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LncRNA MALAT1 cessation antagonizes hypoxia/reoxygenation injury in hepatocytes by inhibiting apoptosis and inflammation via the HMGB1-TLR4 axis

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ABSTRACT

Hepatic ischemia-reperfusion (I/R) injury frequently occurs after liver transplantation, stroke, and trauma, resulting in organ dysfunction and failure. Hepatocyte apoptosis and inflammation are identified as the hallmarks of liver I/R injury. Long non-coding RNA (lncRNA) metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is induced following hypoxia or ischemic stimulation, and exerts the contradictory roles in various injury progression. However, its role and mechanism lying beneath hepatic I/R remains ill defined. In this study, elevation of MALAT1 expression was corroborated in human hepatocytes under hypoxia/reoxygenation (H/R) H/R condition. Of interest, depression of MALAT1 blunted H/R-inhibited cell viability, and counteracted lactate dehydrogenase (LDH) and malondialdehyde release. Additionally, MALAT1 cessation antagonized H/R-evoked cell apoptosis and caspase-3 activity. Simultaneously, the increased inflammatory reaction triggered by H/R stimulation was also abrogated following MALAT1 suppression by reducing pro-inflammatory cytokine transcripts and productions including IL-1β and TNF-α. Mechanistically, H/R exposure activated the pathway of high-mobility group box1 (HMGB1)-TLR4, which was muted after MALAT1 inhibition. More importantly, elevation of HMGB1 reversed MALAT1 down-regulation-mediated inhibition in cell injury and inflammation. Moreover, blocking the TLR4 signaling also ameliorated H/R-evoked hepatocyte apoptosis and inflammatory response. Consequently, these data suggest that MALAT1 may aggravate hepatic I/R injury by regulating the HMGB1-TLR4-triggered cell apoptosis and inflammation, implying a promising therapeutic strategy to fight liver I/R injury.

1. Introduction

Hepatic ischemia and reperfusion (I/R) injury is a common and inevitable clinical complication of hepatic transplantation, liver resection and hemorrhagic shock(Zhai et al., 2013). More precisely, in a case of liver transplantation, occurrence of I/R injury evoked up to 10% of early graft failure and higher incidence of rejection in acute and long-term. It is generally believed that I/R damage is negatively associated with patient survival and prognosis(Foley et al., 2011). Although mortality attributable to acute hepatic event has declined substantially with advance in current reperfusion therapies, liver I/R damage remains a major unsolved public health problem with the elusive mechanism.

During liver I/R injury, ischemic insult not only incurs in direct cellular damage, but also evokes acute inflammatory response that will further aggravates hepatocellular injury, organ dysfunction and failure. Abundant report confirms that toll-like receptor-4 (TLR-4)-triggered innate immune response is evoked upon hepatic I/R injury and resulting in inflammatory cytokine production by activating the nuclear factor kappa B (NF-κB)(He et al., 2016; Yang et al., 2017). Convincing research has corroborated the potential therapeutic efficacy against hepatic I/R injury by targeting TLR4-mediated inflammation(He et al., 2016; Kadono et al., 2017). High-mobility group box1 (HMGB1), a common pro-inflammatory mediator, is recently identified as a key endogenous TLR-4 ligand in various tissues, such as kidney, lung and liver(Tsung et al., 2005; Yang et al., 2013; Zhang et al., 2016). Recent evidence demonstrates that HMGB1 exerts the critical roles in pathogenic progression of hepatic I/R injury in a TLR4-dependent manner (Tsung et al., 2005; Yu et al., 2017).

Long non-coding RNAs (lncRNAs) are generally defined as the class

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of non-protein-coding RNAs that exceed 200 nucleotides in length. Specifically, lncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) has become a subject of interest due to its pivotal roles in various progression of diseases associated with inflammation, injury and cancer(Gao et al., 2017; Gordon et al., 2018; Yu et al., 2015; Zhao et al., 2017). MALAT1 is localized on chromosome 11q13.1. and is validated to be widely expressed in many tissues. Though abundant evidence has been performed, the role of MALAT1 in I/R injury is contradictory. For instance, MALAT1 is induced by hypoxia or ischemic stimulation, which enhances NLRP3 inflammasome expression and myocardial I/R injury; whilst, its down-regulation ameliorates the injury process(Yu et al., 2018). Conversely, MALAT1 protects brain microvascular endothelial cell injury against oxygen-glucose deprivation/reoxygenation-mimic I/R injury(Li et al., 2017). Up to now, the function of MALAT1 in liver I/R injury is still undefined. The injury of liver cells induced by hypoxia/reoxygenation is widely applied as an *in vitro* model for studying hepatic I/R injury(He et al., 2016; Tsung et al., 2005). Thus, we aimed to investigate the role of MALAT1 in hepatocyte apoptosis and inflammation during simulated ischemia–reperfusion injury by exposing to hypoxia/reoxygenation (H/R) condition. Additionally, the underlying molecular mechanism was also explored.

2. Materials and methods

2.1. Cell culture

Normal human hepatocyte cell line HL- 7702 was applied and obtained from the Chinese Academy of Science Type Culture Collection (Shanghai, China). Cells were grown in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and antibiotics consisted of 100 U/mL penicillin and 100 μg/ml streptomycin. For incubation, all cells were housed at 37 ˚C in a 5% CO2 incubator.

2.2. Construction of HMGB1 recombinant plasmid

Total RNA from HL-7702 was prepared using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), and then was applied to generate the first cDNA by the SuperScript II First Strand Synthesis System (Invitrogen). Human full-length HMGB1 cDNA was then performed by the PCR amplification. After enzyme digestion, the obtained cDNA was inserted into pCDNA3.1(+) construct (Invitrogen) to construct the recombinant pcDNA-HMGB1 plasmid. For transfection, 0.5 μg of recombinant vector was transfected into HL-7702 cells with 70–80% confluence using the Lipofectamine 2000 (Invitrogen). The empty plasmid-transfected group was defined as the negative control.

2.3. Knockdown of target gene expression by siRNA transfection

To specially knock down expression of MALAT1 and TLR4, a siRNA-based technique was carried out. The siRNA sequences targeting MALAT1, TLR4 and the corresponding scramble siRNA were designed as formerly reported(Liu et al., 2016; Wang et al., 2016). The scramble siRNA was defined as the negative control. All siRNA sequences were synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). For transfection, 50 nM of siRNA and negative control were transfected into cells using Lipofectamine 2000 (Invitrogen). The transfected efficiency was then evaluated by qRT-PCR and western blotting at 48 h after incubation.

2.4. Model of simulated ischemia/reperfusion (H/R) in vitro

HL-7702 cells were preconditioned with pcDNA-HMGB1, pcDNA vector, si-MALAT1, si-TLR4 or si-NC, and then were subjected to hypoxia/re-oxygenation to simulate ischemia/reperfusion injury. For H/R exposure, cells were incubated in a chamber containing 1% O2, 94% N2 and 5% CO2 for 2, 4, 8, 12 and 24 h to mimic ischemia. Subsequently,

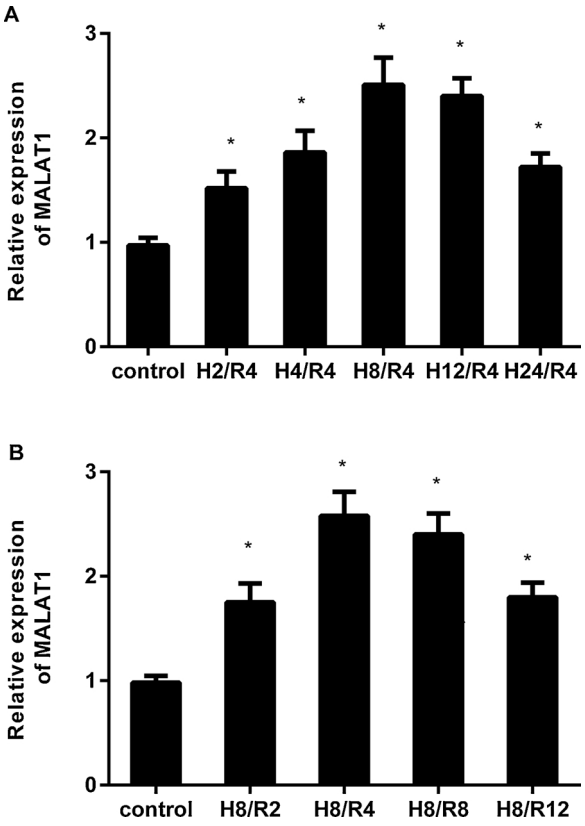

Fig. 1. High expression of MALAT1 in hepatocytes upon H/R. (A–B) Human hepatocytes were exposed to hypoxia (H) for 2, 4, 8, 12 and 24 h and re-oxygenation (R) for 2, 4, 8 and 12 h. Then, the expression of MALAT1 was detected by qRT-PCR. $^{*}P < 0.05$.

re-oxygenation was conducted in RPMI-1640 medium supplemented with 10% FBS for 2, 4, 8 and 12 h to simulate reperfusion. All procedures were performed at 37℃.

2.5. RNA extraction and quantitative RT-PCR (qRT-PCR)

Cells under various si-RNA transfections were exposed to H/R. Total RNA was prepared using the TRIzol Reagent (Invitrogen), and then was applied to generate the first cDNA using the SuperScript II First Strand Synthesis System (Invitrogen). After that, qRT-PCR was conducted to detect the transcription levels of MALAT1, IL-1β and TNF-α using the SYBR Premix Ex Taq™ II Kit (Takara Bio Inc., Otsu, Japan) in accordance with the manufacturer's guidelines. To amplify the target of MALAT1, the specific primer was referred as previous reported(Wang et al., 2016). Whilst, the specific oligonucleotide primers were as follows: IL-1β (sense, 5′-CAAAGGCGGCCAGGATATAA-3′; anti-sense, 5′-CTAGGG ATTGAGTCCACATTCAG-3′), TNF-α (sense, 5′-GGATGGATGGAGGTGA AAGTAG-3′; anti-sense, 5′-TGATCCTGAAGAGGAGAGAGAA-3′), and HMGB1 (5′-CACTCTCTGCCTGCATCTAATC-3′; anti-sense, 5′-GCTATC TTTCCCTCCCATCAC-3′). All specimens were analyzed on the ABI PRISM 7000 sequence detection system (Applied Biosystems) to calculate the relative expression of target gens using the $2^{-\Delta\Delta Ct}$ method. For normalization, β-actin was used as a internal standard.

2.6. Assay of cell viability

Cells were seeded into a 96-well plate at the density of 1×10^4 cells/well in a volume of 200 μl. Cells transfected with various siRNA were incubated under H/R condition. Then, of 0.5 mg/ml MTT solution (100 μl, Sigma, St. Louis, MO, USA) was supplemented into the culture medium for 3 h. After removing the supernatants, 100 μl of DMSO was

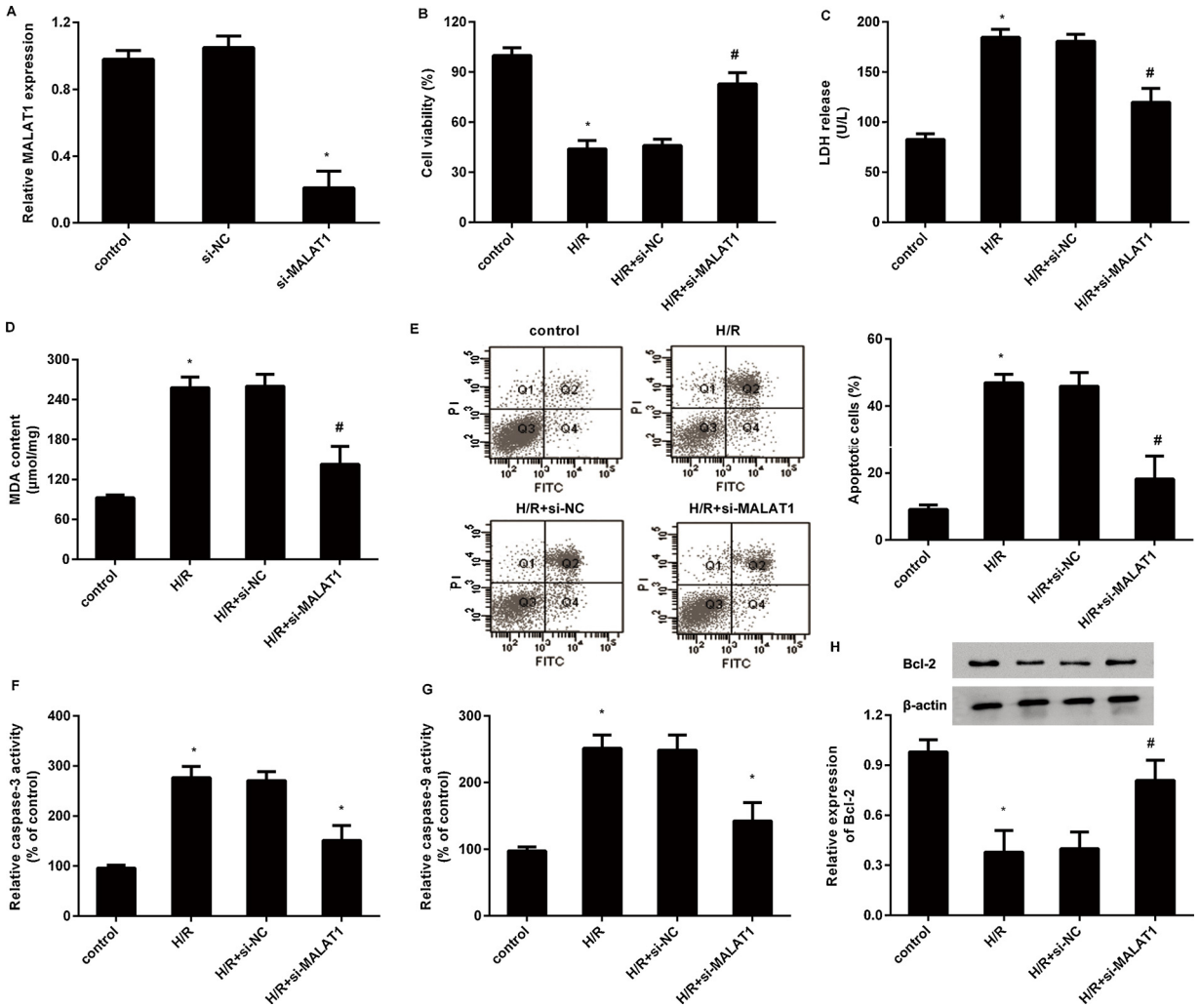

Fig. 2. Effects of MALAT1 inhibition on cell injury under H/R exposure. (A) After transfection with si-MALAT1 or si-NC, efficacy was evaluated by qRT-PCR analysis. (B) Cells were preconditioning with si-MALAT1, prior to incubation under H/R condition. Then, cell viability was analyzed by MTT. (C–F) The subsequent effects on LDH release (C), MDA production (D), cell apoptosis (E) and caspase-3 activity (F) were evaluated. (G) The activity of caspase-9 was determined by a commercial kits. (H) The expression of Bcl-2 protein was evaluated by western blotting. $^{*}P < 0.05$ vs. control group. $^{\#}P < 0.05$ vs. H/R-treated group.

performed to dissolve the formazan precipitate. The absorbance per well was determined at 570 nm using the microplate reader. Relative cell viability was shown as the absorbance percentage of the treated group to that of control group.

2.7. Lactate dehydrogenase (LDH) release analysis

The contents of LDH in cells were conducted using the commercial LDH Detection kit (Beyotime Biotechnology, Shanghai, China) according to the protocol provided by the manufacturers. After treatment with the indicated conditions, 120 μl of culture supernatants were seeded into 96-well plate, and 60 μl of LDH detection solution was added for further incubation under dark. Approximately 30 min later, the absorbance at 490 nm was measured to calculate LDH levels.

2.8. Evaluation of malondialdehyde (MDA) content

Cells under various treatments were lysed with RIPA lysis buffer, and then were centrifuged at 1600 g for 10 min. The collected supernatants were mixed with 200 μl of MDA reaction solution at 100℃ for 15 min. Then, the samples were cold and centrifuged. The absorbance was captured at 532 nm to detect the content of MDA.

2.9. Estimation of cell apoptosis

The double staining with Annexin V/Propidium iodide (PI) was performed to assess cell apoptosis. Briefly, cells under various groups were rinsed and re-suspended with 500 μl Binding buffer. Then, 10 μl Annexin V-FITC and 5 μl PI (Beyotime) were supplemented into each well for incubation at room temperature. After 15 min reaction under dark, the percentages of apoptotic cells were analyzed by a FACScan flow cytometer (BD Biosciences, San Jose, CA).

2.10. Measurement of caspase-3 and caspase-9 activity

Following treatment with the lysis buffer, cells in various groups were centrifuged for 15 min at 4 ˚C. Then, the activity of caspase-3 and caspase-9 in supernatants was determined in line with the instructions of the commercial Kits (Beyotime). The specific substrates of Ac-DEVD-pNA for caspase-3 and Ac-LEHD-pNA for caspase-9 were added. Then, the absorbance at 405 nm was captured to evaluate the activity of caspase-3 and caspase-9.

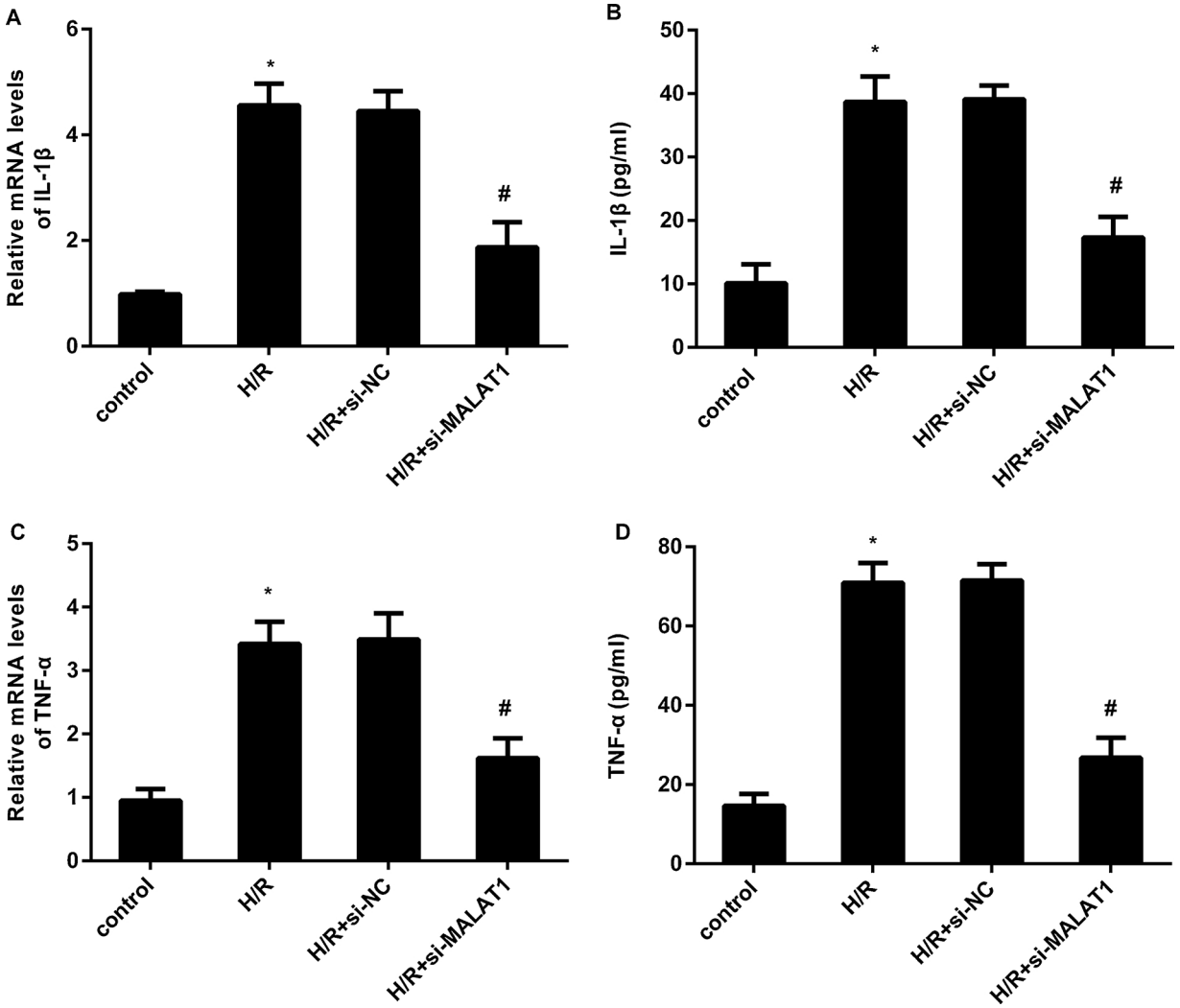

Fig. 3. Depression of MALAT1 weakened H/R-induced inflammation. (A) Cells were pre-transfected with si-MALAT1, and then were exposed to H/R. After that, the mRNA levels of pro-inflammatory cytokine IL-1β were determined. (B) The contents of IL-1β in supernatants were analyzed by ELISA. (C, D) The transcript (C) and production (D) of TNF-α were evaluated after MALAT1 suppression in cells upon H/R condition. $^{*}P < 0.05$ vs. control group. $^{\#}P < 0.05$ vs. H/R-treated group.

2.11. Detection of inflammatory cytokine levels and HMGB1 release by ELISA assay

After collection, cells were lysed and centrifuged. The contents of inflammatory cytokines IL-1β and TNF-α in supernatants were detected using the commercial ELISA kits (Invitrogen). The concentration of HMGB1 in supernatants were measured using a HMGB1 ELISA kits (Shanghai future industrial co., LTD, Shanghai, China). All experiments were carried out as per the manufacturer's standard protocols.

2.12. Western blotting analysis

To extract total protein, RIPA buffer was introduced, and the protein concentration was then determined using the BCA Protein Detection Kit (Pierce, Rockford, IL, USA). The equal contents of protein were loaded onto 12% SDS-PAGE to separate protein, followed by the transferred to the PVDF membrane (Millipore, Billerica, MA, USA). After incubation with 5% non-fat milk to interdict the non-specific bind, the primary antibodies against human HMGB1, TLR4 and p-p65NF-κB (all from Abcam, Cambridge, UK, USA) were added and incubated at 4 °C overnight. Then, horseradish peroxidase-conjugated secondary antibodies were supplemented for further incubation for 1 h. The immunoreactive bands were ultimately visualized by the ECL chemiluminescent detection system(Beyotime). The protein expression was normalized to β-actin. The immunostained proteins were analyzed by Gel DocTM XR imaging system (Bio-Rad Laboratories, Hercules, CA, USA), and band intensities were quantified by Image J software.

2.13. Statistical analysis

All results were obtained from at least three independent experiments and presented as the mean ± standard deviation (SD). The statistical calculations were conducted using SPSS 19.0 (SPSS Inc., Chicago, IL). Student's *t*-test was used to compare the difference between two groups. The one-way ANOVA was introduced for the comparison of three or more groups, followed by the post-hoc Student-Newman–Keuls (SNK) test.

3. Results

3.1. Exposure to H/R elicits the up-regulation of MALAT1 in human hepatocytes

To gain insight into the potential function of MALAT1 in the progression of liver I/R injury, human normal hepatocyte cell line HL7702 was exposed to hypoxia–reoxygenation (H/R) to simulate I/R injury *in*

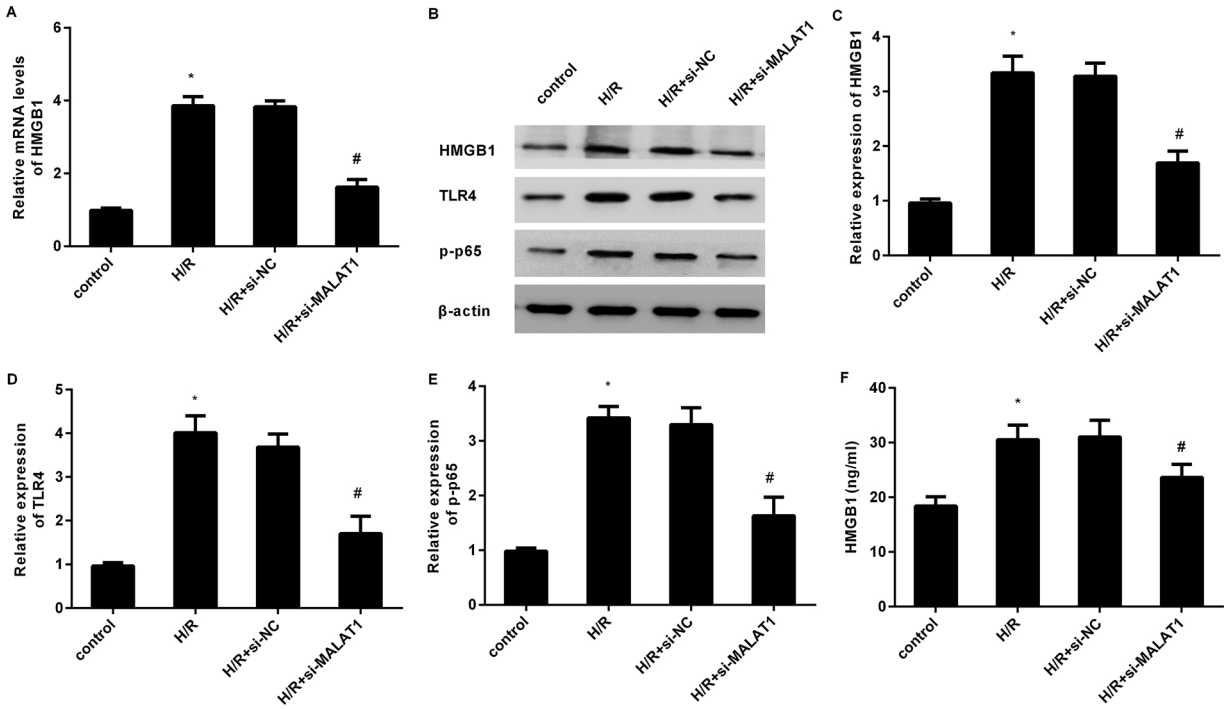

Fig. 4. MALAT1 cessation counteracted the activation of HMGB1-TLR4 signaling triggered by H/R exposure. (A) Following transfection with si-MALAT1, hepatocytes were cultured under H/R condition. The expression of MALAT1 was determined by qRT-PCR. (B) Then, the protein levels of HMGB1, TLR4 and p-p65 NF-κB were measured by western blotting. (C–E) The quantified analysis of HMG1 (C), TLR4 (D) and p-p65 NF-κB (E) proteins were performed by Image J software. (F) The concentration of HMGB1 in supernatants was measured by a commercial ELISA kit. $^{*}P < 0.05$. $^{\#}P < 0.05$.

vitro. QRT-PCR analysis corroborated that the expression of MALAT1 was increased following the increased times for hypoxia and subsequent reoxygenation relative to the control group, and reached into a point after hypoxia for 8 h and reoxygenation for 4 h (Fig. 1A). Furthermore, after exposure to hypoxia for 8 h, the expression of MALAT1 was elevated following the increasing times for reoxygenation, and the highst levels of MALAT1 was found in H8/R4 groups (Fig. 1B). Therefore, the hypoxia for 8 and reoxygenation for 4 h was chosen for the subsequent experiments.

3.2. Cessation of MALAT1 protects against H/R-induced injury and apoptosis

We next assessed the effect of MALAT1 on H/R-evoked hepatocyte cell injury. As presented in Fig. 2A, transfection with si-MALAT1 notably inhibited the expression of MALAT1 in HL7702 cells. In striking contrast, cessation of MALAT1 greatly suppressed the adverse effect of H/R on cell viability (Fig. 2B). Furthermore, in contrast to the control group, H/R stimulation enhanced LDH release into supernatants, which was reversed after MALAT1 deficiency (Fig. 2C). Simultaneously, inhibition of MALAT1 also antagonized H/R-increased production of MDA, a marker for cell oxidative stress (Fig. 2D). Further analysis confirmed that suppression of MALAT1 weakened H/R-triggered cell apoptosis (Fig. 2E). Analogously, H/R exposure-evoked increase in caspase-3 activity was also abrogated when cells were pre-transfected with si-MALAT1 (Fig. 2F). Additionally, knockdown of MALAT1 inhibited H/R-increased caspase-9 activity in contrast to H/R groups (Fig. 2G). Simultaneously, the expression of anti-apoptotic protein Bcl-2 was suppressed when cells were exposed to H/R condition, which was reversed following MALAT1 depression (Fig. 2H).

3.3. MALAT1 deficiency inhibits the inflammatory response in hepatocytes upon H/R condition

Convincing evidence indicates that hepatic I/R injury can activate the inflammatory response, which will further aggravate hepatocellular damage(Inoue et al., 2013; van Golen et al., 2013). We next evaluated the role of MALAT1 inhibition in H/R-induced inflammation. As shown in Fig. 3A, the high transcript levels of inflammatory cytokine IL-1β were validated in HL 7702 cells upon H/R condition. Notably, suppression of MALAT1 adversely weakened the increase in IL-1β mRNA levels. Moreover, transfection with si-MALAT1 offset the augment production of IL-1β in cells upon H/R condition (Fig. 3B). Similarly, H/R-evoked high expression of TNF-α mRNA decreased after MALAT1 down-regulation (Fig. 3C). Concomitantly, the high release of TNF-α triggered by H/R were also attenuated following si-MALAT1 transfection (Fig. 3D).

3.4. Down-regulation of MALAT1 mutes H/R-triggered activation of the HMGB1-TLR4 signaling

The HMGB1-TLR4 pathway has been widely implicated in various I/R progression, including liver I/R injury(Tsung et al., 2005; Zhang et al., 2016). To explore the mechanism underlying MALAT1 cessation-mediated hepatoprotective effect, western blotting assay was performed to detect the activation of the HMGB1-TLR4 signaling. As presented in Fig. 4A, the high levels of HMGB1 mRNA were observed in hepatocytes upon H/R condition, which was attenuated following MALAT1 suppression. Furthermore, H/R treatment obviously induced approximately 3.12-fold increase in HMGB1 protein expression (Fig. 4B and C), which was reversed after MALAT1 silencing. Furthermore, the high expression of TLR4 (Fig. 4B and D) and its downstream p-p65 NF-κB (Fig. 4B and E) were also impeded when cells upon H/R were pretreated with si-MALAT1 knockdown. Additionally, H/R-evoked release of HMGB1 into supernatants of hepatocytes were inhibited after MALAT1 down-regulation (Fig. 4F). These findings suggest that MALAT1 suppression blunts the activation of the HMGB1-TLR4 signaling induced by H/R exposure.

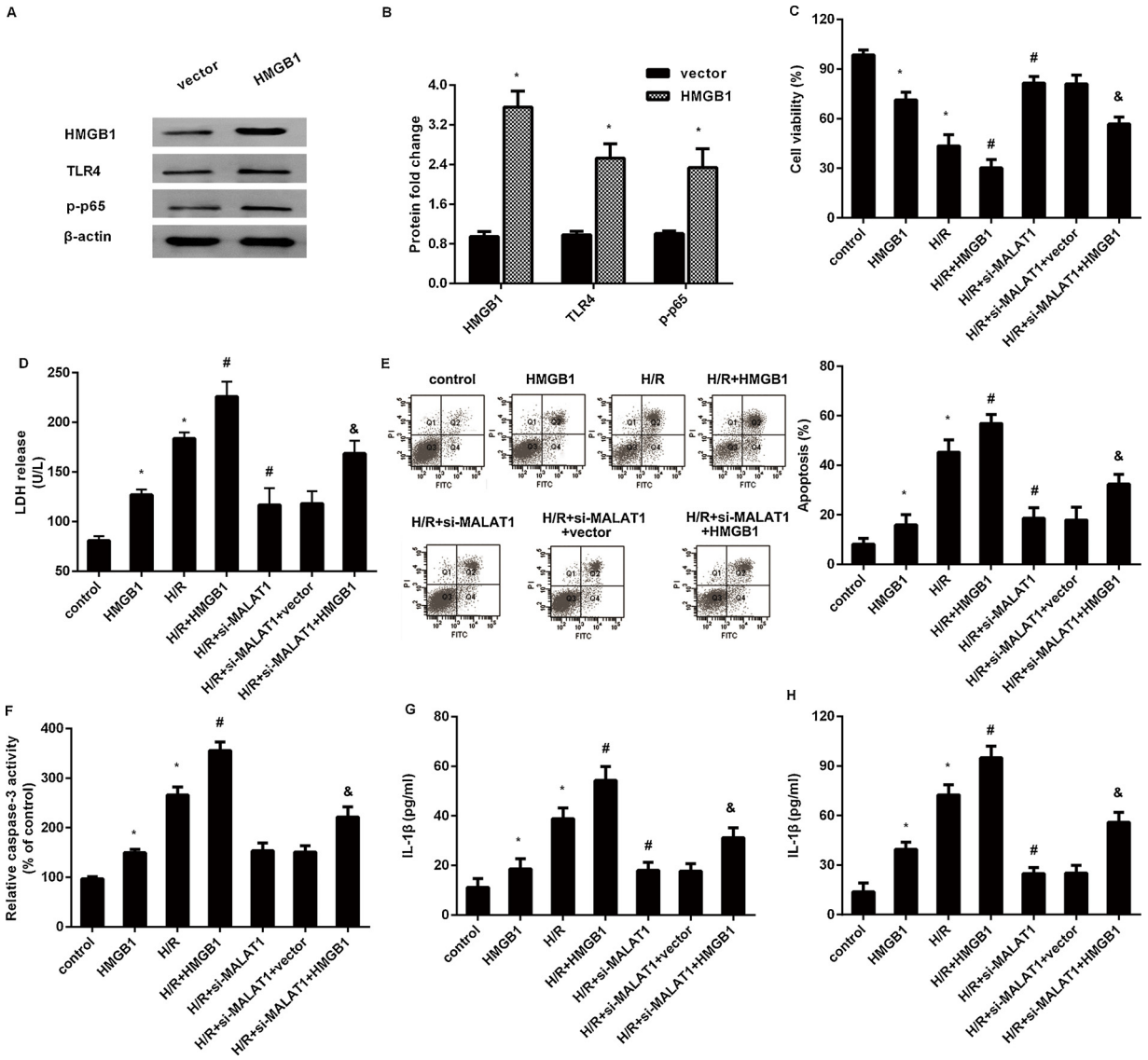

Fig. 5. Elevation of HMGB1 reversed MALAT1 suppression-mediated hepatoprotective function. (A) Effects of recombinant HMGB1 plasmid transfection on the expression of HMGB1 and subsequent activation of TLR4 signaling. (B) The quantified evaluation of HMGB1, TLR4 and p-p65 NF-κB protein expression. (C) Cells with HMGB1 overexpression were treated with si-MALAT1 transfection, then cell viability upon H/R exposure was detected. (D–F) The subsequent effects on LDH release (D), cell apoptosis (E) and caspase-3 activity (F) were carried out. (G, H) The contents of IL-1β (G) and TNF-α (H) in supernatants from various groups were measured using the ELISA kits. $^{*}P < 0.05$ vs. control group. $^{\#}P < 0.05$ vs. H/R-treated group. $^{\&}P < 0.05$ vs. H/R + si-MALAT1 groups.

3.5. Enhancement of HMGB1 expression reverses the protective effects of MALAT1 suppression against H/R-evoked hepatocyte injury and inflammation

To elucidate the question whether MALAT1 suppression protect against liver I/R injury by regulating the HMGB1 signaling, we increased the protein expression of HMGB1 by transfection with a recombinant pcDNA-HMGB1 plasmid (Fig. 5A and 5B). Furthermore, HMGB1 elevation also enhanced the subsequent expression of TLR4 and p-p65 NF-κB (Fig. 5A and B). More importantly, the protective role of MALAT1 cessation in ameliorating H/R-inhibited cell viability was muted following HMGB1 overexpression (Fig. 5C). Simultaneously, MALAT1 suppression dampened H/R-triggered LDH release, which was offset by HMGB1 elevation (Fig. 5D). Additionally, enhancement of HMGB1 expression also abrogated the inhibitory effects of MALAT1 inhibition on H/R-evoked cell apoptosis (Fig. 5E) and caspase-3 activity (Fig. 5F). Furthermore, MALAT1 cessation-mediated suppression in IL-1β (Fig. 5G) and TNF-α (Fig. 5H) triggered by H/R stimulation was also overturned following HMGB1 overexpression.

3.6. Blocking the TLR4 signaling attenuates the effects of H/R on cell injury and inflammation

We further clarified the role of TLR4 signaling in hepatocyte SI/T injury. As shown in Fig. 6A, transfection with si-TLR4 notably restrained TLR4 expression, concomitant with the reduction in the expression of subsequent p-p65 NF-κB. Functional assay corroborated that blocking the TLR4 signaling attenuated the adverse effect of H/R on cell viability (Fig. 6B). Furthermore, inhibition of TLR4 pathway also antagonized H/R-induced LDH release (Fig. 6C) and cell apoptosis (Fig. 6D). Additionally, the high generation of inflammatory cytokines IL-1βand TNF-α (Fig. 6E) was repressed after TLR4 depression.

4. Discussion

Hepatic ischemia-reperfusion (I/R) injury ranks as the initial culprit that constitutes a worldwide threat for liver transplantation failure. This pathogenesis predisposes graft to dysfunction, resulting in poor prognosis(Foley et al., 2011; Zhai et al., 2013). Nevertheless, the exact

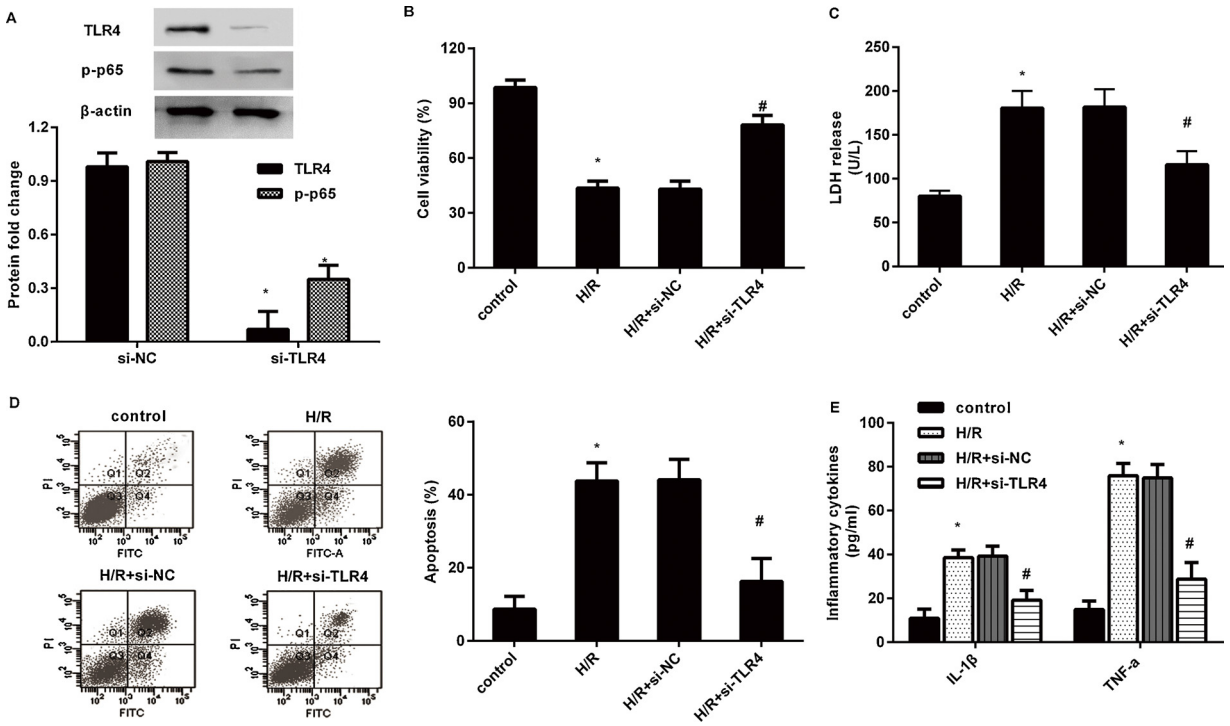

Fig. 6. Blocking the TLR4 signaling reversed H/R-evoked cell apoptosis and inflammation. (A) Cells were transfected with si-TLR4, and the transfected efficacy was subsequently evaluated. Quantified results of TLR4 and p-p65 NF-κB expression. (B–D) After silencing the TLR4 expression, the effects of H/R on cell viability (B), LDH release (C) and cell apoptosis (D) were analyzed. (E) ELISA assay was performed to determine the concentrations of IL-1β and TNF-α in TLR4-inhibited cells upon H/R condition. $^{*}P < 0.05$. $^{\#}P < 0.05$.

mechanisms orchestrating the progression of hepatic I/R injury remains poorly defined. Recent report has highlighted the pivotal roles of lncRNAs in various tissue I/R injury(Zhao et al., 2017). In the present study, we unveiled the role of MALAT1 in liver I/R injury *in vitro*, and substantiated the high expression of MALAT1 in human hepatocyte upon H/R condition. More importantly, cessation of MALAT1 antagonized H/R-induced cell apoptosis and inflammation. Moreover, the HMGB1-TLR4 pathway might account for these processes.

Hepatocyte apoptosis is proverbially characterization of hepatic I/R injury. It is a fact that liver ischemia initiates shortly cell apoptosis, which will be further amplified after reperfusion. Convincing evidence documents that muting the apoptotic process can minimize liver injury evoked by I/R, and slow the risk of hepatic failure(Tao et al., 2014; Zhang et al., 2017). MALAT1 is an evolutionarily conserved lncRNA and exerts the critical roles in a wide spectrum of biological activities in regulating oxidative stress injury, cancer and cardiovascular disease (Gao et al., 2017; Gordon et al., 2018; Yu et al., 2015; Zhao et al., 2017). Intriguingly, recent study has corroborated that depletion of MALAT1 blunts liver fibrosis by suppressing hepatic stellate cell activation and collagen deposits(Yu et al., 2015). Furthermore, MALAT1 promotes hepatic steatosis and insulin resistance(Yan et al., 2016). Here, similar with previous report in heart(Zhao et al., 2017), the current study confirmed the high expression of MALAT1 in hepatocytes upon H/R stimulation. More importantly, cessation of MALAT1 blunted H/R-inhibited cell viability, but dampened H/R-triggered LDH and MDA release, both the markers of cell injury. Additionally, cells transfected with MALAT1 were less susceptible to H/R-triggered cell apoptosis and caspase-3 activity. These finding convers the potential protective role of MALAT1 inhibition in hepatic I/R injury. Analogously, silencing the high expression of MALAT1 attenuated cardiomyocyte apoptosis in myocardial I/R injury(Zhao et al., 2017). Inversely, MALAT1 may act as a potent autophagy inducer to protect brain microvascular endothelial cell against oxygen-glucose deprivation/reoxygenation-induced damage(Li et al., 2017).

Liver inflammation principally occurs in acute hepatic failure, a proverbial life-threatening disease. During the progression of hepatic I/R injury, hepatocyte apoptosis will trigger an inflammatory response, which further aggravates cell injury. Acceptably, convincing evidence supports the potential strategy against hepatic I/R injury by slowing the inflammation(Tao et al., 2014; Zhang et al., 2017). For instance, methylprednisolone treatment minimizes liver I/R injury through inhibiting inflammation and apoptosis(Zhang et al., 2017). Recent study corroborated that MALAT1 was endowed to regulate inflammatory response in various pathogenic processes(Gordon et al., 2018). For experiment on LPS-induced septic cardiomyocytes, MALAT1 enhances inflammatory cytokine TNF-α expression and subsequent cell apoptosis (Zhuang et al., 2017). Furthermore, MALAT1 also acts as a positive regulator of inflammation in diabetic complications(Gordon et al., 2018). These observations led us to postulate whether MALAT1 also involves in H/R-induced inflammatory response. As expected, suppression of MALAT1 counteracted the transcription and production of L-1β and TNF-α in hepatocytes upon H/R condition. Analogously, MALAT1 elevated hyperglycaemia-induced inflammatory mediators IL-6 and TNF-α expression(Puthanveetil et al., 2015). Of interest, in macrophages, knockdown of MALAT1 increased LPS-triggered expression of IL-6 and TNF-α expression(Zhao et al., 2016).

We next deciphered the mechanism lying beneath the process of MALAT1 depression-mediated hepatoprotective role, and confirmed that the activation of HMGB1-TLR4 signaling in H/R-exposed hepatocytes were overturned following MALAT1 depression. TLR4 is widely present in many kinds of cells in liver and is proved to be associated with the progression of liver I/R injury(Kadono et al., 2017; Yang et al., 2013). Increasing evidence has documented that deficiency of TLR4 signaling exerts the beneficial effects on ameliorating hepatic I/R damage(He et al., 2016; Kadono et al., 2017). Recently, HMGB1 has been identified as a endogenous TLR-4 ligand in liver and act as a mediator to participate in injury and inflammation in hepatic I/R injury(Tsung et al., 2005). Blocking the HMGB1-TLR4 pathway ameliorates liver

warm I/R-induced lung injury(Yu et al., 2017). The latest research has substantiated that MALAT1 elevates HMGB1 expression in several carcinomas(Gao et al., 2017). Hence, we were compelled to investigate the correlation between this pathway and MALAT1-regulated hepatocyte I/R injury and inflammation. In striking contrast, HMGB1 elevation blunted the protective function of MALAT1 inhibition against H/R-induced hepatocyte apoptosis and inflammation. Additionally, abrogating the TLR4 signaling attenuated H/R-evoked hepatocyte injury and inflammation. These findings suggest that MALAT1 may regulate H/R-induced cell injury and inflammation in hepatocytes by mediating the HMGB1-TLR4 signaling. Intriguingly, targeting the HMGB1-TLR4 pathway also alleviated renal I/R inflammatory injury(Zhang et al., 2016). However, HMGB1 overexpression could not fully abrogated the effects of MALAT1 knockdown on attenuating hepatocyte injury and inflammatory response. Whether other pathways are also involved in this processes, which needs to be further explored in our future.

Collectively, the present research highlighted the high expression of MALAT1 in hepatocytes upon H/R treatment. Notably, depression of MALAT1 afforded a prominent hepatoprotective intervention in response to H/R-induced cell injury and inflammatory response by regulating the HMGB1-TLR4 signaling. These findings may elucidate the mechanism about how MALAT1 aggravates the development of hepatic I/R by promoting cell injury and inflammation. Consequently, these findings support a notion that targeting MALAT1 may be a novel strategy to fight hepatic I/R damage. However, whether the similar finding will be confirmed in primary hepatocytes. Can MALAT1 depression acquire the ideal effects *in vivo* via the HMGB1-TLR4 signaling? These questions will be r elaborated in our next plan.

Declaration of interest

None.

Compliance with ethical standards

No human and animal experiments were performed.

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References

Foley, D.P., Leverson, G., Mezrich, J.D., et al., 2011. Hepatic ischemia reperfusion injury: an independent predictor of long-term patient and graft survival after liver transplantation. J. Surg. Res. 165 332-332.
Gao, D., Lv, A.E., Li, H.P., et al., 2017. LncRNA MALAT-1 elevates hmgb1 to promote autophagy resulting in inhibition of tumor cell apoptosis in multiple myeloma. J. Cell. Biochem. 118, 3341–3348.
Gordon, A.D., Biswas, S., Feng, B., et al., 2018. MALAT1: a regulator of inflammatory cytokines in diabetic complications. Endocrinol. Diabetes Metab. e00010.
He, D., Guo, Z., Pu, J.L., et al., 2016. Resveratrol preconditioning protects hepatocytes against hepatic ischemia reperfusion injury via Toll-like receptor 4/nuclear factor-kappaB signaling pathway in vitro and in vivo. Int. Immunopharmacol 35, 201–209.
Inoue, Y., Yasuda, Y., Takahashi, M., 2013. Role of the inflammasome in inflammatory responses and subsequent injury after hepatic ischemia-reperfusion injury. Hepatology 58, 2212.
Kadono, K., Uchida, Y., Hirao, H., et al., 2017. Thrombomodulin attenuates inflammatory damage due to liver ischemia and reperfusion injury in mice in toll-Like receptor 4-Dependent manner. Am. J. Transplant. 17, 69–80.
Li, Z., Li, J., Tang, N., 2017. Long noncoding RNA Malat1 is a potent autophagy inducer protecting brain microvascular endothelial cells against oxygen-glucose deprivation/reoxygenation-induced injury by sponging miR-26b and upregulating ULK2 expression. Neuroscience 354, 1–10.
Liu, Y., Li, T., Xu, Y., et al., 2016. Effects of TLR4 gene silencing on the proliferation and apotosis of hepatocarcinoma HEPG2 cells. Oncol. Lett. 11, 3054–3060.
Puthanveetil, P., Chen, S., Feng, B., et al., 2015. Long non-coding RNA MALAT1 regulates hyperglycaemia induced inflammatory process in the endothelial cells. J. Cell. Mol. Med. 19, 1418–1425.
Tao, T., Chen, F., Bo, L., et al., 2014. Ginsenoside Rg1 protects mouse liver against ischemia-reperfusion injury through anti-inflammatory and anti-apoptosis properties. J. Surg. Res. 191, 231–238.
Tsung, A., Sahai, R., Tanaka, H., et al., 2005. The nuclear factor HMGB1 mediates hepatic injury after murine liver ischemia-reperfusion. J. Exp. Med. 201, 1135–1143.
van Golen, R.F., Reiniers, M.J., Olthof, P.B., et al., 2013. Sterile inflammation in hepatic ischemia/reperfusion injury: present concepts and potential therapeutics. J. Gastroenterol. Hepatol. 28, 394–400.
Wang, S.H., Zhang, W.J., Wu, X.C., et al., 2016. Long non-coding RNA Malat1 promotes gallbladder cancer development by acting as a molecular sponge to regulate miR-206. Oncotarget 7, 37857–37867.
Yan, C., Chen, J., Chen, N., 2016. Long noncoding RNA MALAT1 promotes hepatic steatosis and insulin resistance by increasing nuclear SREBP-1c protein stability. Sci. Rep. 6, 22640.
Yang, Z., Deng, Y., Su, D., et al., 2013. TLR4 as receptor for HMGB1-mediated acute lung injury after liver ischemia/reperfusion injury. Lab. Invest. 93, 792–800.
Yang, H., Zhou, H., Zhuang, L., et al., 2017. Plasma membrane-bound G protein-coupled bile acid receptor attenuates liver ischemia/reperfusion injury via the inhibition of toll-like receptor 4 signaling in mice. Liver Transpl. 23, 63–74.
Yu, F., Lu, Z., Cai, J., et al., 2015. MALAT1 functions as a competing endogenous RNA to mediate Rac1 expression by sequestering miR-101b in liver fibrosis. Cell Cycle 14, 3885–3896.
Yu, Z., Tong, Y., Zhang, R., et al., 2017. Saquinavir ameliorates liver warm ischemia-reperfusion-induced lung injury via HMGB-1- and P38/JNK-mediated TLR-4-Dependent signaling pathways. Mediators Inflamm. 2017, 7083528.
Yu, S.Y., Dong, B., Tang, L., et al., 2018. LncRNA MALAT1 sponges miR-133 to promote NLRP3 inflammasome expression in ischemia-reperfusion injured heart. Int. J. Cardiol. 254, 50.
Zhai, Y., Petrowsky, H., Hong, J.C., et al., 2013. Ischaemia–reperfusion injury in liver transplantation—from bench to bedside. Nat. Rev. Gastroenterol. Hepatol. 10, 79–89.
Zhang, J., Xia, J., Zhang, Y., et al., 2016. HMGB1-TLR4 signaling participates in renal ischemia reperfusion injury and could be attenuated by dexamethasone-mediated inhibition of the ERK/NF-kappaB pathway. Am. J. Transl. Res. 8, 4054–4067.
Zhang, P., Zhang, M., Wan, M., et al., 2017. Tamoxifen attenuates lipopolysaccharide/galactosamine-induced acute liver failure by antagonizing hepatic inflammation and apoptosis. Immunol. Invest. 46, 284–294.
Zhao, G., Su, Z., Song, D., et al., 2016. The long noncoding RNA MALAT1 regulates the lipopolysaccharide-induced inflammatory response through its interaction with NF-kappaB. FEBS Lett. 590, 2884–2895.
Zhao, Z.H., Hao, W., Meng, Q.T., et al., 2017. Long non-coding RNA MALAT1 functions as a mediator in cardioprotective effects of fentanyl in myocardial ischemia-reperfusion injury. Cell Biol. Int. 41, 62–70.
Zhuang, Y.T., Xu, D.Y., Wang, G.Y., et al., 2017. IL-6 induced lncRNA MALAT1 enhances TNF-alpha expression in LPS-induced septic cardiomyocytes via activation of SAA3. Eur. Rev. Med. Pharmacol. Sci. 21, 302–309.

Glossary

I/R: ischemia-reperfusion
lncRNA: long non-coding RNA
MALAT1: metastasis-associated lung adenocarcinoma transcript 1
H/R: hypoxia/reoxygenation
LDH: lactate dehydrogenase
HMGB1: high-mobility group box1
TLR-4: toll-like receptor-4
NF- B: nuclear factor kappa B
FBS: fetal bovine serum
MDA: malondialdehyde