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Overexpression of the long noncoding RNA NEAT1 protects against As_2O_3-induced injury of cardiomyocyte by inhibiting the miR-124/NF-κB signaling pathway

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Abstract. – OBJECTIVE: Arsenic trioxide (As_2O_3) an evident effect in the treatment of acute promyelocytic leukemia and other malignant tumors in recent years. However, more and more studies have found that the cardiac toxicity of As_2O_3 was increased, limiting its wide clinical application. This study aims to explore the molecule mechanisms of As_2O_3 on cardiomyocyte injury.

MATERIALS AND METHODS: The cardiomyocyte injury under As_2O_3 was detected by MTT assay. The levels of NEAT1 and miR-124 were examined by RT-PCR. The functions of NEAT1 and miR-124 at H9c2 cell injury under As_2O_3 were detected by cell transfection of the overexpression or repression. The expression levels of inflammation factors, apoptosis genes and NF-κB signals were measured by Western blot in H9c2 cell lines under As_2O_3. The luciferase assay detected the direct interaction between NEAT1 and miR-124.

RESULTS: The overexpression of NEAT1 decreased the H9c2 cells injury under As_2O_3. The levels of IL-1β, IL-6, TNF-α were upregulated after NEAT1 overexpression. Moreover, the luciferase assay results showed NEAT1 was directly interacting with miR-124. Silencing of miR-124 significantly increased the H9c2 cell survival under As_2O_3 by repressing NF-κB signaling pathway. Furthermore, the overexpression of NEAT1 markedly increased H9c2 cells survival under As_2O_3, while the miR-124 could reverse the effects. Finally, NEAT1 regulated the H9c2 cells As_2O_3 injury by repressing the miR-124, NF-kappa B expressions and inflammatory response.

CONCLUSIONS: According to the results, we found that long non-coding RNA NEAT1 regulated the expression of inflammatory factors to protect cardiomyocytes from As_2O_3 damage by inhibiting miR-124/NF-kappa B signaling pathway. It provides a novel potential treatment strategy for As_2O_3 cardiomyocytes injury.

Key Words:
LncRNA NEAT1, MiR-124, NF-κB signaling pathway, Arsenic trioxide, Cardiomyocyte injury.

Introduction

Arsenic trioxide (As_2O_3) is the main effective component of arsenic in traditional Chinese medicine. And it is also one of the most effective drugs in the treatment of acute promyelocytic leukemia (APL). It has been determined by NCCN in the United States as the first choice for the recurrence of APL[1]. In recent years, As_2O_3 has also made good achievements in the treatment of gastric cancer, liver cancer, lung cancer, lymphoma, breast cancer, cervical cancer and other malignant tumors[2]. However, the toxic effects of As_2O_3 have also increased gradually, such as cardiac toxicity, humoral retention, digestive tract symptoms, and so on[3]. In particular, the toxic effect of the heart limits the wide application of As_2O_3 in clinic[4]. The reports showed that 92.9%-100% of leukemia patients treated with arsenic were accompanied by cardiac toxicity[4]. More than half of the patients would have prolonged Q-T interval, sinus tachycardia, tachycardia, and even lead to death in severe cases[5]. Therefore, it is necessary to understand the cardiotoxicity mechanism of As_2O_3 deeply. At present, it is believed that the cardiotoxicity of As_2O_3 is mainly caused by the change of cardiac ion channels, oxidative stress injury and cardiomyocyte apoptosis[6], but the detailed molecular mechanism is still unclear.

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Arsenic trioxide, as an anticancer drug, is safe, effective and not easy to produce drug resistance. In order to solve the cardiotoxicity of arsenic trioxide, the researchers screened a variety of compounds that could alleviate the toxicity of the drug. For example, flavonoids can prevent cardiac toxicity caused by arsenic trioxide[7]. Morphine reduces the toxic effect of arsenic trioxide on H9c2 cardiomyocytes[8]. Omega-3 fatty acids protect the body from cardiac toxicity caused by arsenic trioxide *in vivo* and *in vitro*[9]. In addition, arsenic trioxide inhibits the proliferation of human pluripotent stem cells and cardiomyocyte differentiation[10]. And its toxicity affects the normal development of the heart [11]. At present, the molecular mechanism of As_2O_3 inducing myocardial cytotoxicity is less studied.

Long non-coding RNA is a kind of RNA, which is more than 200 nucleotides in length but does not have protein-coding function. It plays an important role in a variety of human diseases, including the process of cardiomyocyte injury [12,13]. For example, UCA1 reduced H9c2 injury, which is induced by hypoxia and glucose deprivation by decreasing miR-122 level[14]. TUG1 protects cardiomyocytes from ischemia-reperfusion injury by inhibiting HMGB1[15]. Inhibition of miR-7-5p expression by MEG3 leads to myocardial ischemia-reperfusion injury[16]. HIF1A-AS1 regulates SOCS2 expression by adsorbing miR-204 to promote ventricular remodeling after myocardial ischemia/reperfusion injury[17]. ANRIL regulates miR-7-5p/SIRT1 axis to protect H9c2 cells from hypoxia-induced injury[18]. Therefore, the further study of the role of lncRNAs in cardiomyocyte As_2O_3 toxicity is expected to provide a new idea for the prevention, diagnosis and treatment of As_2O_3 cardiotoxicity.

Long non-coding RNA NEAT1 (nuclear paraspeckle assembly transcript 1) has been reported to be associated with the development of multiple cancers and is often abnormally expressed in different types of solid tumors such as lung cancer, esophageal cancer, colorectal cancer, and liver cancer[19]. In particular the upregulation of NEAT1 regulated TLR4/NF-κB signaling and promoted the proliferation of lung adenocarcinoma cells[20]. As a competitive endogenous RNA (ceRNA), NEAT1, has the main role, which is that to inhibit the expression of microRNA in tumors, thus hindering the translation ability of transcripts of downstream carcinogenic targets, and finally promoting carcinogenesis[19]. In addition to playing a role in the development of cancer, NEAT1 also plays an important role in a variety of diseases. Chen et al[21] reported that NEAT1 plays an important role in sepsis-induced acute renal injury by regulating miR-204/NF- κB pathway. In addition, NEAT1 also plays an important role in cardiac cell injury such as vascular smooth muscle cell transformation[22], myocardial ischemia-reperfusion injury[23]. However, the function and mechanism of arsenic trioxide in cardiomyocyte toxicity are unknown.

This study aimed to investigate the function and mechanism of long non-coding RNA NEAT1 in arsenic trioxide toxicity of cardiomyocytes. Further, we studied the miRNA combined with NEAT1 and the related downstream signal pathways to explore the specific molecular mechanism of NEAT1 in the process of arsenic trioxide toxicity in cardiomyocytes. This study provides the theory and strategy of preventing, detecting and taking effective measures for the cardiotoxicity in the tumor treatment of As_2O_3.

Materials and Methods

Cell Culture and Treatment

H9c2 rat ventricular cardiomyocytes were purchased from Cell Library of the Chinese Academy of Sciences (Shanghai, China) and maintained in Dulbecco's Modified Eagle's Medium (DMEM) media (HyClone, South-Logan, UT, USA) supplemented with 15% fetal bovine serum (FBS; HyClone), 100 Ul/mL penicillin/ 100 μg/mL streptomycin (Sigma-Aldrich, St. Louis, MO, USA) (PH7.2-7.4) and cultured at 37° C in a CO_2 incubator (5% CO_2 and 95% air, 95% humidity). The cells were cultured by passage after fusion of 70% to 80%. As_2O_3 at 10 μM concentration was used to treat H9c2 cells for 24 h after cell growth to 70% fusion, and the model was referred to Zhang et al[24].

Lentivirus Infection

Full-length sequence of NEAT1 was cloned into lentivirus vector to construct NEAT1-overexpression vector (lenti-NEAT1). LV-NEAT1 or LV-NC was transfected into H9c2 cells and the infected cells were collected at 72 h after transfection. Next, puromycin was used to screen cells for at least 1 week to obtain the stable NEAT1-overexpression cells. The transfected and screened methods of miR-124 inhibitor, miR-124 mimic or miR-NC were the same as described above[24].

MTT Assay

Toxicity of As_2O_3 to H9c2 cells was evaluated by MTT assay. Briefly, H9c2 cells or transfected H9c2 cells were seeded in 96-well plates at density of 5×10^4 cells/well and cultured at 37° C in a CO_2 incubator (5% CO_2 and 95% air, 95% humidity) for 24 h. The cells were cultured according to the treatment described above. 10 μM As_2O_3 was added for culture at 0 h, 24 h, 48 h and 72 h, respectively. The MTT solution (dark) was added after supernatant discarding and PBS washing. 150 μl dimethyl sulfoxide (DMSO) was added and centrifuged for 15 min after incubation for 4 h. Plates were gently shaken to dissolve blue formazan crystals. The absorbance was tested at 490 nm using a microplate reader (Thermo Scientific, Vario Skan Flash, Waltham, MA, USA). Cell survival rate (%) =OD (experimental group)/OD (blank control group) ×100%. Cell growth curve was drawn, and the experiment was repeated three times.

RT-PCR Assays

Real-Time qRT-PCR (Quantitative Reverse Transcription PCR) amplification was performed using stem-loop primers; PCR primers were designed by Ribobio (Guangzhou, China) and the primer sequences are shown in Table I. Cells in each treatment group were collected. Total RNA was extracted with TRIzol and template cDNA was synthesized with reverse transcription kit (TaKaRa, Otsu, Shiga, Japan). The PCR system was using the UItraSYBR Green qPCR Mixture reagents (TaKaRa) in a Real-Time PCR System (BioRad, Hercules, CA, USA). Each sample was repeated 3 times and amplified by the following protocol: 30 sec at 95° C for the initial denaturation, followed by 95° C for 5 sec and 60° C for 30 sec for 40 cycles. After the reaction, the amplification curve and dissolution curve of PCR were confirmed to meet the requirements. Ct value was automatically output in the instrument software, and the $2^{-\Delta\Delta CT}$ method was used to calculate relative expression levels. GAPDH served as an endogenous control.

Western Blot Assays

Western blot analysis was used to assess the protein levels of inflammation factors, NF-κB signals and apoptosis signals. The cells or transfected with lncNC, lncNEAT1, miR-NC, miR-124 mimic, miR-124 inhibitor, were lysed by RIPA lysis buffer (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The samples were centrifuged at 12000 g for 10 min at 4° C and the concentrations were determined by the BCA Protein Assay Kit (Beyotime, Shanghai, China). Total proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Beyotime, China) and transferred to 0.22 μm polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% fetal bovine serum (FBS) buffer for 2 h and incubated overnight with primary antibodies (p65 1:1000, IL-1β 1:500, IL-6 1:1000, TNF-α 1:800, Bax 1:1000, Bcl-2 1:2000, cleaved PARP1 1:800, β-actin 1:3000) (Cell Signaling Technology, Danvers, MA, USA) at 4°C after washed triple by TBST. The β-actin was served as an endogenous control. After TBST washing, the chemiluminescence (ECL) reagent (Cell Signaling Technology, Danvers, MA, USA) was exposed to detect the target protein straps and the ImageJ software was used to evaluate the signals of each protein band.

Table I. Primers used for RT-PCR.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
GAPDH	CTCCTCCACCTTTGACGCTG	TCCTCTTGTGCTCTTGCTGG
U6	CTCGCTTCGGCAGCACATATACT	ACGCTTCACGAATTTGCGTGTC
NEAT1	TGGCTAGCTCAGGGCTTCAG	TCTCCTTGCCAAGCTTCCTTC
miR-124	CTAGTCTAGAGTCGCTGTTATCTCAT-TGTCTG	CGCGGATCCTCTGCTTCTGTCACAG-AATC
Bax	ACTAAAGTGCCCGAGCTGAT	ATGGTGAGTGAGGCAGTGAG
Bcl-2	GGCATCTTCTCCTTCCAGC	TCCCAGCCTCCGTTATCC
PARP1	GCGGAGAAGACATTGGGTGA	ACCATCTTCTTGGACAGGCG
IL-1β	GGACCTTCCAGGATGAGGACAT	GCTCATGGAGAATATCACTTGTTGG
IL-6	GACTGATGTTGTTGACAGCCACTGC	AGCCACTCCTTCTGTGACTCTAACT
TNF-α	CATGATCCGAGATGTGGAACTGGC	CTGGCTCAGCCACTCCAGC
IkBα	GACGAGGATTACGAGCAGAT	CCTGGTAGGTTACTCTGTTG
p65	GCCTCTGGCGAATGGCTTTA	TGCTTCGGCTGTTCGATGAT

Luciferase Reporter Assay

TargetScan website was used to search for the potential target miRNAs of NEAT1 (or p65). The partial sequences of NEAT1 containing the putative binding sites of miR-124 were amplified by PCR and cloned to construct NEAT1 wild-type (WT) reporter vector. Site-Directed Mutagenesis System (Thermo Fisher Scientific) was used to construct NEAT1 mutant-type (MUT) reporter vector with mutant miR-124 binding sites. Then the constructed reporter vector was transfected into H9c2 cells, respectively, together with miR-124 mimics or miR-NC. 48 h post-transfection, luciferase activity of the cells was assayed using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Statistical Analysis

The data were analyzed using SPSS 20.0 (SPSS Inc. IBM, Armonk, NY, USA) and reported as mean ± standard deviation of the number of experiments indicated. For all the measurements, one-way ANOVA analysis followed by a Student's *t*-test was used to assess the statistical significance of the difference between each group. A statistically significant difference was considered at the level of $p < 0.05$.

Results

Effect of Sodium Trioxide on the Proliferation of H9c2 Cell

To explore the function of NEAT1 in cardiomyocytes As_2O_3 injury, we first constructed a cell model of As_2O_3 injury in H9c2 cells. It was reported that the concentration of As_2O_3 in cardiomyocytes injury model was generally 10 μmol/L. In order to analyze the inhibitory effect of As_2O_3 on the proliferation of H9c2 cells, we used the MTT assay to detect the proliferation of H9c2 cells in 0 h, 24 h, 48 h, 72 h at 10 μmol/L treatment condition. The results showed that compared with the control group, the survival of H9c2 cells was significantly inhibited in the cardiomyocytes model of As_2O_3 injury (Figure 1A) ($p<0.05$). Furthermore, we also detected the expression of Bax, cleaved PARP1 and Bcl-2 in As_2O_3 injury cells. The result showed that Bax, cleaved PARP1 levels were increased significantly, and the Bcl-2 level was significantly reduced in H9c2 myocardial injury cells (Figure 1B-1C) ($p<0.05$). These findings indicated that the H9c2 cell model of As_2O_3 injury was constructed successfully.

It is reported that lncRNA NEAT1 plays an important role in cardiac toxicity. Liu et al[25] reported that NEAT1 upregulation inhibited doxorubicin-induced cardiotoxicity in mice. Wang et al[26] showed that NEAT1 attenuates sepsis-induced myocardial injury by regulating the TLR2/NF-κB signaling pathway. These studies have shown that NEAT1 plays a protective role in myocardial cell injury, but it is also found that NEAT1 promotes myocardial cell damage. In myocardial ischemia-reperfusion injury, the down-regulation of NEAT1 promoted the cell proliferation and inhibited apoptosis by targeting miR-193a[27]. Ruan et al[28] showed NEAT1 regulated the miR-27b/PINK1 and aggravated myocardial ischemia-reperfusion injury in diabetes mellitus. In order to confirm that NEAT1 can be used as one of the therapeutic targets to alleviate the injury of As_2O_3 cardiomyocytes, we have to explore the role of NEAT1 in cardiomyocytes damaged by As_2O_3. Therefore, we detected the expression of NEAT1 in As_2O_3 injury cell and the results showed that the expression of NEAT1 was significantly down-regulated in H9c2 injury cells (Figure 1D) ($p<0.05$). These results suggested that NEAT1 may play a protective role in cardiomyocytes injury by As_2O_3.

NEAT1 Overexpression Attenuated the Inflammatory Response

Concerning the molecular mechanism of NEAT1 protecting cardiomyocytes from As_2O_3 damage, we constructed a cardiomyocyte line with overexpression of NEAT1 (Figure 2A) ($p<0.05$). 10 μmol/L As_2O_3 was added to the H9c2 cells after transfection of lnc-NC or lnc-NEAT1. The MTT assay results showed that overexpression of NEAT1 alleviated the inhibitory effect of As_2O_3 on the proliferation of H9c2 cells compared with the control (Figure 2B) ($p<0.05$). Gast et al[29] have shown that NEAT1 regulates the immune cell function and is suppressed in patients with early myocardial infarction. With regard to investigate whether there is an immune response in the process of protecting cardiomyocytes by NEAT1, we detected the expression of IL-1β, IL-6, TNF-α in the As_2O_3 cell model after overexpression of NEAT1. The results showed that overexpression of NEAT1 significantly decreased the transcription and translation of IL-1β, IL-6 and TNF-α compared with the control (Figure 2C-D) ($p<0.05$). These results suggested

that NEAT1 protects H9c2 cells from As_2O_3 injury by inhibiting the expression of inflammation factors.

MiR-124 was a Target of NEAT1

To explore the molecular mechanisms of NEAT1 that attenuated the As_2O_3 injury in cardiomyocytes, we found about 80 miRNAs interacting with NEAT1 through starBase v2.0 database. In those miRNAs data, we found that miR-124 has been reported to be associated with cardiomyocyte survival and the up-regulation of miR-124 aggravated hypoxia injury in H9c2 cells[30]. We found the binding sequence of NEAT1 to miR-124 in the database and designed the NEAT1 mutation sequence corresponding to the binding site (Figure 3A). The luciferase result showed that the NEAT1 did bind to miR-124 (Figure 3B). With the overexpression of NEAT1, the miR-124 level was significantly down-regulated (Figure 3C). Besides, we also detected that the expression of miR-124 was significantly increased in As_2O_3 injury H9c2 cells (Figure 1D). These results suggested that miR-124 is the directly targeted gene of NEAT1 and NEAT1 may regulate the survival of As_2O_3 injury H9c2 cells by regulating miR-124.

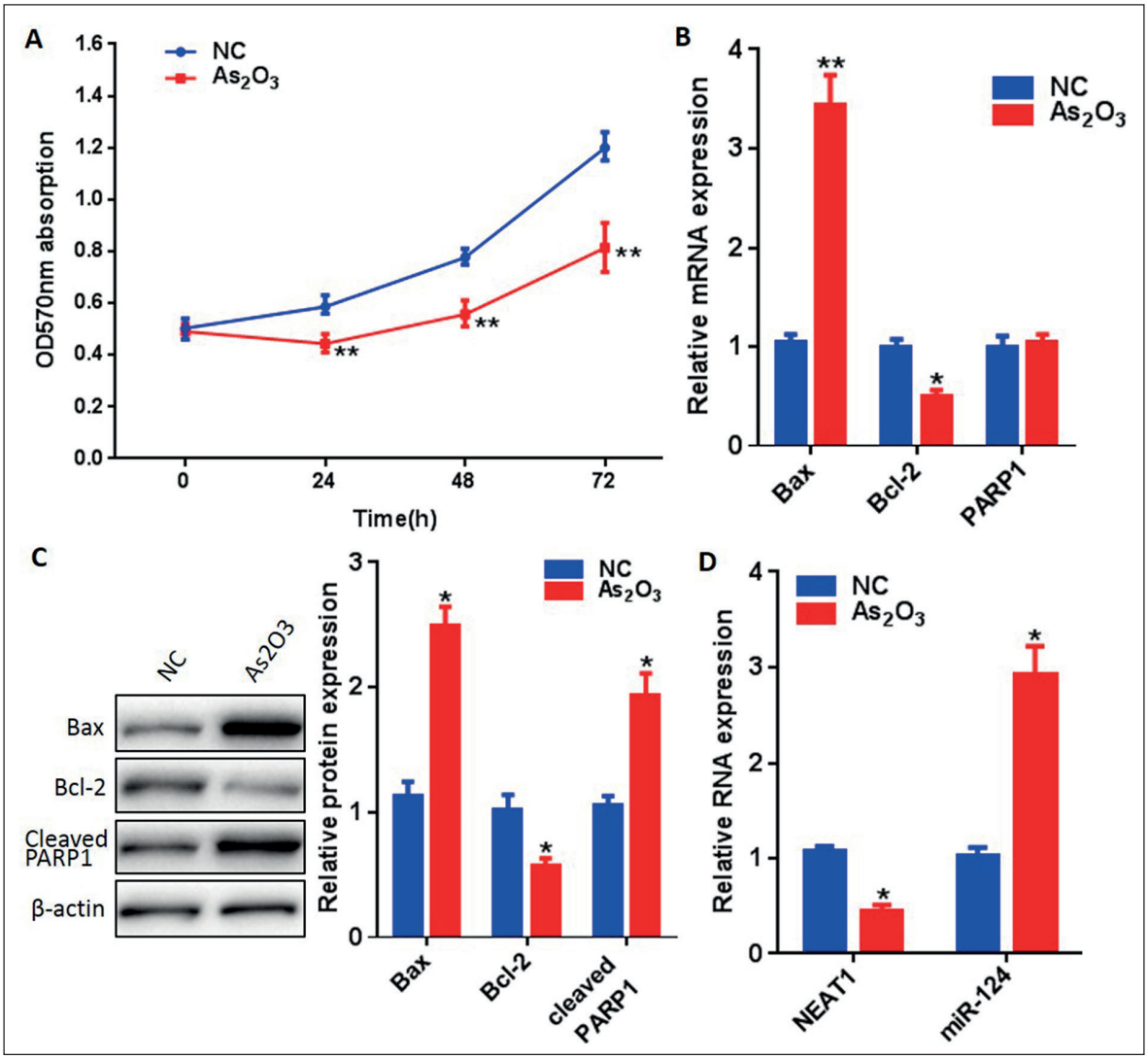

Figure 1. The H9c2 myocardial injury cell model by As_2O_3 was constructed successfully. **(A)** The inhibition on growth of the H9c2 cells *in vitro* after treatment with As_2O_3 for 24 h by MTT assay. **(B-C)** The levels of Bax, cleaved PARP1 and Bcl-2 in As_2O_3 injury cells were detected by RT-PCR and Western blot. **(D)** The expressions of NEAT1 and miR-124 in As_2O_3 injury cells were detected by RT-PCR. Data are present the mean ± SD of three independent experiments. *p<0.05, **p<0.01, *vs.* the control group, same as below.

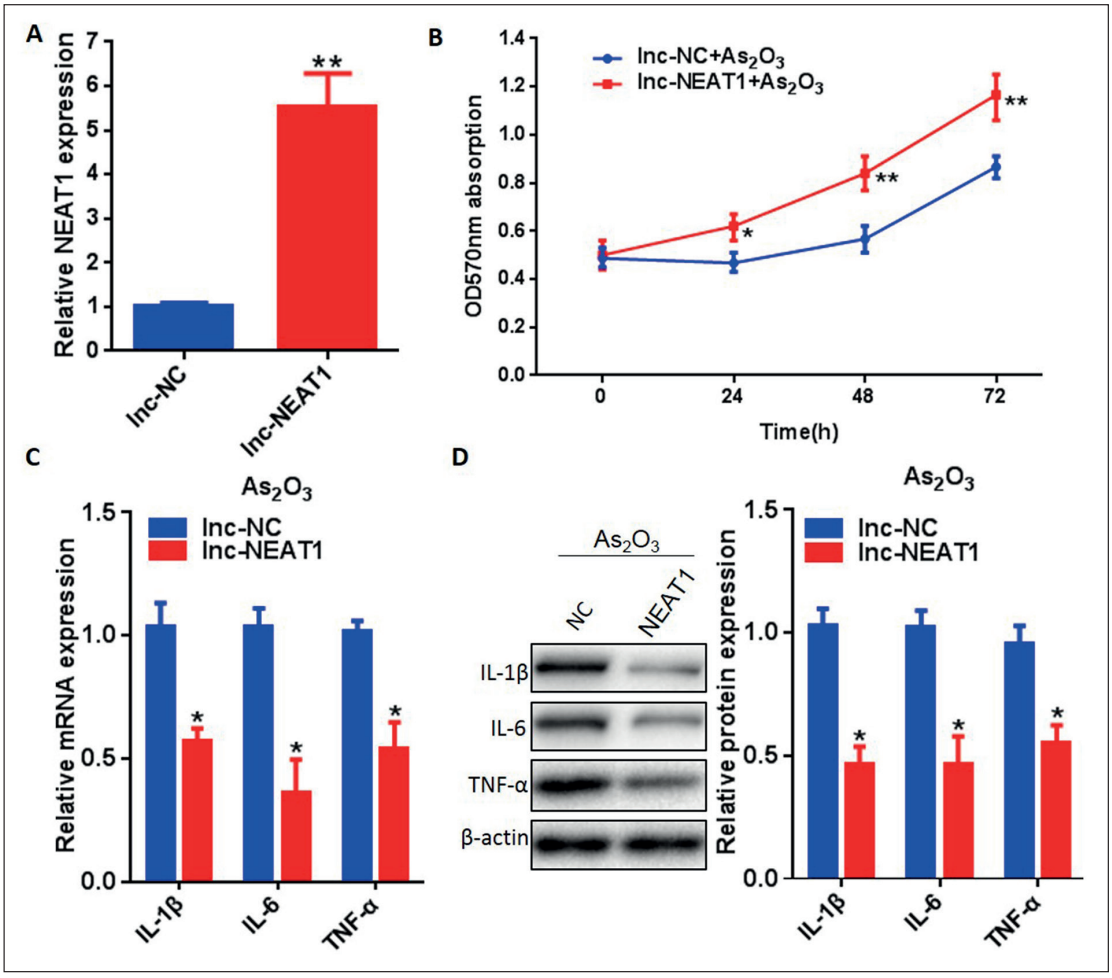

Figure 2. The expression of NEAT1 protects H9c2 cells from As_2O_3 injury**. (A)** The NEAT1 levels in H9c2 cells after transfected was detected by RT-PCR. **(B)** The growth of NEAT1 overexpression H9c2 cells after treatment with As_2O_3 for 24h by MTT assay. **(C-D)** The expressions of IL-1β, IL-6 and TNF-α in As_2O_3 cell model after overexpression of NEAT1 were detected by RT-PCR and Western blot. Data are present the mean ± SD of three independent experiments. *p<0.05, **p<0.01.

MiR-124 Repression Attenuated the Inflammatory Response

To detect the role of miR-124 in H9c2 cells damaged by As_2O_3, we constructed a myocardial cell line with the inhibition of miR-124 expression (Figure 4A) (p<0.05). 10 μmol/L As_2O_3 was added to the H9c2 cells after transfection of miR-NC or miR-124 inhibitor. The MTT assay results showed that inhibition of miR-124 promoted the cell survival and decreased the inhibitory effect of As_2O_3 on H9c2 cells (Figure 4B) (p<0.05). To investigate the changes of inflammatory factors during the inhibition of cell survival by miR-124, we observed the expressions of IL-1β, IL-6 and TNF-α by RT-PCR and Western blot. The results showed that the IL-1β, IL-6 and TNF-α levels were decreased significantly in H9c2 injured cells (Figure 4C) (p<0.05). These results indicated miR-124 upregulated the expression of inflammatory factors and then promoted the injury of H9c2 cells induced by As_2O_3.

MiR-124 Upregulation Activated the NF-κB Signaling Pathway

In addition, we also detected the molecular mechanism of miR-124 promoting As_2O_3 damage to H9c2 cell. Some researchers have shown that miR-124 directly activated the NF-κB signal and regulated cell proliferation, immunosuppression [31,32]. To study whether miR-124 affected the H9c2

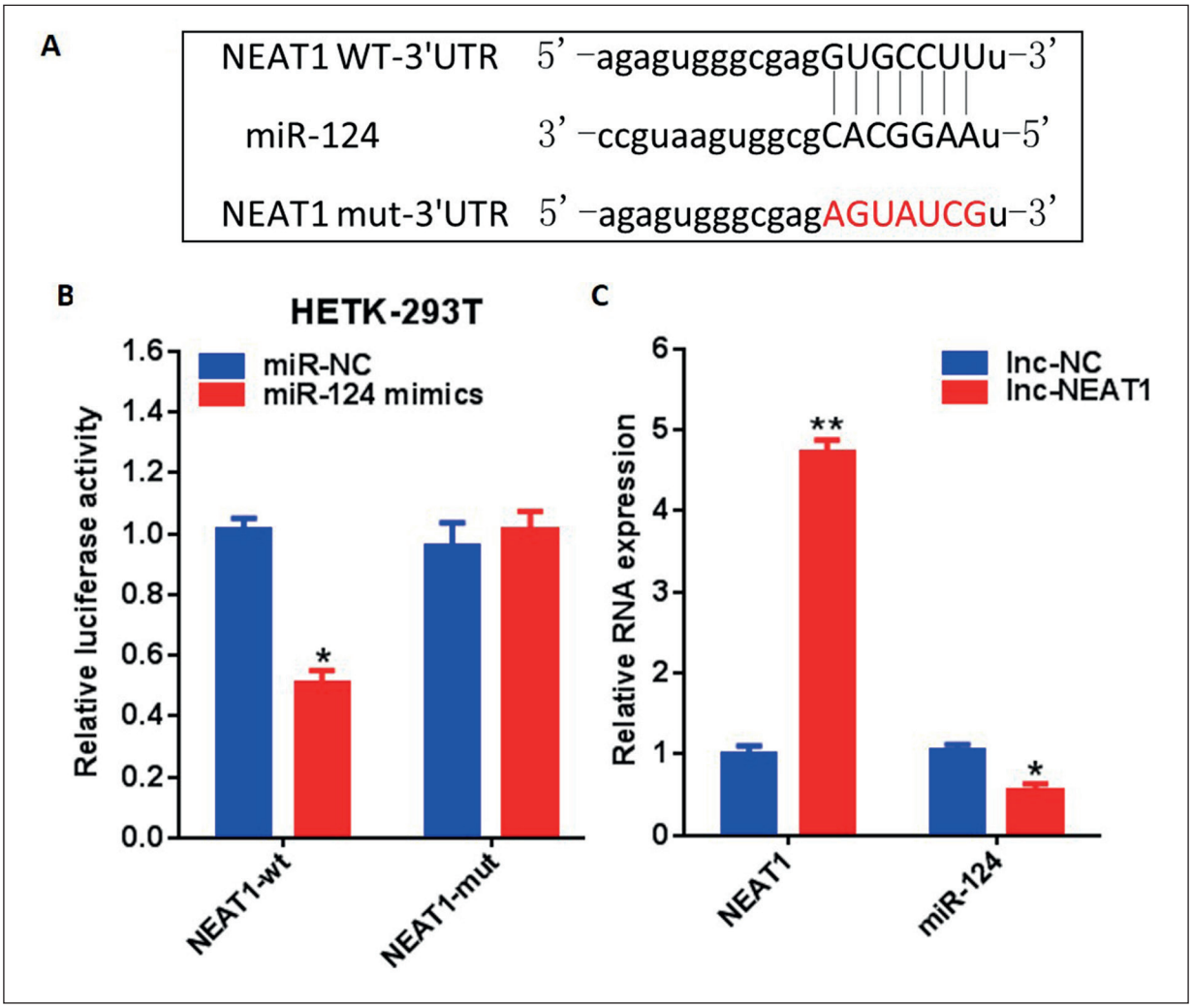

Figure 3. The NEAT1 directly binding with miR-124. **(A)** The binding sequence of NEAT1 to miR-124 by starBase v2.0 database. **(B)** The NEAT1 directly binding with miR-124 by luciferase assay. **(C)** The level of miR-124 in NEAT1 overexpression H9c2 cells was detected by RT-PCR. Data are present the mean ± SD of three independent experiments. *$p<0.05$, **$p<0.01$.

cells injury by regulating NF-κB signaling pathway, we detected the interaction and expression of NF-κB in miR-124 overexpression cells. The luciferase assay result showed miR-124 binding with NF-κB (Figure 5A-B) ($p<0.05$). The NF-κB level was significantly down-regulated in cardiomyocytes after overexpression of miR-124 (Figure 5C-D) ($p<0.05$). Moreover, we also observed the expressions of NF-κB signals in H9c2 cells damaged by As_2O_3 and it was showed that the NF-κB signals were activated in injured H9c2 cells (Figure 5E-F) ($p<0.05$). These results suggested that As_2O_3 activated NF-κB signaling by up-regulating miR-124 level and promoted H9c2 cells apoptosis.

MiR-124 Reversed NEAT1-Mediated the Inflammatory and Apoptosis Caused by Activated NF-κB

With the aim to show that NEAT1 protected H9c2 cells from As_2O_3 by inhibiting miR-124/NF-κB signal axis, we upregulated miR-124 levels in NEAT1 overexpressed cells to test whether miR-124 could reverse the protective effect of NEAT1. The MTT assay results showed that the overexpression of NEAT1 attenuated the As_2O_3 injury of cardiomyocytes, while the effects were reversed after miR-124 overexpression (Figure 6A) ($p<0.05$). The results of RT-PCR and Western blot indicated that the expression of NF-κB signals was inhi-

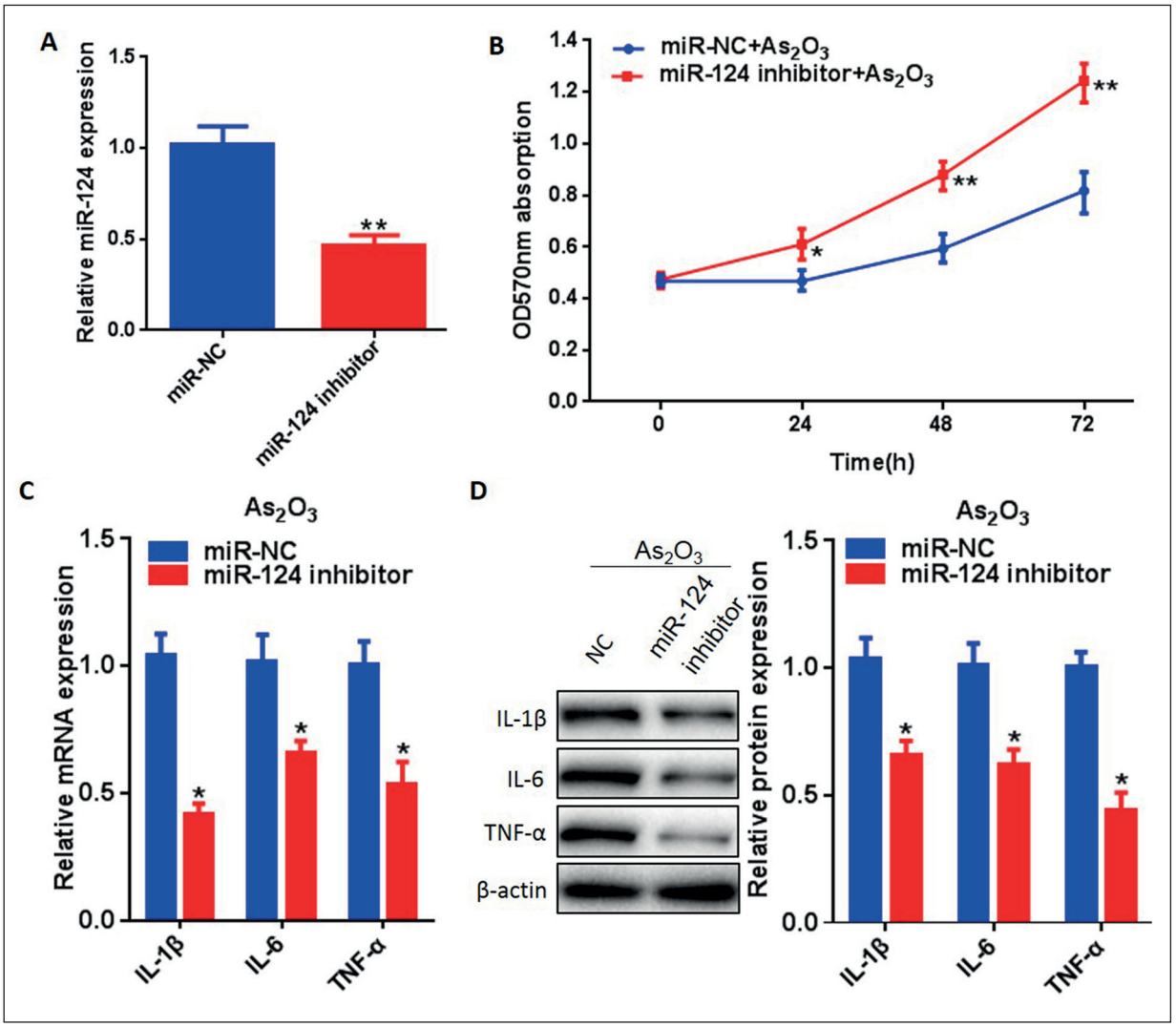

Figure 4. The expression of miR-124 promoted the H9c2 cells injury by As_2O_3. **(A)** The miR-124 levels in H9c2 cells after transfected was detected by RT-PCR. **(B)** The H9c2 cells survival of miR-124 repression after treatment with As_2O_3 for 24h by MTT assay. **(C-D)** The levels of IL-1β, IL-6 and TNF-α in As_2O_3 cell model after inhibition of miR-124 were detected by RT-PCR and Western blot. Data are present the mean ± SD of three independent experiments. *p<0.05, **p<0.01.

bited after NEAT1 upregulation, while the overexpression of miR-124 promoted the expression of NF-κB signals in As_2O_3 injured H9c2 cells (Figure 6B-C) (p<0.05). At the same time, the expressions of IL-1β, IL-6 and TNF-α were significantly down-regulated after NEAT1 overexpression, while miR-124 promoted the expression of these inflammatory factors (Figure 6D-E) (p<0.05). The above results suggested that NEAT1 regulated the expression of inflammatory factors to protect H9c2 cells from As_2O_3 damage by inhibiting miR-124/NF-κB signaling pathway.

Discussion

It is reported that the treatment of various cancers with As_2O_3 is increasing day by day[33,34], but its short-term or long-term toxicity is at the same time more common. Cardiac toxicity is the most apparent in various toxicity by As_2O_3[4]. However, the mechanism of cardiotoxicity induced by As_2O_3 is still unclear. More and more evidence has reported that NEAT1 plays some crucial roles in the formation and progression of a series of cancers. Recent studies also reveal that NEAT1 plays an important role in the process

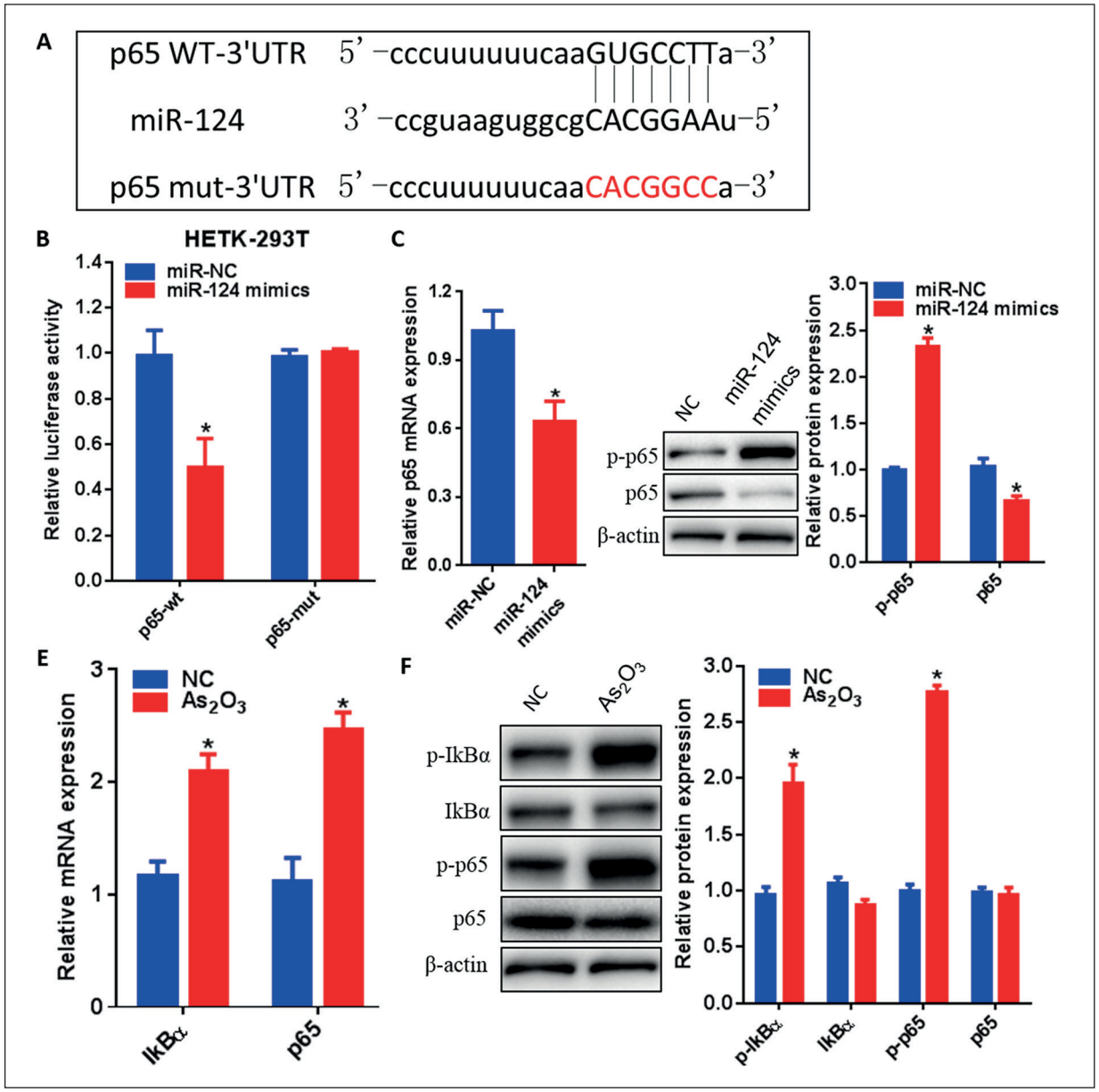

Figure 5. As_2O_3 activated NF-κB signaling by regulating miR-124. **(A)** The binding sequence of p65 to miR-124. **(B)** The p65 directly binding with miR-124 by luciferase assay. **(C-D)** The p65 levels were detected by RT-PCR and Western blot in H9c2 cells after miR-124 overexpression. **(E-F)** The expressions of NF-κB signals including IkBα and p65 in As_2O_3 cell model were detected by RT-PCR and Western blot. Data are present the mean ± SD of three independent experiments. *p<0.05, **p<0.01.

of cardiomyocyte injury[23]. Therefore, making a better understanding of NEAT1 may be useful as special markers and therapeutic targets in the diagnosis and treatment of cardiomyocyte As_2O_3 damage.

In this study, the function and molecular mechanism of NEAT1 in arsenic trioxide cardiomyocyte injury model were detected and discussed. Our results showed that the overexpression of NEAT1 decreased the H9c2 cells apoptosis and upregulated the inflammation response under As_2O_3. The role of NEAT1 was caused by interacting with miR-124 and the downregulation of miR-124 increased the H9c2 cell survival significantly by repressing NF-κB signaling pathway under As_2O_3. Our research showed NEAT1 may be effective protection and therapeutic target for As_2O_3 injury.

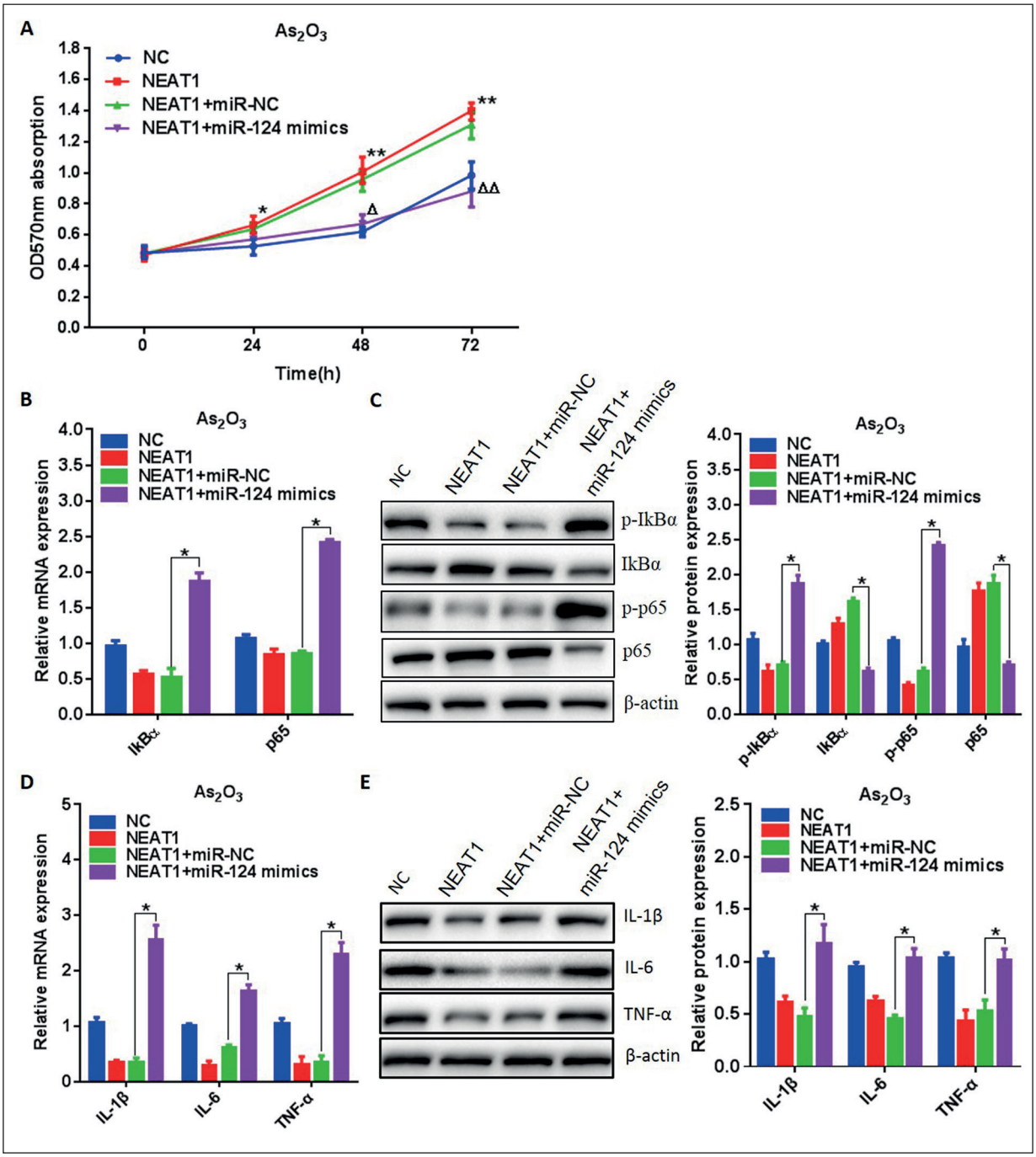

Figure 6. NEAT1 protects H9c2 cells from As_2O_3 damage by inhibiting miR-124/NF-κB signal axis. **(A)** The H9c2 cells survival of NEAT1 adding miR-124 expression after treatment with As_2O_3 for 24h by MTT assay. **(B-C)** The IkBα and p65 levels were detected by RT-PCR and Western blot in H9c2 cells with NEAT1 adding miR-124 overexpression after treatment with As_2O_3. **(D-E)** The expressions of IL-1β, IL-6 and TNF-α were detected by RT-PCR and Western blot in H9c2 cells with NEAT1 adding miR-124 overexpression after treatment with As_2O_3. Data are present the mean ± SD of three independent experiments. *p<0.05, **p<0.01.

As we know, arsenic and its various forms have been used in ancient Chinese medicine for more than 2000 years. Arsenic agents become more and more important because of the therapeutic effects on diseases to cancer[35]. The ability of arsenic, especially arsenic trioxide, to treat acute promyelocytic leukemia has fundamentally changed the people's understanding for the poi-

son. But the cardiac toxicity of arsenic trioxide is still making the clinic application in trouble. In our results, NEAT1 is down-regulated, miR-124 expression is increased and NF-κB signaling pathway is activated after As_2O_3 treatment in cardiomyocytes, promoting the inflammatory response and apoptosis process of cardiomyocytes. Our research shows another molecular mechanism of arsenic trioxide cardiotoxicity.

It is reported that NEAT1 plays an important role in heart injury. NEAT1 inhibits cardiomyocyte apoptosis by regulating BCL2L12/miR-125a-5p expression[36]. In doxorubicin induced cardiotoxic mice model, NEAT1 up-regulated the let-7f-2-3p level to prevent cardiac injury process[25]. NEAT1 is down-regulated in patients with early myocardial infarction[29]. In our study, NEAT1 promotes an inflammatory response in cardiomyocytes and protects the normal survival of cardiomyocytes. This is quite similar to the results of other investigations above, which NEAT1 protect cardiomyocytes.

MiR-124 as a tumor inhibitor, has been confirmed in a variety of cancers. For example, miR-124 increases the chemosensitivity of human esophageal cancer cells by regulating the expression of CDK4[37]. MiR-124-3p can inhibit bladder cancer by targeting DNA methyltransferase B[38]. In non-small cell lung cancer, miR-124 inhibits the growth of cancer cells and enhances radiation-induced apoptosis by inhibiting STAT3[39]. Moreover, MiR-124 also inhibits the growth of pancreatic ductal carcinoma through regulating lactic acid metabolism[40]. In addition, it plays a role in other diseases such as traumatic brain injury by regulating inflammation processes[41]. miR-124 reduces skin inflammation by inhibiting the innate immune response[42]. Zhao et al[43] have also shown that miR-124 increased intestinal inflammation. In this study, miR-124 is upregulated and activated NF-κB signal, then increasing the inflammatory response and decreasing the As_2O_3 injury process of cardiomyocytes. Our results confirmed that miR-124 can promote inflammation in cardiomyocytes.

In recent years, a large number of studies have reported that NEAT1 may be used as the ceRNA to antagonize the function of miRNA and played an important role in the occurrence and development of many cancers[19]. In human breast cancer, NEAT1 promotes cell growth by regulating miR-211/HMGA2[44]. Similarly, NEAT1 accelerates tumor progress by inhibiting E2F3 in non-small cell lung cancer[45]. It also promotes the transformation of epithelial cells into stroma by regulating the miR-204/ZEB1 axis in NPC[46]. In our results, we found that NEAT1 interacts with miR-124 directly and inhibits the expression of miR-124. This is consistent with previous papers[47,48].

Traditional Chinese medicine (TCM) has the characteristics of being multi-component and multi-target medicine. It has an incomparable protective effect on the mechanism[49] of cardiotoxicity caused by As_2O_3. Therefore, the research and development of targeted drugs in the later stage focus on the combination of Chinese medicinal materials with anti-inflammatory effects and As_2O_3, which can maximize its anti-tumor characteristics while minimizing cardiac toxicity. This makes As_2O_3 more useful to human beings. In our research, NEAT1 modulates the inflammatory response to protects cardiomyocytes from As_2O_3 damage, providing a new strategy for the development of targeted drugs.

Conclusions

Taken together, long non-coding RNA NEAT1 inhibited the expression of miR-124/NF-κB signal and regulated the inflammatory response to attenuate the cardiomyocytes damage by arsenic trioxide *in vitro*. Our study provides an important insight for understanding the cardiac toxicity molecular mechanisms of arsenic trioxide and also a new theoretical basis and therapeutic strategy for the research and development of targeting drugs with cardiac protection.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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