Indian Journal of Experimental Biology
Vol. 61, August 2023, pp. 622-629
DOI: 10.56042/ijeb.v61i08.3674

Circ_0004214 prevents human cardiomyocytes from doxorubicin induced cardiotoxicity by governing the miR-22-3p/GATA4 pathway

Lin Yang[1#], Ya Nan Liu[2#], Yi Gu[3], Lan Zhu[4]* & Qi Guo[5]*

[1]Intensive Care Unit; [2]Department of Obstetrics and Gynecology;[3]Nursing department of Obstetrics and Gynecology;
[4]Nursing department of Emergency synthetic ward;[5]Department of Radiotherapy,
Second Affiliated Hospital of Soochow University, Suzhou - 215006, Jiangsu, China

Received 19 April 2022; *revised 22 June 2023*

In a clinical setting, the likelihood of doxorubicin (DOX) causing cardiotoxicity is high. However, the underlying mechanism remains obscure. In this study, we investigated whether DOX toxicity is associated with the deregulation of circular RNA_0004212 (circ_0004214). Circ_0004214, microRNA-22-3p (miR-22-3p), and GATA binding protein 4 (GATA4) expression in human cardiomyocyte AC16 cells was detected via RT-qPCR. Lactate dehydrogenase (LDH) release, reactive oxygen species (ROS) production, malondialdehyde (MDA) content, and 4-hydroxynonenal (4-HNE) content were assessed using corresponding commercial kits. Cell viability and apoptosis were analyzed using cell counting kit-8 (CCK-8) and flow cytometry assays. Western blot assay was used to evaluate apoptosis-related markers and GATA4 protein levels. Dual-luciferase reporter validated the relationship between miR-22-3p and circ_0004214 or GATA4. Declined circ_0004214 was viewed in DOX-treated AC16 cells. DOX treatment weakened cell viability, and promoted oxidative stress and apoptosis, which was ameliorated via circ_0004214 overexpression. In addition, circ_0004214 promoted GATA4 expression by decoying miR-22-3p. Overall, the results have demonstrated that circ_0004214 protects against DOX-induced cardiotoxicity via governing miR-22-3p/GATA4 pathway, and thereby reveal promising therapeutic strategies against cardiotoxicity.

Keywords: Anticancer, circular RNAs, GATA binding protein, microRNA, Reactive oxygen species (ROS)

Doxorubicin (DOX), the most widely used antitumor drug, to prevents the proliferation of cancer cells by inhibiting DNA topoisomerase II (TOP2)[1]. However, the use of DOX is accompanied by serious side effects, leading to various diseases, such as dilated cardiomyopathy and heart failure[2,3]. The cardiotoxicity of DOX is characterized by cardiomyocyte apoptosis or other forms of cell death, accompanied by a series of oxidative stress[1,4]. Understanding the exact mechanism of cardiotoxicity caused by DOX may help improving its clinical applications.

Previous studies have documented that non-coding RNAs (ncRNAs), such as circular RNAs (circRNAs) and microRNAs (miRNAs), are associated with DOX-inducedcardiotoxicity in cardiomyocytes[5,6]. CircRNAs are well-characterized by closed-loop structures, which makes them more stable than linear RNA molecules[7]. Furthermore, circRNAs exhibit a wide range of biological functions and play pivotal roles in multiple human diseases[8]. Interestingly, previous studies have stated that RNA-binding protein largely alleviate DOX-triggered toxic effects via the regulation of numerous circRNAs[9], suggesting that circRNAs are involved in DOX-induced cardiotoxicity. Circ_0004214 (circ-AMOTL1) is produced by the human angiomotin-like 1 gene (AMOTL1), and was previously reported to alleviate DOX-induced cardiomyopathy and cardiomyocytes death in mice by ectopic expression of circ_0004214[10], which largely attracted our interest. Yet, the detailed effects of circ_0004214 on DOX-triggered cardiotoxicity are poorly defined.

It has been widely explored that miRNAs are regarded as biomarkers for DOX cardiotoxicity[11,12]. Already, miR-22-3p has been documented to be upregulated in hearts from DOX-treated murine, and its deficiency largely suppressed DOX-triggered cardiotoxicity[13]. Though circ_0004214 is known to possess some binding sites with miR-22-3p, their interactions in DOX-induced cardiotoxicity have not been addressed.

*Correspondence:
Phone: +86 15150139687
E-Mail: guoqi456258@163.com

As negative regulators of gene expression, miRNAs govern function by interacting with their 3'UTR[14]. For example, reduced miR-22-3p attenuated DOX-evoked oxidative stress and cardiomyocyte apoptosis by modulating sirtuin 1[13]. Bioinformatics analyses have shown that there were still numerous mRNAs targeted by miR-22-3p, such as GATA binding protein 4 (GATA4). GATA4 was exposed to inhibit DOX-induced autophagy and cardiomyocyte death[15], suggesting that GATA4 prevents DOX-induced cardiotoxicity. The role of GATA4 in the circ_0004214-mediated miR-22-3p regulatory networks needs demonstrating. Hence, in this study, we tried to understand the mechanism of circ_0004214 that determines the interactions among circ_0004214, miR-22-3p, and GATA4 in DOX-treated cardiomyocytes (AC16).

Materials and Methods

Cells treatment

In this research, Bena Culture Collection (Beijing, China) offered human ventricular cardiomyocytes (AC16), which were maintained in DMEM medium and 10% FBS (Sigma-Aldrich, St. Louis, MO, USA), setting at 37°C supplemented with 5% CO_2 in a humidified atmosphere.

Doxorubicin (DOX; Sigma-Aldrich) dissolved in DMSO was used to administer 1×10^6 AC16 cells at a final concentration of 0, 1, 2, 3 or 4 μM for 24 h. Meanwhile, AC16 cells were exposed to 4 μM DOX for 0-24 h and then collected for other analyses. In function experiments, 4 μM DOX for 24 h was applied to maintain the Ac16 cell phenotype.

Cell counting kit-8 (CCK-8 assay

After maintaining for 48 h, 2×10^3 cells in 96-well plates were exposed to 10 μL CCK-8 reagent (Dojindo, Kumamoto, Japan) and incubated for 4 h. To examine cell viability, samples were measured using a microplate reader at 450 nm.

RT-qPCR

After being isolated from a canonical Trizol (Invitrogen, Paisley Scotland, UK), RNA was reversely transcribed into cDNA according to HiScript III 1st Strand cDNA Synthesis Kit or using miRNA 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China). qPCR amplification was implemented based on SYBR Green Master Mix (Vazyme). $2^{-\Delta\Delta CT}$ method analyzed gene expression, and β-actin or U6 was used as an internal control. Primer details are given in Table 1.

Table 1 — Primer details

circ_0004214	F: 5'-ACGAGATGGTCAAGCCCTAC-3' R: 5'-TCGGAACCTCTCATTTCCAC-3'
miR-22-3p	F: 5'-GCCGAGAAGCTGCCAGTTGAAG-3'R: 5'-CAGTGCGTGTCGTGGAGT-3'
GATA4	F: 5'-CGACACCCCAATCTCGATATG-3' R: 5'-GTTGCACAGATAGTGACCCGT-3'
U6	F: 5'-CGCTTCGGCAGCACATATACTA-3' R: 5'-CGCTTCACGAATTTGCGTGTCA-3'
β-actin	F: 5'-CTCCATCCTGGCCTCGCTGT-3' R: 5'-GCTGTCACCTTCACCGTTCC-3'

Oligonucleotides, vectors, and cell transfection

Sequence of circ_0004214 was cloned into pCD5-ciR vector for circ_0004214 overexpression, and Geneseed (Guangzhou, China) supplied fusion vector (circ_0004214) and Vector. The mimics/inhibitors miR-22-3p (miR-22-3p/anti-miR-22-3p), and matched controls were supplied by Ribobio (Guangzhou, China). Genepharma (Shanghai, China) provided si-GATA4 for GATA4 knockdown and si-NC. Based on Lipofectamine 3000, transfection of AC16 cells was performed for 48 h. Then, transfection efficiency was tested using RT- qPCR or Western blot.

Measurement of LDH, ROS, MDA, and 4-HNE

LDH release (a cytotoxic assay), ROS production, MDA content, and 4-HNE content were determined using LDH assay kit (ab65396; Abcam, Cambridge, MA, USA), ROS/superoxide detection assay kit (ab139476; Abcam), MDA assay kit (ab118970; Abcam), and lipid peroxidation assay kit (ab238538; Abcam), respectively.

Flow cytometry assay

Simply put, AC16 cells with treatment or transfections were digested with trypsin and then resuspended into 1×annexin V Binding Buffer with annexin V-FITC and PI (P-CA-201; Procell, Wuhan, China) for 1h in the dark. After incubation, the sample status was examined using a flow cytometer equipped with CellQuest software.

Western blot assay

Using RIPA lysis buffer IV (Sangon Biotech, Shanghai, China), total proteins were prepared, followed by quantification using BCA protein assay kit (Sangon Biotech). After being subjected to separating gel and blotted to the membrane (Bio-Rad), proteins were blocked in BLOT-QuickBlocker (Sangon Biotech). The membranes were probed with the specific primary antibodies in this experiment (Abcam): anti-Bax (ab182733), anti-Bcl-2 (ab182858), anti-cleaved caspase-3 (ab214430), anti-GATA4 (ab256782), anti-β-actin (ab8227) at 4°C

overnight, followed by incubation with HRP-conjugated secondary antibody (ab205718) for 1 h. The protein signals were detected with High sensitive Plus ECL luminescence reagent (Sangon Biotech).

Dual-luciferase reporter

First of all, circBank (http://www.circbank.cn/) or starbase v3.0 predicted miR-22-3p between circ_0004214 or GATA4. Then, WT and MUT sequences of circ_0004214 and GATA4 3'UTR were included in the PGL4 vector. Fusion reporter plasmids were introduced with miR-22-3p or miR-NC into AC16 cells for 48 h, followed by analysis using the dual-luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical analysis

Data were collected and processed usingGraphPad Prism 7.0 and shown as mean ± SD at least three times. Difference analysis was performed in different groups using Student's '*t*' test or ANOVA. $P<0.05$ was regarded as statistically significant.

Results

DOX weakened AC16 cell viability and depleted circ_0004214 expression

AC16 cells were exposed to DOX (0, 1, 2, 3 and 4 μM) for 24 h or treated with 4 μM DOX at various times. Cell viability was significantly declined via DOX in a dose/time-dependent manner (Fig. 1 A & B). Interestingly, circ_0004214 was strikingly decreased by DOX in a dose/time-dependent manner (Fig. 1 C & D). Overall, DOX-triggered cardiotoxicity might be related to circ_0004214 deregulation.

Circ_0004214 overexpression alleviated DOX-induced cardiotoxicity in AC16 cells

Enhanced circ_0004214 could effectively enhance circ_0004214 content in AC16 cells (Fig. 2A). DOX-depleted cell viability was largely recovered via the overexpression of circ_0004214 (Fig. 2B). To assess oxidative stress, we examined the levels of LDH, ROS, MDA and 4-HNE. As a result, LDH release (a cytotoxic assay), ROS production, MDA content, and 4-HNE content were strikingly promoted by DOX

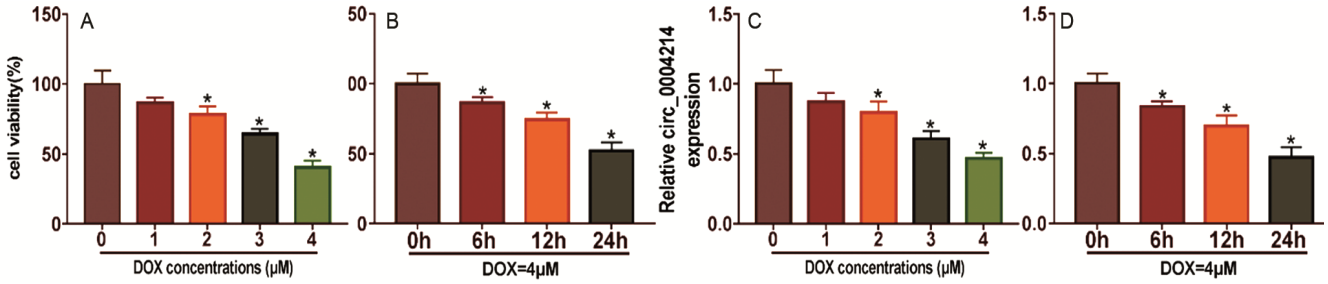

Fig. 1 — (A & B) DOX decreased cell viability and inhibition of circ_0004214 expression. Cell viability detected using CCK-8 after exposure to (A) DOX (0, 1, 2, 3 and 4 μM) for 24 h; (B) 4 μM DOX for (0, 6, 12 and 24 h); and (C & D) qPCR analysis of circ_0004214 in AC16 cells exposed to (C) DOX at various doses for 24 h, and (D) 4 μM DOX for different times (0, 6, 12 and 24 h). [*$P<0.05$]

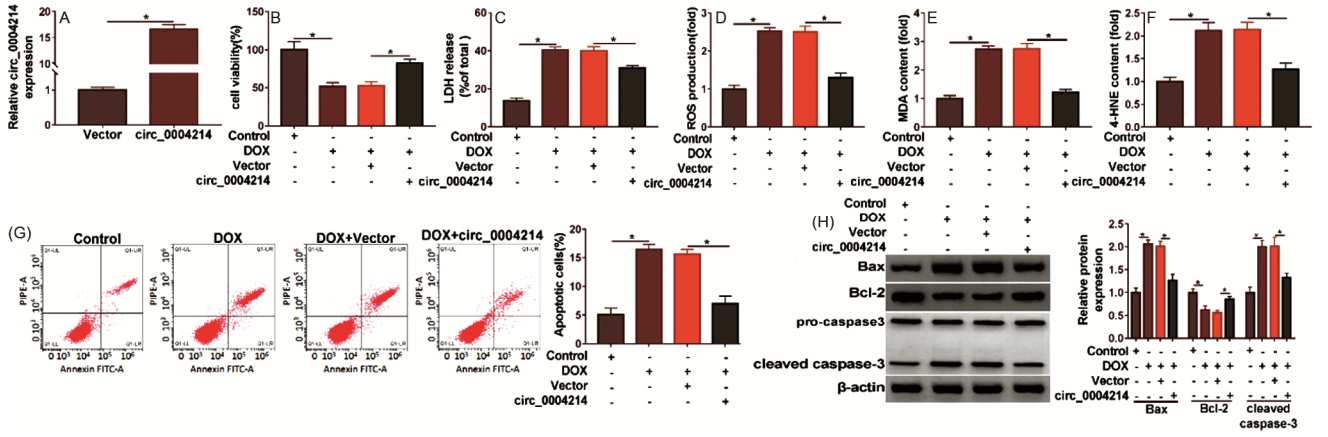

Fig. 2 — DOX-induced AC16 cell cardiotoxicity was alleviated by circ_0004214 overexpression. (A) The efficiency of circ_0004214 overexpression was measured using qPCR. Transfected cells were exposed to DOX for subsequent experiments. (B) Cell viability was examined by CCK-8 assay. (C-F) LDH release, ROS production, MDA content and 4-HNE content were assessed via commercial kits. (G and H) Apoptosis and corrected factors were determined based on flow cytometry and western blot. *$P<0.05$

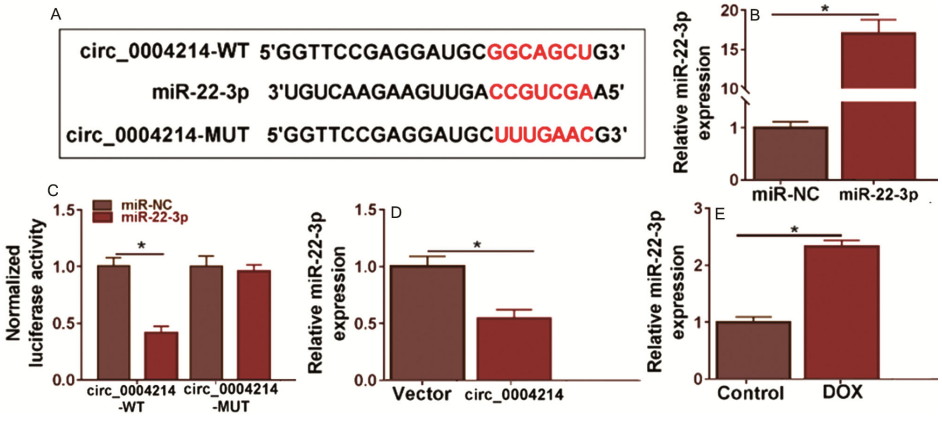

Fig. 3 — circ_0004214 sequesters miR-22-3p. (A) circBank exhibited their binding. (B) qPCR analyzed the efficiency of miR-22-3p mimic. (C) Their relationship was confirmed using a dual-luciferase reporter. (D) The impact of circ_0004214 overexpression on miR-22-3p was detected via qPCR. (E) miR-22-3p in AC16 cells treated with DOX or Control was monitored via qPCR. *P<0.05

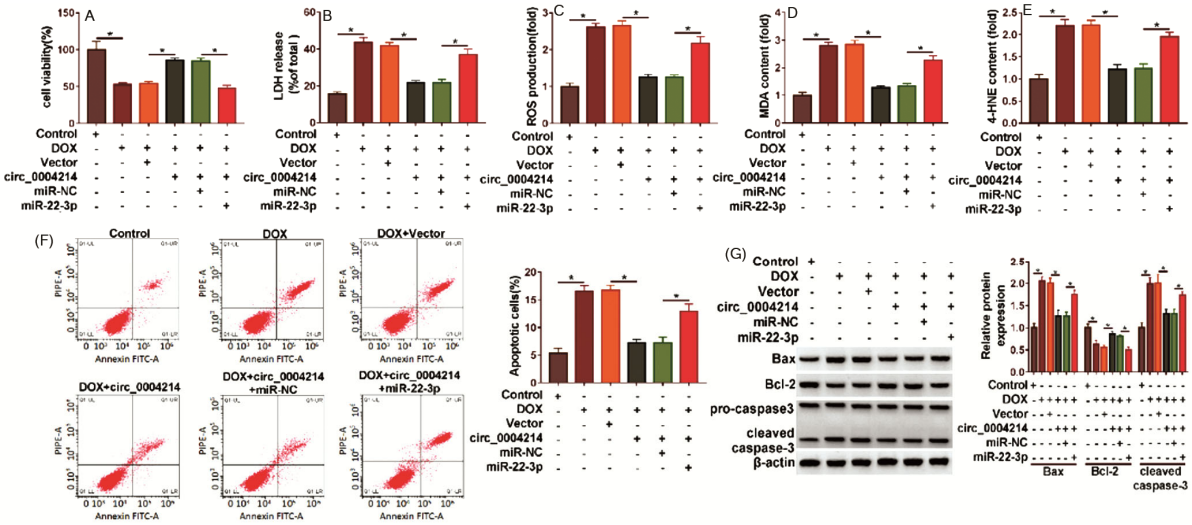

Fig. 4 — Circ_0004214 overexpression alleviated DOX-triggered AC16 cell cardiotoxicity via the inhibition of miR-22-3p. AC16 cells were transfected with circ_0004214 alone or circ_0004214+miR-22-3p under DOX exposure. (A) Cell viability was examined using CCK-8 assay. (B-E) Matched commercial kits were used to examine LDH release, ROS production, MDA content, and 4-HNE content. (F and G) Flow cytometry and western blot assessed apoptosis and related proteins. *P<0.05

but largely repressed by circ_0004214 overexpression (Fig. 2 C-F). DOX-caused AC16 cell apoptosis was blocked via circ_0004214 upregulation (Fig. 2G). In addition, DOX exposure elevated Bax and cleaved caspase-3 protein levels and reduced Bcl-2 in AC16 cells. However, these influences were abolished via circ_0004214 overexpression (Fig. 2H).

Circ_0004214 bound to miR-22-3p

To see whether circ_0004214 played its role by acting as the sponge of miRNAs, we used bioinformatics tools to identify the targets of circ_0004214. circBank showed circ_0004214 bound miR-22-3p (Fig. 3A). As displayed in Fig. 3B, miR-22-3p mimic could effectively enrich miR-22-3p expression. Then, the miR-22-3p mimic could notably reduce the luciferase activities of the circ_0004214-WT vector, rather than the circ_0004214-MUT vector (Fig. 3C). Besides, miR-22-3p was decreased by circ_0004214 upregulation and improved after DOX treatment in AC16 cells (Fig. 3 D & E). The data suggested that circ_0004214 acted as the miR-22-3p sponge.

Circ_0004214 overexpression alleviated DOX-induced cardiotoxicity by suppressing miR-22-3p

Subsequently, we verified whether circ_0004214 sponged miR-22-3p to regulate DOX-induced cardiotoxicity. Circ_0004214 overexpression-triggered increase in the viability of DOX-treated AC16 cell was repressed by the reintroduction of miR-22-3p (Fig. 4A). LDH release, ROS production, MDA

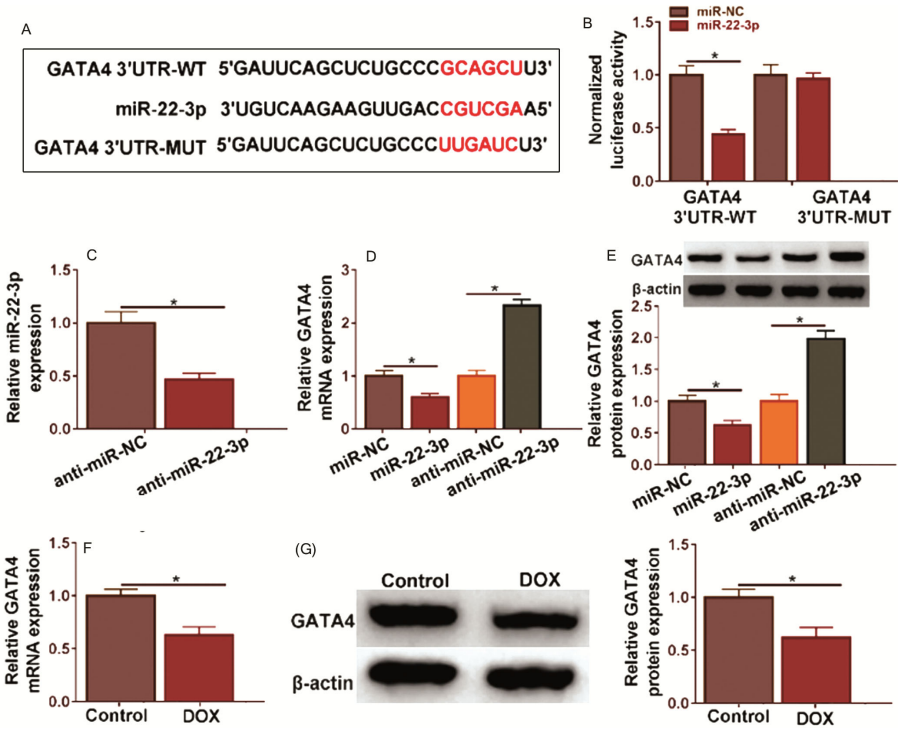

Fig. 5 — miR-22-3p directly targeted GATA4. (A and B) Their binding was displayed using Starbase v3.0 and validated using a dual-luciferase reporter. (C) Qpcr analysis of efficiency of miR-22-3p inhibitor. (D and E) GATA4 in AC16 cells with miR-22-3p restoration or inhibition was examined. (F and G) qPCR and western blot analysis of GATA4 in AC16 cells treated with DOX or Control. *P<0.05

content, and 4-HNE content inhibited by circ_0004214 upregulation were notably strengthened by miR-22-3p overexpression in DOX-treated AC16 cells (Fig. 4 B-E). Circ_0004214-caused repression in apoptosis was recovered via miR-22-3p mimic in DOX-treated AC16 cells (Fig. 4F). Besides, Bax and cleaved caspase-3 protein levels were significantly weakened in DOX-treated AC16 cells via circ_000421, which was restored by miR-22-3p, and Bcl-2 protein level was an opposite trend (Fig. 4G). Overall, circ_0004214 overexpression alleviated DOX-mediated cardiotoxicity by sponging miR-22-3p in AC16 cells.

GATA4 was a target of miR-22-3p

MiRNA is well-known to regulate gene expression by binding with their 3'UTR. Considering this, we used bioinformatics tools to identify the targets of miR-22-3p. MiR-22-3p bound GATA4 3'UTR were presented (Fig. 5A). MiR-22-3p mimic effectively diminish luciferase activities of GATA4 3'UTR-WT vector but not GATA4 3'UTR-MUT vector (Fig. 5B). The miR-22-3p was effectively reduced via anti-miR-22-3p (Fig. 5C). Besides, GAPA4 expression was notably declined via miR-22-3p promotion but heightened via miR-22-3p inhibition (Fig. 5 D & E). Interestingly, GATA4 was strikingly reduced in DOX-treated AC16 cells (Fig. 5 F & G). Overall, miR-22-3p targeted GATA4.

MiR-22-3p inhibition ameliorated DOX-inducedcardiotoxicity by increasing GATA4 expression in AC16 cells

To further determine interactions between miR-22-3p and GATA4, rescue assays were conducted. First, we examined the efficiency of si-GATA4 and found that si-GATA4 transfection effectively reduced GATA4 expression (Fig. 6 A & B). Functionally, miR-22-3p improved cell viability in DOX-treated AC16 cells, which was repressed via GATA4 knockdown (Fig. 6C). MiR-22-3p inhibitor repressed LDH release, ROS production, MDA content, and 4-HNE content in DOX-treated AC16 cells, which was substantially restored via si-GATA4 (Fig. 6 D-G). In addition, miR-22-3p inhibition-induced cell apoptosis repression in DOX-treated AC16 cells, which was partly promoted via GATA4 deficiency (Fig. 6H). Besides, Bax and cleaved caspase-3 were inhibited by miR-22-3p inhibition, and combined GATA4 knockdown recovered their levels. However, Bcl-2 was the opposite trend in tumor cells (Fig. 6I). Together, miR-22-3p inhibition ameliorated DOX-induced cardiotoxicity by increasing GATA4 expression in AC16 cells.

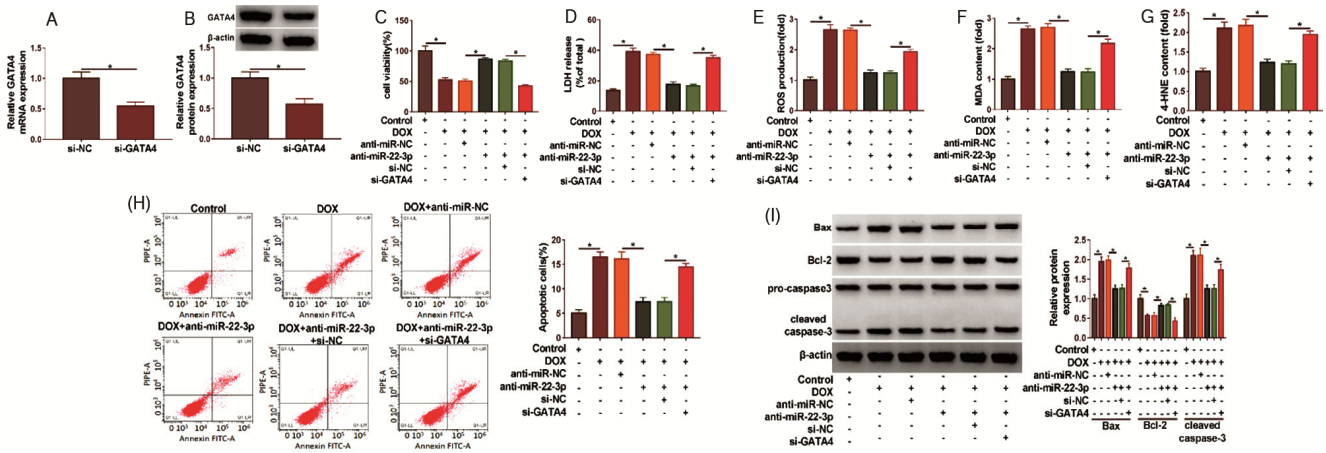

Fig. 6 — DOX-induced AC16 cell cardiotoxicity was regulated via miR-22-3p/GATA4. (A and B) The efficiency of GATA4 interference was checked via qPCR and western blot. AC16 cells were transfected with anti-miR-22-3p alone or anti-miR-22-3p+si-GATA4 under DOX treatment. (C) CCK-8 analysis of cell viability. (D-G) Commercial kits determinedLDH release, ROS production, MDA content, and 4-HNE content. (H and I) Cell apoptosis and associated factors were determined by flow cytometry and western blot. **P*<0.05

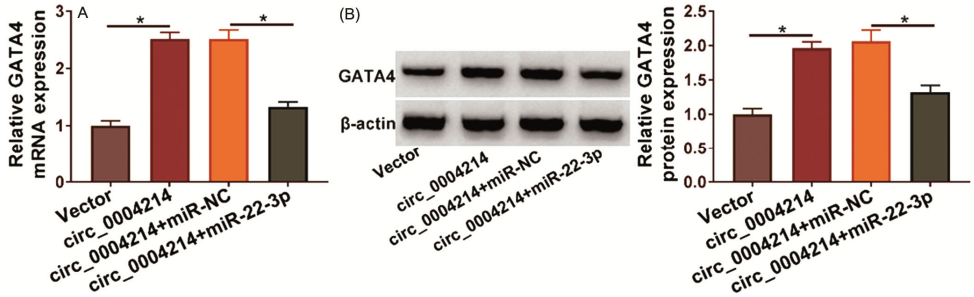

Fig. 7 — Circ_0004214 promoted GATA4 expression via decoying miR-22-3p. (A and B) qPCR and western blot analysis of GATA4 in AC16 cells transfected with circ_0004214, Vector, circ_0004214+miR-22-3p or circ_0004214+miR-NC. **P*<0.05.

Circ_0004214 competitively bound miR-22-3p to upregulate GATA4 expression

Furthermore, GATA4 content was strikingly enhanced in AC16 cells overexpressed circ_0004214, which were largely impaired via miR-22-3p upregulation (Fig. 7 A & B). Together, circ_0004214 promoted GATA4 content via sponging miR-22-3p.

Discussion

In the present study, we mainly discovered that circ_0004214 was decreased in DOX-treated AC16 cells. DOX treatment significantly weakened cell viability, and promoted oxidative stress and apoptosis, which were reversed via circ_0004214 overexpression. In addition, circ_0004214 functioned as a miR-22-3p sponge to increase GATA4 expression. Circ_0004214 overexpression alleviated DOX-induced cardiotoxicity by activating GATA4 via targeting miR-22-3p, providing novel insights into DOX-induced cardiotoxicity.

The cumulative and dose-dependent cardiac toxicity of DOX is a leading concern in cancer therapeutic practices[16]. Studies supported cardiomyocyte death caused by apoptosis and necrosis is a major cause and mechanism in cardiotoxicity. Besides, cell autophagy, aging, and reactive oxygen species (ROS) production were also involved in this process[16,17]. Consistent with these findings, we found that DOX inhibited cell viability and promoted cell apoptosis. Numerous studies reported that excessive ROS production promoted the accumulation of LDH, MDA, and 4-HNE[18]. In our data, DOX-induced LDH release, ROS production, MDA content, and 4-HNE content, suggested that DOX promoted oxidative stress in AC16 cells.

A previous study maintained that the reintroduction of circ_0004214 prevented DOX-induced cardiomyocyte death[10]. Here, we found DOX treatment inhibited the expression of circ_0004214. Circ_0004214 promotes the development of human cancers[19,20]. Unluckily, circ_0004214 in DOX cardiac toxicity and associated mechanisms remain unclear. Our present study illustrated that circ_0004214 overexpression alleviated DOX-induced cardiotoxicity, including

recovering cell viability, blocking cell apoptosis and oxidative stress. The data hinted that circ_0004214 played a protective role against DOX-induced cardiotoxicity.

For mechanism analysis, we characterized miR-22-3p as a target of circ_0004214. MiR-22 was reported to be upregulated during cardiomyocyte differentiation and cardiac hypertrophy[21], suggesting that miR-22 was linked to heart dysfunction. It was also reported miR-22 was highly expressed in DOX-treated murine hearts, and the inhibition of miR-22 repressed DOX-induced cardiotoxicity[13]. Here, circ_0004214 overexpression mitigated DOX-induced cardiotoxicity, while miR-22-3p promoted cell viability, repressed cell apoptosis and oxidative stress.

GATA4 expressed was downregulated by DOX in H9C2 cardiomyocytes[5]. In function, GATA4 inhibited DOX-induced autophagy and cell death in cardiomyocytes, thus alleviating cardiac toxicity and improving cardiac functions[15,22]. Also, GATA4 overexpression blocked DOX-induced cardiomyocyte apoptosis[23]. Given the gradual clarity of GATA4 functions in DOX-induced cardiotoxicity, we found GATA4 knockdown reversed the repression of miR-22-3p inhibition on DOX-triggered cardiotoxicity. More importantly, circ_0004214 functioned as a miR-22-3p sponge to increase the expression of GATA4, thereby preventing DOX-induced cardiotoxicity.

Conclusion

Collectively, circ_0004214 is a vital regulator responding to doxorubicin (DOX) induced cardiotoxicity. DOX triggers cardiotoxicity, including cell viability inhibition, cell apoptosis, and oxidative stress by downregulating circ_0004214 via circ_0004214-mediated miR-22-3p/GATA4 signaling pathway. Our above finding provides novel avenues to improve clinical applications of DOX. However, this study has some limitations. Precisely, we did not conduct animal experiments or any clinical tests. Moreover, we did not examine the effects of DOX and circ_0004214 in other cells to see whether they also show similar responses to cardiomyocytes.

Acknowledgement

The current work was supported by suRui-E emergency medicine fund of Peking Union Medical College Hospital (Grant NO.:14040).

Conflicts of interest

Authors declare no competing interests.

References

1 Tolani D, Wilcox J, Shyam S & Bansal N, Cardio-oncology for Pediatric and Adolescent/Young Adult Patients. *Curr Treat Options Oncol*, 24 (2023) 1052.
2 Kim D, Jang G, Hwang J, Wei X, Kim H, Son J, Rhee SJ, Yun KH, Oh SK, Oh CM & Park R, Combined Therapy of Low-Dose Angiotensin Receptor-Neprilysin Inhibitor and Sodium-Glucose Cotransporter-2 Inhibitor Prevents Doxorubicin-Induced Cardiac Dysfunction in Rodent Model with Minimal Adverse Effects. *Pharmaceutics*, 14 (2022) 2629.
3 He L, Liu F & Li J, Mitochondrial Sirtuins and Doxorubicin-induced Cardiotoxicity. *Cardiovasc Toxicol*, 21 (2021) 179.
4 Christidi E & Brunham LR, Regulated cell death pathways in doxorubicin-induced cardiotoxicity. *Cell Death Dis*, 12 (2021) 339.
5 Yarmohammadi F, Ebrahimian Z & Karimi G, MicroRNAs target the PI3K/Akt/p53 and the Sirt1/Nrf2 signaling pathways in doxorubicin-induced cardiotoxicity. *J Biochem Mol Toxicol*, 37 (2023) e23261.
6 Wang X, Cheng Z, Xu J, Feng M, Zhang H, Zhang L & Qian L, Circular RNA Arhgap12 modulates doxorubicin-induced cardiotoxicity by sponging miR-135a-5p. *Life Sci*, 265 (2021) 118788.
7 Zhou B, Xue J, Wu R, Meng H, Li R, Mo Z, Zhai H, Chen X, Liu R, Lai G, Chen X, Li T & Zheng S, CREBZF mRNA nanoparticles suppress breast cancer progression through a positive feedback loop boosted by circPAPD4. *J Exp Clin Cancer Res*, 42 (2023) 138.
8 Ma Z, Sun ZL & Liu M, CRBP-HFEF: Prediction of RBP-Binding Sites on circRNAs Based on Hierarchical Feature Expansion and Fusion. *Interdiscip Sci*, (2023)
9 Christidi E & Brunham LR, Regulated cell death pathways in doxorubicin-induced cardiotoxicity. *Cell Death Dis*, 12 (2021) 339.
10 Shi JQ, Ma Y, Zhang Y, Chen ZJ & Jia G, [Effects of titanium dioxide nanoparticles on circRNA expression profiles in human hepatocellular carcinoma cells HepG2]. *Beijing Da Xue Xue Bao Yi Xue Ban*, 55 (2023) 392.
11 Rosenfeld R, Riondino S, Formica V, Torino F, Martuscelli E & Roselli M, MiRNAs and circRNAs for the Diagnosis of Anthracycline-Induced Cardiotoxicity in Breast Cancer Patients: A Narrative Review. *J Pers Med*, 12 (2022) 1059.
12 Kuang Z, Wu J, Tan Y, Zhu G, Li J & Wu M, MicroRNA in the Diagnosis and Treatment of Doxorubicin-Induced Cardiotoxicity. *Biomolecules*, 13 (2023) 568.
13 Wang A J, Zhang J, Xiao M, Wang S, Wang B J, Guo Y, Tang Y & Gu J, Molecular mechanisms of doxorubicin-induced cardiotoxicity: novel roles of sirtuin 1-mediated signaling pathways. *Cell Mol Life Sci*, 78 (2021) 3105.
14 Anbazhagan R, Kavarthapu R, Dale R, Campbell K, Faucz FR& Dufau ML, miRNA Expression Profiles of Mouse Round Spermatids in GRTH/DDX25-Mediated Spermiogenesis: mRNA-miRNA Network Analysis. *Cells*, 12 (2023) 756.
15 Wu YP, Zhang S, Xin YF, Gu LQ, Xu XZ, Zhang CD & You ZQ, Evidences for the mechanism of Shenmai injection antagonizing doxorubicin-induced cardiotoxicity. *Phytomedicine*, 88 (2021) 153597.

16 Li D, Yang Y, Wang S, He X, Liu M, Bai B, Tian C, Sun R, Yu T & Chu X, Role of acetylation in doxorubicin-induced cardiotoxicity. *Redox Biol*, 46 (2021) 102089.

17 Sun X, Zhou L, Han Y, Yang Q, Li X, Xin B, Chi M, Wang Y & Guo C, Scutellarin Attenuates Doxorubicin-Induced Cardiotoxicity by Inhibiting Myocardial Fibrosis, Apoptosis and Autophagy in Rats. *Chem Biodivers*, 20 (2023) e202200450.

18 Yarmohammadi F, Rezaee R & Karimi G, Natural compounds against doxorubicin-induced cardiotoxicity: A review on the involvement of Nrf2/ARE signaling pathway. *Phytother Res*, 35 (2021) 1163.

19 Wei J, Li M, Xue C, Chen S, Zheng L, Deng H, Tang F, Li G, Xiong W, Zeng Z & Zhou M, Understanding the roles and regulation patterns of circRNA on its host gene in tumorigenesis and tumor progression. *J Exp Clin Cancer Res*, 42 (2023) 86.

20 Sadlak J, Joshi I, Prószyński TJ & Kischel A, CircAMOTL1 RNA and AMOTL1 Protein: Complex Functions of AMOTL1 Gene Products. *Int J Mol Sci*, 24 (2023) 2103.

21 Li G, Shao Y, Guo HC, Zhi Y, Qiao B, Ma K, Du J, Lai YQ & Li Y, MicroRNA-27b-3p down-regulates FGF1 and aggravates pathological cardiac remodelling. *Cardiovasc Res*, 118 (2022) 2139.

22 Kawano I & Adamcova M, MicroRNAs in doxorubicin-induced cardiotoxicity: The DNA damage response. *Front Pharmacol*, 13 (2022) 1055911.

23 Wu S, Lan J, Li L, Wang X, Tong M, Fu L, Zhang Y, Xu J, Chen X, Chen H, Li R, Wu Y, Xin J, Yan X, Li H, Xue K, Li X, Zhuo C & Jiang W, Sirt6 protects cardiomyocytes against doxorubicin-induced cardiotoxicity by inhibiting P53/Fas-dependent cell death and augmenting endogenous antioxidant defense mechanisms. *Cell Biol Toxicol*, 39 (2023) 237.