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*Original Article*

# Ameliorative effects of miR-186 on cisplatin-triggered acute kidney injury via targeting ZEB1

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**Abstract:** Cisplatin is a commonly used chemotherapy drug in cancers, which can lead to acute kidney injury (AKI). AKI can occur in almost one third of tumor patients, who receive cisplatin treatment. microRNAs (miRNAs) are significant tools in regulating the expression of crucial factors in multiple diseases, but little is known about their biological roles in AKI. As exhibited, miR-186 has been observed to be down-regulated in tumors. Our study concentrated on the function of miR-186 in cisplatin-triggered AKI. Here, we reported miR-186 was considerably decreased in the serum samples from AKI patients compared with those from the healthy controls. Additionally, we found in NRK-52E cells exposed to 6 mM cisplatin, miR-186 was greatly decreased time-dependently. Meanwhile, an AKI model in rats was successfully set in our study. Levels of serum creatinine and blood urea nitrogen were significantly induced by cisplatin exposure. In AKI rat models, miR-186 exhibited a rapid decrease in both the serum and the kidney tissues. Then, miR-186 overexpression improved NRK-52E cell proliferation and protected NRK-52E cells against cisplatin-triggered apoptosis. Furthermore, ZEB1 was identified and confirmed as a target gene of miR-186. It has been demonstrated that ZEB1 exerts crucial roles in the development of AKI. As evidenced in our current study, ZEB1 was remarkably elevated in AKI patients and AKI rat models. Moreover, ZEB1 was induced by indicated doses of cisplatin in different time periods in NRK-52E cells. ZEB1 inhibition rescued the reduced proliferation and increased apoptosis of NRK-52E cells. In conclusion, loss miR-186 expression contributed to cisplatin-induced AKI, partly through targeting ZEB1. miR-186 might be provided as an effective biomarker for AKI via targeting ZEB1.

**Keywords:** Acute kidney injury (AKI), miR-186, ZEB1

## Introduction

Recently, AKI has been reported a serious health problem worldwide, which contributes to an increasing morbidity and mortality [1, 2]. As a recent study has pointed out, more than 13.3 million patients are affected by AKI and about 1.7 million deaths have been observed each year [3]. So far, still no effective therapies are used to treat AKI progression and the clinical efforts to promote AKI patients' outcomes are greatly limited [4]. Hence, investigating the progression of AKI and identifying the possible therapeutic targets is significant.

MicroRNAs are endogenous and small non-coding RNA molecules with about 21-25 nts [5]. MicroRNAs can normally bind with the 3'-UTR of their targeting mRNA transcripts, which can result in translation inhibition, silencing, or degradation of mRNA [6-8]. Recently, miRNAs have been recognized as potential therapeutic targets for AKI [9, 10]. For example, miR-146b can promote mesenchymal stem cell repair in AKI progression [11]. Inhibition of miR-182-5p can ameliorate ischemic AKI [12]. miR-140-5p can attenuate oxidative stress progression in AKI induced by cisplatin via the activation of Nrf2/ARE [13]. In AKI, miR-34a can depress autophagy of tubular epithelial cells [14]. However, the function of miR-186 remains poorly known in AKI progression.

This research investigated the roles of miR-186 in cisplatin-induced AKI. miR-186 was inhibited in AKI patients. Meanwhile, cisplatin was used to treat NRK-52E cells and the rats, which stimulated AKI. We found miR-186 was significantly reduced and then, miR-186 was overexpressed in NRK-52E cells. We observed that cell viability and apoptosis altered by cisplatin was greatly reversed by miR-186 overexpression.

For another, we focused onthe possible regulatory mechanism of miR-186 in AKI. ZEB1 was predicted as its downstream target, which was found to be obviously increased in AKI. Therefore, it was implied that miR-186 exhibited an inhibitory role in cisplatin-induced AKI via target ZEB1, which might offer a potential therapeutic target to treat AKI.

## Materials and methods

### *Patient and samples*

Blood samples from the healthy controls and AKI patients were obtained from Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University. All the patients involved in the study provided the written informed consent. Both the study and consent were approved by the Ethics Committee of Qingpu Branch of Zhongshan Hospital Affiliated to Fudan University.

### *Cell culture*

NRK52E cell line was obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in HG-DMEM with 5% FBS, penicillin, and streptomycin at 37°C with 5% $CO_2$.

### *Animals*

Male Wistar rats were purchased from Shanghai Animal Laboratory Center. Animals were kept under standard conditions with 12 h light/12 h dark cycle. Free access to food and water was provided to the rats. After fasting for 12 hours, AKI was triggered in rats using an intraperitoneal injection of 6 mg/kg cisplatin. Rats were kept under the Guide for the Care and Use of Laboratory Animals of the National Academy of Sciences. All animal experiments were based on the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

### *Histology of kidney tissues*

Kidneys were fixed using 10% neutral buffered formalin. After sectioned, dehydrated and embedded, the sections were stained with HE (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China).

### *Biochemistry measurements of serum samples*

Blood samples from the sacrificed rats were collected and kept using heparinized tubes. In order to obtain plasma, blood was centrifuged at 14,000 g for 10 minutes. Levels of BUN and Cr were tested using detection kits (Nanjing Jian cheng Institute of Biotechnology, Nanjing, China).

### *Immunohistochemical staining*

4 μm thick sections of the kidneys from the rats were mounted. Then, the slides were deparaffinized and rehydrated. Citrate buffer was used to pretreat the sections for 20 minutes. Sections were immersed using PBS added with 3% $H_2O_2$ for 10 minutes. Rabbit polyclonal anti-ZEB1 (dilution 1:100) was used to incubate the sections at 4°C for a whole night. Subsequently, sections were rinsed using PBS, incubated with goat anti-rabbit IgG and then treated with 3,30-diaminobenzidine chromogen.

### *Cell transfection*

The miR-186 mimics, miR-186 inhibitors, ZEB1 siRNA and their corresponding NCs were synthesized by GenePharma (Shanghai, China) and were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

### *CCK-8 assay*

Cell viability was evaluated using CCK8 assay (Promega, Madison, WI, USA). The transfected cells were seeded in 96-well plates for a whole night. 10 μL CCK8 solution was added with the cells for 3 hours. The absorbance was tested using a microplate reader.

### *EdU incorporation assay*

EdU detection kit (RiboBio, Guangzhou, PR, China) was carried out to detect cell proliferation. 50 μM of EdU was used and the cells were incubated for 2 hours. Afterwards, cells were fixed using 4% paraformaldehyde for 30 minutes. All the cells were labeled by anti-EdU working reagents. 0.5% Triton X-100 was used and then 5 μg/mL Hoechst33342 was employed. A fluorescent microscopy was utilized.

### *Cell apoptosis assay*

Cell apoptosis was tested using the Annexin V: FITC Apoptosis Detection Kit II (BD Biosciences, San Jose, CA). Briefly, cells were re-suspended in binding buffer. 2 μL of Annexin V-FITC was added to incubate the cells. 400 μL of PBS and 1 μL PI were added before detection. Flow

**Table 1.** Primers used for real-time PCR

| Genes | Forward (5'-3') | Reverse (5'-3') |
|---|---|---|
| GAPDH | CAAGGTCATCCATGACAACTTTG | GTCCACCACCCTGTTGCTGTAG |
| U6 | CTCGCTTCGGCAGCACA | AACGCTTCACGAATTTGCGT |
| ZEB1 | GCCAATAAGCAAACGATTCTG | TTTGGCTGGATCACTTTCAAG |
| miR-186 | GCGGCGCAAAGAATTCTCCT | GTGCAGGGTCCGAGGT |

cytometry (BD Biosciences, San Jose, CA) was employed to observe the apoptotic cells in the analysis.

*TUNEL apoptosis assay*

TUNEL detection kit (Promega, Madison, WI, USA) was performed to detect cell apoptosis. After paraffin-embedded, deparaffinized and dehydrated, sections were incubated with 20 μg/ml Proteinase K, rinsed using 0.3% Triton X-100 for 10 minutes. Then, mixture of TUNEL reaction was used to incubate the sections for 1 hour. HRP conjugated streptavidin was used for 30 minutes. Then, 0.04% DAB and 0.03% $H_2O_2$ were utilized for visualization.

*qRT-PCR*

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). miR-186 was determined by the mirVana™ qRT-PCR microRNA Detection kit (Ambion, Austin, TX, USA). PrimeScript® RT reagent kit (TaKaRa, Dalian, China) was employed to synthesize cDNAs. ZEB1 mRNA expression was measured using SYBR Green Real-time PCR. QRT-PCR was conducted utilizing the ABI 7500 Fast Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The relative gene expression was assessed by $2^{-\Delta\Delta Ct}$. The primers used in this work are exhibited in **Table 1**.

*Western blot analysis*

Equal protein extracts were separated on 10% SDS-PAGE and transferred onto PVDF membranes. Then, the membrane was incubated with the primary antibodies at 4°C for a whole night. Next day, secondary antibodies were used to incubate the membranes for 2 hours at room temperature. The following primary antibodies were used: mouse anti-GAPDH (Abcam, Cambridge, UK), rabbit anti-ZEB1 antibody (Abcam, Cambridge, UK). An enhanced chemiluminescence system was used to visualize the protein signals.

*ELISA assay*

Cox-2, IL-1β, IL-6 and TNF-α protein concentration in the supernatants of NRK52E cell cultures was determined using the corresponding ELISA Kit (Solarbio, Beijing, China).

*Luciferase assay*

3'-UTR of ZEB1 was amplified using PCR from genomic DNA. The predicted target sites were mutated using QuikChange Site Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA). WT-ZEB1 and MUT-ZEB1 were cloned into a pGL3 luciferase promoter vector (Promega, Madison, WI, USA). Cells were co-transfected with the WT-ZEB1/MUT-ZEB1 construct and miR-186 mimics or inhibitors. The luciferase activity was determined using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

*Statistical analysis*

Data are exhibited as the mean ± SD. Statistical analysis was carried out using SPSS 22.0 software (SPSS, Chicago, IL, USA). Statistical differences between different groups were assessed using one-way analysis of variance (ANOVA) and Student's t-test. Significance was considered at $P<0.05$.

## Results

*Down-regulation of miR-186 in AKI*

Firstly, to test the effect of miR-186 in renal disease, serum from healthy controls (n = 100) and AKI patients (n = 100) was obtained. qRT-PCR was carried out to analyze the expression of miR-186. As exhibited in **Figure 1A**, miR-186 was obviously decreased in AKI patients. For another, we observed miR-186 was also repressed in NRK-52E cells exposed to cisplatin treatment at various time points especially at 24 hours (**Figure 1B**).

Then, an AKI rat model was established using 6 mg/kg cisplatin administration through intraperitoneal injections. As displayed in **Figure 1C** and **1D**, serum Cr and BUN levels were greatly triggered after cisplatin treatment). Then, we observed that cisplatin significantly induced kidney injury as evidence with HE staining and

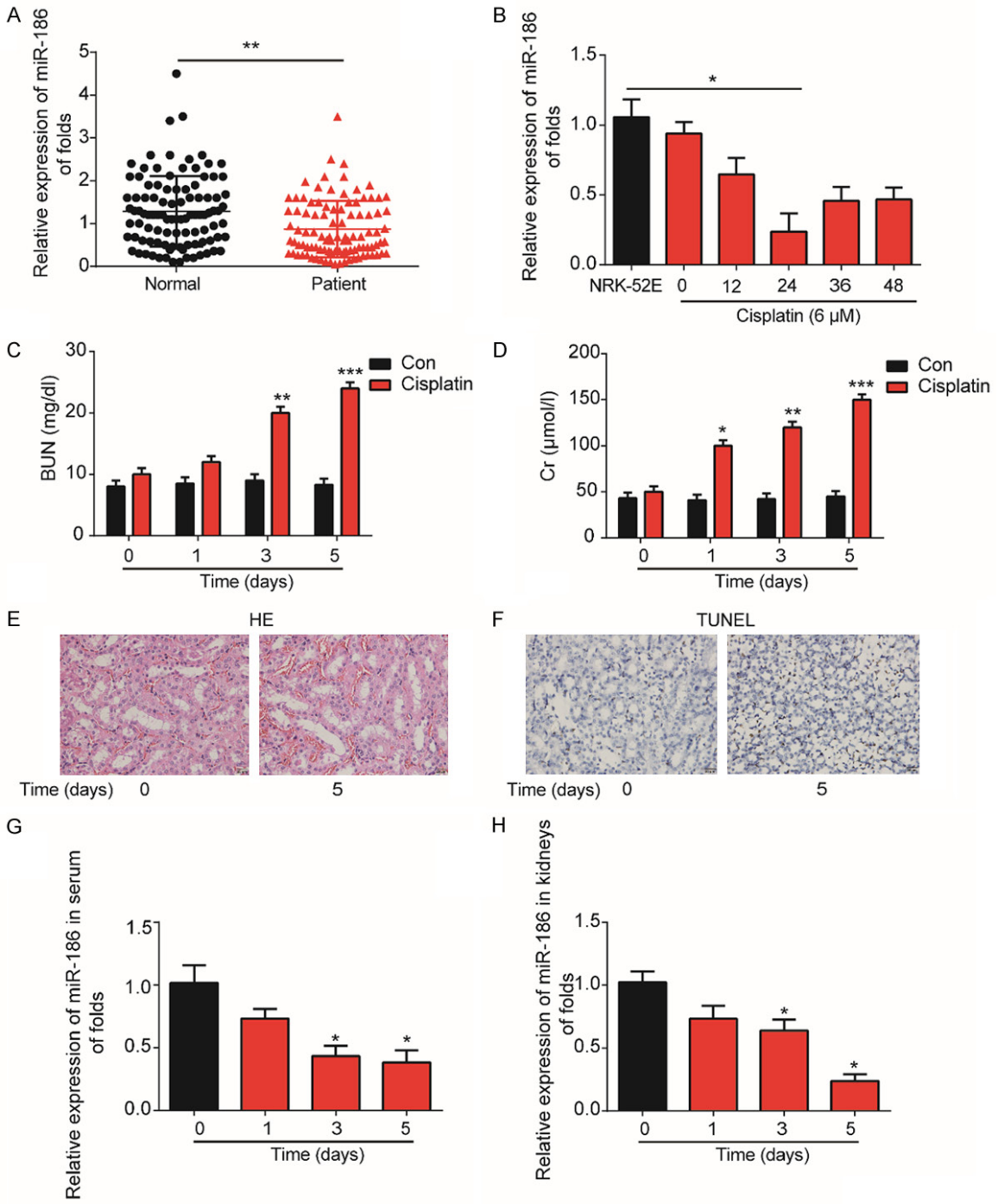
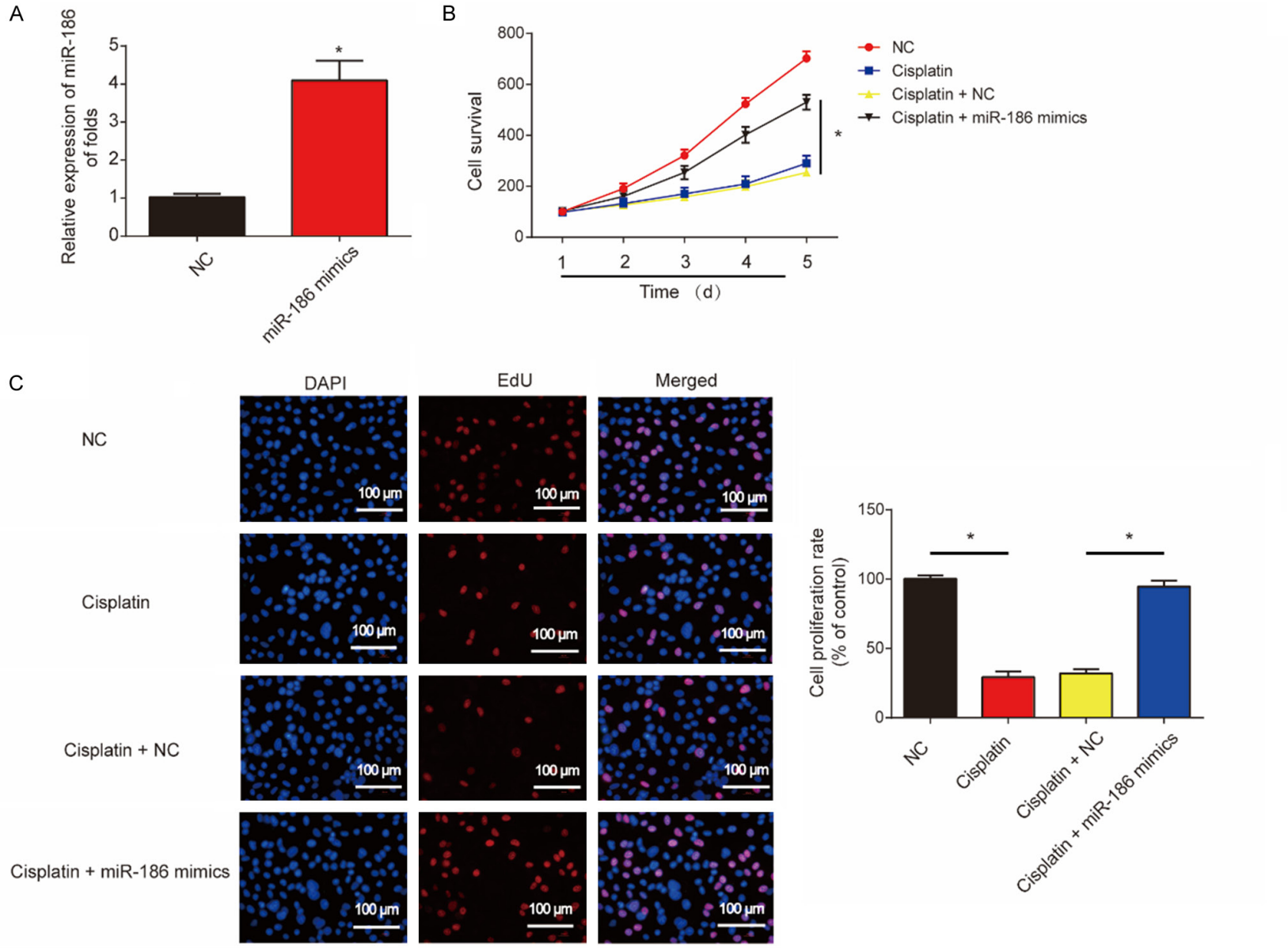



**Figure 1.** Identification of down-regulated miR-186 in AKI. A. Analysis of miR-186 in the serum from healthy controls and patients with AKI. U6 served as a loading control. B. miR-186 expression in NRK-52E cells treated with 6 μM cisplatin for various periods. Normal NRK-52E cells were employed as a control. C. Serum Cr levels on days 0-5 after cisplatin treatment. D. BUN levels on days 0-5 after cisplatin treatment. E. Representative micrographs of renal histologic findings. Scale bars = 20 μm. F. Analysis of apoptosis using TUNEL assay in renal tissues from AKI rats. G. miR-186 expression in rat serum at indicated time points after injection with cisplatin. H. miR-186 expression in kidneys of rats at indicated time points after injection with a low dose of cisplatin. n = 10 in each group. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

TUNEL assay in **Figure 1E** and **1F**. Furthermore, the serum and the renal tissue miR-186 levels in the AKI rats were elevated by cisplatin (**Figure 1G** and **1H**). These data suggested miR-186


A
Relative expression of miR-186 of folds
NC
miR-186 mimics
B
Cell survival
Time (d)
NC
Cisplatin
Cisplatin + NC
Cisplatin + miR-186 mimics
C
DAPI
EdU
Merged
NC
Cisplatin
Cisplatin + NC
Cisplatin + miR-186 mimics
100 μm
Cell proliferation rate (% of control)
NC
Cisplatin
Cisplatin + NC
Cisplatin + miR-186 mimics

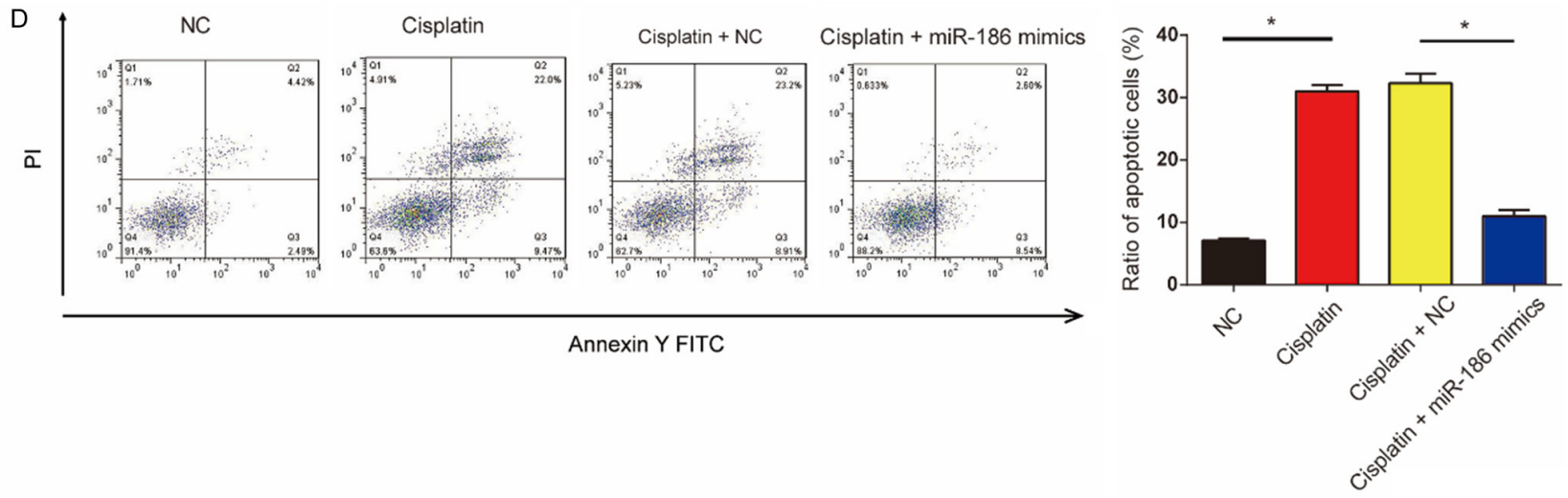



**Figure 2.** Overexpression of miR-186 alleviated cisplatin-induced renal injury in vitro. (A) miR-186 expression in NRK-52E cells transfected with miR-186 mimics or the corresponding negative controls for 48 hours. CCK-8 assay was used to detect cell viability (B) EdU assay was employed to test cell proliferation. Scale bars = 100 μm. (C) Flow cytometry was utilized to detect cell apoptosis (D) NRK-52E cells were transfected with miR-186 mimics or the corresponding negative controls for 48 hours. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

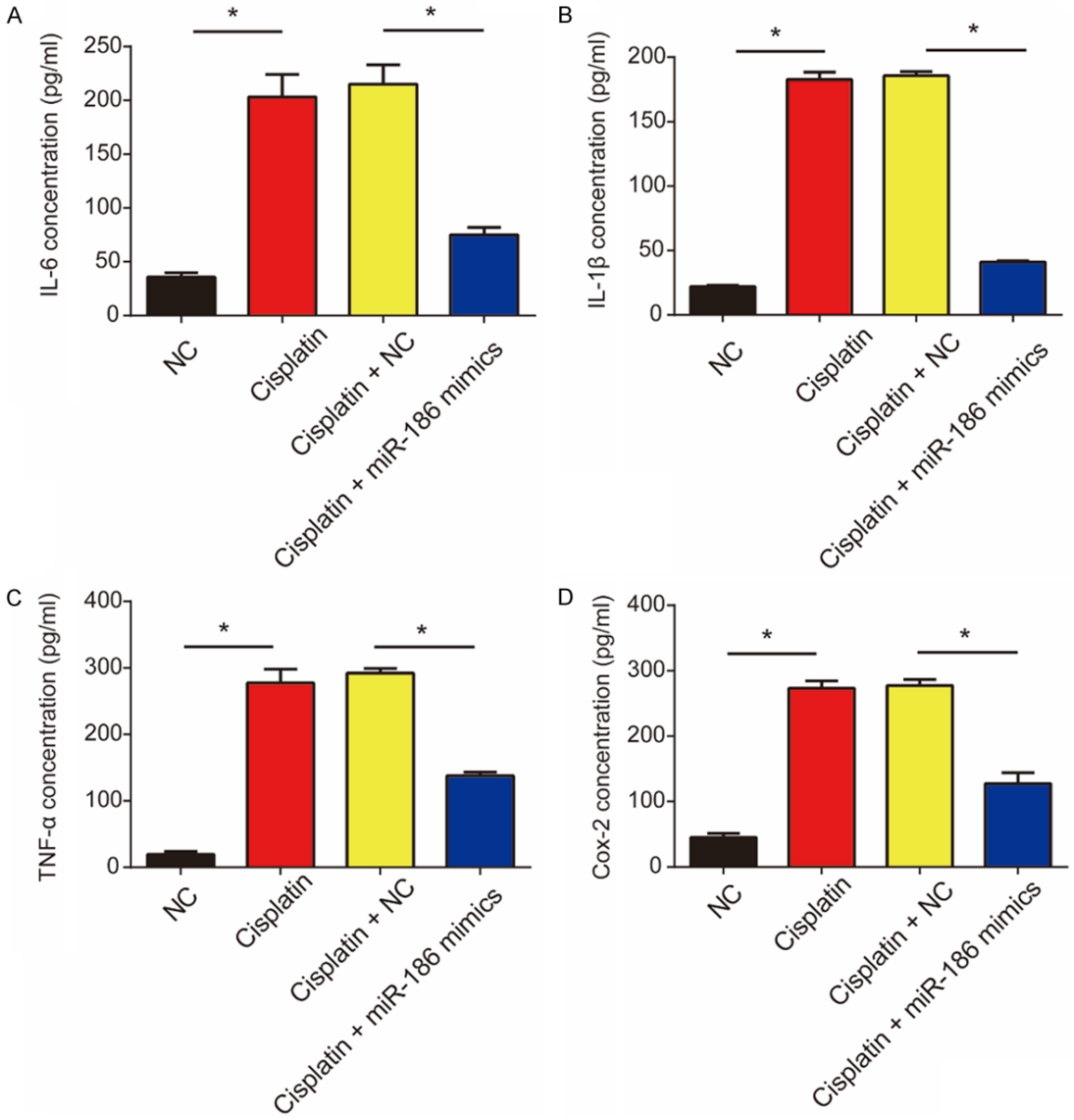



**Figure 3.** Increase of miR-186 repressed the secretion of inflammatory cytokines. A-D. ELISA assay was carried out to examine the concentrations of inflammatory cytokines (IL-6, IL-1β, TNF-α and Cox-2) in NRK-52E cells. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

was inhibited in AKI. These data indicated loss of miR-186 was involved in AKI.

*The effects of miR-186 on the proliferation and apoptosis of NRK-52E cells*

Furthermore, to explore the potential role of miR-186 in cisplatin-induced AKI, NRK-52E cells were transfected with miR-186 mimics. The efficiency of the mimics was verified in NRK-52E cells and miR-186 was greatly increased by the mimics (**Figure 2A**). As demonstrated in **Figure 2B**, cisplatin obviously inhibited the cell survival and the mimics improved NRK-52E cell survival, which was consistent with the data in **Figure 2C** as evidence by EdU assay. Additionally, after transfection for 48 hours, miR-186 mimics rescued the number of apoptotic cells restrained by cisplatin as indicated by the flow cytometry assay (**Figure 2D**). These indicated miR-186 promoted NRK-52E cell proliferation and reduced cell apoptosis.

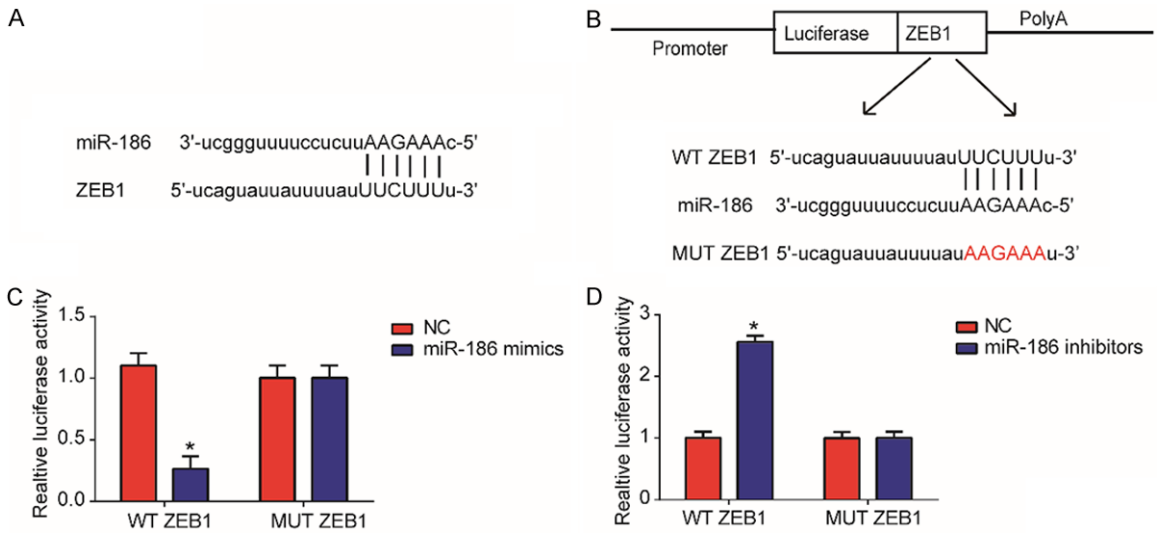



**Figure 4.** ZEB1 was a direct target of miR-186. A. The binding correlation between miR-186 and ZEB1. B. The luciferase reporter constructs containing the wild type (WT-ZEB1) or mutant ZEB1 (MUT-ZEB1) sequence. C. WT-ZEB1 or MUT-ZEB1 was co-transfected into NRK-52E cells with miR-186 mimics or their corresponding negative controls. D. WT-ZEB1 or MUT-ZEB1 was co-transfected into NRK-52E cells with miR-186 inhibitors or their corresponding negative controls. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

*Up-regulation of miR-186 repressed inflammatory response*

Next, cell inflammatory response was evaluated by detecting inflammatory cytokines. IL-6, IL-1β, TNF-α and Cox-2 protein expression could be triggered by cisplatin. Increase of miR-186 reversed this phenomenon (**Figure 3A-D**). These data implied that increase of miR-186 repressed inflammatory response induced by cisplatin.

*ZEB1 was a downstream target of miR-186*

Next, by searching the online bioinformatics analysis http://starbase.sysu.edu.cn/, several probable targets of miR-186 were identified, among which the E-cadherin transcriptional repressor ZEB1 was concentrated on due to its significant effects in the process of AKI. Then, we verified the targeting of ZEB1 and miR-186 in tubular epithelial cells. In **Figure 4A**, binding regions between miR-186 and ZEB1 were exhibited. Besides this, luciferase reporter plasmids of WT-ZEB1 and MUT-ZEB1 were displayed in **Figure 4B**. Subsequently, co-transfection of WT-ZEB1 with miR-186 mimics suppressed the reporter activity in NRK-52E cells (**Figure 4C**). Reversely, co-transfection of WT-ZEB1 with miR-186 inhibitors enhanced the reporter activity (**Figure 4D**). The results showed that miR-186 directly targeted ZEB1.

*Up-regulation of ZEB1 in AKI*

Furthermore, ZEB1 mRNA expression was strong increased in the serum samples of AKI patients (**Figure 5A**). Additionally, in NRK-52E cells, ZEB1 mRNA and protein level was triggered by cisplatin (**Figure 5B** and **5C**). In AKI rat models, we found that ZEB1 was remarkably up-regulated in the serum and the renal tissues (**Figure 5D** and **5E**). Apart from these, IHC staining indicated that ZEB1 was also strongly stained in AKI rat kidney tissues (**Figure 5F**). These data manifested that ZEB1 was up-regulated in AKI rat models.

*Knockdown of ZEB1 reversed the effects of cisplatin on NRK-52E cells*

In **Figure 6A** and **6B**, ZEB1 expression in NRK-52E cells were inhibited by miR-186 mimics. In addition, the efficiency of ZEB1 siRNA was validated in **Figure 6C**. As displayed in **Figure 6D**, loss of ZEB1 greatly increased NRK-52E cell proliferation, which was reduced by the incubation of cisplatin. Apoptosis were repressed by the decreased of ZEB1 as proved by the flow cytometry assay (**Figure 6E**). These indicated

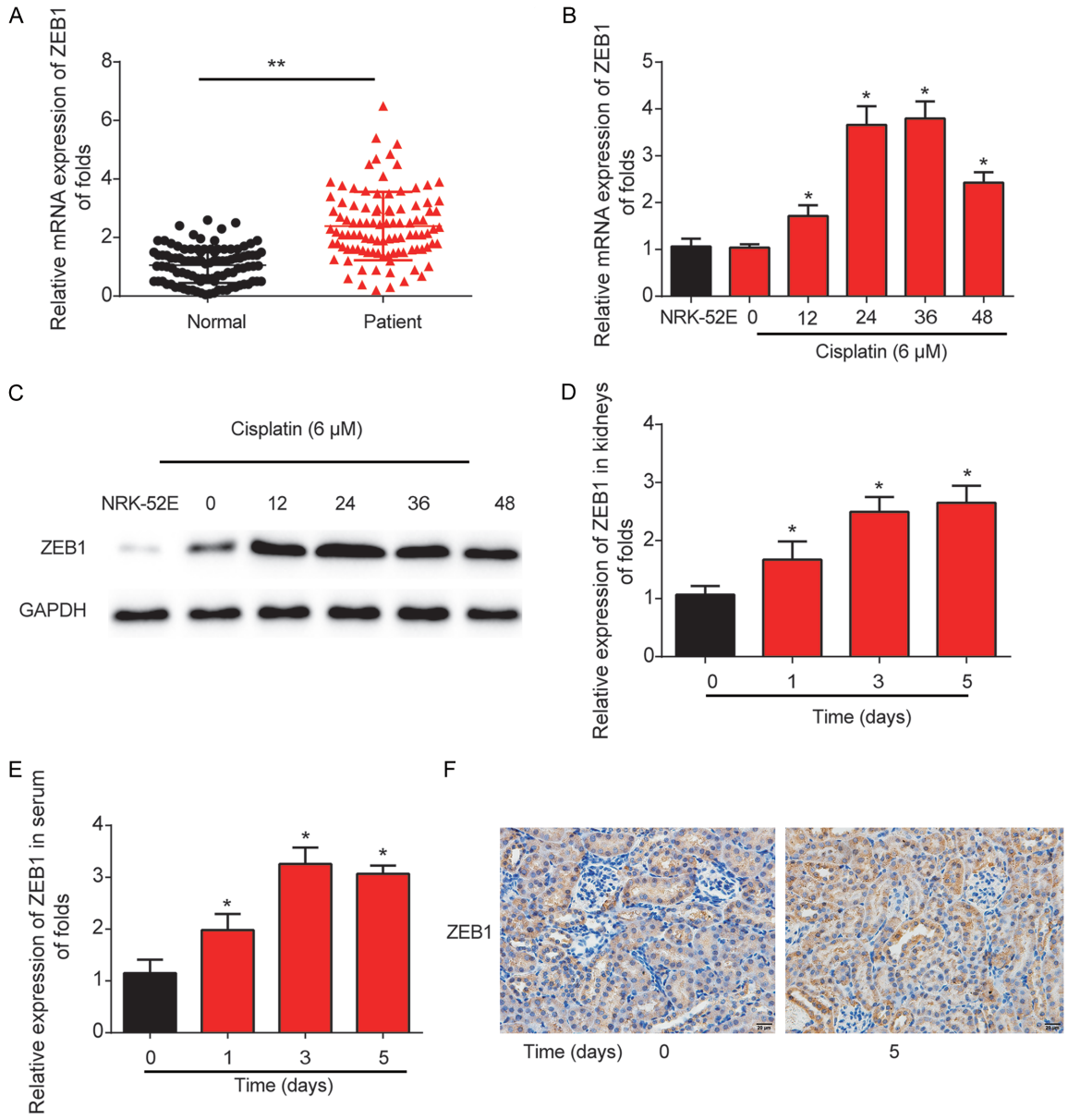



**Figure 5.** Identification of up-regulated ZEB1 in AKI. A. Analysis of ZEB1 mRNA expression in serum from healthy controls and patients with AKI. GAPDH served as a loading control. B. ZEB1 mRNA expression in NRK-52E cells treated with 6 mM cisplatin for various periods. Normal NRK-52E cells were employed as a control. C. ZEB1 protein expression in NRK-52E cells treated with 6 mM cisplatin for various periods. D. ZEB1 mRNA expression in rat serum at indicated time points after injection with cisplatin. E. ZEB1 mRNA expression in kidneys of rats at indicated time points after injection with a low dose of cisplatin. F. IHC of ZEB1 in AKI rats kidney tissues. Scale bars = 20 μm. Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

that knockdown of ZEB1 could reverse the effects of cisplatin on the proliferation and apoptosis of NRK-52E cells. In addition, we displayed that induced IL-6, IL-1β, TNF-α and Cox-2 protein expression was depressed by loss of ZEB1 in vitro (**Figure 6F-I**). These data revealed that decrease of ZEB1 inhibited the inflammatory response triggered by cisplatin.

## Discussion

Recently, increasing microRNAs have been identified in AKI and the research on miRNA has become popular in AKI [15-17]. Increasing miRNAs are essential for maintaining the stability of kidneys [18]. For example, miR-17 family can promote the progression of polycystic kidney

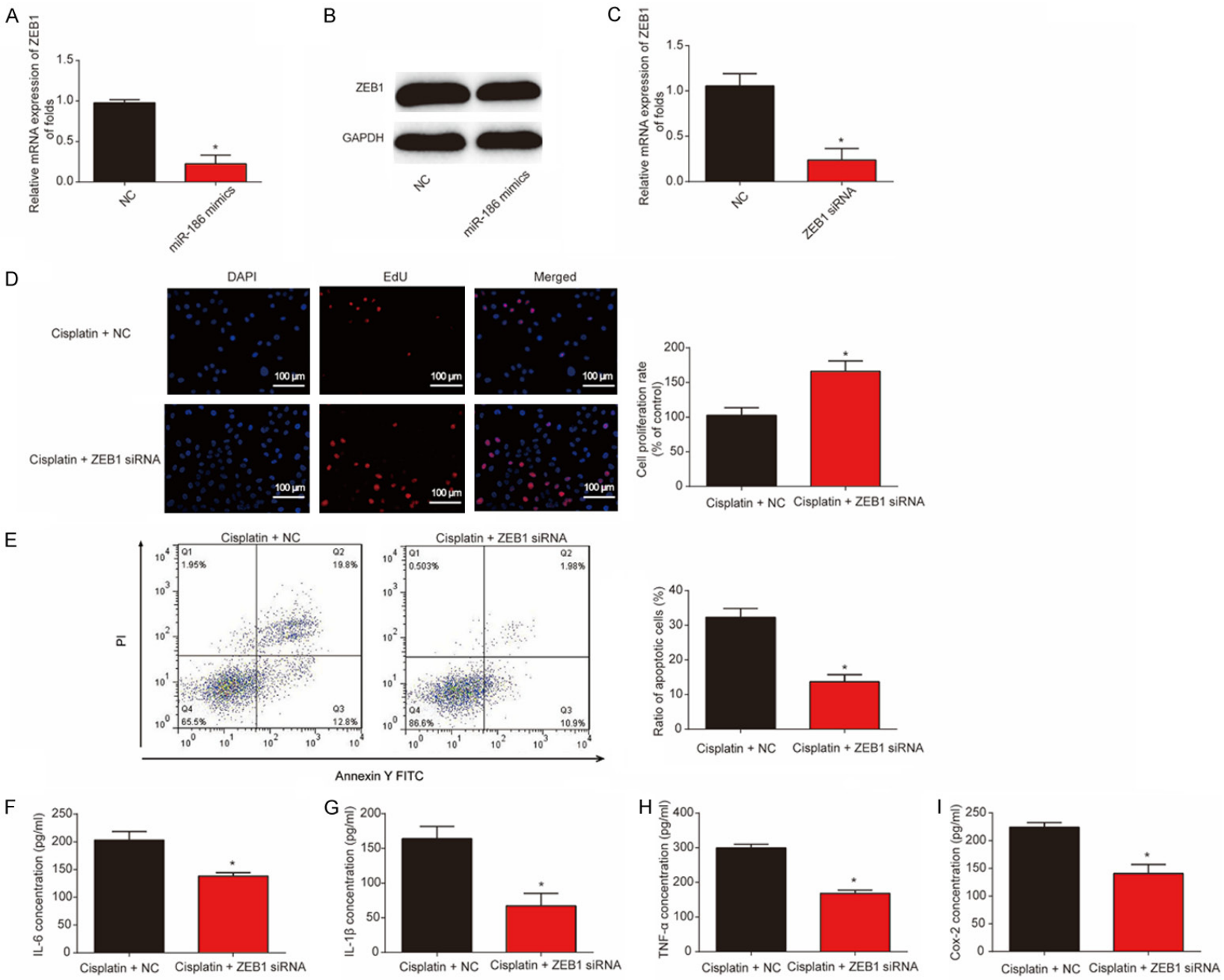

A
Relative mRNA expression of ZEB1 of folds
1.5
1.0
0.5
0.0
*
NC
miR-186 mimics
B
ZEB1
GAPDH
NC
miR-186 mimics
C
Relative mRNA expression of ZEB1 of folds
1.5
1.0
0.5
0.0
*
NC
ZEB1 siRNA
D
DAPI
EdU
Merged
Cisplatin + NC
Cisplatin + ZEB1 siRNA
100 μm
Cell proliferation rate (% of control)
200
150
100
50
0
*
Cisplatin + NC
Cisplatin + ZEB1 siRNA
E
Cisplatin + NC
Cisplatin + ZEB1 siRNA
PI
Annexin Y FITC
Q1
1.95%
Q2
19.8%
Q4
65.5%
Q3
12.8%
Q1
0.503%
Q2
1.98%
Q4
86.6%
Q3
10.9%
Ratio of apoptotic cells (%)
40
30
20
10
0
*
Cisplatin + NC
Cisplatin + ZEB1 siRNA
F
IL-6 concentration (pg/ml)
250
200
150
100
50
0
*
Cisplatin + NC
Cisplatin + ZEB1 siRNA
G
IL-1β concentration (pg/ml)
200
150
100
50
0
*
Cisplatin + NC
Cisplatin + ZEB1 siRNA
H
TNF-α concentration (pg/ml)
400
300
200
100
0
*
Cisplatin + NC
Cisplatin + ZEB1 siRNA
I
Cox-2 concentration (pg/ml)
250
200
150
100
50
0
*
Cisplatin + NC
Cisplatin + ZEB1 siRNA

**Figure 6.** Knockdown of ZEB1 repressed cisplatin-triggered renal injury in vitro. A. ZEB1 mRNA expression in NRK-52E cells transfected with miR-186 mimics or the corresponding negative controls for 48 hours. B. ZEB1 protein expression in NRK-52E cells transfected with miR-186 mimics or the corresponding negative controls for 48 hours. C. ZEB1 mRNA expression in NRK-52E cells. Cells were transfected with ZEB1 siRNA. D. EdU assay was used to test cell proliferation. NRK-52E cells were incubated with 6 μM cisplatin and then transfected with ZEB1 siRNA. Scale bars = 100 μm. E. Flow cytometry was carried out to detect cell apoptosis. F-I. ELISA assay was carried out to examine the concentrations of inflammatory cytokines (IL-6, IL-1β, TNF-α and Cox-2). Three independent experiments were carried out. Error bars stand for the mean ± SD of at least triplicate experiments. *P<0.05.

disease via modulating mitochondrial metabolism [19]. miR-106b-5p can inhibit kidney injuries after deep hypothermic circulatory arrest [20]. miR-375 can inhibit the progression of kidney cancer through triggering apoptosis and modulating PDK1 [21].

Here, in our present study, we investigated the biological role of miR-186 in cisplatin-induced AKI. We revealed the down-regulated miR-186 expression in AKI patients, and cisplatin-incubated NRK-52E cells and AKI rat models. We reported that overexpressing miR-186 could reverse the effects of cisplatin on NRK-52E cells proliferation and apoptosis. Moreover, miR-186 regulated its downstream target ZEB1. Knockdown of ZEB1 greatly increased NRK-52E cell proliferation and restrained the apoptosis, which were caused by indicated dose of cisplatin.

In recent years, miR-186 has been identified as a crucial cancer modulator in cancers. For instance, miR-186 inhibits prostate cancer cell proliferation by targeting GOLPH3 [22]. miR-186 can repress aerobic glycolysis in gastric cancer through regulating HIF-1α [23]. In addition, miR-186 can restrain NSCLC progression by targteing Yin Yang 1 [24]. In renal cell carcinoma, miR-186 can suppress cell proliferation and metastasis via regulating Sentrin-Specific Protease 1 [25]. In membranous nephropathy, loss of miR-186 contributes to the apoptosis of podocytes [26]. Here, in our study, we reported miR-186 was greatly decreased in AKI patients and AKI cell models induced by cisplatin. Besides, in AKI rat models, we also proved that miR-186 was obviously reduced. Then, miR-186 was overexpressed in NRK-52E cells and we observed that miR-186 greatly increased cell proliferation and repressed the apoptosis in NRK-52E cells.

ZEB1 is located on human chromosome 10 [27]. ZEB1 can control EMT process and its aberrant expression has been reported in many cancers [28]. Recent study has reported that ZEB1 is a potential regulator of Six2 in metanephric mesenchyme cell development [29]. miR-302a-3p may regulate renal EMT in diabetic kidney disease via targeting ZEB1 [30]. miR-205 can depress HK-2 cells EMT process through down-regulating ZEB1 expression [31]. miR-200 family can modulate TGF-β1-triggered renal tubular EMT by targeting ZEB1 [32]. Here, ZEB1 was predicted as the target of miR-186. We found that ZEB1 was significantly elevated in AKI. In addition, we reported that knockdown of ZEB1 was able to up-regulate NRK-52E cell proliferation and reduce the apoptosis, which were resulted from cisplatin treatment. Based on the online analysis, miR-186 has many target genes and ZEB1 was confirmed as one of them. The detailed effect of ZEB1 needed to be well studied in AKI. In addition, since other targets of miR-186 may also accelerate AKI, in our future study, more investigation is warranted.

In conclusion, our study indicated that miR-186 overexpression in cisplatin-incubated NRK-52E cells rescued the reduced cell viability and promoted cell apoptosis resulted from cisplatin exposure. These data implied miR-186 was a potential therapeutic target for treating AKI via targeting ZEB1.

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## Disclosure of conflict of interest

None.

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