I/R+Oprm1 group. The Sham group and the I/R group were administered with the intragastrical injection of saline, while the I/R+Oprm1 group was given a 30μl injection of recombinant AAV9-Oprm1 around the infarction region. The rats of the I/R group and the I/R+Oprm1 group was given the I/R surgical protocol, while the Sham group was given the same procedure except for the ligation.

2.2 Myocardial Ischemia/Reperfusion Model

Surgical protocols were conducted as previously described[16]. After opening the chest and showing the heart via an incision in the fourth intercostal space under anesthesia, the left anterior descending coronary artery was temporarily ligated for 30 minutes with a 6–0 suture. Then the ligation was released, and the heart was checked for blood flow. Sham operation was conducted with similar without the ligature.

2.3 Infarct size assessment

As previously described, the infarct size of the heart was assessed with the TTC-staining technique and digital measurement with Image-Pro Plus software (Media Cybernetics)[17].

2.4 Echocardiography

As previously described, echocardiography was performed blindly four days after the surgery (VisualSonics Vevo 2100, M-mode, and 30MHz probe). The rats were under consciousness and examined at the mid-papillary level. LV dimensions in diastole and systole, especially the ejection fraction (EF) was measured with standard procedures and calculations[18].

2.5 Microassay analysis

We applied Mouse LncRNA Expression Array V3.0 chip (Aksomics, Shanghai, China) to examine differentially expressed lncRNA after MIRI. Total lncRNAs were extracted for chip hybridization. Fluorescence intensity values represented the lncRNA expression levels. The differential expression criteria were determined as fold change >2, $P < 0.05$.

2.6 Histology and TUNEL staining

Hematoxylin and eosin (HE) and TUNEL staining were conducted as previously described[19]. Image-Pro Plus software (Media Cybernetics) was used to detect the apoptosis rates of TUNEL sections.

2.7 CSE activity and H_2S concentration

As previously described, CSE activity was measured with an ELISA kit (ml037623, Shanghai Enzyme-linked Biotechnology Co., Ltd.). H_2S concentration was measured with the endogenous hydrogen sulfide test kit (Nanjing Xinfan Biotechnology Co., Ltd.)[14].

2.8 Luciferase reporter assay

As previously described[20], 293T cells were seeded in a 24-well plate. After 24 hours, miR-30b-5p mimics or miR-NC was cotransfected with Oprm1-wt (wild type) or Oprm1-mut (mutant) into 293T cells using Lipofectamine 3000 (Invitrogen), dual-luciferase reporter assay system (Promega) were applied at 48 hours after transfection according to the manufacturer's instructions.

2.9 Cell culture and H/R protocol

H9c2 cardiomyocytes were purchased from Shanghai Gefen Biotechnology. The cell was cultured in DMEM supplemented with 10% FBS with standard protocols. The H/R procedure was conducted as follows: the culture plates were placed in the hypoxia chamber (1% O2, 5% CO2, and 94% N2) for 24 h, following by reoxygenation (5% CO2 and 95% air) with maintenance medium for 6 h.

2.10 Gene transfection

As previously described, lncRNA Oprm1, miR-30b-5p mimics were synthesized by Shanghai GenePharma (Shanghai, China). H9c2 cardiomyocytes were transfected with Oprm1 or miR-30b-5p mimics when cell confluence reached 70%. After transfection for 48 h, the cells were exposed to the H/R procedure[8].

2.11 Cell viability

The MTT assay was used to test the cardiomyocyte viability. The absorbance of the solution was determined at 570 nm at 0, 3, 6, 9, 12, 18, and 24 h after reoxygenation.

2.12 Apoptosis detection in vitro

According to the manufacturer's instructions, flow cytometry (FACS Calibur™, BD Biosciences, CA, USA) was adopted to measure the percentage of early apoptosis of four groups after H/R procedure with the Annexin V-FITC/PI apoptosis kit (Invitrogen).

2.13 Key protein activity

According to the manufacturer's instructions (RapidBio Lab, USA), the ROS and LDH activity was measured in the cardiac homogenates and cell supernatant. According to the manufacturer's instructions (BioVision, USA), the Caspase-3, -8, and -9 Fluorometric Assay Kits were used to determine the enzyme's activity of the cardiac homogenates and cell supernatant. According to the manufacturer's instructions (Sigma-Aldrich, USA), the antioxidant enzyme of the SOD Assay kit was applied to measure activities in heart homogenates and cell supernatant.

2.14qRT-PCR

Total RNA was extracted using TRIzol (Takara). 1000 ng total RNA was reversely transcribed into cDNA using the Prime-Script TMRT reagent kit (Takara). qRT-PCR was performed using SYBR green (Takara) and normalized to GAPDH expression. LncRNA Oprm1, miR-30b-5p, CSE were examined.

2.15 Western blotting

Western blotting was conducted as previously described[21]. Primary antibodies were purchased from Abcam: CSE, ab96755; p-Akt 1/2/3, ab8805; Akt 1/2/3, ab179463; HIF-1α, ab51608; Bnip3 ab219609; Bcl-2, ab185002; Bax: ab-32503; Bcl-xl, ab32370; Caspase-3, ab197202; Caspase-9, ab52298; and β-actin, ab8226. Image lab software (BIO-RAD, USA) was used to quantify the grayscale value of the bands.

2.16 Statistical analysis

We used GraphPad Prism 7.0 to analyze the data. The continuous data were expressed as the mean ± standard deviation (SD). One-way ANOVA followed by Post-hoc t Turkey's test was adopted to compare the variables of different groups. A P-value of less than 0.05 was considered as statistical significance.

3. Results

3.1 Overexpression of lncRNA Oprm1 mitigated MIRI and preserved the cardiac function in vivo

As shown in Figure 1A, we examined the differentially expressed lncRNAs between the sham group and I/R group with LncRNA Microarray v3.0 chip analysis, which demonstrated that lncRNA Oprm1 was most down-regulated. qRT-PCR also showed that Oprm1was significantly lower in I/R group than the sham group. However, when administered with AAV9-Oprm1, the lncRNA Oprm1 in heart tissue was significantly increased (Figure 1B). We observed the general condition of heart (Figure 1E, 1F), and found that the I/R group and the I/R+Oprm1 group suffered myocardial infarction, and then we compared the myocardial infarct size of different groups of rats, which demonstrated that the infarct size of the I/R+ Oprm1 was significantly lower than that of the I/R group ($P<0.001$). Echocardiography was used to evaluate the cardiac function (Figure 1C, 1D), showing that the I/R+ Oprm1 group had significantly higher EF than the I/R group ($P<0.001$), although it was lower than the Sham group ($P<0.001$).

3.2 LncRNA Oprm1 mitigated myocardial injury and inhibited apoptosis in vivo

Compared with the Sham group, HE staining also demonstrated that the myocardium was severely injured in the I/R group, manifested in myocardial edema, intense eosinophilic change, as well as contraction band anomaly, which was significantly reduced by Oprm1 (Figure 2A). We confirmed the apoptosis of three groups with TUNEL staining, shown in Figure 2B. The results showed that apoptosis was obviously activated in the I/R group, while the Oprm1overexpression inhibited the apoptosis procedure induced by the I/R stimulation. Quantitative results showed that the apoptosis rates (TUNEL positive cell rates) of the I/R group and the I/R+ Oprm1 group were higher than that of the Sham group ($P<0.001$), while the I/R+ Oprm1 group was lower than the I/R group ($P<0.001$), shown in Figure 3C.

3.3 LncRNA Oprm1 increased CSE activity, H_2S production, and reduced oxidative-stress in vivo

We tested the CSE expression level and H_2S concentration in heart tissue, indicating that CSE expression and H_2S concentration was reduced in I/R group, while they were significantly higher in the I/R+ Oprm1 group (Figure 3D, 3E, 3F). We also examined the key biomarkers in the cardiac tissue, including the myocardial injury, the antioxidant, and the apoptosis, shown in Figure 3G-3K. The results showed that LDH, ROS, Caspase-3, Caspase-9 were significantly increased, while SOD was decreased in the I/R group (All $P<0.05$). Compared with the I/R group, pretreatment of Oprm1 reduced the production of LDH, ROS, Caspase-3, Caspase-8, Caspase-9, and increased the SOD concentration (All $P<0.05$).

3.4 LncRNA Oprm1 targeted miR-30b-5p directly

Bioinformatics predicted that lncRNA Oprm1 combined well with miR-30b-5p (Figure 4A). Luciferase reporter assay demonstrated that lncRNA Oprm1 (WT) could significantly lower the luciferase activity in miR-30b-5p, but the mutated lncRNA Oprm1 (MT) had less effect (Figure 4B). We also examined the miR-30b-5p expression levels, which showed that I/R group had significantly higher miR-30b-5p expression levels, while Oprm1 decreased the miR-30b-5p expression level (Figure 3C). These results indicated that lncRNA Oprm1 directly targeted and sponged miR-30b-5p.

3.5 LncRNA Oprm1 protected cardiomyocytes from H/R injury in vitro

To validate the results further in vitro, we subjected H9c2 cells to H/R procedure and found significant cell injury, manifested in the decrease of cell viability and apoptosis rates, shown in Figure 4A. We examined the cell viability at 0, 3, 6, 9, 12, 18, and 24 h after reoxygenation using an MTT assay. Compared with the control group, the other three groups all demonstrated a significant decrease. The pretreatment of Oprm1 rescued the cell viability reduced by H/R stimulation. However, this improvement could be stopped by miR-30b-5p mimics. Following results in vivo, CSE expression level and H_2S concentration were also increased by Oprm1 administration. However, the miR-30b-5p mimics also reversed the increments (Figure 4C, 4D).

Also, we found that the LDH of the H/R+ Oprm1 group was lower than that of the H/R group, and the H/R+ Oprm1+ miR-30b-5p mimics group (both $P<0.001$), shown in Figure 4E. Similar to the viability results, the apoptosis rate of the H/R group was significantly higher than the control group ($P<0.001$), while H/R+ Oprm1 group was lower than the H/R group ($P<0.001$). The apoptosis biomarker also demonstrated similar trends as the apoptosis rates, including the Caspase-3, Caspase-9 (Figure 4F, 4G). We also explored the ROS concentrations in cell supernatants, which showed that the ROS activity was significantly higher for H/R group ($P<0.001$), while it was lower in the H/R+ Oprm1 group ($P<0.001$). However, the protecting effect of reduced ROS was hindered by miR-30b-5p mimics ($P<0.001$).

3.6 PI3K/Akt and HIF-1α pathway in vitro

We explored the classic pathway of PI3K/Akt and HIF-1α in the H/R cell model as well as the impact of Oprm1 and miR-30b-5p mimics, shown in Figure 5. We found that the PI3K/Akt was muted in H/R model, while the HIF-1α and Bnip3 were up-regulated, resulting in the activation of apoptosis pathways. The transfection of Oprm1 significantly up-regulated the CSE expression and endogenous H_2S production, which greatly enhanced phosphorylation of Akt and inhibited the expression of HIF-1α and downstream signaling molecules. This effect could be reversed by miR-30b-5p mimics.

4. Discussion

MIRI is now considered a major cause leading to the worse prognosis of AMI patients, despite early revascularization therapies[22]. Numerous studies focusing on MIRI demonstrated that the endogenous ROS caused by I/R and apoptosis could play a vital role in the process of cardiac injury[23,24]. In the present study, we used the rats and H9c2 cells to build the I/R model and found that transfection with lncRNA Oprm1 could mitigate MIRI and reduces apoptosis. We also explored the potential mechanism and found that miR-30b-5p could be sponged by lncRNA Oprm1 and inhibited CSE activity, as well as endogenous H_2S production, which could reduce ROS via PI3K/Akt and HIF-1α pathway.

Despite its non-coding characteristic, lncRNA has shown various biological activities, and the most important one is to combine with microRNA, also known as sponge function, and competing for endogenous RNAs (ceRNA)[25]. The first step to determine the key lncRNA is to find the differentially

expressed lncRNAs. In the present study, we used the Microassay and found several differentially expressed ones. Long noncoding RNA (lncRNA) TUG1 (taurine-up-regulated gene 1) was highly expressed in I/R model. Shan and colleagues also found that lncRNA TUG1 may function as a competing endogenous RNA (ceRNA) for miR-145 to induce cell damage[20]. LncRNA AK139328 is another potential therapeutic target for MIRI, which is highly expressed in I/R model. Yu demonstrated that Silencing lncRNA AK139328 significantly increased miR-204-3p expression and inhibited cardiomyocyte autophagy, thereby attenuating MIRI[26]. LncRNA Oprm1 was mostly differentially expressed and decreased in I/R model. We tried to rescue the Oprm1 by transfection with AAV9-Oprm1 in the infarct area, and the results were promising. The MIRI was attenuated and the apoptosis was reduced.

Further investigation demonstrated these effects could possibly be attributed to the up-regulation of CSE and increased production of H_2S. The previous study has revealed a novel molecular mechanism for endogenous H_2S production in the heart at the miRNA level and demonstrates the therapeutic potential of miR-30 family inhibition for ischemic heart diseases by increasing H_2S production[14]. Bioinformatic analysis and luciferase assay in the present study demonstrated a tight relationship between lncRNA Oprm1 and miR-30b-5p. We believed the overexpression of lncRNA Oprm1significantly decreased miR-30b-5p level via sponge effect, while the inhibition of CSE by miR-30b-5p was therefore canceled. Massive endogenous H_2S was produced and played a protective effect.

Previous studies have proven that H_2S is protective in the I/R injury of multiple organs, especially for the heart[27]. Snijder found that administration of H_2S is protective when administered during a cardiac ischemic insult by inducing a hypometabolic state in mice[28]. Another study demonstrated that H_2S could attenuate myocardial hypoxia-reoxygenation injury by inhibiting autophagy via mTOR activation[29]. The present study also found that pretreatment of NaHS (precursor of H_2S) had a significant cardioprotective effect. In the animal model, the infarct size was significantly reduced, with myocardial injury biomarker sharply decreased. In vitro, the cell viability was increased by the H_2S, standing for cell integrity and vitality. Due to the well-established efficacy, H_2S has been developed into different formulations for preclinical practice[30]. Apoptosis and oxidative stress were closely linked with each other. Oxidative stress contributed to I/R injury via significantly increased oxidant metabolites exerting a toxic effect on cell metabolism[31]. One of the most critical characteristics of oxidative stress is the over-production of ROS, which outweighed the effect of antioxidants[32]. ROS is massively produced in I/R injury, especially at the reperfusion phase. Several studies found that ROS is elevated in the I/R injury model, and associated with apoptosis[33,34]. We also examined the ROS level in cardiac tissue of I/R rats and supernatants of H/R H9c2 cells and found that ROS was significantly increased, which conformed with previous studies.

What's more, we also demonstrated that exogenous H_2S could reduce the production of ROS and increase the antioxidant concentration of SOD. As a result, the apoptosis rates in both rats and cells decreased sharply in the I/R+Oprm1 group and H/R+ Oprm1 group. Kang and his colleagues found that MiR-1 regulated H_2S protection of cardiomyocytes against I/R-induced apoptosis by stimulating Bcl-

2[35]. We both demonstrated the down-regulated effect of H_2S on apoptosis induced by I/R as well as the cardio-protection. In the present study, the biomarkers of apoptosis, including Caspase-3, Caspase-8, Caspase-9, were all down-regulated by H_2S. Apoptosis is a complicated and accurately-mediated process involving numerous molecules and pathways[23]. Studies have found the PI3K/Akt pathway is associated with cardiomyocytes apoptosis induced by I/R[36,37]. Some investigation found that HIF-1α participated in I/R injury in multiple organs as a critical modulator[38,39]. Liu and his colleagues found that DMY decreased the apoptosis and necrosis by I/R treatment, and PI3K/Akt and HIF-1α plays a crucial role in protection during this process. However, there was limited evidence about H_2S and PI3K/Akt pathway. We also examined the potential pathways involved in the cardioprotection effect of H_2S. Western blot results demonstrated that PI3K/Akt was activated and HIF-1α was inhibited for the I/R+ Oprm1 group in vitro and vivo.

To validate our hypothesis, we applied the miR-30b-5p mimics in cell experiments, which demonstrated that miR-30b-5p mimics could reverse the effect of Oprm1 completely. Figure 6 showed the schematic pathways involving in this study. Several limitations of this study must be noted. The transgenic animal might be more convincing if used as the animal model. Direct injection of AAV9-Oprm1 at the infarct area might be affected by the activity. There might be other pathways affecting the MIRI, which was not found in the present study, which requires further investigation.

5. Conclusions

lncRNA Oprm1 competitively combines with miR-30b-5p, which down-regulates the expression of CSE. When administered with lncRNA Oprm1, increased endogenous H_2S can reduce apoptosis and protect the myocardium from I/R injury via activating PI3K/Akt pathway and inhibiting HIF1-α activity.

Abbreviations

LncRNA, long-non coding RNA

CSE, cystathionine-γ-lyase

I/R, Ischemia/reperfusion

H/R, Hypoxia/reoxygenation

AMI, acute myocardial infarction

CABG, coronary artery bypass graft

MIRI, myocardial ischemia/reperfusion injury

H_2S, hydrogen sulfide

NO, nitric oxide

CO, carbon monoxide

ROS, reactive oxygen species

EF, ejection fraction

SOD, Superoxide Dismutase

LDH, Lactate dehydrogenase

Declarations

Ethics approval and consent to participate: This study was approved by the Ethical Committee of Tianjin Third Central Hospital. This study also complied with the Declaration of Helsinki, and signed, written informed consent was obtained from all subjects included in this study.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Y.Z. and S.C. designed and performed the experiments including biological analysis and western blot analysis and wrote the manuscript; S.Y. performed the animal study and analysis; J.F. and A.L. performed the cell experiments and analysis; S.C. reviewed and edited the manuscript. All authors approved the final manuscript.

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Figures

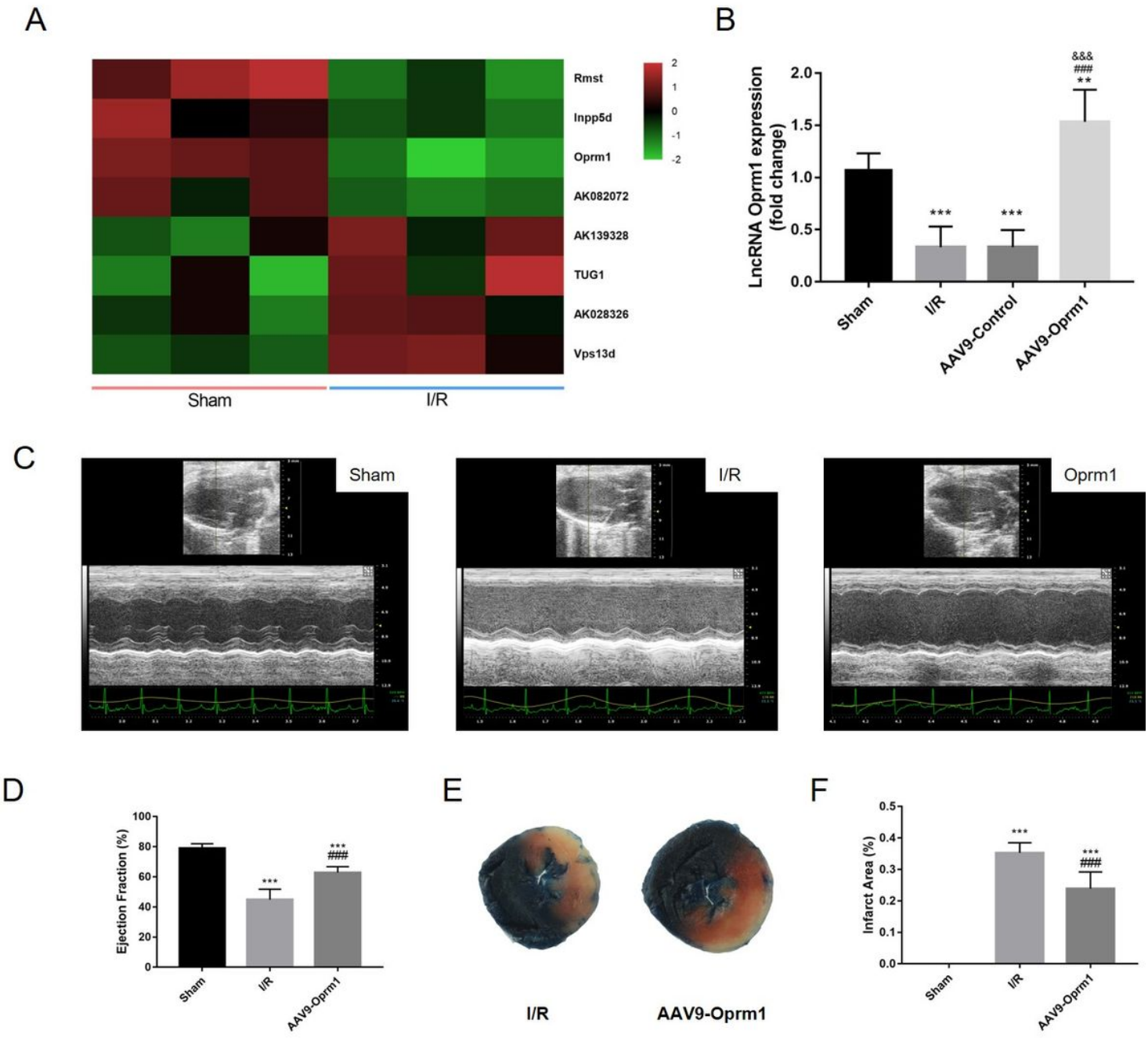

Figure 1

Overexpression of lncRNA Oprm1 reduced infarct size and preserver the left ventricular systolic function in vivo. A Microarray v3.0 chip analysis showed the differentially expressed lncRNAs between the Sham group and the I/R group. B LncRNA Oprm1 expression level was reduced in I/R group and increased by AAV9-Oprm1 injection. C Representative echocardiography results of three groups. D Ejection fraction (EF) measurements of three groups. E TTC assay shows the infarct size of the three groups. F Quantitative results of infarct size. *P<0.05 vs. Sham, **P<0.01 vs. Sham, ***P<0.001 vs. Sham; #P<0.05 vs. I/R, ##P<0.01 vs. I/R, ###P<0.001 vs. I/R.

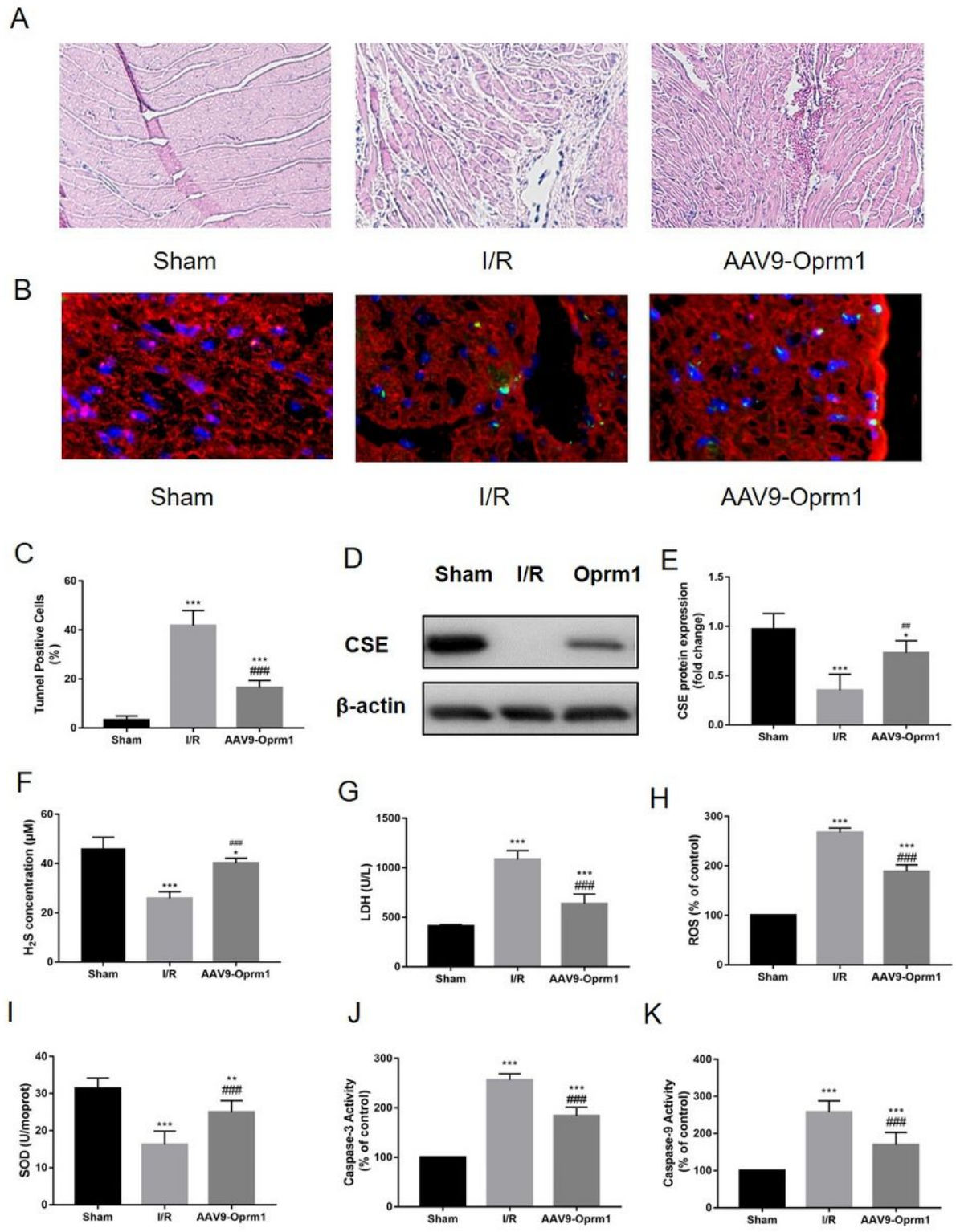

Figure 3

Overexpression of lncRNA Oprm1 inhibited I/R injury, apoptosis and increased CSE expression, H2S production. A Representative HE staining section of three groups. B Representative sections of TUNEL staining images; C Quantitative results of TUNEL positive cell rate; D Representative WB bands of CSE; E quantitative results of CSE protein expression level; F H2S concentration of different groups; G LDH concentration of different groups;; H ROS concentration of different groups;; I SOD concentration of

different groups;; J Caspase-3 activity of different groups;. K Caspase-9 activity concentration of different groups. *P<0.05 vs. Sham, **P<0.01 vs. Sham, ***P<0.001 vs. Sham; #P<0.05 vs. I/R, ##P<0.01 vs. I/R, ###P<0.001 vs. I/R.

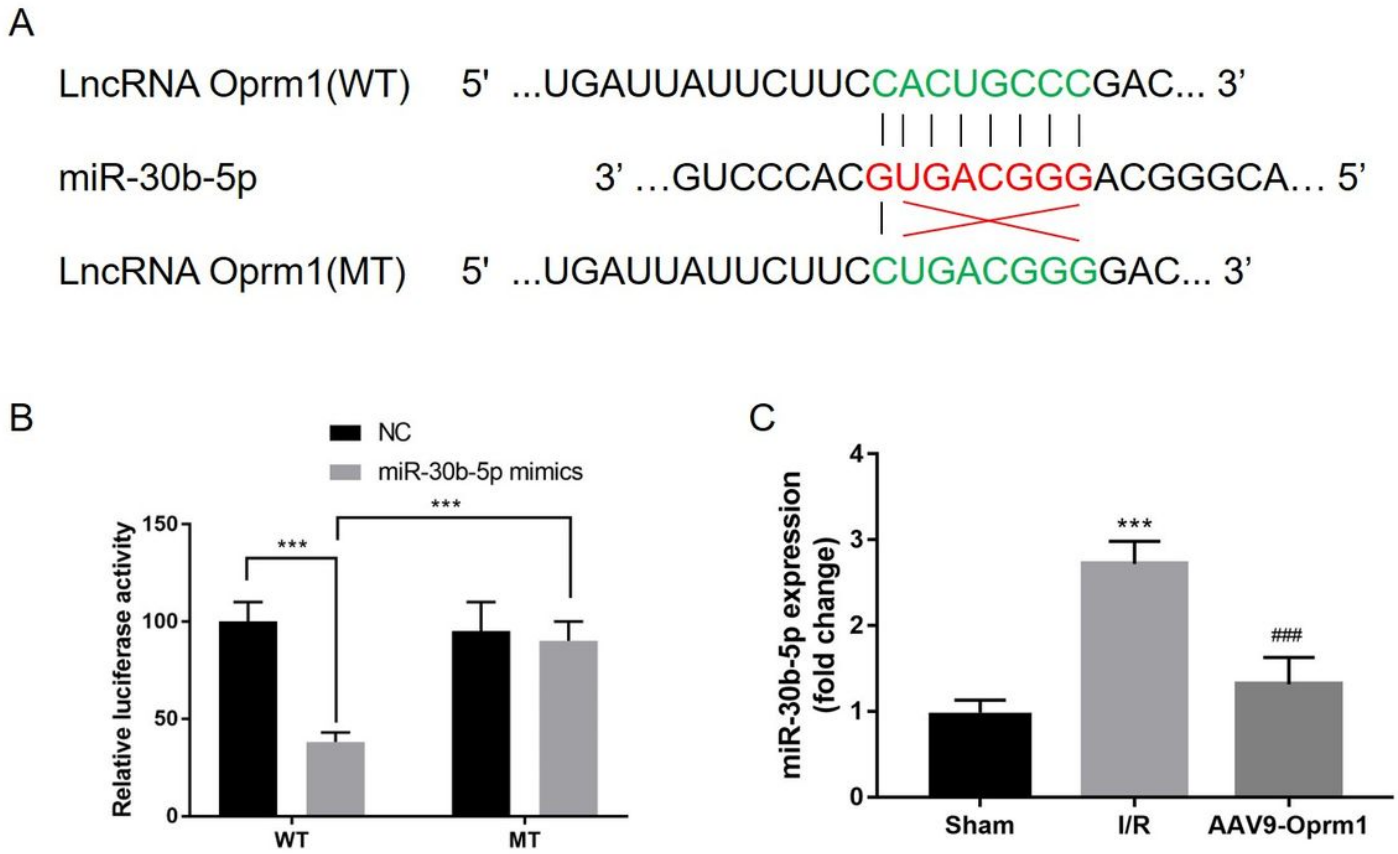

Figure 5

LncRNA Oprm1 directly targeted miR-30b-5p. A The potential binding sequence was predicted using Target Scan Software; B Luciferase activity was detected to evaluate the binding potential between Oprm1 and miR-30b-5p; C miR-30b-5p was negatively regulated by Oprm1using qPCR. ***P<0.001 vs. Sham; ###P <0.001 vs. I/R.

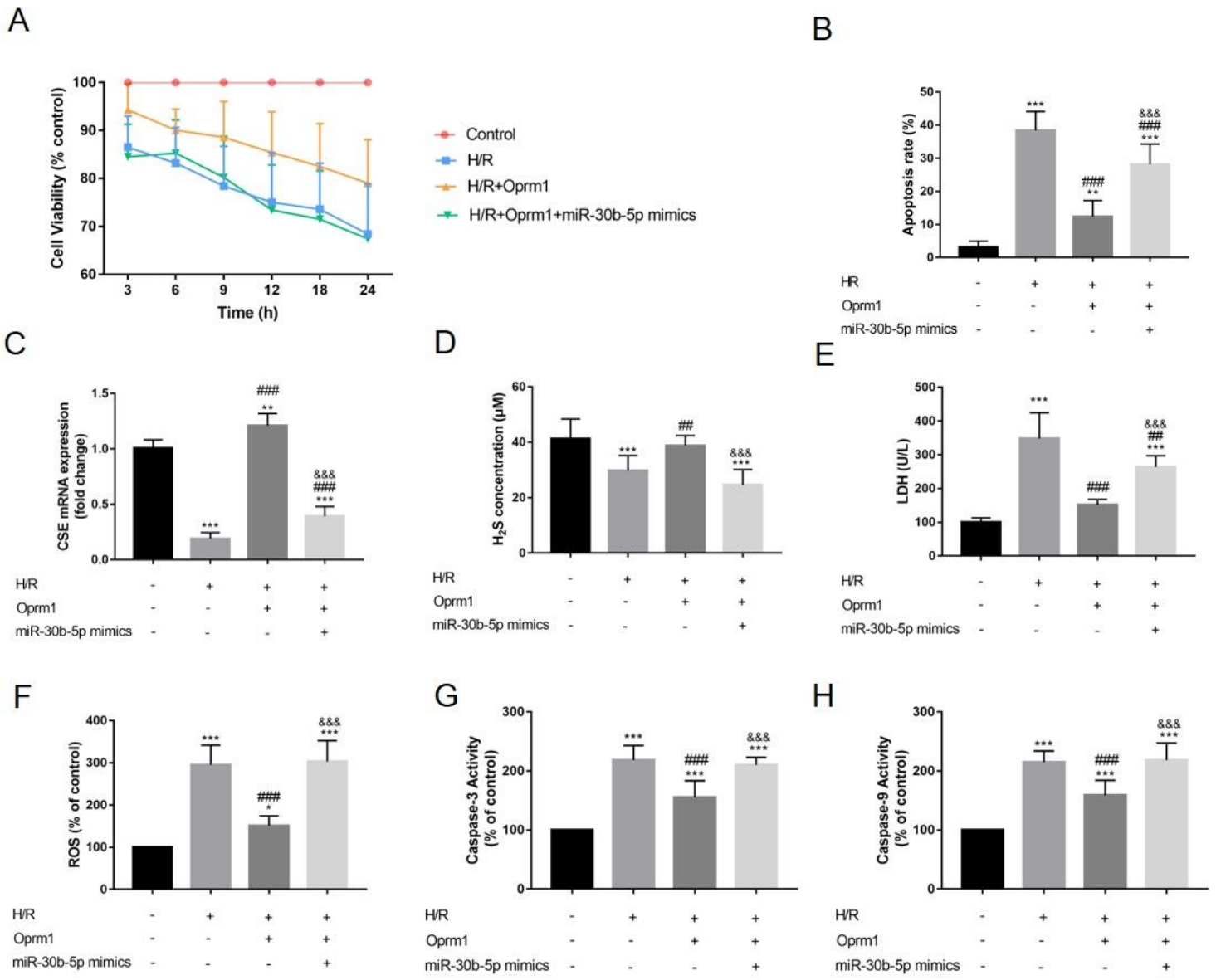

Figure 7

Overexpression of lncRNA Oprm1 protected H9c2 cardiomyocytes from H/R injury in vitro. A Cell viability changes of four groups. B Apoptosis rates of cells assayed by flow cytometry. C CSE mRNA expression level of four groups; D H2S concentration in cell supernatants; E LDH concentration in cell supernatants. F ROS concentration in cell supernatants. G Caspase-3 activity in cell supernatants. H Caspase-9 activity concentration in cell supernatants. *P<0.05 vs. control, **P<0.01 vs. control, ***P<0.001 vs. control; #P<0.05 vs. H/R, ##P<0.01 vs. H/R, ###P<0.001 vs. H/R; &P<0.05 vs. H/R+H2S, & &P<0.01 vs. H/R+H2S, & & &P<0.001 vs. H/R+H2S.

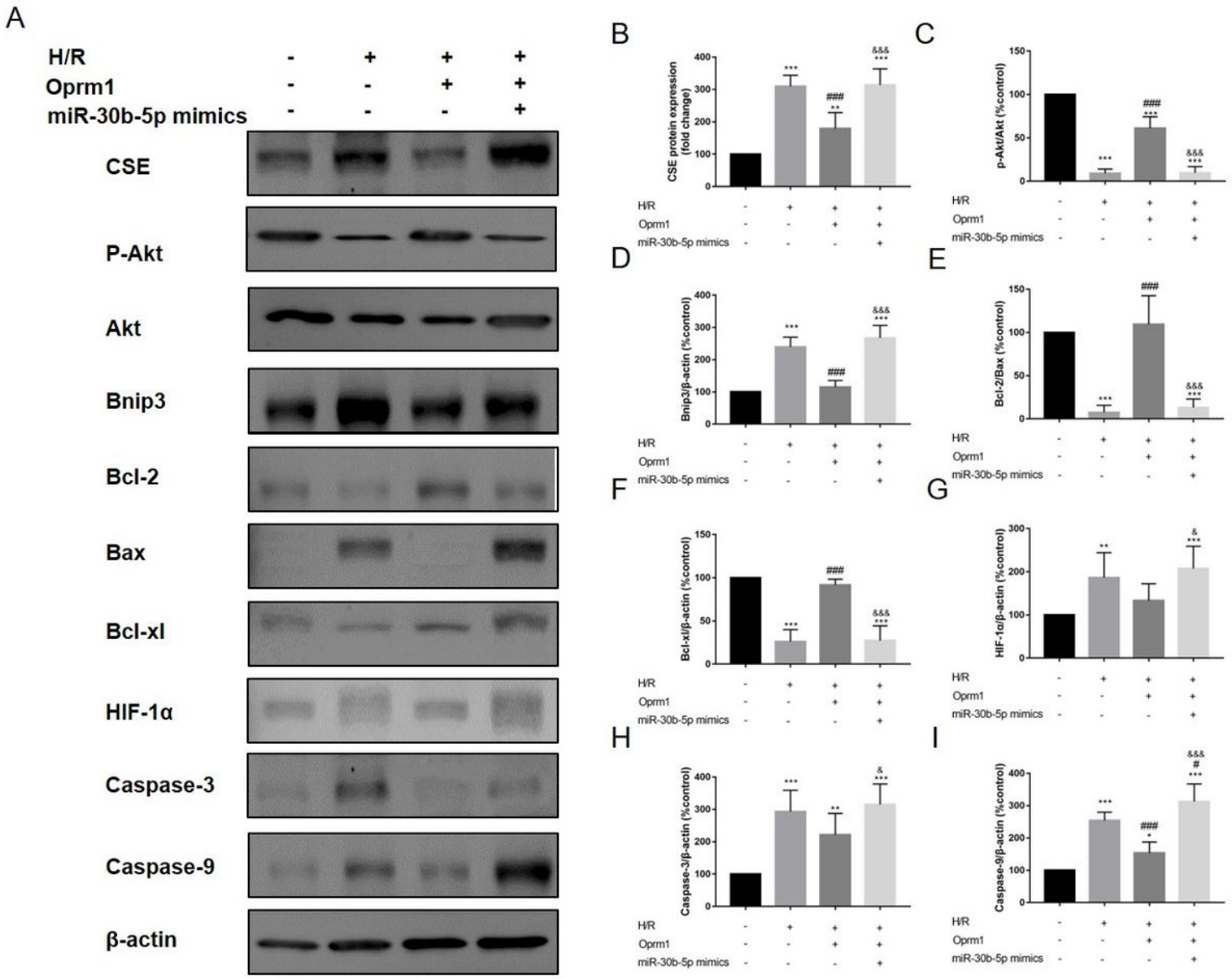

Figure 9

LncRNA Oprm1 ActivateD the PI3K/Akt pathway and inhibits HIF-1α pathway in vitro. A WB bands of PI3K/Akt and HIF-1α pathways. B Quantitative results of CSE protein expression by WB; C Quantitative results of p-Akt/Akt ratio assayed by WB; D Quantitative results of Bnip3 assayed by WB; E Quantitative results of the Bcl-2/Bax ratio assayed by WB; F Quantitative results of Bcl-xl assayed by WB; G Quantitative results of HIF-1α assayed by WB; H Quantitative results of Caspase-3 assayed by WB. I Quantitative results of Caspase-9 assayed by WB. *P<0.05 vs. control, **P<0.01 vs. control, ***P<0.001 vs. control; #P<0.05 vs. H/R, ##P<0.01 vs. H/R, ###P<0.001 vs. H/R; &P<0.05 vs. H/R+Oprm1, & &P<0.01 vs. H/R+ Oprm1, & & &P<0.001 vs. H/R+ Oprm1.

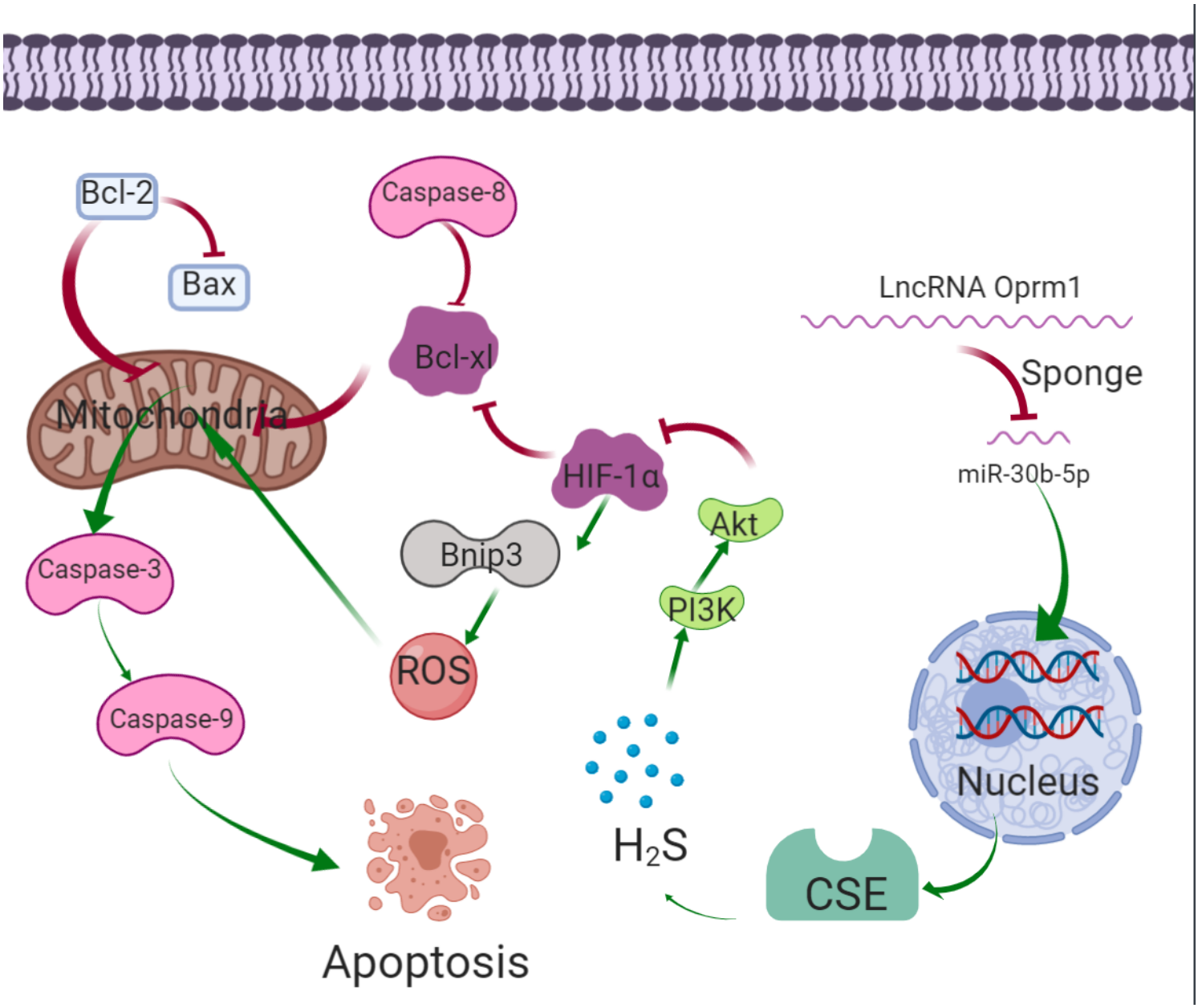

Figure 11

Schematic diagram of the signaling pathway for the effect of LncRNA Oprm1 on MIRI via CSE activation and H2S production.