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ORIGINAL RESEARCH

Diagnostic Value and Regulatory Mechanism of miR-322-5p in Coronary Heart Disease

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Background: Coronary heart disease (CHD) is a heart condition caused by narrowed or blocked coronary arteries. The miR-322-5p is closely related to inflammation and vascular diseases, yet its role in CHD remains unknown.

Objective: This study focused on investigating the clinical significance of miR-322-5p and its regulatory mechanism in CHD.

Materials and Methods: This study enrolled 160 CHD patients and 130 healthy individuals. The expression of miR-322-5p and TRAF6 was measured by RT-qPCR. The correlation between miR-322-5p and CHD was evaluated via Pearson correlation analysis. The clinical predictive performance of miR-322-5p was assessed by ROC analysis. Cell viability was assessed using the CCK-8 assay while apoptosis was analyzed by flow cytometry. Inflammatory cytokine levels were determined by ELISA.

Results: MiR-322-5p was significantly downregulated in patients with CHD and exhibited high diagnostic accuracy for CHD with an AUC of 0.882. The declined miR-322-5p was negatively correlated with Gensini score ($r = -0.611$) and CRP ($r = -0.646$), but positively associated with HDL-C ($r = 0.598$). Although miR-322-5p was reduced under pathological conditions, its upregulation enhanced cell viability and inhibited both apoptosis and inflammatory factors. TRAF6, a direct target of miR-322-5p, was negatively regulated by miR-322-5p ($r = -0.683$), and high levels of TRAF6 aggravated CHD.

Conclusion: The declined miR-322-5p in CHD presented high diagnostic value. Reduced miR-322-5p exacerbated the CHD by inhibiting cell viability, enhancing apoptosis and inflammation through negatively regulating the TRAF6.

Keywords: miR-322-5p, coronary heart disease, apoptosis, inflammation

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Introduction

Coronary heart disease (CHD) is a heart condition caused by narrowed or blocked coronary arteries, leading to heart muscle ischemia or infarction. It is the disease with the highest incidence and mortality globally.[1] Reports show that the prevalence in people over 60 years old is as high as 27.8%.[2] Studies also indicate that the incidence and mortality rates are increasing annually.[3] Currently, significant progress has been made in the treatment of CHD in both techniques and medications. However, challenges still remain in adverse drug reactions,[4] microcirculatory disorders,[5] myocardial no-reflow,[6] reperfusion injury,[7] as well as in-stent restenosis.[8] Therefore, developing specific molecular markers may provide a promising approach for early identification and personalized treatment of CHD, although further validation in clinical settings is required.

MicroRNAs (miRNAs) have been proved to mediate gene expression, at the post-transcriptional level, via binding to the 3'UTR of target mRNAs, promoting degradation or inhibiting translation.[9] As a result, miRNAs play a significant role in the development and progression of acute and chronic CHD. For example, a bioinformatics analysis has confirmed that miR-22-3p is a potential biomarker for CHD.[10] A clinical study revealed that serum miR-183-5p correlates positively with the severity of CHD.[11] Mechanistic studies have further underscored the critical role of miRNAs in

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CHD. Research has manifested that miR-223-3p regulates endothelial cell apoptosis in CHD.[12] The miR-148a-3p has been proven to reduce vascular endothelial cell injury by downregulating PCSK9 and inhibiting the NF-κB pathway.[13] It has been reported that miR-181c-5p improves myocardial pathology and reduce cell apoptosis by enhancing SIRT1 and reducing acetylated p65 levels in CHD.[14] These findings highlight the potential of miRNAs in the early diagnosis and treatment of CHD.

In recent years, emerging reports have highlighted the broad regulatory role of miR-322-5p in diseases associated with vessels as well as inflammatory. For instance, miR-322-5p has been shown to regulate lipopolysaccharide-induced acute kidney injury in mice.[15] In the context of neuroinflammation, research has demonstrated a significant down-regulation of miR-322-5p, coinciding with elevated levels of pro-inflammatory cytokines.[16] In studies on vascular diseases, miR-322-5p has been linked to the instability of atherosclerotic plaques.[17] There is also evidence that miR-322-5p is associated with the formation of carotid atherosclerotic plaques.[18] In addition, mechanistic investigations have further revealed that miR-322-5p is involved in modulating vascular smooth muscle cell proliferation, migration as well as the angiogenesis.[19] Nevertheless, the precise role and underlying mechanisms of miR-322-5p in CHD remain to be fully elucidated.

This study investigated the clinical significance of miR-322-5p in CHD patients and explored the underlying mechanisms in the coronary artery endothelial cells treated with ox-LDL, with the goal of providing a theoretical foundation for the early diagnosis and targeted treatment of CHD.

Materials and Methods

Enrolled Population

This study recruited 160 patients with CHD and 130 healthy individuals from author's institution. A total of 160 patients with CHD were recruited from the inpatient ward and outpatient clinic of the Department of Cardiology in our hospital. They were diagnosed with stable CHD or unstable angina pectoris (non-acute myocardial infarction) with coronary artery stenosis ≥ 50%. Meanwhile, 130 healthy controls were volunteers recruited from the Physical Examination Center of our hospital, who were excluded from coronary artery disease by electrocardiogram, blood lipid test and coronary CT, and had no cardiovascular risk factors such as hypertension and diabetes mellitus.

Exclusion criteria were: (1) severe heart failure or myocardial infarction; (2) malignant tumors; (3) acute infections. In addition, the study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of The Second Hospital of Hebei Medical University before the study began. The written informed consent has been obtained from the participants involved. All peripheral blood samples from participants were centrifuged (3700 rpm for 15min) one hour after collection, and subsequently the supernatant was stored at −80°C in new tubes.

Cell Culture and Transfection

The coronary artery endothelial cells were cultured in a sterile environment maintained at 37°C with 5% CO_2, with routine procedures for cell culture and passage. Before passage, cells were washed with room-temperature PBS and digested with 0.25% trypsin-EDTA buffer. The complete culture medium included 94% endothelial cell medium, 5% FBS as well as endothelial cell growth supplement (1%). In addition, ox-LDL was employed to simulate the inflammatory pathological state of CHD as reported previously.[20,21] After passage, cells were placed in serum-free medium for 12 hours and followed by treatment with ox-LDL (100 μg/mL) for 24 hours when 80% confluence was reached.

The expression level of miR-322-5p was artificially upregulated by transient transfection with miR-322-5p mimic (miR-mimic) with negative control (miR-NC). Moreover, the recombinant plasmid with the full TRAF6 sequence was used for its overexpression (oe-TRAF6), with the empty vector as negative control (oe-NC). Lipofectamine was utilized to encapsulate small nucleic acid molecules and transport them into cells with good growth conditions. The effect on gene regulation was verified by subsequent qPCR.

RT-qPCR

The transfected cells or collected serum samples were processed using the Trizol method to obtain RNA. Soluble RNA was promptly reversely transcribed into the more stable cDNA following the determination of its concentration and purity. The expression level of miR-322-5p and TRAF6 were measured by a quantitative analysis kit and then calculated by $2^{-\Delta\Delta CT}$ method, normalized to reference gene GAPDH.

Cell Viability

The viability of cultured coronary artery endothelial cells invitro was measured by CCK-8 kit. Cells in optimal growth conditions were seeded into 96-well plates and wells containing only culture medium served as blank controls. Following cultivation, CCK-8 solution (10 μL) was added to each well and incubated under culture conditions for 2 hours. The absorbance of each well was measured at 450 nm using a microplate reader.

Cell Apoptosis

Adherent cells were harvested following trypsinization and resuspended in PBS, washing three times. After adjusting the cell concentration, Annexin V-FITC and PI solution were added and incubated in dark for ten minutes at room temperature. The stained cells were subsequently analyzed using a flow cytometer to determine the apoptosis rate.

Measurement of Inflammatory Factors

The inflammatory factors including interleukin-6 (IL-6), interleukin-1β (IL-1β) as well as tumor necrosis factor-α (TNF-α) in the supernatant were detected by ELISA assay based on the specific reaction between antigen and antibody. The ELISA plate was initially coated with specific antibody overnight followed by blocking with filtered 10% FBS. Diluted samples and standards were then added respectively for incubation. After washing, the enzyme-conjugated antibody was introduced for subsequent color development reaction. The OD value at 450 nm was measured via a microplate reader. The concentration of the samples was determined based on a standard curve.

Dual-Luciferase Reporter Assay

Dual-luciferase reporter assays were performed in coronary artery endothelial cells under normal culture conditions (without ox-LDL treatment) to verify the direct binding between miR-322-5p and TRAF6, independent of pathological stimulation. Specifically, the 3’UTR sequences of wild-type (WT) or mutant (MUT) TRAF6 were amplified by PCR, then inserted into the pmirGL3 vector via enzymatic digestion and ligation to create recombinant plasmids. These plasmids were severally transformed into competent cells, and positive clones were selected using antibiotics. After extraction, the recombinant plasmids were co-transfected with miR-322-5p mimic (miR-mimic) or inhibitor (miR-inhibitor) into cells with negative control (miR-NC). After 48 hours, luciferase activity was measured to assess the regulation of TRAF6 expression by miR-322-5p. The relative luciferase activity was calculated as the ratio of firefly luciferase activity to Renilla luciferase activity. Each group was performed in 3 independent biological replicates, with 3 technical replicates per biological replicate.

Statistical Analysis

Both SPSS Statistics 23.0 and GraphPad Prism 7.0 were utilized for data processing. Group comparisons were performed using *t*-tests or one-way ANOVA or chi-square tests. The diagnostic potential of miR-322-5p was assessed via receiver operating characteristic (ROC) curves, and its correlation with CHD was examined through Pearson correlation analysis. A p-value below 0.05 was considered statistically significant.

Results

Clinical Characteristics

The mean age of patients with CHD was 60.51 ± 8.99 years, comprising 98 males and 62 females. In the Control group the average age was 61.81 ± 7.47 years, with 75 males and 55 females. The body mass index (BMI) in the CHD group

Table 1 Basic Characteristics of Study Population

Indicators	Control (n=130)	CHD (n=160)	*P* values
Age, years	61.81 ± 7.47	60.51 ± 8.99	0.761
BMI, Kg/m^2	23.40±2.51	23.92±2.86	0.108
Gender			0.539
Male	75 (57.69%)	98 (61.25%)	
Female	55 (42.31%)	62 (38.75%)	
Smoking	49 (16.69%)	70 (23.75%)	0.297
Diabetes	28 (21.54%)	47 (29.38%)	0.130
Hypertension	59 (45.38%)	92 (57.50%)	0.040
Hyperlipidemia	51 (39.23%)	83 (51.88%)	0.032
TC (mmol/L)	4.66 ± 1.06	4.69 ±1.02	0.776
LDL-C (mmol/L)	2.75 ± 0.71	2.91 ± 0.76	0.063
HDL-C (mmol/L)	1.30 ± 0.29	1.20 ±0.28	0.004
Gensini score	2.41 ± 1.20	37.87 ±12.78	< 0.001
CRP (mg/L)	3.42 ± 1.53	7.48 ± 3.37	< 0.001
NT-proBNP (pg/mL)	234.44 ± 88.59	606.55 ± 162.58	< 0.001

Abbreviations: CHD, coronary heart disease; BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; CRP, C-reactive protein; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

was 23.92±2.86 kg/m^2, which was comparable to that in the Control group (23.40±2.51 kg/m^2). In the CHD group, 23.75% of individuals had a smoking history, compared to 16.69% in the Control group. In the CHD group, 29.38% had diabetes, whereas this proportion was 21.54% in the control group. Blood tests showed no significant differences in total cholesterol and low-density lipoprotein cholesterol levels between the groups. On the contrary, in the CHD group, 57.50% had hypertension, significantly higher than that of the Control group ($P = 0.040$). Similarly, 51.88% had hyperlipidemia, compared to 39.23% in the control group ($P = 0.032$). Additionally, the CHD group had significantly lower HDL-C ($P = 0.004$) and higher CRP levels ($P < 0.001$). Additionally, the Gensini score in the CHD group exhibited significant increase ($P < 0.001$). The level of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in the CHD group was significantly higher than that in the control group ($P < 0.001$). The above indicators were summarized in Table 1.

Expression Level of miR-322-5p and Diagnostic Performance

Calculation on the expression level of miR-322-5p confirmed a distinct decline in patients with CHD compared to the Control (Figure 1, $P < 0.001$). The abnormal expression suggested a potential participation role of miR-322-5p in CHD.

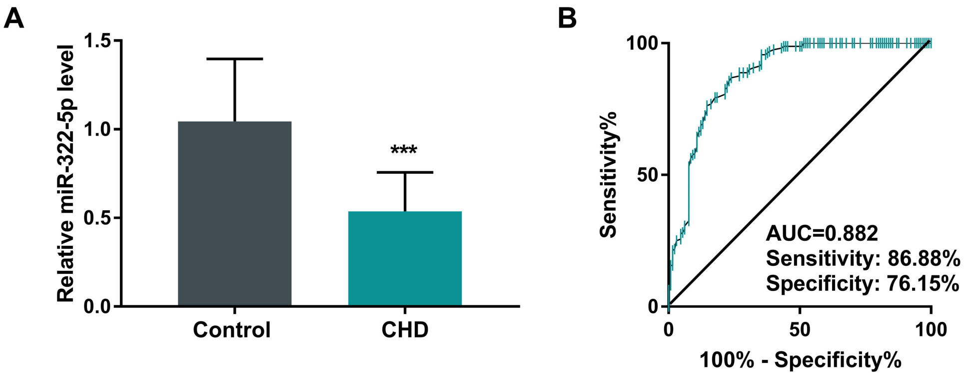

Figure 1 Expression level and diagnostic ability of miR-322-5p. (**A**) Relative expression of miR-322-5p in CHD group (n = 160) compared to the control (n = 130). (**B**) ROC curve of miR-322-5p. ***P <0.001.

The ensuing ROC curve was established to evaluate its diagnostic capacity for CHD. The area under the curve was 0.882, with a sensitivity of 86.88% and specificity of 76.15% (Figure 1B).

Correlation Analysis Between miR-322-5p and CHD

Both the Gensini score and the SYNTAX score are quantitative indicators for the severity of coronary artery lesions. It was found in the study that miR-322-5p was significantly negatively correlated with both the Gensini score ($r=-0.611$) and the SYNTAX score ($r=-0.654$) (Figures 2A and B, $P<0.001$), suggesting that its expression level decreases with the increase in the severity of coronary artery lesions. Moreover, the relative miR-322-5p level was observed to be directly proportional to the concentration of HDL-C in the blood of CHD patients (Figure 2C, $r = 0.598$, $P < 0.001$), indicating a close association between miR-322-5p and the risk of CHD. Meanwhile, the downregulation of miR-322-5p was moderately negatively correlated with the increase in C-reactive protein (CRP) level (Figure 2D, $r=-0.646$, $P<0.001$), which further supports its correlation with the inflammatory process of CHD. Notably, miR-322-5p was also moderately negatively correlated with NT-proBNP, a biomarker of myocardial injury (Figure 2E, $r=-0.505$, $P<0.001$), suggesting that it may be involved in the regulation of myocardial function injury in CHD patients.

The Effect of miR-322-5p on Cell Viability and Apoptosis

Subsequent exploration principally probed the regulatory role of miR-322-5p in CHD. Coronary artery endothelial cells were treated with ox-LDL (100 µg/mL) to simulate pathological conditions. Under pathological conditions, the expression of miR-322-5p was significantly reduced, aligning with the findings from patients (Figure 3A, $P < 0.001$). Moreover, its expression level could be artificially increased by transfecting miR-322-5p mimics ($P < 0.01$). Cell viability tests showed that ox-LDL treatment prominently reduced cell viability, which was reserved by subsequent transfection with miR-322-5p mimics (Figure 3B, $P < 0.001$). Apoptosis assays revealed that ox-LDL significantly increased apoptosis rates while miR-322-5p upregulation effectively attenuated apoptosis (Figure 3C, $P < 0.001$).

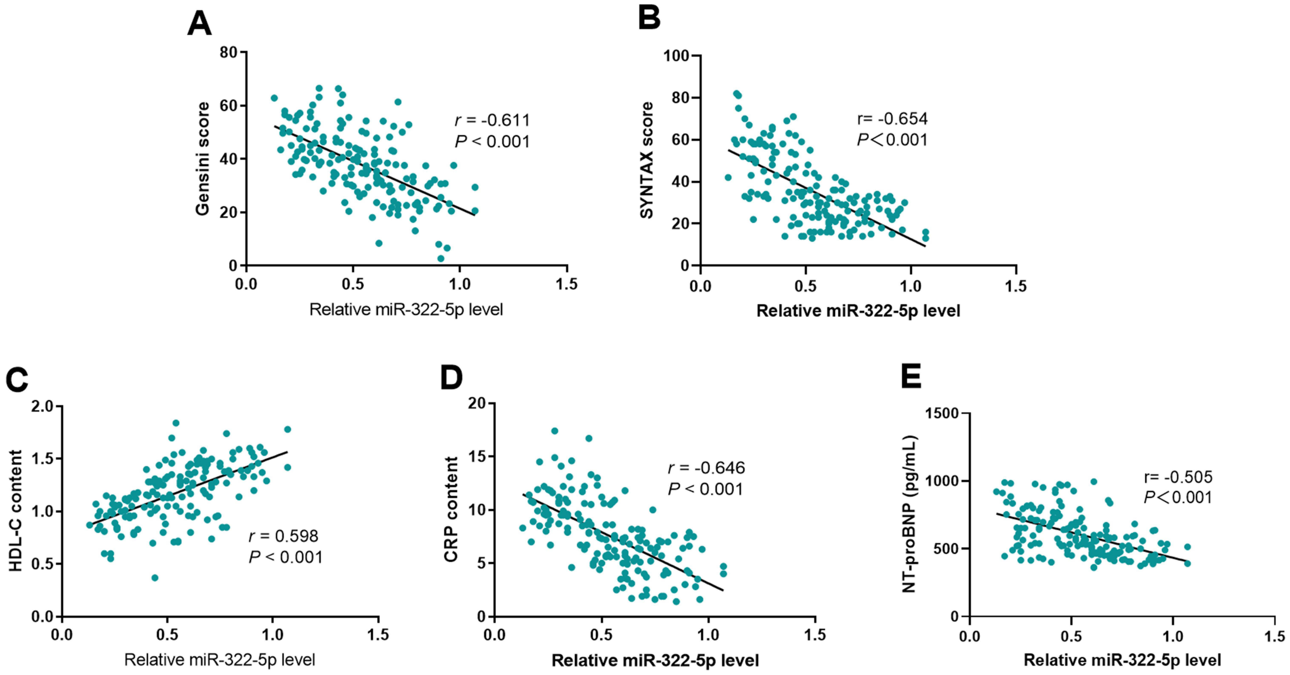

Figure 2 Correlation analysis of miR-322-5p with CHD. (**A**) Correlation between miR-322-5p and Gensini score. (**B**) Correlation between miR-322-5p and SYNTAX score. (**C**) Correlation between miR-322-5p and HDL-C. (**D**) Correlation between miR-322-5p and CRP. (**E**) Correlation between miR-322-5p and NT-proBNP.

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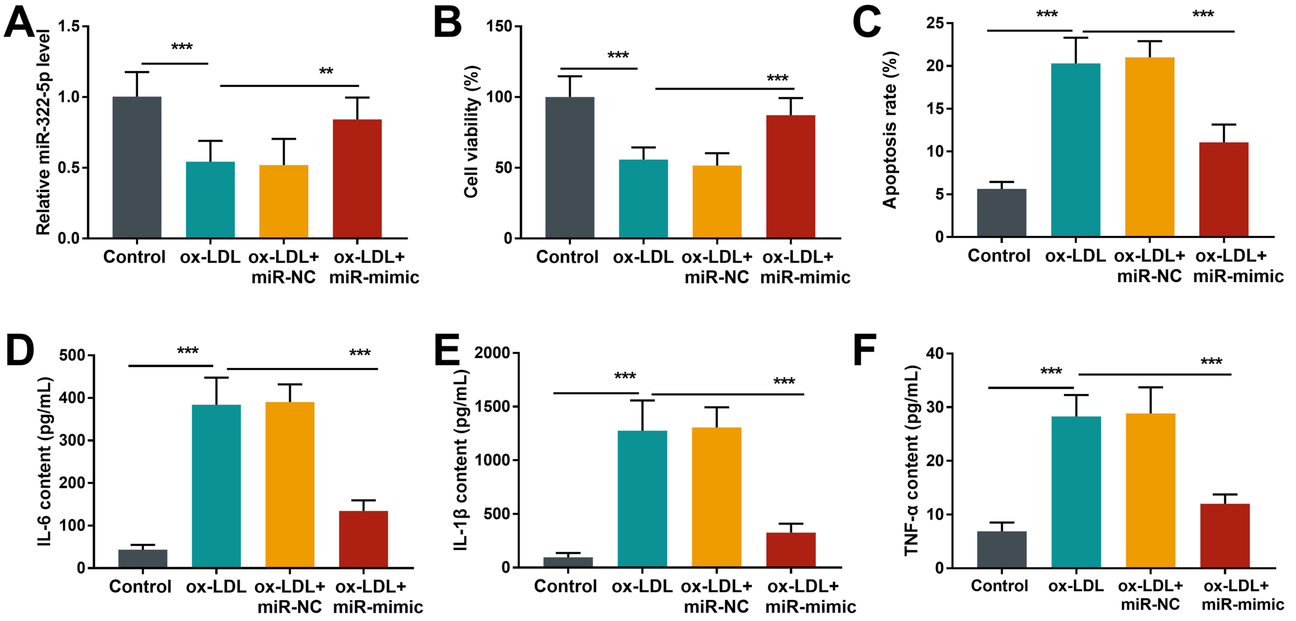

Figure 3 The effect of miR-322-5p on cell viability, apoptosis and inflammation. (**A**) Relative expression of miR-322-5p. (**B**) The regulatory effects of miR-322-5p on cell viability. (**C**) The regulatory effects of miR-322-5p on cell apoptosis. (**D**) The regulatory effects of miR-322-5p on IL-6. (**E**) The regulatory effects of miR-322-5p on IL-1β. (**F**) The regulatory effects of miR-322-5p on TNF-α. ***P* <0.01. ****P* <0.001.

The Effect of miR-322-5p on Inflammation

Inflammation is pivotal in the development and progression of CHD. Under pathological conditions, we observed a significant elevation in the levels of IL-6, IL-1β as well as TNF-α in the cell supernatant ($P < 0.001$). Surprisingly, this abnormally enhanced inflammatory level could be effectively mitigated by miR-322-5p upregulation (Figure 3D–F, $P < 0.001$).

The Target Relationship Between miR-322-5p and TRAF6

The downstream target of miR-322-5p was predicted in the ENCORI database and further verification was done by the dual-luciferase reporter system. TRAF6 was thought to possess binding sites for miR-322-5p (Figure 4A, $P < 0.001$). Data manifested that in the WT-TRAF6 group, the luciferase activity was regulated by miR-322-5p levels, with both upregulation and downregulation of miR-322-5p affecting it (Figure 4B). However, after TRAF6 mutation, the activity of

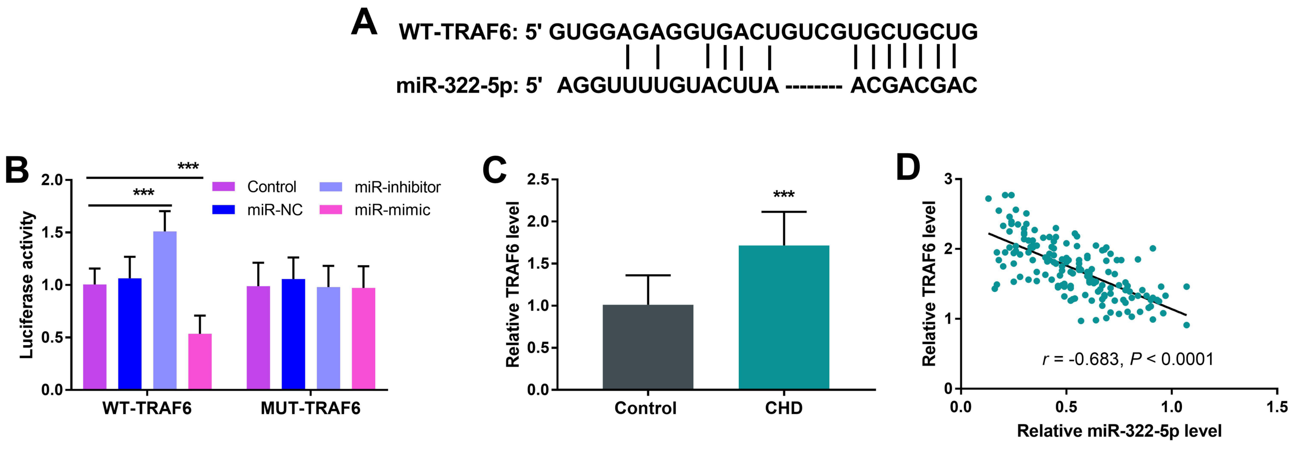

Figure 4 The target relationship between miR-322-5p and TRAF6. (**A**) Binding sites between TRAF6 and miR-322-5p. (**B**) The luciferase activity in WT-TRAF6 and MUT-TRAF6 groups. (**C**) Expression level of TRAF6 in CHD group. (**D**) Correlation analysis of TRAF6 and miR-322-5p in patients with CHD. ****P* <0.001.

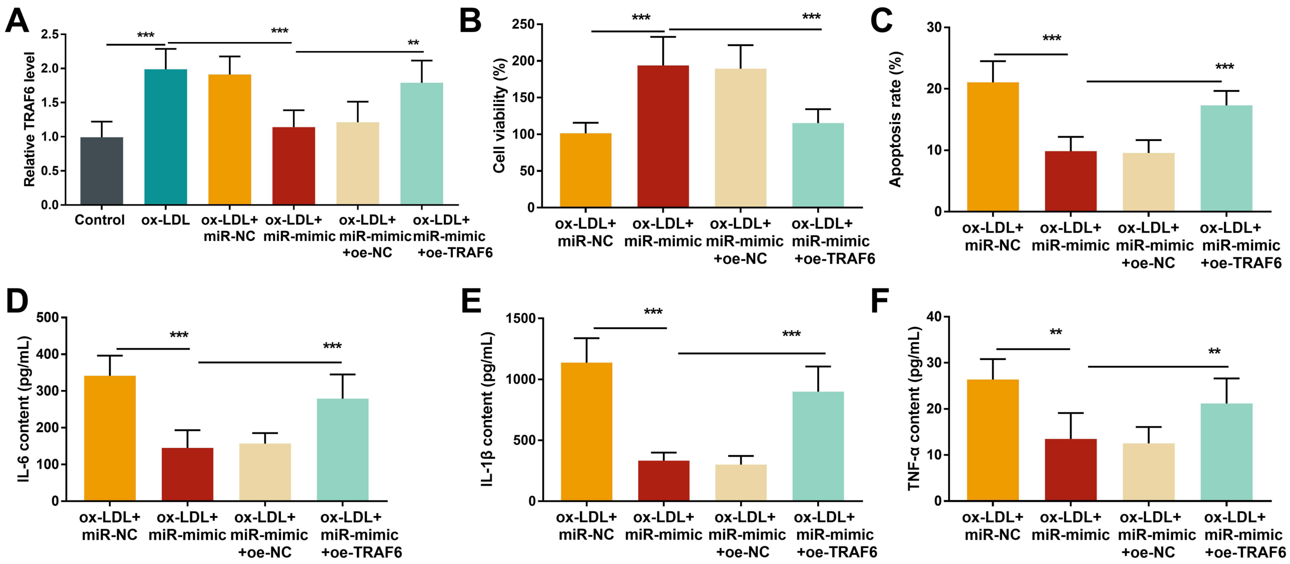

Figure 5 The regulatory role of TRAF6 on cell viability, apoptosis and inflammation. (**A**) Expression level of TRAF6. (**B**) The regulatory role of TRAF6 on cell viability. (**C**) The regulatory role of TRAF6 on apoptosis. (**D**) The regulatory role of TRAF6 on IL-6. (**E**) The regulatory role of TRAF6 on IL-1β. (**F**) The regulatory role of TRAF6 on TNF-α. ***P* <0.01, ****P* <0.001.

luciferase was no longer influenced by miR-322-5p levels (Figure 4B). In addition, it was observed that the TRAF6 was significantly increased in patients with CHD compared to the Control (Figure 4C, $P < 0.001$). Subsequent Pearson analysis confirmed that the expression of TRAF6 was negatively correlated to miR-322-5p level in patients with CHD (Figure 4D, $r = -0.683$, $P < 0.0001$).

The Regulatory Effect of miR-322-5p on TRAF6

To confirm the regulatory effect of miR-322-5p on TRAF6, we conducted a rescue experiment. As shown in Figure 5A, in ox-LDL-induced cells, the significantly elevated TRAF6 was markedly inhibited by miR-322-5p upregulation. However, the TRAF6 overexpression reversed the inhibitory effect of miR-322-5p ($P < 0.001$). The effects of differentially expressed TRAF6 on cells were also investigated. Cell viability under pathological conditions was enhanced by miR-322-5p upregulation, which was rescued by TRAF6 overexpression (Figure 5B, $P < 0.001$). Conversely, the enhanced apoptosis rate was reduced by miR-322-5p upregulation and then was reversed by TRAF6 overexpression (Figure 5C, $P < 0.001$). It was also observed that highly expressed TRAF6 reversed the reduced inflammatory factors caused by upregulated miR-322-5p, including IL-6, IL-1β and TNF-α (Figure 5D–F, $P < 0.01$).

Discussion

CHD remains a leading cause of global morbidity and mortality, with unmet needs in early diagnosis and targeted therapy. Research on specific miRNAs as potential biomarkers for CHD offers a new perspective for early diagnosis and treatment. This study explored the clinical significance and regulatory mechanism of miR-322-5p in CHD. The results showed that the expression of miR-322-5p was significantly decreased in CHD patients, which had high diagnostic value for CHD. In addition, its expression level was negatively correlated with Gensini score and CRP, and positively correlated with HDL-C. Further cellular experiments demonstrated that upregulation of miR-322-5p could reverse ox-LDL-induced coronary endothelial cell injury, exerting a protective effect by enhancing cell viability, inhibiting apoptosis, and reducing the release of inflammatory factors. Mechanistically, miR-322-5p directly targets and negatively regulates TRAF6, while high expression of TRAF6 exacerbates the pathological progression of CHD.

Data from our research indicated that miR-322-5p was distinctly declined in patients with CHD and presented high accuracy for the recognition of CHD. This highlighted its potential as a biomarker for CHD. Similar findings have been reported before. For example, miR-322-5p is downregulated in rats with pulmonary arterial hypertension.[22] In acute myocardial infarction, low miR-322-5p can serve as an early diagnostic marker and monitoring miR-322-5p expression contributes to assessing disease severity and prognosis.[23] Moreover, our subsequent findings further supported the above conclusion that declined miR-322-5p was negatively correlated to both Gensini score and CRP, while it was positively associated with HDL-C. Increasing studies have confirmed that HDL-C is negatively correlated with the risk of CHD,[24] and elevated CRP levels are positively correlated with increased CHD risk.[25] Additionally, the Gensini score has been shown to be closely related to the severity of CHD.[26]

The SYNTAX score is an anatomical factor-based scoring system for coronary arteries, which can comprehensively reflect the complexity of coronary artery lesions in patients with stable coronary artery disease.[27] Pearson correlation analysis further confirmed the strong link between miR-322-5p and CHD. These results suggest that lower expression level of miR-322-5p is associated with higher inflammation levels and more severe coronary artery lesions.

Our further research focused on the regulatory role of miR-322-5p in CHD and found that under pathological conditions, cell apoptosis and inflammation levels were significantly increased. However, upregulation of miR-322-5p enhanced cell viability, and inhibited apoptosis and the level of inflammatory factors (IL-6, IL-1β, and TNF-α). Similar findings have been reported in previous publications. In myocardial ischemia/reperfusion injury, miR-322 is downregulated, leading to increased apoptosis and inflammation, and reduced cell viability.[28] In myocardial infarction and OGD-stimulated rat cardiomyocytes, miR-322-5p is downregulated, while its overexpression can inhibit apoptosis, enhance both cell viability and cardiac function.[29] In the acute lung injury model, miR-322-5p is downregulated, with elevated levels of IL-1β, IL-6, and TNF-α.[30] These results show that increasing miR-322-5p can improve cell viability and reduce inflammation, highlighting the pivotal regulatory role in CHD. In addition, it is worth noting that this study found a significant increase in the level of NT-proBNP in patients with CHD. This result is consistent with the clinical characteristics of NT-proBNP — as a marker secreted by ventricular myocytes when pressure or volume load increases, its elevated level usually indicates myocardial remodeling or impaired cardiac function.[31] Further analysis showed that the increase in NT-proBNP in the CHD group was moderately negatively correlated with the downregulation of miR-322-5p, suggesting that low expression of miR-322-5p may be one of the potential molecular factors associated with the increase in NT-proBNP. This provides a new clue for subsequent exploration of the role of miR-322-5p in cardiac function protection in CHD patients.

TRAF6 is a key signaling protein involved in many inflammatory and immune responses.[32] Recent studies suggest that high levels of TRAF6 may play a role in the progression of CHD. It has been declared that TRAF6 mRNA levels are much higher in patients with coronary artery disease than in those without it.[33] In addition, abnormally expressed TRAF6 can also regulate cell death and inflammation. For instance, in atherosclerosis, elevated TRAF6 increases the death of macrophage and the release pro-inflammatory factors, making plaques less stable.[34] TRAF6 knockdown has been confirmed to suppress the expression of IL-6 and TNF-α levels.[35] A cardiovascular study also confirms that TRAF6 inhibitors improve endothelial function and reduce inflammation and oxidative stress.[36] Our results showed that TRAF6 was negatively regulated by miR-322-5p and high levels of TRAF6 aggravated the CHD and it could be a promising target for treatment. At the level of clinical translation, speckle tracking echocardiography enables the simultaneous assessment of miRNA expression levels and myocardial strain parameters, providing an effective means to analyze the association between abnormal myocardial strain parameters and imbalanced miRNA expression.[37] This offers systematic and comprehensive evidence support for research on the pathological mechanisms of CHD, clinical diagnosis, and evaluation of treatment responses, and is of great significance for promoting the translation of basic research in the field of CHD into precise clinical applications.

Furthermore, TRAF6 can affect cell survival and function through various signaling pathways. Results from myocardial ischemia/reperfusion injury show that high TRAF6 expression can worsen damage to heart muscle and blood vessel cells by activating inflammatory pathways like NF-κB.[38] Our results indicate that miR-322-5p exerts a protective effect by regulating TRAF6. However, whether it blocks these inflammatory pathways by inhibiting TRAF6 requires further verification. In subsequent studies, we will explore and clarify the specific molecular mechanisms by which TRAF6 mediates CHD pathology, and improve the regulatory network of “miR-322-5p→TRAF6→inflammatory/apoptotic pathways”.

This study has some limitations. The single-center design may limit the generalizability of our findings. Although we find that miR-322-5p regulates TRAF6 in vitro, its exact mechanism and therapeutic potential in CHD need confirmation through in vivo experiments. In addition, we do not conduct a long-term follow-up to assess miR-322-5p's prognostic value. Future research will focus on clinical translation. Next, we will first standardize miR-322-5p detection, verify its reliability as a CHD diagnostic marker via multi-center large-sample studies, and build a combined diagnostic model to enhance early identification. Mechanistically, we will further analyze downstream pathways of the miR-322-5p/TRAF6 axis and use animal models to optimize synergistic interventions with targeted delivery systems and TRAF6 inhibitors. Ultimately, clinical trials will promote its development as a clinically applicable diagnostic tool and therapeutic target, translating basic research into clinical application.

In conclusion, miR-322-5p is a potential diagnostic marker for CHD and exerts a protective role in CHD through the miR-322-5p/TRAF6 axis.

Disclosure

The authors report no conflicts of interest in this work.

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