Desflurane protects against liver ischemia/reperfusion injury via regulating miR-135b-5p

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Abstract

Background: A number of anesthetics have protective effect against ischemia-reperfusion (I/R) injury, including desflurane. But the function and molecular mechanism of desflurane in liver I/R injury have not been fully understood. The aim of this study was to investigate the effect of desflurane on liver I/R injury and further investigated the molecular mechanisms involving in miR-135b-5p.

Methods: The models of liver I/R injury in rats were established, and received desflurane treatment throughout the injury. Serum alanine transaminase (ALT) and aspartate transaminase (AST) were measured and compared between groups. H/R-induced cell model in L02 was established, and were treated with desflurane before hypoxia. Quantitative real-time polymerase chain reaction was performed to determine the expression of miR-135b-5p in different groups. The cell apoptosis was detected using flow cytometry assay. Western blot was used for the measurement of protein levels.

Results: I/R significantly increased serum levels of ALT and AST in rats, which were reversed by desflurane treatment. Desflurane also significantly attenuated the increase of cell apoptosis induced by I/R *in* both *vivo* and *vitro*. MiR-135b-5p significantly reversed the protective effect of desflurane against liver I/R injury. Additionally, Janus protein tyrosine kinase (JAK)2 was shown to be a target gene of miR-135b-5p, and miR-135b-5p overexpression significantly decreased the protein levels of p-JAK2, JAK2, p-STAT3.

Conclusion: Desflurane attenuated liver I/R injury through regulating miR-135b-5p, and JAK2 was the target gene of mIR-135b-5p. These findings provide references for further development of therapeutic strategies in liver injury.

Keywords: Desflurane; Hypoxia/reoxygenation; Janus protein tyrosine kinase 2; Liver ischemia-reperfusioninjury; MiR-135b-5p

1. INTRODUCTION

Liver ischemia-reperfusion (I/R) injury is a serious complication associated with a number of clinical conditions, such as liver resection and transplantation and hypovolemic shock, which can be very detrimental to the health condition of patients.[1] Liver I/R includes two types, cold ischemia and warm ischemia.[2] Liver I/R injury is considered to be induced by the reperfusion of blood flow and hypoxia accentuation in ischemic tissues, as it exacerbates the metabolic dysfunction of tissues rather than restoring their function.[3] Despite advances have been made in techniques of surgical and liver preservation, liver I/R injury remains as a server problem during liver surgery. Thus, it is of great significance to explore the mechanisms of live I/R injury and develop a new kind of therapeutics.

Recently, a number of anesthetics have been proposed to be protective against I/R injury.[4] And currently the most interesting aspect lies in the area of volatile anesthetic agents, which have been supported to ameliorate I/R injury in various tissues, including heart, brain, kidney, liver, intestine, and lung.[5–11] Volatile anesthetics have a certain of immunomodulatory effect, which can inhibit the expression of intercellular adhesion molecule and alleviate the inflammation reaction.[12,13] Desflurane is a fluorine-containing volatile anesthetic, which can inhibit the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and reduce the expression of inflammatory factors.[14] It is reported that desflurane affects the expression of adhesion molecules involved in the multistep process of neutrophil recruitment, and ameliorates I/R injury via regulating the expression of ICAM-1, VCAM-1, and E-selectin.[15] Recently, Mangus et al[16] reviewed the anesthesia and perioperative records, and measured the serum alanine aminotransferase (ALT) and total bilirubin levels of the enrolled patients who have undergone liver transplantation. The authors suggested that administration of desflurane can provide some early hepatoprotection against I/R injury.[16] But the underlying molecular mechanisms are still poorly understood.

MicroRNAs (miRNAs) are a class of small and noncoding RNA molecules, which contain 22 nucleotides in length. It has been regarded as the posttranscriptional regulators for gene expression, which can bind to the 3′ untranslated region (3′-UTR) of target messenger RNAs (mRNAs).[17] It is widely suggested that miRNAs play a crucial role in various biological processes, especially in tumor development.[18–20] Recently, a number of miRNAs have been found to play a crucial role in liver I/R injury. MiR-125b was shown to be downregulated in hypoxia/reoxygenation (H/R) cells, it may protect liver from I/R injury through inhibiting TRAF6 and the NF-κB signal pathway.[21] MiR-155 was reported to be upregulated during liver I/R,

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Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 38-45.

Received April 30, 2020; accepted July 17, 2020.

doi: 10.1097/JCMA.0000000000000427.

and overexpressed miR-155 aggravated liver injury via targeting SOCS1.[22] Zhi et al[23] reported that the expression of miR-135b-5p was upregulated in the rat liver exposed to hypoxia, suggesting that hypoxia significantly regulated the miR-135b-5p expression in the liver, and miR-135b-5p might be associated with hypoxia induced liver injury. A major study reported that in the I/R mice exposed to sevoflurane, the miR-135b-5p expression in myocardial tissues decreased significantly compared with the model mice without anesthesia treatment.[24] Considering the crucial role of miR-135-5p in the protective mechanism of anesthesia in myocardial I/R injury, we further explored its role in liver I/R injury.

Therefore, in the present study, we aimed to verify the effect of desflurane on liver I/R injury and further investigated the molecular mechanisms involving in miR-135b-5p.

2. METHODS

2.1. Animals

Male Sprague-Dawley (SD) rats (n = 48) with weight of 250 to 300 g were purchased from Beijing Experimental Animal Center (Beijing, China). All animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Administrative Panel on Laboratory Animal Care of Shengli Oilfield Central Hospital.

All rats were randomly divided into 6 groups as follows: (1) sham group (n = 8): rats were anesthetized by an intraperitoneal injection of 40 mg/kg pentobarbital sodium (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China), then underwent the similar abdominal surgery of I/R group without liver ischemia. (2) Liver I/R group (I/R group, n = 8): as previous reports described, a rat model of segmental (70%) liver I/R was established.[25,26] Rats were anesthetized by an intraperitoneal injection of 40 mg/kg pentobarbital sodium (Sinopharm Chemical Reagent Co., Ltd.). The portal structures of the left and median lobes of the liver were subsequently isolated, and liver ischemia (70%) was performed using a microaneurysm clamp placed on the hepatic artery and portal vein to block the blood supply to these lobes, which was lasted for 60 minutes. Subsequently, the clamp was removed and reperfusion was initiated for 120 minutes. Finally, the abdominal incision was closed with a 4-0 black silk suture. (3) I/R + desflurane group (desflurane treatment group, n = 8): rats received desflurane treatment from 3 minutes before reperfusion, until 2 minutes after the reperfusion, maintaining the end-tidal desflurane concentration of 1 minimum alveolar concentration (MAC). (4) I/R + saline group (n = 8): the same dosage of saline instead of desflurane was given to rats from 3 minutes before reperfusion, until 2 minutes after the reperfusion. (5) I/R + desflurane + miR-NC group (n = 8): rats were given miR-NC (5′-UUCUCCGAACGUGUCACGUTT-3′) by intraperitoneal injection for 7 days before ischemia. (6) I/R + desflurane + miR-135b-5p agomir group (n = 8): the rats were given miR-135b-5p agomir (5′-UAUGGCUUUUCAUUCCUAUGUGA-3′; 20 μL of 500 pmol miR-135b-5p agomir/day) by intraperitoneal injection for 7 days before ischemia. After the surgery, tissues and serum of rats in different groups were collected for further analysis.

2.2. Alanine transaminase/aspartate transaminase assessment

Serum alanine transaminase (ALT) and aspartate transaminase (AST) were determined by an automated analyzer (Hitachi, Tokyo, Japan), to measure the degree of liver I/R injury.

2.3. Cell culture and H/R model

Human normal liver cells L02 were obtained from the Chinese Academy of Sciences Shanghai Institutes for Biological Sciences Cell resource center (Shanghai, China), and cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Grand Island, NY, USA) with 10% fetal bovine serum (Hyclone, South Logan, UT, USA), and incubated at 37°C in a humidified incubator with 5% CO_2. For H/R group, to simulate ischemia, L02 cells were exposed to hypoxic with a gas mixture of 95% N_2 and 5% CO_2 at a flow rate of 10 L/min for 5 minutes, then incubated in a hypoxic environment of 1% O_2 at 37°C for 8 hours, cells were cultured with serum-free medium. After ischemia and hypoxic, normal medium was added, and cells were cultured in a normal incubator for 24 hours. For H/R plus desflurane group, in which cells were incubated in similar conditions with H/R group, and were treated with desflurane for 15 minutes before hypoxia: desflurane mixture containing 7.5 vol. % and comprising 1 MAC of desflurane in a closed chamber. Cells cultured in a normal incubator were included as a control group.

2.4. Cell transfection

MiR-135b-5p mimics, and the negative controls (mimic-NC) were purchased from Gene-Pharma (Shanghai, China). The miT-135b-5p overexpression study was performed using miR-135b-5p mimics (5′-UAUGGCUUUUUAUUCCUAUGUGA-3′) and mimic NC (5′-UCACAACCUCCUAGAAAGAGUAGA-3′). L-02 cells were cultured to 30% to 50% confluence and transfected with miR-135b-5p mimics or miR-NC using Lipofectamine 2000 according to the manufacturer's instructions. Subsequent experiments including H/R, and apoptosis were performed 24 hours after miRNA transfection.

2.5. RNA extraction and quantitative real-time polymerase chain reaction

Trizol Reagent (Invitrogen, Carlsbad, CA, USA) was applied for the total RNA extraction. RNA was reverse transcribed into cDNA using the miScript Reverse Transcription Kit (QIAGEN, Germany). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using SYBR green I Master Mix kit (Invitrogen) and 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) to estimate the gene expression. U6 was used as an internal reference for data normalization of mRNA levels. The relative expressions of miR-135b-5p and Janus protein tyrosine kinase (JAK)2 mRNA were determined by the $2^{-\Delta\Delta Ct}$ method. U6 and GAPDH were used as reference control respectively. The primer sequences were as follows: miR-135b-5p forward, 5′-GGTATGGCTTTTCATTCCT-3′ and reverse, 5′-CAGTGCGTGTCGTGGAGT-3′; U6 forward, 5′-GCTTCGGCAGCACATATACTAAAAT-3′ and reverse, 5′-CGCTTCACGAATTTGCGTGTCAT-3′; JAK2 forward 5′-GGGAGGTGGTCGCTGTAAAA-3′ and reverse 5′-ACCAGCACTGTAGCACACT-3′; GAPDH forward, 5′-CTTTGGTATCGTGGAAGGACTC-3′ and reverse, 5′-GTAGAGGCAGGGATGATGTTCT-3′.

2.6. Flow cytometry

The Annexin V-FITC/PI apoptosis detection kit was used to determine the cell apoptosis, according to the manufacturer's instructions. The harvested cells were resuspended in 200 μL binding buffer, then incubated with 5 μL Annexin V-FITC and 5 μL PI in the dark for 15 minutes. The stained cells were analyzed by flow cytometry (FACS Calibur, BD Biosciences, San Jose, CA, USA).

2.7. Luciferase reporter assay

The predicted 3′-UTRs sequence of JAK2 interacting with miR-135b-5p and mutant sequences within the predicted target sites were synthesized and inserted into the pRL-TK control vector (Promega, Madison, WI, USA). L02 cells were co-transfected

with JAK2-3′UTR-Wt reporter vector or JAK2-3′UTR-Mut and miR-135b-5p mimic or its negative control (mimic NC). pRL-TK expressing Renilla luciferase was co-transfected as an internal control. The luciferase activities were measured using Dual-Luciferase Reporter Assay kit (Promega) according to the manufacturer's instructions.

2.8. Western blot

Protein was extracted from cell lysate based on the manufacturer's instructions with the application of Radio Immunoprecipitation Assay Lysis Buffer (Beyotime, Shanghai, China). A BCA method (Beyotime, Beijing, China) was used for the quantification of protein concentration. After blocking in TBST buffer (TBS buffer with 0.1% Tween-20) containing 5% BSA for 1 hour at room temperature, protein bands were successively incubated with primary antibodies at 4°C overnight and secondary antibodies for 1 hour at room temperature. Then, protein bands were washed by TBST buffer for three times every 5 minutes and visualized using Immobilon western (Millipore Co., Bedford, MA, USA). The primary antibodies against JAK2, p-JAK2, signal transducer and activator of transcription (STAT)3, P-STAT3, and GAPDH were purchased from Cell Signaling Technology (Beverly, MA, USA). All secondary antibodies were purchased from Invitrogen.

2.9. Statistics analysis

Experiments were performed in triplicate and data were expressed as mean ± SD. Student's *t* test was used for the difference analysis between groups. All the data analysis was conducted using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, CA, USA). A p value <0.05 was considered to indicate a statistically significant difference.

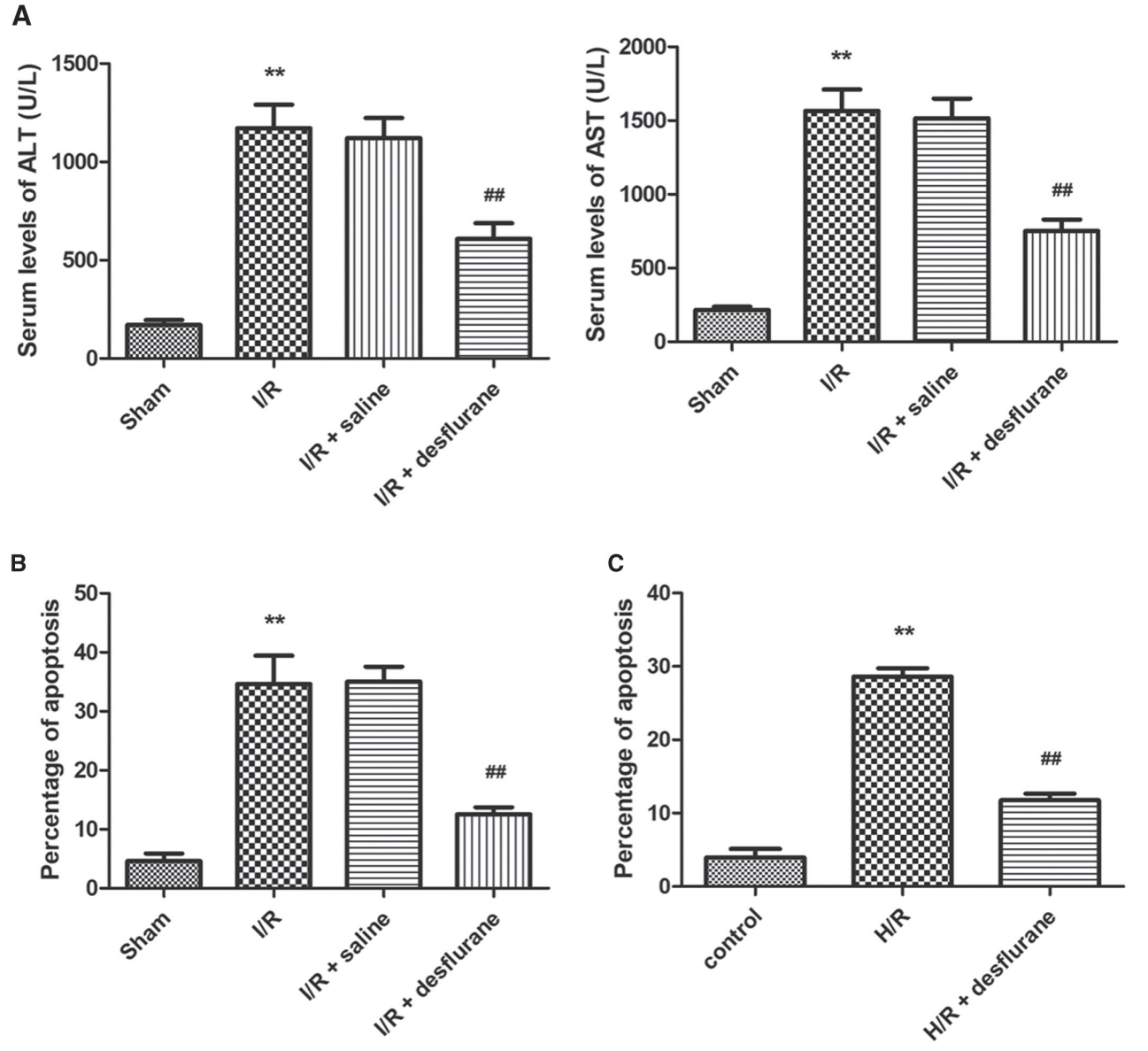

Fig. 1 Desflurane attenuated liver I/R injury. A, Serum transaminase levels of ALT and AST were detected in liver tissue of rats (**$p < 0.01$, compared with sham group; ##$p < 0.01$, compared with I/R group). B, The cell apoptosis of different groups in rats (**$p < 0.01$, compared with sham group; ##$p < 0.01$, compared with I/R group). C, The cell apoptosis of different groups in L02 cells (**$p < 0.01$, compared with control group; ##$p < 0.01$, compared with H/R group). ALT=alanine transaminase; AST=aspartate transaminase; H/R=hypoxia/reoxygenation; I/R=ischemia-reperfusion.

3. RESULTS

3.1. Desflurane attenuated liver I/R injury

To explore the role of desflurane in liver I/R injury, the serum transaminase levels of ALT and AST were detected. It was observed that compared with sham group, serum levels of ALT and AST were increased significantly in rats underwent liver I/R surgery ($p < 0.01$, Fig. 1A). These results suggested that a rat model of I/R was constructed successfully in this study. Additionally, treatment with desflurane throughout the surgery period inhibited the increase of ALT and AST levels induced by I/R, and the differences reached significant level ($p < 0.01$, Fig. 1A). Furthermore, the cell apoptosis of different groups was also detected by flow cytometry in rats. We found that I/R significantly increased the cell apoptosis compared with the sham group, but desflurane treatment effectively inhibited the increase of cell apoptosis induced by I/R ($p < 0.01$, Fig. 1B). We further verified the results in L02 cells. H/R-induced cell model in L02 was established, and was treated with desflurane before hypoxia. The flow cytometry assay results suggested that H/R significantly promoted the cell apoptosis compared with control group ($p < 0.01$, Fig. 1C), indicating that H/R-induced cell model in L02 was established successfully. It was also observed that desflurane pretreatment significantly inhibited cell apoptosis compared with H/R group ($p < 0.01$, Fig. 1C). These findings suggested that desflurane attenuated liver I/R injury.

3.2. Desflurane regulated the expression changes of miR-135b-5p induced by I/R

We further investigated the expression changes of miR-135b-5p induced by desflurane, and the expression level of miR-135b-5p in both liver tissue of rats and L02 cells were measured using qRT-PCR. I/R increased the level of miR-135b-5p significantly in both rats and L02 cells, which were reversed by the pretreatment with desflurane ($p < 0.01$, Fig. 2). These findings suggested that miR-135b-5p played a crucial role in liver I/R injury, desflurane might attenuate liver I/R injury through regulating miR-135b-5p expression.

3.3. MiR-135b-5p reversed the protective effect of desflurane on liver I/R injury

To detect the effect of miR-135b-5p on the protective function of desflurane in H/R cells, the expression level of miR-135b-5p was regulated via transfecting miR-135b-5p mimics. The transfection efficiency was calculated by qRT-PCR, and the results demonstrated that miR-135b-5p mimics transfection led to a dramatically increase in its expression level compared with H/R + desflurane group ($p < 0.01$, Fig. 3A). Then, we detected the effect of miR-135b-5p on the protective function of desflurane against H/R induced cell apoptosi. Flow cytometry assay showed that desflurane pretreatment inhibited the cell apoptosis induced by H/R, but miR-135b-5p overexpression abrogated the protective effect of desflurane on H/R treated cells ($p < 0.01$, Fig. 3B).

We further verified the results in I/R rats, and the I/R rats were treated with miR-135b-5p agomir or miR-NC by intraperitoneal injection. The transfection efficiency was calculated by qRT-PCR, and the results demonstrated that miR-135b-5p injection led to a dramatically increase in its expression compared with I/R + desflurane group ($p < 0.01$, Fig. 3C). As shown in Fig. 3D, E, desflurane treatment reduced the serum levels of ALT and AST compared with I/R group, but miR-135b-5p agomir injection significantly increased the levels of ALT and AST compared with I/R + desflurane ($p < 0.01$). Additionally, the results of flow cytometry assay showed that desflurane treatment inhibited the cell apoptosis induced by liver I/R in rats, but miR-135b-5p agomir injection significantly reversed the inhibition of cell apoptosis ($p < 0.01$, Fig. 3F). By generalizing the above findings, we concluded that that miR-135b-5p reversed the protective effect of desflurane against liver I/R injury.

3.4. JAK2 was a potential target gene of miR-135b-5p

Bioinformatics analysis results indicated that the 3′-UTR of JAK2 mRNA contains a binding site for miR-135b-5p, suggesting that JAK2 was one of the candidate genes of miR-135b-5p (Fig. 4A). To validate this prediction, the luciferase reporter gene assay was applied to measure the relationship between miR-135b-5p and JAK2. The results showed that miR-135b-5p mimics significantly suppressed the luciferase activity of the 3'-UTR wild type of JAK2 ($p < 0.05$, Fig. 4B), indicating that JAK2 was a target gene of miR-135b-5p. Additionally, the mRNA and protein levels of JAK2 and STAT3 were further detected. The qRT-PCR results indicated that there was a significant reduction of the JAK2 mRNA level in miR-135b-5p mimic transfection cells ($p < 0.001$, Fig. 4C). As shown in Fig. 4D, miR-135b-5p overexpression significantly decreased the protein levels of p-JAK2, JAK2, and p-STAT3 ($p < 0.05$).

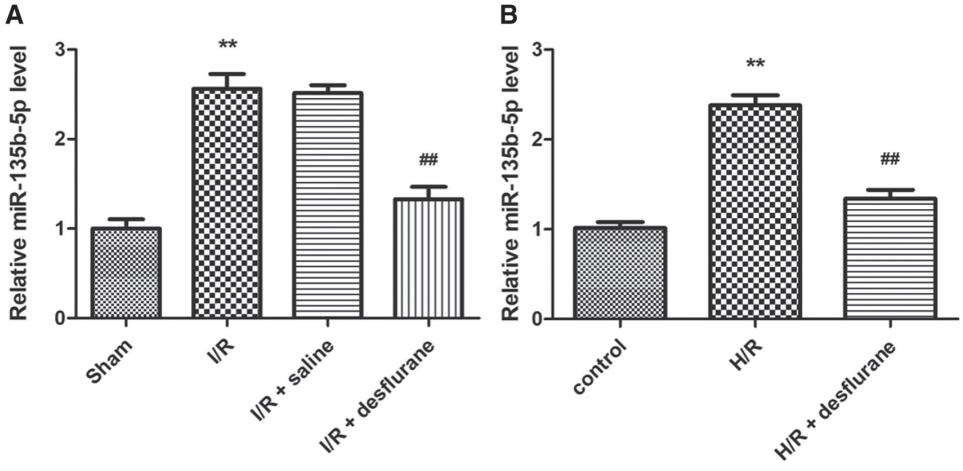

Fig. 2 The effect of desflurane on the expression of miR-135b-5p. A, The level of miR-135b-5p in liver tissue of rats (**$p < 0.01$, compared with sham group; ##$p < 0.01$, compared with the I/R group). B, The level of miR-135b-5p in different cell groups (**$p < 0.01$, compared with control group; ##$p < 0.01$, compared with the H/R group). H/R=hypoxia/reoxygenation; I/R=ischemia-reperfusion.

4. DISCUSSION

Liver I/R injury is proved to be a complex pathophysiological process, and several mechanisms have been reported to be involved in its process, including reactive oxygen species, proinflammatory cytokines and chemokines as well as Kupffer cells, and so on.[27–29] Among them, impaired balance between products of oxidative stress and the levels of antioxidant enzyme activities might be an important mechanism underlying the occurrence of I/R. Although the underlying mechanisms of liver I/R injury are still unknown, multiple drugs and approaches have been developed to alleviate liver I/R injury, including anesthetics.

Desflurane is a fluorine-containing volatile anesthetic, which can be used to inhibit the activation of NF-κB and reduce the expression of inflammatory factors.[30] In the present study, the

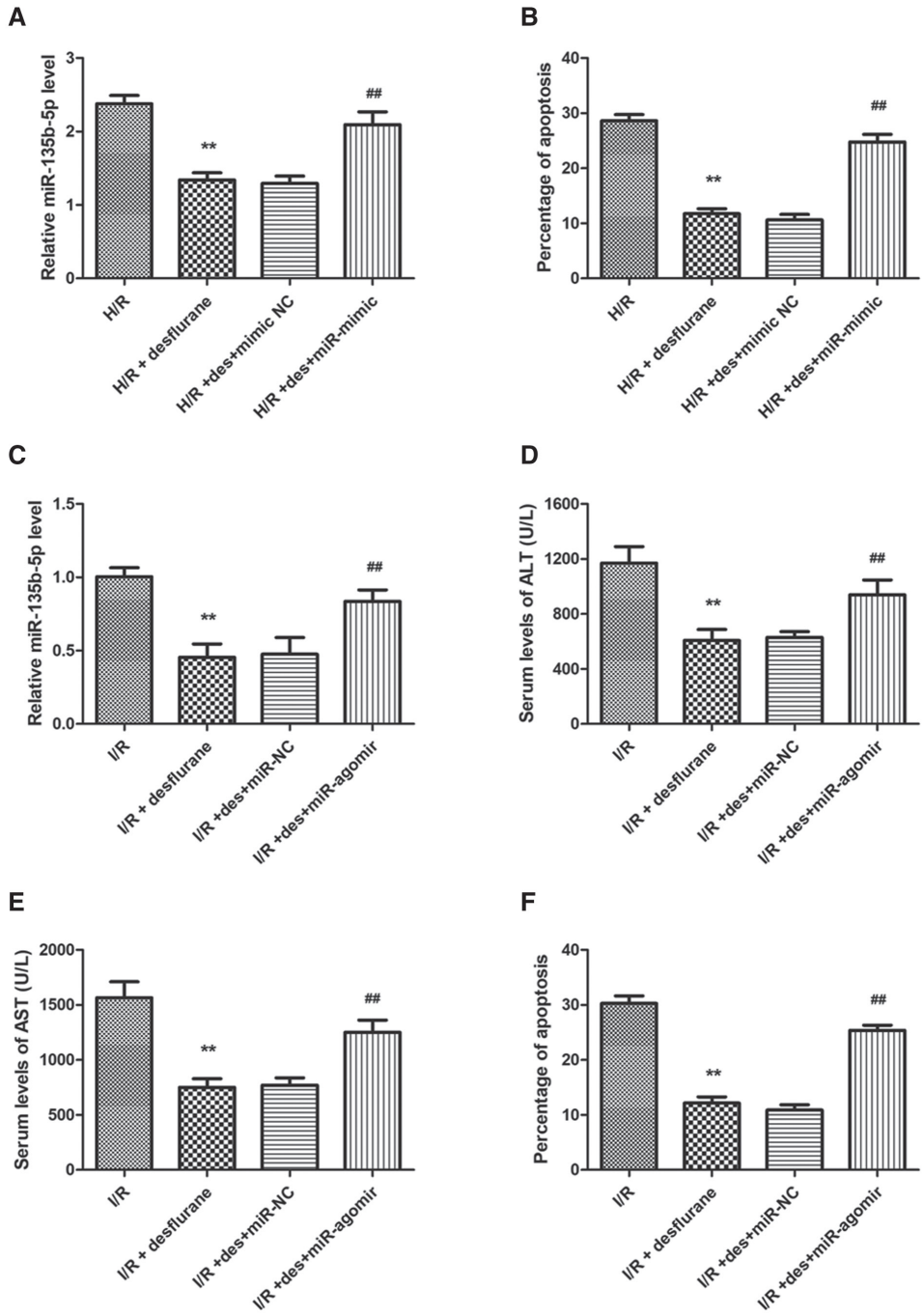

Fig. 3 The effect of miR-135b-5p on the protective function of desflurane in H/R cells. A and B, The level of miR-135b-5p and cell apoptosis in different cell groups (**$p < 0.01$, compared with the H/R group; ##$p < 0.01$, compared with the H/R + desflurane group). C, The level of miR-135b-5p in liver tissue of rats (**$p < 0.01$, compared with the I/R group; ##$p < 0.01$, compared with the I/R + desflurane group); D and E, Serum transaminase levels of ALT and AST were detected in liver tissue of rats (**$p < 0.01$, compared with I/R group; ##$p < 0.01$, compared with I/R + desflurane group). F, The cell apoptosis of different groups in rats (**$p < 0.01$, compared with the I/R group; ##$p < 0.01$, compared with I/R + desflurane group). ALT=alanine transaminase; AST=aspartate transaminase; H/R=hypoxia/reoxygenation; I/R=ischemia-reperfusion.

role of desflurane in liver I/R injury was explored *in vivo* and *vitro*. *In vitro* experiment, it was noted that desflurane treatment inhibited the increase of ALT and AST levels induced by I/R in rats. Besides, the liver cell apoptosis was also markedly inhibited by desflurane treatment. Additionally, in H/R cell models, the cell apoptosis induced by H/R was inhibited by desflurane pretreatment. Thus, we concluded that desflurane attenuated liver I/R injury. As Mangus et al[16] reported, liver transplantation (LT) recipient and organ donor data were extracted retrospectively for all LTs, and their serum ALT and AST levels, survival and outcomes were recorded and compared, which suggested that administration of desflurane may provide some early hepatoprotection against I/R injury. Savran et al[4] reported that desflurane has the protective effect on I/R injury in living-donor kidney transplant recipients. All evidences demonstrated the crucial role of desflurane in liver I/R injury. By generalizing the above findings, we concluded that desflurane attenuated liver I/R injury.

Recently, a number of miRNAs have been found to paly crucial role in liver I/R injury, such as miR-125b, miR-155, miR-182-5p, and so on.[21,22,31] In the current study, the miR-135b-5p level was significantly increased in the human liver H/R cells models. Zhi et al[23] reported that the hypoxia significantly increased the level of miR-135b-5p in the liver, which supported our findings. But another study reported by Qiang et al[32] has presented contradictory results that miR-135-5p might protect neurons against OGD/R-induced injury in the mouse hippocampal neuronal cells. Considering the contradictory results, we further verified the results *in vivo* experiments, and it was confirmed that the level of miR-135b-5p was increased remarkably in the liver tissues of liver I/R rats, which was consistent

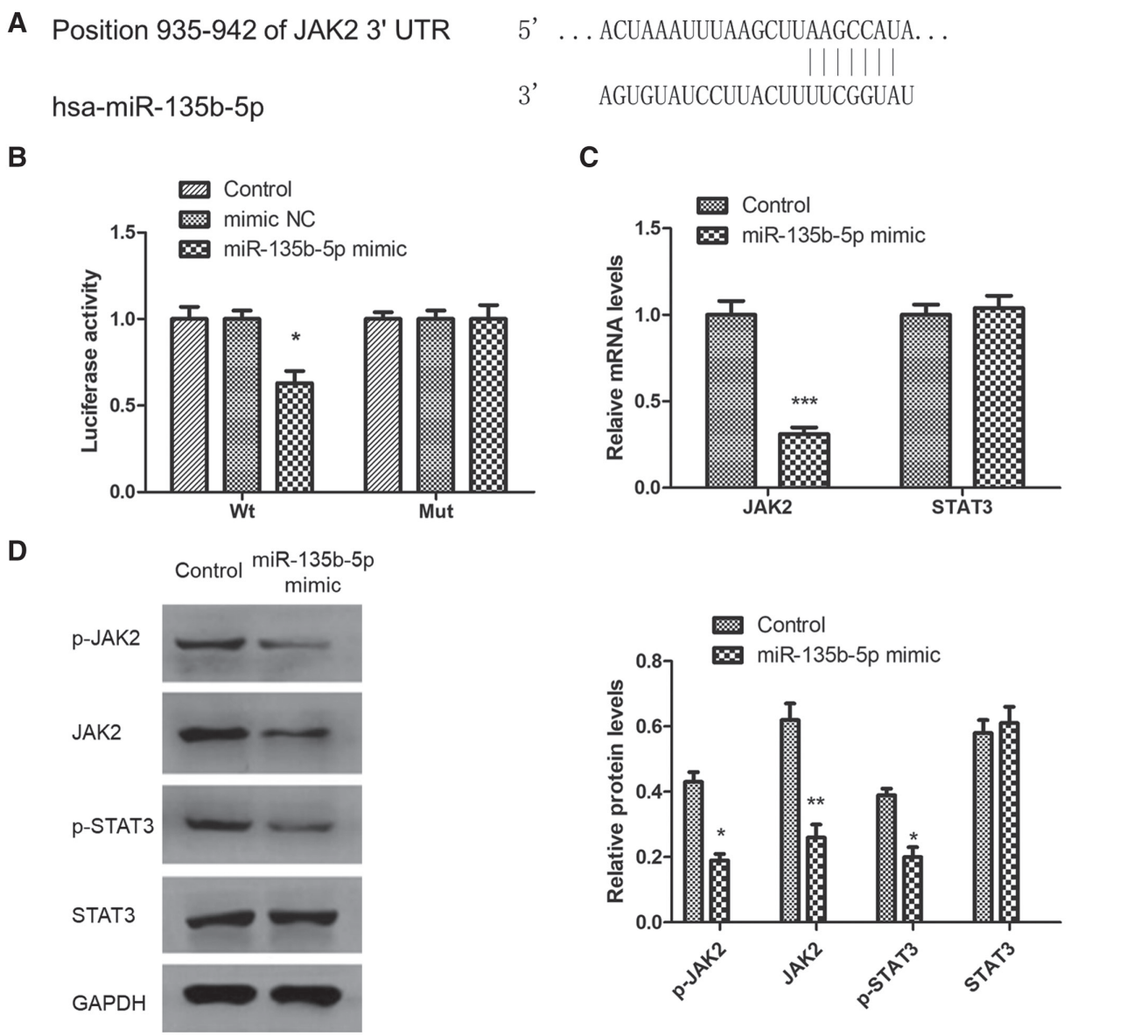

Fig. 4 JAK2 was a target gene of miR-135b-5p. A, The biological online prediction showed that the 3′-UTR of JAK2 contained a miR-135b-5p binding site. B, MiR-135b-5p mimics significantly suppressed the luciferase activity of the 3′-UTR wild type of JAK2, which was not observed under the mutant JAK2 3′-UTR expression. C, MiR-135b-5p overexpression significantly decreased the mRNA levels of JAK2. D, MiR-135b-5p overexpression significantly decreased the protein levels of p-JAK2, JAK2, p-STAT3, but the STAT3 level showed no significant difference between two groups. *$p < 0.05$, compared with the control group. 3′-UTR=3′ untranslated region; JAK/STAT=Janus protein tyrosine kinase/signal transducer and activator of transcription.

with results observed in human liver H/R cells. Then, we further investigated the effect of desflurane on the expression of miR-135b-5p. It was found that after desflurane treatment, the expression level of miR-135b-5p increased significantly in the liver tissues of I/R rats. Additionally, in the H/R cell model, desflurane treatment also downregulated the miR-135b-5p level. We inferred that miR-135b-5p may participate in the protective effect of desflurane on liver I/R injury. Therefore, we further explored the contribution that miR-135b-5p makes to the protective effect of desflurane on the liver I/R injury. Firs, miR-135b-5p mimics were transfected into L02 cells to upregulate the miR-135b-5p expression. It was noted that miR-135b-5p overexpression abrogated the protective effect of desflurane on H/R cells. We further verified the results in I/R rats, and the I/R rats were treated with miR-135b-5p agomir. We found that desflurane treatment reduced the levels of ALT and AST, but the influence was reversed by miR-135b-5p agomir injection. Additionally, miR-135b-5p agomir injection was also found to reverse the inhibition of cell apoptosis induced by desflurane. Taken together, we concluded that miR-135b-5p reversed the protective effect of desflurane in liver I/R injury, it is possible that desflurane attenuated liver I/R injury via downregulating the miR-135b-5p expression.

The JAK/STAT pathway is one of the most important and active signaling pathways in cells, and it has been reported to be involved in various physiological processes, such as cell growth, differentiation, and immune function.[1,33] As reported by a major study, inhibition of miR-135b-5p can protect against the myocardial I/R injury, and JAK2 was shown to be its target gene in the progress.[24] Furthermore, Sima et al[34] reported that JAK2/STAT3 pathway made great contribution to the protective effect of devoflurane on liver I/R injury in rats. Considering the crucial role of miR-135-5p and JAK2/STAT3 pathway in the protective role of anesthesia in I/R injury, we further explored their role in liver I/R injury. In the present study, the bioinformatics analysis and luciferase reporter gene assay indicated that JAK2 was a target gene of miR-135b-5p. We inferred that desflurane attenuated liver I/R injury via regulating the miR-135b-5p expression, and JAK2 was the target gene of miR-135b-5p in the process. The JAK/STAT signaling pathway is responsible for the signal transduction from the plasma membrane to the nucleus, and exerts a crucial influence on the pathogenesis of I/R injury.[35,36] The JAK family contains several members, including JAK1, JAK2, JAK3 and TYK, while the STAT family consists of STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. But the JAK2/STAT3 signal pathway draws much attention, which have been reported to have a significant impact on several conditions such as cardiac, renal and cerebral I/R injury.[37–39] Recently, Sima et al[34] reported that sevoflurane can improve liver I/R injury and reduce liver immune inflammation in rats, and the mechanism is probably related to the activation of JAK2/STAT3 pathway. The present results showed that desflurane attenuated liver I/R injury via regulating the miR-135b-5p expression, and JAK2 was found to be the target gene of miR-135b-5p. Furthermore, it is also observed that miR-135b-5p overexpression significantly decreased the levels of p-JAK2, JAK2, P-STAT3. Thus, we deduced that the JAK2/STAT3 signaling pathway might play a crucial role in the protective effect of desflurane on liver I/R injury, but further research is needed to verify the hypothesis.

In conclusion, the present results suggested that desflurane attenuated liver I/R injury through downregulation of miR-135b-5p, and JAK2 was the target gene of miR-135b-5p. These findings could provide insight into the molecular mechanisms whereby desflurane attenuated the liver I/R injury, and provide new avenues for further development of therapeutic strategies in liver injury.

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