Original Article

LncRNA-Airn alleviates acute liver injury by inhibiting hepatocyte apoptosis via the NF-κB signaling pathway

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Abstract
Acute liver injury is a common and serious syndrome caused by multiple factors and unclear pathogenesis. If the injury persists, liver injury can lead to cirrhosis and liver failure and ultimately results in the development of liver cancer. Emerging evidence has indicated that long noncoding RNAs (lncRNAs) play an important role in the development of liver injury. However, the role of antisense Igf2r RNA (Airn) in acute liver injury and its underlying mechanism remain largely unclear. In this study, we show that Airn is upregulated in liver tissue and primary hepatocytes from an acute liver injury mouse model. Consistently, Airn is also overexpressed in serum samples of patients with acute-on-chronic liver failure and is negatively correlated with the Model for End-Stage Liver Disease (MELD) score. Moreover, gene knockout and rescue assays reveal that Airn alleviates CCl4-induced liver injury by inhibiting hepatocyte apoptosis and oxidative stress in vivo. Further investigation reveals that Airn decreases H2O2-induced hepatocyte apoptosis in vitro. Mechanistically, we reveal that Airn represses CCl4-induced enhancement of phosphorylation of p65 and IκBα, suggesting that Airn inhibits hepatocyte apoptosis by inactivating the NF-κB pathway. In conclusion, our results demonstrate that Airn can alleviate acute liver injury by inhibiting hepatocyte apoptosis via inactivating the NF-κB signaling pathway, and Airn could be a potential biomarker for acute liver injury.

Key words  acute liver injury, lncRNA, apoptosis, oxidative stress, NF-κB signaling

Introduction
Acute liver injury is a common pathological basis for the development and progression of many liver diseases [1]. If the injury persists, when the extent of hepatocyte death exceeds the regenerative capacity of the liver, liver injury can progress to cirrhosis and liver failure, ultimately resulting in the development of liver cancer [2,3]. It has been found that aggravated hepatocyte apoptosis in acute liver injury is induced by sepsis, and that the inhibition of apoptosis could efficiently alleviate acute liver injury [4]. Another study revealed that shikonin attenuates acetaminophen (APAP)-induced acute liver injury via inhibiting oxidative stress [5]. Consequently, these studies indicated that inhibiting oxidative stress and hepatocyte apoptosis is important to alleviate acute liver injury.

Long noncoding RNAs (lncRNAs) are a class of non-protein-coding RNA transcripts that are longer than 200 nt and are involved in numerous physiological and pathological processes, including epigenetic regulation, transcription, and posttranscriptional regulation. lncRNAs have been found to play a key role in liver diseases [6,7]. For example, downregulation of IncRNA LINC00472 amelio-
rates sepsis-induced acute hepatic injury by regulating the miR-373-3p/TRIM8 axis [8]. LncRNA XIST silencing protects against sepsis-induced acute liver injury via inhibition of BRD4 expression [9]. LncRNA-Airn, an antisense RNA of IGF2R, is an imprinted and paternally expressed gene [10]. Recent studies have found that Airn plays an important role in many diseases. Knockdown of LncRNA-Airn restrained hepatocellular carcinoma (HCC) cell proliferation and boosted cell apoptosis by restraining the CUL4A-mediated ubiquitination of STAT1 protein [11]. It has been reported that Airn locates upstream of Igf2 bp2 to control the translation of many mRNAs, including genes involved in apoptosis and directly related to cell survival in cardiomyocytes [12]. Nevertheless, the underlying role of Airn in acute liver injury has not been investigated.

In the present study, we found that Airn was upregulated in CCl₄-induced acute liver injury and serum samples of patients with acute-on-chronic liver failure (ACLF). Further experiments revealed that Airn could alleviate CCl₄-induced ALI by mitigating liver apoptosis and oxidative stress. Mechanistically, Airn represses CCl₄ or H₂O₂-induced upregulation of phos-p65 and phos-IκBα, indicating that Airn alleviates hepatocyte apoptosis via inactivating the NF-κB signaling pathway. Our results indicated that Airn might play a protective role and could be a potential biomarker for acute liver injury.

Materials and Methods

Study population

In total, we collected serum samples from 27 healthy people and 31 patients diagnosed with acute-on-chronic liver failure at Tianjin Third Central Hospital (Tianjin, China). All subjects were of the same ethnicity. Clinical and pathological characteristics, including age, sex, alanine transaminase (ALT) and aspartate aminotransferase (AST), were recorded and summarized in Supplementary Table S1. The study was approved by the local Ethical Committee of Tianjin Third Central Hospital. Written informed consent was obtained from each patient according to the policies of the committee. The study methodologies conformed to the standards set by the Declaration of Helsinki.

In vivo animal study

All animal experiments were approved by the Animal Experiments Ethical Committee of Nankai University (Tianjin, China). Airn-knockout C57BL/6N mice generated by using the CRISPR/Cas9 system were obtained from Cyagen (Suzhou, China). All Balb/c male mice aged at eight weeks with a body weight of approximately 20 g were obtained from the Institute of Laboratory Animal Sciences, CAMS and PUMC (Beijing, China). All mice were housed in a specific-pathogen-free environment with 12/12-h dark/light cycles and had free access to chow and water. After one week of acclimatization, the acute liver injury mouse model was established. Forty-eight male mice were randomly divided into four groups: (1) WT mice treated with olive oil (WT, n = 10), (2) WT mice injected i.p. with CCl₄ (WT + CCl₄, n = 10), (3) Airn-KO mice injected i.p. with olive oil (Airn-KO, n = 10), and (4) Airn-KO mice injected i.p. with CCl₄ (Airn-KO + CCl₄, n = 10). They were administered with 20% CCl₄ (v/v) dissolved in olive oil (1 mL/kg body weight). Twenty-four hours later, all mice were sacrificed under anesthesia with 3% sodium pentobarbital (45 mg/kg, i.p.). Liver tissues and serum were collected for analysis.

For the rescue experiment, adeno-associated virus (AAV8) vectors were used to overexpress Airn in mice, and AAV8-GFP was used as a control. AAV8-GFP and AAV8-Airn were produced by AVA-293 cells and purified by using iodixanol density gradient ultracentrifugations. Forty male Airn-KO mice were divided into four groups: (1) Airn-KO mice treated with olive oil in combination with injection of AAV8-GFP (Airn-KO + AAV8-GFP, n = 10), (2) Airn-KO mice treated with CCl₄ in combination with injection of AAV8-GFP (Airn-KO + AAV8-GFP + CCl₄, n = 10), (3) Airn-KO mice treated with olive oil in combination with injection of AAV8-Airn (Airn-KO + AAV8-Airn, n = 10), and (4) Airn-KO mice treated with CCl₄ in combination with injection of AAV8-Airn (Airn-KO + AAV8-Airn + CCl₄, n = 10). Mice were injected with AAV8-GFP or AAV8-Airn (1 × 10¹² pfu/mouse) via the tail vein 2 weeks before oil or CCl₄ injection. CCl₄ was diluted to 20% (v/v) with olive oil before use, and used at 1 mL/kg body weight. After 24 h, all mice were sacrificed under anesthesia with 3% sodium pentobarbital (45 mg/kg, i.p.). Liver specimens and serum were obtained for subsequent analysis.

Isolation and culture of primary hepatocytes

Primary hepatocytes were isolated from the 8-week-old male mice by sequential in situ perfusion with 30 mL of SC1 solution and then with 30 mL of 0.05% collagenase IV (Sigma-Aldrich) solution. Cells were collected after centrifugation at 50 g for 4 min three times. Cell viability was determined by the trypan blue exclusion method. Mouse primary hepatocytes were plated onto collagen-coated six-well plates at a density of 2 × 10⁵ cells/well. Primary HCs were cultured in high-glucose Dulbecco’s modified Eagle’s medium (Invitrogen, Carlsbad, USA) containing 10% fetal bovine serum (FBS; Gibco, Gaithersburg, USA) and 1% penicillin/streptomycin (Gibco) and maintained in a humidified incubator with 5% CO₂ at 37°C.

Hematoxylin and eosin (H&E) staining

The liver specimens were fixed in 10% formalin for 2 days, dehydrated with a graded series of ethanol, and embedded in paraffin. For hematoxylin and eosin (H&E) staining, sections (5 μm) were stained with hematoxylin for 5 min at room temperature, and after extensive wash slices were stained with eosin for 1 min at room temperature. After that, the sections were dehydrated in gradient ethanol, transparently treated with xylene, and sealed with neutral gum. Images from randomly selected areas were captured at 100× magnification under a light microscope.

Measurement of serum biochemical markers

Blood samples were collected from CCl₄-treated mice, and serum levels of AST, ALT, and lactate dehydrogenase (LDH) were measured using commercially available diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The final data are presented as units/liter (U/L).
**Determination of SOD, CAT and GSH activities**

Superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) activities in serum samples or mouse livers were analyzed using a SOD assay kit, a CAT assay kit and a GSH assay kit (Jiancheng Bioengineering Institute), respectively. All experiments were carried out according to the manufacturer’s instructions.

**TUNEL assay**

For the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining assay, an in situ cell detection kit (Roche, Basel, Switzerland) was used according to the manufacturer’s protocol. After dewaxing and rehydration, sections were treated with 3% H2O2 and subsequently permeabilized with proteinase K. DNAse I was used as a positive control, and a TUNEL reaction mixture lacking terminal transferase (TdT) was used as a negative control. Samples were randomly selected and analyzed by light microscopy.

**Small interfering RNA (siRNA) transfection**

Mouse primary hepatocytes were plated onto collagen-coated six-well plates at a density of 2×10^4 cells/well. Then, the cells were transfected for 48 h with siRNA-Airn or siRNA-control. After that, total RNA and protein were extracted from the cells. Small interfering RNA (siRNA)-targeting Airn (siAirn) and negative control siRNA (NC) were designed and synthesized by GenePharma (Shanghai, China), and the sequences are as follows: siAirn (mouse), sense 5′-CGUCACCAUGUGUCCUUTT-3′ and antisense 5′-AAAGGACACAUUGUGACGCGT-3′; and NC (mouse), sense 5′-UUUCUCGGAACUGUCAGGT-3′, and antisense 5′-ACGUGACACUGUCGAGAATT-3′.

**Western blot analysis**

Liver tissues or primary hepatocytes were lysed with cell lysis buffer (Cell Signaling Technology, Beverly, USA) supplemented with protease inhibitor cocktail, 1% phenylmethylsulfonyl fluoride (PMSF) and 1% phosphatase inhibitor. A BCA protein quantitative kit (Bio-Rad Laboratories, Hercules, USA) was used to detect the protein concentration. The resulting protein was boiled with SDS-PAGE sample buffer, separated by 12% SDS-PAGE, and transferred to a PVDF membrane (Millipore, Billerica, USA). The PVDF membrane in the dark room for 3–5 min prior to exposure. The PVDF membrane was blocked with 5% skimmed milk in TBST for 1 h at room temperature and then incubated with primary antibodies at 4°C overnight. The primary antibodies used in this study are as follows: Caspase-3 (9664S, 1:1000; Cell Signaling Technology), phospho-IκBα (mouse monoclonal 4814S, 1:1000; Cell Signaling Technology), GAPDH (mouse monoclonal ab8245, 1:8000; Abcam). Then, all membranes were incubated with the HRP-conjugated secondary antibody for 1 h at room temperature. Signals were visualized using enhanced chemiluminescence (ECL) reagent (WBKLS0500; Millipore) and quantified. using ECL (WBKLS0500; Millipore) and quantified. using ECL (WBKLS0500; Millipore) and quantified.

**Quantitative real-time polymerase chain reaction (qPCR)**

qPCR analysis was performed as described previously [13]. In brief, total RNA was extracted from liver tissues or cells with Trizol reagent (Takara, Dalian, China), and all RNA was digested with DNase I (Takara). Next, the first-strand cDNA was synthesized using AMV Reverse Transcriptase (Thermo Fisher Scientific, Basingstoke, UK) according to the manufacturer’s instructions. The sequences of the primers are shown in Table 1.

**Hoechst/PI double-staining**

Cell apoptosis was analyzed using a Hoechst 33342-propidium iodide (PI) double-staining apoptosis-detection kit (SuoLaibao Technology, Beijing, China) according to the manufacturer’s instructions. Briefly, primary hepatocytes were plated in 6-well plates. Treated cells were washed with ice-cold PBS, and then 1 mL reaction buffer was added to each well. Then, 5 μL Hoechst 33342 solution and 5 μL PI solution were added and incubated for 30 min at 4°C. Next, the wells were washed with 1 × PBS to remove excess stain. The cells were observed under a fluorescence microscope (Olympus, Tokyo, Japan).

**Dual-luciferase reporter assay**

Primary hepatocytes were seeded in 6-well plates at 5×10^5 cells/well and cotransfected with 500 ng pNF-κB-Luc (Human Fenghui Biotechnology, Changsha, China) and 50 ng pRL-TK (Hunan Fenghui Biotechnology) using Lipofectamine 3000 (Invitrogen, Carlsbad, USA) for 24 h. Lentivirus-Airn was transfected for another 48 h, followed by treatment with 5% H2O2 for 3 h. Luciferase assays were performed using the Dual-luciferase reporter assay system (Promega, Madison, USA) following the manufacturer’s instructions. The relative firefly luciferase activity was normalized to Renilla luciferase activity.

**Statistical analysis**

All statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, USA). Comparisons were performed using either Student’s t test (between two groups) or one-way analysis of variance (among more than two groups), followed by post hoc comparison test. *P* <

### Table 1. Sequences of primers used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward sequence (5’→3’)</th>
<th>Reverse sequence (5’→3’)</th>
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<tr>
<td>GAPDH (mouse)</td>
<td>GCATGGAAGTCCTGTCATGAG</td>
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<td>GAAGGACGACGAGCCGACT</td>
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<tr>
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<td>TCCAGATTTCGAGACATCC</td>
</tr>
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<td>ACCCAAGGATCCTGAGATGG</td>
<td>TTCAGCTCAGGAGATCAGTT</td>
</tr>
<tr>
<td>Airn (human)</td>
<td>GGAAAAGGGGATTCGCTTGTT</td>
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</tr>
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**Shao et al. Acta Biochim Biophys Sin 2022**
0.05 was considered significantly different.

**Results**

**Airn is upregulated in acutely injured livers**

We have previously identified systemic variations of lncRNAs from fibrotic and normal mouse livers with microarray [13]. In that study, we found that Airn was significantly upregulated in fibrotic liver according to the microarray data. Since liver fibrosis is a consequence of wound healing caused by acute or chronic liver injury, we initially detected the level of Airn in a mouse model of acute liver injury. The constructed acute liver injury mouse models were confirmed and showed that the most severe injury was at 24 h after injection of CCl₄ (Figure 1A–E). qPCR analysis showed that Airn was dramatically increased in CCl₄-induced acute injured liver tissues (Figure 1F). The primary hepatocytes were subsequently isolated from the injured liver to detect the expression of Airn,

![Figure 1. CCl₄-induced acute liver injury mouse model and the expression of Airn](image)

Mice were injected with CCl₄ (olive oil as a control) to induce acute liver injury and were sacrificed at different time points. Macroscopic appearance and H&E staining of liver tissues from CCl₄-treated mice (A). The areas of liver damage for H&E staining (B), serum ALT (C), AST (D), and LDH (E) were detected. qPCR analysis of the Airn transcript in injured liver tissues (F) and primary HCs (G). Primary HCs were isolated from the livers of Balb/c mice and treated with 3% H₂O₂ for 3 h. Then, the expressions of Airn, IL-6, Mcp-1, and TNF-α were detected by qPCR (H). qPCR analysis of the Airn transcript in serum samples of healthy people and patients with ACLF (I). The correlation between the Airn level and the MELD score of ACLF patients was assessed using Pearson's correlation analysis, n = 58 (J). Data are expressed as the mean ± SD from at least three experiments, n = 6. *P < 0.05 vs control.
which showed an enhanced level of this molecule (Figure 1G). To confirm this finding in vitro, primary hepatocytes were treated with H₂O₂ to generate an acute hepatocytic injury model, which was used for the detection of Airn, and the qPCR results indicated that Airn was overexpressed (Figure 1H). As the human homolog of the mouse Airn has been identified [14], we then detected the serum level of Airn in patients with acute-on-chronic liver failure, and the result demonstrated that Airn was increased in these patients compared with that in healthy people (Figure 1I). Moreover, the Airn level was negatively correlated with the MELD score of ACLF patients (Figure 1J). Taken together, these data revealed that Airn was upregulated in acutely injured liver and hepatocytes and could be a potential marker for acute liver injury.

Deletion of Airn aggravates CCl₄-induced acute liver injury

To investigate the function of Airn during acute liver injury in vivo, Airn-KO mice were generated using the CRISPR/Cas9 system. As shown in Figure 1A–E, liver injury reached the greatest severity after administration of CCl₄ for 24 h, accompanied by a peak level of Airn. We therefore selected this time point to investigate the biological function of Airn. The expression of Airn was enhanced in CCl₄-treated WT mice but not in Airn-KO mice (Figure 2A). The macroscopic examination and H&E staining showed that the CCl₄-treated WT group developed severe liver damage, while knockout of Airn notably aggravated CCl₄-induced liver injury (Figure 2B). In addition, serum levels of ALT, AST and LDH were significantly increased after CCl₄ injection, however, knockout of Airn further increase of these enzymes in the CCl₄-treated group (Figure 2C–E). Taken together, these results indicated that Airn deficiency aggravates CCl₄-induced acute liver injury.

Airn alleviates CCl₄-induced acute liver injury and oxidative stress

To investigate whether Airn could alleviate CCl₄-induced acute liver injury in vivo, a rescue experiment was performed. The qPCR results confirmed that Airn was overexpressed (Figure 3A), and macroscopic examination and H&E staining showed that overexpression of Airn attenuated CCl₄-induced acute liver injury (Figure 3B). In addition, overexpression of Airn reduced CCl₄-induced ALT, AST and LDH levels (Figure 3C–E), suggesting that Airn alleviates CCl₄-induced acute liver injury.

It has been reported that CCl₄-induced liver injury is always accompanied by serious oxidative stress [15]. To explore whether Airn alleviates hepatic injury induced by CCl₄ through inhibition of hepatic oxidative stress, we next measured the levels of oxidative stress-associated indexes. As shown in Figure 4A–C, the levels of SOD, GSH and CAT were decreased after CCl₄ treatment, and knockout of Airn aggravated CCl₄-induced oxidative stress. However, forced expression of Airn increased CCl₄-reduced levels of SOD, GSH and CAT (Figure 4D–F). Taken together, these results suggested that Airn alleviates CCl₄-induced oxidative stress.

Airn inhibits hepatocyte apoptosis

CCl₄-induced liver injury is accompanied by increased hepatocyte apoptosis [16]. Thus, we detected whether Airn is involved in this process. Western blot analysis showed that the expressions of the proapoptotic proteins BAX, cleaved Caspase-3 and PARP1 were decreased upon Airn overexpression (Figure 5A,B). To further elucidate the role of Airn in hepatocyte apoptosis, a TUNEL apoptosis detection assay was performed. The results showed that CCl₄ exposure led to greatly increased numbers of TUNEL-positive hepatocytes, while the numbers were further increased when Airn was knocked out and decreased after overexpression of Airn (Figure 5C,D). In an in vitro study, we used siRNA to silence the expression of Airn and lentivirus to overexpress Airn in primary hepatocytes.
Next, we examined the changes in apoptosis-related genes at the protein level. Western blot analysis demonstrated that the proapoptotic proteins BAX and cleaved Caspase-3 were upregulated after H$_2$O$_2$ treatment, and the expression of the antiapoptotic protein Bcl-2 was downregulated, while the H$_2$O$_2$-induced increase in apoptosis was aggravated when Airn was knocked down. In contrast, overexpression of Airn mitigated H$_2$O$_2$-induced apoptosis (Figure 6C,D). To further assess the influence of
Airn inhibits hepatocyte apoptosis through NF-κB signaling

Next, we investigated the mechanisms of Airn in hepatocyte apoptosis. It has been extensively reported that NF-κB has proinflammatory functions and promotes apoptosis [16]. Therefore, further experiments were performed to investigate whether Airn is involved in NF-κB signaling. The results revealed that phos-p65 and phos-κBα, which are degraded and subsequently enable NF-κB dimers to translocate into the nucleus to regulate gene expression, were markedly increased after CCl₄ treatment. Knockout of Airn aggravated CCl₄-induced increase of phos-p65 and phos-κBα (Figure 7A). On the other hand, CCl₄-induced increase of phos-p65 and phos-κBα was decreased after overexpression of Airn (Figure 7B). In vitro, silencing Airn promoted H₂O₂-induced upregulation of phos-p65 and phos-κBα. Overexpression of Airn decreased the H₂O₂-induced increase of phos-p65 and phos-κBα (Figure 7C). Moreover, BAY11-7082, an antagonist of NF-κB, was used to further confirm that Airn is involved in NF-κB signaling.

Airn inhibits hepatocyte apoptosis through NF-κB signaling.

Discussion

Acute liver injury results in the massive death or loss-of-function of hepatocytes, and severe or persistent liver injury eventually leads to liver failure, which represents a stage of sudden deterioration of liver function that is characterized by cell necrosis, inflammation and oxidative damage [17,18]. It is a severe clinical syndrome caused by many factors, such as drugs, viruses, alcohol, and autoimmunity [19]. A recent study also revealed that intestinal flora disorder inhibited the recovery of liver function to aggravate acute liver injury [20]. In this study, CCl₄ was used to induce acute liver injury in a mouse model, and we found that Airn was upregulated in injured mouse livers and primary hepatocytes. Consistently, Airn was also overexpressed in serum samples of patients with acute-on-chronic liver failure and negatively correlated with the MELD score. Furthermore, Airn could alleviate CCl₄-induced acute liver injury by
reducing hepatocyte apoptosis and oxidative stress. Mechanistically, Airn represses CCl$_4$- and H$_2$O$_2$-induced increase of phos-p65 and phos-IκBα, suggesting that Airn inhibits hepatocyte apoptosis via NF-κB signaling.

CCL$_4$ is a well-known hepatotoxin that causes serious liver injury and cell death through death pathways such as apoptosis and necrosis, and prolonged exposure to CCL$_4$ leads to liver failure, cirrhosis and hepatocellular carcinoma [21]. Its principal mechan-

Figure 6. Airn inhibits H$_2$O$_2$-induced hepatocyte apoptosis in vitro Primary HCs were transfected with siRNA or lentivirus for 48 h, followed by treatment with 3% H$_2$O$_2$ for another 3 h. (A,B) The mRNA level of Airn was detected by qPCR. (C,D) The protein levels of apoptosis-related genes were detected by western blot analysis. GAPDH was used as an internal control. (E) Hoechst/PI double-staining was used to detect cell apoptosis. Scale bar: 100 μm. Data are expressed as the mean ± SD from at least three experiments. *P < 0.05, siAirn vs siRNA-control or LV-Airn vs LV-control or NC + H$_2$O$_2$ vs NC or LV-con + H$_2$O$_2$ vs LV-con; #P < 0.05, siAirn + H$_2$O$_2$ vs NC + H$_2$O$_2$ or LV-Airn + H$_2$O$_2$ vs LV-con + H$_2$O$_2$.
Figure 7. Airn inhibits hepatocyte apoptosis through NF-κB signaling. 

(A) Mice were divided into four groups. The protein levels of p65, phospho-p65, IκBα and phospho-IκBα were detected by western blot analysis. GAPDH was used as an internal control. (B) Airn-KO mice were divided into four groups. The protein levels of p65, phospho-p65, IκBα and phospho-IκBα were detected by western blot analysis. GAPDH was used as an internal control. (C) Primary HCs were transfected with siRNA or lentivirus for 48 h, followed by treatment with 3% H2O2 for another 3 h. The protein levels of p65, phospho-p65, IκBα and phospho-IκBα were detected by western blot analysis. GAPDH was used as an internal control. (D,E) Primary hepatocytes were transfected with siRNA for 48 h, and then treated with 3% H2O2 or 10 μM of the NF-κB antagonist BAY11-7082 for another 3 h. The protein levels of PARP1, Bcl-2, BAX and cleaved caspase-3 were determined by western blot analysis. GAPDH was used as an internal control. (F) Airn and treated with 3% H2O2; LV-con or NC vs WT or Airn-KO; AAV8-GFP or NC vs Airn-KO + AAV8-GFP + CCl4 vs Airn-KO + AAV8-GFP or NC vs H2O2 vs NC or LV-con + H2O2 vs LV-con or NC + H2O2 + BAY vs NC + H2O2; *P < 0.05, WT + CCl4 vs WT or Airn-KO + AAV8-GFP + CCl4 vs Airn-KO + AAV8-GFP or NC vs H2O2 vs NC or LV-con + H2O2 vs LV-con or NC + H2O2 + BAY vs NC + H2O2 + BAY vs NC + H2O2 + BAY. Values were analyzed by Student’s t test. *P < 0.05, WT + CCl4 vs WT or Airn-KO + AAV8-GFP + CCl4 vs Airn-KO + AAV8-GFP or NC vs H2O2 vs NC or LV-con + H2O2 vs LV-con or NC + H2O2 + BAY vs NC + H2O2; *P < 0.05, Airn-KO + CCl4 vs WT + CCl4 or Airn-KO + AAV8-Airn + CCl4 vs Airn-KO + AAV8-GFP + CCl4 or siAirn + H2O2 vs NC + H2O2 or LV-Airn + H2O2 vs LV-con + H2O2 or siAirn + H2O2 + BAY vs NC + H2O2 + BAY.

LncRNA-Airn alleviates acute liver injury by promoting apoptosis [29]. Another study revealed that glutamine treatment effectively attenuated oxidative stress, ameliorated CCl4-induced liver fibrosis and suppressed TGF-β1-induced EMT progression and apoptosis [15]. The most common resident cell types in the liver are hepatocytes and account for 75%–80% of the liver [30]. Hepatocyte apoptosis has emerged as an important pathological mechanism of acute liver injury or liver failure [31]. Effective blockade of hepatocyte apoptosis is one of the important ways to reduce liver damage and maintain hepatic function. For example, a previous study revealed that Parkin deficiency could aggravate chronic alcohol intake-induced liver injury by promoting apoptosis [32]. Some other studies have demonstrated that cellular apoptosis is often coupled with mitochondrial dysfunction, while mitochondrial dysfunction causes ROS overproduction. Excessive ROS can increase mitochondrial membrane permeability, allowing the...
release of cytochrome C into the cytoplasm, followed by the activation of the mitochondria-dependent caspase signaling pathway, which ultimately leads to apoptosis [33]. In this study, CCl\textsubscript{4}-induced hepatocyte apoptosis was aggravated under the condition of Airn knockout but decreased when Airn was overexpressed. Similarly, the levels of SOD, CAT and GSH showed the same results. These findings demonstrated that Airn could increase the levels of SOD, CAT and GSH to strengthen the antioxidant capacity and alleviate CCl\textsubscript{4}-induced hepatocyte apoptosis.

Accumulated evidence indicates that the NF-\kappaB signaling pathway plays an essential role in acute liver injury. Under normal physiological conditions, NF-\kappaB and I\kappaB exist in the cytoplasm as inactive complexes; when the liver is damaged, with the action of the kinases IKK\textalpha and IKK\textbeta, I\kappaB is phosphorylated and dissociated from NF-\kappaB, and NF-\kappaB transfers into the nucleus, activates the expressions of a series of cell inflammatory factors, and promotes necrosis and apoptosis of liver cells [23,24]. It has been reported that knockdown of either STING or IRF3 leads to a significant reduction in FFA-induced hepatic inflammation and apoptosis, as evidenced by modulation of NF-\kappaB signaling pathway [34]. In addition, liver kinase B1 (LKB1) suppresses the activation of the NLRP3 inflammasome by blocking the NF-\kappaB signaling pathway, leading to alleviation of the inflammatory response to hypoxia/reoxygenation injury [35]. Tp4 inhibits hepatic apoptosis and fibrosis induced by lincRNA-p21 via suppressing the PI3K-AKT-NF-\kappaB pathway [36]. Some studies have reported that inhibiting NF-\kappaB p65 and I\kappaB\alpha phosphorylation can block the activation of the NF-\kappaB pathway, thereby reducing the expressions of inflammatory factors and the occurrence of apoptosis, which is important for the prevention of CCl\textsubscript{4}-induced acute liver injury in mice [37–39]. Our results verified that Airn suppressed the activation of NF-\kappaB signaling in vivo and in vitro. Moreover, by using an NF-\kappaB antagonist, we demonstrated that Airn inhibits hepatocyte apoptosis through NF-\kappaB signaling, as revealed by luciferase reporter assay.

In summary, our research demonstrated that Airn could alleviate CCl\textsubscript{4}-induced acute liver injury by reducing hepatocyte apoptosis and oxidative stress. Mechanistically, Airn decreases hepatocyte apoptosis by inactivating the NF-\kappaB signaling pathway (Figure 8). This study suggests that Airn may play a protective role and be used as a potential biomarker in acute liver injury.

**Supplementary Data**

Supplementary data is available at Acta Biochimica et Biophysica Sinica online.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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Shao et al. Acta Biochim Biophys Sin 2022