

Hippophae rhamnoides extract alleviates diabetic cardiomyopathy via the LncRNA NEAT1/miR-147a axis

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Abstract: Background: The incidence of diabetic cardiomyopathy (DCM) continues to rise. Although the pathogenesis of DCM has been progressively elucidated, effective treatment options remain insufficient. **Objectives:** To investigate the involvement of LncRNA NEAT1 in the protective effect of *Hippophae rhamnoides* extract on DCM. **Methods:** A total of 229 subjects were included, including 127 individuals with DCM. The cardiac condition of the patients was observed using electrocardiography and 5 mL of fasting venous blood was collected. ROC curves assessed the diagnostic value of LncRNA NEAT1 and logistic regression analysis was conducted to identify factors contributing to DCM occurrence. High-glucose (HG) models of H9c2 and HL-1 cells were constructed using 50 mM and 25 mM glucose, respectively. Cells were pretreated with 20 μ M SBU to protect them. RT-qPCR detected gene expression and flow cytometry was employed to observe cell apoptosis. **Results:** DCM patients exhibited obvious increases in blood glucose and myocardial injury markers. Upregulation of LncRNA NEAT1 was detected in DCM patients and HG-treated cardiomyocytes. LncRNA NEAT1 was identified as a risk factor for the occurrence of DCM. SBU pretreatment was able to downregulate LncRNA NEAT1 expression in damaged cardiomyocytes. oe-LncRNA NEAT1 induced cardiomyocyte apoptosis and increased the expression of myocardial injury markers and oxidative stress levels. Meanwhile, miR-147a was downregulated in DCM patients. miR-147a acted as a negative regulatory factor in SBU-mediated protection of HG cardiomyocytes from damage and could counteract the effects of oe-LncRNA NEAT1 on cardiomyocytes. **Conclusion:** LncRNA NEAT1 participates in the protective effect of SBU on damaged cardiomyocytes through miR-147a.

Keywords: Diabetic cardiomyopathy; Diagnosis; *Hippophae rhamnoides*; LncRNA NEAT1; miR-147a

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INTRODUCTION

Diabetic cardiomyopathy (DCM) is a relatively serious metabolic syndrome. It is caused by structural changes in the myocardium resulting from long-term exposure to high glucose levels in patients (Brownlee 2005, Swiatkiewicz *et al.* 2024). This can lead to ventricular dysfunction (Lam 2015) and even heart failure (Pham *et al.* 2015, Boonman-de Winter *et al.* 2012). While diabetes is not the only mechanism that induces cardiomyopathy, the coexistence of the two conditions has sparked research interest (Marfella *et al.* 2021). However, the causes of cardiovascular lesions induced by diabetes are complex and require ongoing investigation (Xiao *et al.* 2023). Studies have shown that around 50% of people with diabetes die from cardiovascular disease and around 10%-30% of people with heart failure have a history of diabetes

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(Einarson *et al.* 2018). It has been found that patients with advanced diabetes exhibit severe ventricular diastolic dysfunction and diffuse interstitial fibrosis, which more easily trigger systemic inflammatory responses (Lee *et al.* 2023). Carmen's team found that DCM is associated with poor prognosis in patients (Zulet *et al.* 2024). This indicates that the pathogenic mechanism of myocardial glucotoxicity cannot be ignored (Battault *et al.* 2020). It has been reported that microvascular disease is an independent factor for atrial fibrillation in adults with diabetes (Kaze *et al.* 2022).

These findings suggest that exploring the mechanisms of diabetic microvascular disease is imperative in order to establish diagnostic criteria and therapeutic targets for DCM. *Hippophae rhamnoides* is a deciduous shrub native to the Eurasian continent. In 1977, the "Pharmacopoeia of the People's Republic of China" included *Hippophae rhamnoides* and recognized its attribute of being both

medicinal and edible. *Hippophae rhamnoides* contains hundreds of nutrients (Zhang *et al.* 2025) and bioactive compounds and its juice is rich in amino acids, vitamins, fatty acids and trace elements (Wang *et al.* 2022). Sea buckthorn extract contains flavonoids, tannins, sterols and serotonin, among other compounds, which exhibit extensive pharmacological activities, especially with regard to cardioprotection (Ma *et al.* 2020). It has been reported that flavonoids from *Hippophae rhamnoides* and their derivatives contribute to the treatment of DCM through multiple pathways (Zhang *et al.* 2025). It has been reported that rhamnoside can inhibit apoptosis of H9c2 cells by scavenging reactive oxygen species (Sun *et al.* 2012).

Long non-coding RNAs (LncRNAs) play an important regulatory role in DCM disease (Taylor *et al.* 2024). Through bioinformatics analysis, a significant downregulation of LncRNA NEAT1 in DCM mice treated with sea buckthorn extract is reported (Safavi *et al.* 2025). Researchers have discovered that LncRNA NEAT1 exacerbates reperfusion injury in patients with diabetic myocardial ischemia (Ruan *et al.* 2019). Researchers also detected an upregulation of LncRNA NEAT1 in patients with coronary atherosclerotic heart disease. (Zhang *et al.* 2020). Nevertheless, research into the regulatory role of LncRNA NEAT1 in sea buckthorn extract-mediated protection in DCM remains limited.

MATERIALS AND METHODS

Patient enrollment

This was a case-control study comparing gene expression levels between DCM patients and healthy controls; no randomization was applied. A total of 229 subjects were included in the study and they were comprised of 127 patients with DCM and 102 healthy individuals. All subjects were either hospitalized for treatment or underwent health examinations at PLA Navy Characteristic Medical Center Hospital from May 2022 to April 2023. Fasting peripheral venous blood samples of 5 mL were collected from the patients and cardiac function was assessed using echocardiography (Philips EPIQ7C, Philips Healthcare, USA). According to the American Diabetes Association (ADA)(Muthu, Sukumaran and Sundararajan 2022) and the European Society of Cardiology (ESC) (Ozdemir and European Society of 2014) Guidelines, meeting the following criteria is required for a definitive diagnosis of DCM:

(1) Diagnosis of diabetes

Fasting blood glucose ≥ 7.0 mmol/L, or glycated hemoglobin HbA1c $\geq 6.5\%$, or random blood glucose ≥ 11.1 mmol/L.

(2) Diagnosis of cardiomyopathy

Diastolic dysfunction: Early (E wave) to late (A wave) filling velocity ratio (E/A) < 0.8 in mitral valve diastolic flow; systolic dysfunction: Left ventricular ejection fraction (LVEF) $< 50\%$.

(3) Key exclusion criteria

Other cardiac diseases (coronary artery disease, valvular disease, congenital heart disease, cardiomyopathy, pericardial disease, sustained arrhythmia), hemochromatosis, thyroid dysfunction, history of chemotherapy/radiotherapy-related myocardial injury.

Additionally, patients must be adults with ≥ 5 years of diabetes duration. No prior cardiac medication or surgical intervention. However, patients with gestational diabetes, diabetic ketoacidosis, autoimmune diseases, severe liver or kidney dysfunction and those in the acute phase were excluded from the study. The study was approved by the Ethics Committee of PLA Navy Characteristic Medical Center Hospital and all subjects or their families signed a written informed consent form. The patient recruitment flowchart is shown in Fig. S1.

Cell culture and transfection

Rat cardiomyocytes (H9C2, SCSP-5211) and mouse cardiomyocytes (HL-1, GNM46) were both purchased from the National Resource Center for Standard and Specialty Experimental Cells. Both cell types were cultured in DMEM supplemented with 10% fetal bovine serum (F0193, Sigma-Aldrich, U.S.). Additionally, the complete medium was supplemented with 1% penicillin-streptomycin solution Hybri-Max (P7539, Sigma-Aldrich, U.S.). Cells were maintained at 37°C in 5% CO₂.

Cell transfection was performed using Lipo8000 reagent (C0533, Beyotime, Shanghai). Cells were seeded into 12-well plates one day before transfection. The following day, the culture plates were replaced with 1 mL of fresh medium and set aside. A clean, sterile centrifuge tube was taken and 50 μ L of cell basal medium, along with 1 μ g of DNA or 40 pmol of siRNA, was added to it. The mixture was thoroughly mixed, then 1.6 μ L of Lipo8000 reagent was added and mixed well. Finally, 50 μ L of the transfection reagent mixture was added to each well and the cells were incubated in a constant-temperature incubator for 24 h.

Construction of a high-glucose cell model

D-Glucose purchased from MedChemExpress was used to establish a high-glucose (HG) model in cardiomyocytes. Due to the differing sensitivities of H9C2 and HL-1 cells to glucose, *in-vitro* experiments were conducted at varying glucose concentrations. For H9c2 cells: Blank control group (Con), 5.5 mM glucose; osmotic control group (OC), 5.5 mM glucose with 50 mM mannitol; HG group, 50 mM

glucose (Dai *et al.* 2025). Additionally, it should be noted that validated concentrations of 5.5 mM glucose had no significant effect on H9c2 cell stress markers (such as Bax/Bcl-2 ratio and Caspase-3 activity), while 25 mM glucose induced mild stress. However, 50 mM glucose significantly increased lncRNA NEAT1 expression and promoted the expression of apoptosis-related genes and the levels of reactive oxygen species (Fig. S2). Therefore, 50 mM was selected as the treatment concentration for H9c2 cells. For HL-1 cells: Con, 5.5 mM glucose; OC, 5.5 mM glucose with 25 mM mannitol; HG group, 25 mM glucose. All cells were treated in solutions of different osmotic pressures for 24 h.

Extraction and processing of sea buckthorn flavonoids (SBU)

SBU was extracted based on previously established methods (Safavi *et al.* 2025). *Hippophae rhamnoides* wood was boiled three times in ether at 70°C, each time for 6 h. After air-drying in a fume hood, the powder was incubated four times in 700 mL/L ethanol at 80°C, each time for 2 h. Subsequently, the incubated material was passed through a D101 macroporous resin column using a concentration gradient (300, 500 mL/L) at a flow rate of 2 mL/min (Yang *et al.* 2017). SBU was recovered by vacuum rotary evaporation. Subsequently, SBU content was preliminarily identified using a plant flavonoid content detection kit (KTB1530, Abbkine, Wuhan). A 30% ethanol solution served as the blank control, while a serially diluted rutin solution acted as the standard.

The absorbance values of the solutions were measured at an excitation wavelength of 502 nm using a UV spectrophotometer (UV-2600, Shimadzu Corporation, Japan) (Wen *et al.* 2022). Additionally, to determine the optimal SBU concentration for protecting cardiomyocytes, experiments across different concentration gradients were conducted. After HG treatment, cells were treated with 0, 5, 10, 20 and 50 µM SBU for 12 h (Zhang *et al.* 2025).

RT-qPCR

Total RNA was extracted from cardiomyocytes using the 5 min Flash Cell RNA Kit (MolPure®, 19231, YEASEN, Shanghai). The quality and concentration of RNA were assessed using an ultraviolet spectrophotometer (Nano-400A, Hangzhou Allsheng Instrument Co., Ltd., China). Prior to quantitative analysis of NEAT1 long non-coding RNA (lncRNA), reverse transcription was performed using FastKing gDNA Dispelling RT Super Mix (KR118, TIANGEN, Beijing). Ultra SYBR Mixture (CW0957, CWBIO, Taizhou) was then used for subsequent quantitative reactions. For reverse transcription of miR-147a, RNAiso for Small RNA (9753, TAKARA, Japan) was used and quantitative polymerase chain reaction (qPCR) was conducted using the MicroRNAs qPCR Kit

(B532461, Sangon, Shanghai). Fluorescence signals from the reactions were collected using a real-time PCR system (CFX96 Touch, Bio-Rad Laboratories, USA). β-actin was used as the internal reference gene for lncRNA NEAT1 and U6 was used as the internal reference gene for miR-147a to normalize the results. Primer information for the reactions described above is provided in table S1.

Flow cytometry

The effects of different treatments on cardiomyocyte apoptosis were assessed using an Annexin V-FITC apoptosis detection kit (C1062S, Beyotime, Shanghai). The old culture medium was first collected into a centrifuge tube and the cell surface was gently washed with PBS. The cardiomyocytes were then digested with 0.25% trypsin for 3 min. The digestion process was stopped by adding the old culture medium. The cells were collected in a centrifuge tube and centrifuged at 1,000 g for 5 minutes to pellet the cells.

The cells were resuspended in 1× binding buffer and the cell concentration was adjusted to 1×10⁶ cells/mL. Then, 100 µL of the cell suspension was added to a centrifuge tube and centrifuged to discard the supernatant. The cells were resuspended in 5 µL of Annexin V-FITC binding solution. Subsequently, 5 µL of Annexin V-FITC and 10 µL of propidium iodide (PI) staining solution were added to the above system and mixed well. The mixture was incubated at room temperature in the dark for 15 min and analyzed by flow cytometry (Attune NxT cytometer, Thermo Fisher Scientific, Waltham, MA, USA) within 1 h.

Detection of cardiac injury markers and oxidative stress levels

The AmonMedCAS Troponin (cTnI) assay kit was used to detect cTnI in cardiomyocytes. The Rat Creatine Kinase MB Isoenzyme ELISA Kit (JL12296, JONLNBIO, Shanghai) is used to detect CK-MB in H9C2 cells and the Mouse CK-MB ELISA Kit (D721065, Songan, Shanghai) is used to detect CK-MB content in HL-1 cells. Reactive oxygen species (ROS) release was assessed using the ROS Detection Kit (S0033S, Beyotime, Shanghai). Malondialdehyde (MDA) is measured using the MDA detection kit from Beyotime, Shanghai. The Total SOD Activity Assay Kit (S0101S, Beyotime, Shanghai) is used to measure SOD levels in cardiomyocytes following various treatments. Each reaction should be performed according to the manufacturer's instructions. To minimize experimental error, the same reaction should be conducted by the same person.

Subcellular localization analysis

Different mRNA expressions were extracted using a nuclear and cytoplasmic RNA separation PARIS kit (AM1921, Invitrogen, Waltham, USA) (Jiang *et al.* 2024).

At least 2×10^4 cells were collected and centrifuged at $800 \times g$ for 7 min at 4°C . The cells were washed with pre-chilled PBS, the supernatant was discarded and the cells were placed on ice. 1 mL of pre-chilled nuclear protein extraction reagent A was added to the pellet in the centrifuge tube. The mixture was intermittently processed with a QL-906 vortex mixer (Kylin-Bell Lab Instruments Co., Ltd., Haimen, China) for 1 min, incubated on ice for 2 min and this step was repeated 3 times. Cell lysis efficiency was checked using trypan blue staining and needed to be greater than 50%. The lysed samples were incubated on ice for 10 min, then centrifuged at $6,500 \times g$ for 5 min at 4°C and the supernatant was collected as the cytoplasmic protein. 1 mL of nuclear RNA wash buffer A was added to the remaining pellet to resuspend it. This was then centrifuged to discard the supernatant. Then, 1 mL of nuclear protein wash buffer B was added to the remaining pellet, vortexed vigorously for 1 min and centrifuged at 4°C to collect the pellet. Then, 100 μL of nuclear RNA extraction reagent B was added to resuspend the pellet.

This was vortexed vigorously for 2 min and incubated on ice for 12 min. This process was repeated three times. The mixture was centrifuged at $16,000 \times g$ for 10 min at 4°C and the supernatant was collected as nuclear protein. Four volumes of dilution buffer were added to the nuclear protein to reduce protein viscosity. Loading buffer ($1\times$) was added to the protein samples, which were boiled for 5 min and then stored at -80°C . Protein expression levels of the target genes were analyzed by Western blotting and the immunoreactive bands were visualized using a ChemiDoc™ MP imaging system (Bio-Rad, Hercules, CA, USA).

Dual luciferase reporter assay

The interaction between the target gene and the gene of interest was examined using the Dual Luciferase Reporter Assay Kit (DL101, Vazyme, Nanjing). First, expression plasmids were constructed for the wild-type and mutant using the pGL3-hRLuc vector. Transfection was carried out using Lipo8000 reagent. After transfection, the old culture medium was discarded and the cells were washed with PBS. Then, 100 μL of cell lysis buffer was added to a 24-well plate and shaken for 5 min. The lysate was transferred to a centrifuge tube and centrifuged at 11,200 rpm for 3 min. The supernatant was transferred to a clean centrifuge tube. Take 100 μL of luciferase substrate, add it to a microplate, then add 20 μL of the cell lysate supernatant, mix well and measure the fluorescence intensity with a fluorescence detector. Next, add 100 μL of *Renilla* substrate to the same system and measure again. Finally, normalize the experimental results using the *Renilla* luciferase luminescence value (Suzuki *et al.* 2026).

Database sources

First, potential target genes implicated in DCM were

identified in the CTD database (CTD, 2025). These results were then cross-referenced with four databases—TargetScan (TargetScan, 2025), miRDB (miRDB, 2025), miRWalk (miRWalk, 2025) and ENCORI (ENCORI, 2025)—to identify overlapping genes. The names of these overlapping genes were entered into the STRING database (STRING, 2025) to construct a protein-protein interaction (PPI) network. Additionally, GO and KEGG enrichment analyses were performed on the overlapping genes using the Micro Bioinformatics platform (Micro Bioinformatics, 2025).

Data analysis

Experimental data were analyzed using SPSS 23.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). To minimize experimental error, six replicate wells were included in each group for validation, resulting in a sample size of six ($n=6$). Additionally, during testing procedures, each sample was subjected to three identical operations. The Kolmogorov–Smirnov test was used to assess data distribution ($P > 0.05$ indicates normality). For data that could not be transformed to meet normality assumptions, the Mann–Whitney U test was applied. Second, Levene's test was employed to assess homogeneity of variance across groups. When heterogeneous variances were detected, Welch's ANOVA was applied. Following variance analysis, multiple comparisons were corrected using the Benjamini–Hochberg method.

The diagnostic value of LncRNA NEAT1 for predicting DCM was evaluated using ROC curves. Logistic regression was used to predict potential risk factors for DCM. The relationship between LncRNA NEAT1 and miR-147a expression in the blood of DCM patients was assessed via Pearson correlation analysis. $P < 0.05$ indicates that the data results are statistically significant. The sample size was determined a priori using G*Power software (version 3.1). A power analysis for a two-group independent means t-test, with a medium effect size (Cohen's $d = 0.5$), a two-sided α of 0.05 and a power of 0.80, indicated that 64 participants per group were required. To account for potential dropouts, we ultimately enrolled 127 DCM patients and 102 healthy controls.

RESULTS

Analysis of basic clinical data of DCM and healthy individuals

The results of the analysis (Table 1) indicate that the average age of the healthy population included in the study was 62.12 ± 14.84 years, while the average age of the DCM patients was 62.68 ± 15.90 years.

Table 1: Comparison of clinical baseline parameters between healthy individuals and patients with diabetic cardiomyopathy (DCM).

Clinical indicators	Heathy (n=102)	DCM (n=127)	P value
Age (years)	62.12±14.84	62.68±15.90	0.785
BMI (kg/m ²)	22.91±3.35	23.01±3.12	0.820
Gender (male%)	45 (44.12)	61 (48.03)	0.595
Medication [n(%)]			0.792
No	52 (50.98)	67 (52.76)	
yes	50 (49.02)	60 (47.24)	
Smoking [n(%)]			0.791
No	51 (50.00)	61 (48.03)	
Yes	51 (50.00)	66 (51.97)	
Duration of hypertension [n(%)]			0.894
No	58 (56.86)	71 (55.91)	
Yes	44 (43.14)	56 (44.09)	
SBP (mmHg)	115.87±13.74	158.83±18.28	<0.0001
DBP (mmHg)	75.47±8.23	76.81±15.86	0.443
HDL-C (mmol/L)	1.36±0.29	1.23±0.24	<0.0001
LDL-C (mmol/L)	3.04±0.29	3.43±0.27	<0.0001
FBG (mmol/L)	5.24±0.89	7.72±1.89	<0.0001
HbA1c (%)	5.09±0.73	9.06±3.08	<0.0001
BNP (pg/ml)	63.94±24.06	125.65±12.13	<0.0001
NT-proBNP (pg/ml)	189.78±67.17	334.52±20.16	<0.0001
LVEF (%)	61.06±7.06	54.35±6.14	<0.0001

Abbreviations: BMI, Body Mass Index. SBP, Systolic Blood Pressure. DBP, Diastolic Blood Pressure. HDL-C, high-density lipoprotein cholesterol. LDL-C, low-density lipoprotein cholesterol. FBG, fasting blood glucose. HbA1c, glycated hemoglobin. BNP, B-type natriuretic peptide. NT-proBNP, N-terminal pro-B-type natriuretic peptide. LVEF, left ventricular ejection fraction. $P < 0.05$ indicates significant differences between groups.

There were no significant differences in body mass index (BMI), gender, or diastolic blood pressure (DBP) between healthy individuals and DCM patients. However, systolic blood pressure (SBP), fasting blood glucose (FBG), hemoglobin (HbA1c), brain natriuretic peptide (BNP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were all significantly higher in DCM patients than in healthy individuals. Left ventricular ejection fraction (LVEF) was significantly lower in DCM patients. These results suggest that the myocardium in patients with DCM is already damaged.

Expression and diagnostic value of LncRNA NEAT1 in DCM patients

The expression of LncRNA NEAT1 in the serum of DCM patients was found to be distinctly upregulated ($P < 0.0001$, Cohen's $d=1.46$, $R^2=0.3508$, Fig. 1A). In DCM patients, LncRNA NEAT1 expression levels were positively correlated with BNP ($r = 0.7443$, $R^2 = 0.5540$, $P < 0.0001$, Fig. 1B) and negatively correlated with LVEF ($r = -0.7860$, $R^2 = 0.6179$, $P < 0.0001$, Fig. 1C). Furthermore, the AUC of LncRNA NEAT1 in predicting DCM was 0.8540 ($P < 0.0001$, 95% CI: 0.8058-0.9022, Fig. 1D), with a sensitivity of 70.87% and a specificity of 86.27%. However, analysis of the existing sample revealed that BNP alone predicted DCM with an AUC of 0.9834 (95% CI: 0.9657-1.000; Fig. S3), a sensitivity of 95.28% and a

specificity of 96.08%. Conversely, LVEF alone yielded an AUC of 0.7657 (95% CI: 0.7044-0.8270; Fig. S3C) for predicting DCM, with a sensitivity of 62.99% and specificity of 79.41%. However, when combined with LncRNA NEAT1 prediction, the AUC for BNP predicting DCM increased to 0.992 and the AUC for LVEF predicting DCM increased to 0.888 (Figs. S3B and S3D). Furthermore, after controlling for potential confounders, logistic regression analysis identified LncRNA NEAT1 ($P=0.004$, OR=2.326) and LVEF ($P=0.041$, OR=0.548) as independent predictors of DCM occurrence (Table 2).

SBU modulates myocardial cell function and oxidative stress levels by regulating LncRNA NEAT1

Preliminary experimental results demonstrated that SBU pretreatment enhanced the viability of damaged H9c2 and HL-1 cardiomyocytes in a concentration-dependent manner (Fig. 2A). Treatment with 20 μ M SBU exhibited an excellent enhancing effect on the activity of cardiomyocytes. Therefore, 20 μ M was selected as the optimal concentration for subsequent experiments. Following high-glucose treatment, LncRNA NEAT1 expression levels were significantly upregulated in cardiomyocytes, whereas SBU pretreatment suppressed this effect ($P < 0.01$, Fig. 2B). Second, HG-induced and oe-NEAT1 transfection increased cardiomyocyte apoptosis levels.

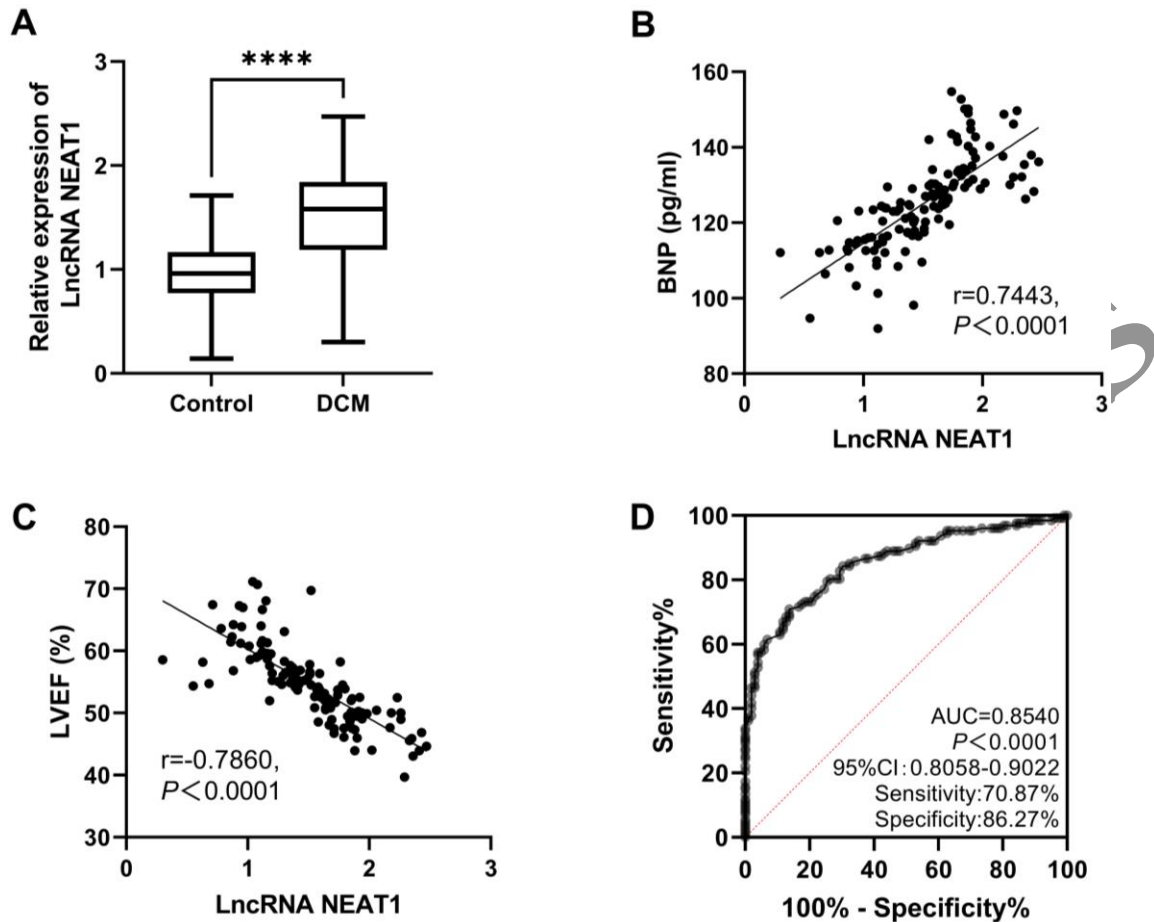


Fig. 1: Expression and diagnostic value of LncRNA NEAT1 in patients with diabetic cardiomyopathy (DCM). (A) Changes in LncRNA NEAT1 expression in serum from DCM patients; (B-C) Correlation analysis between serum LncRNA NEAT1 expression and BNP; (B) and LVEF; (C) in DCM patients; (D) ROC curve predicting the diagnostic value of LncRNA NEAT1 for DCM, where AUC represents the area under the curve and CI represents the confidence interval. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$.

Table 2: Logistic assessment of independent factors influencing the occurrence of DCM.

Clinical indicators	P value	OR	95%CI
LncRNA NEAT1	0.004	2.326	1.300-4.163
Age	0.708	1.117	0.626-1.994
BMI	0.922	0.971	0.546-1.728
Gender	0.690	1.125	0.630-2.011
Medication	0.630	1.151	0.649-2.040
Smoking	0.680	1.127	0.638-1.990
Duration	0.763	0.915	0.515-1.628
SBP	0.470	1.236	0.695-2.198
DBP	0.347	1.358	0.718-2.568
HDL-C	0.136	0.618	0.328-1.163
LDL-C	0.469	1.239	0.694-2.211
FBG	0.335	1.330	0.744-2.377
HbA1c	0.064	1.743	0.969-3.137
LVEF	0.041	0.548	0.308-0.977

Abbreviations: BMI, body mass index. SBP, systolic blood pressure. DBP, diastolic blood pressure. HDL-C, high-density lipoprotein cholesterol. LDL-C, low-density lipoprotein cholesterol. FBG, fasting blood glucose. HbA1c, glycated hemoglobin. LVEF, left ventricular ejection fraction. $P < 0.05$ indicates significant differences between groups.

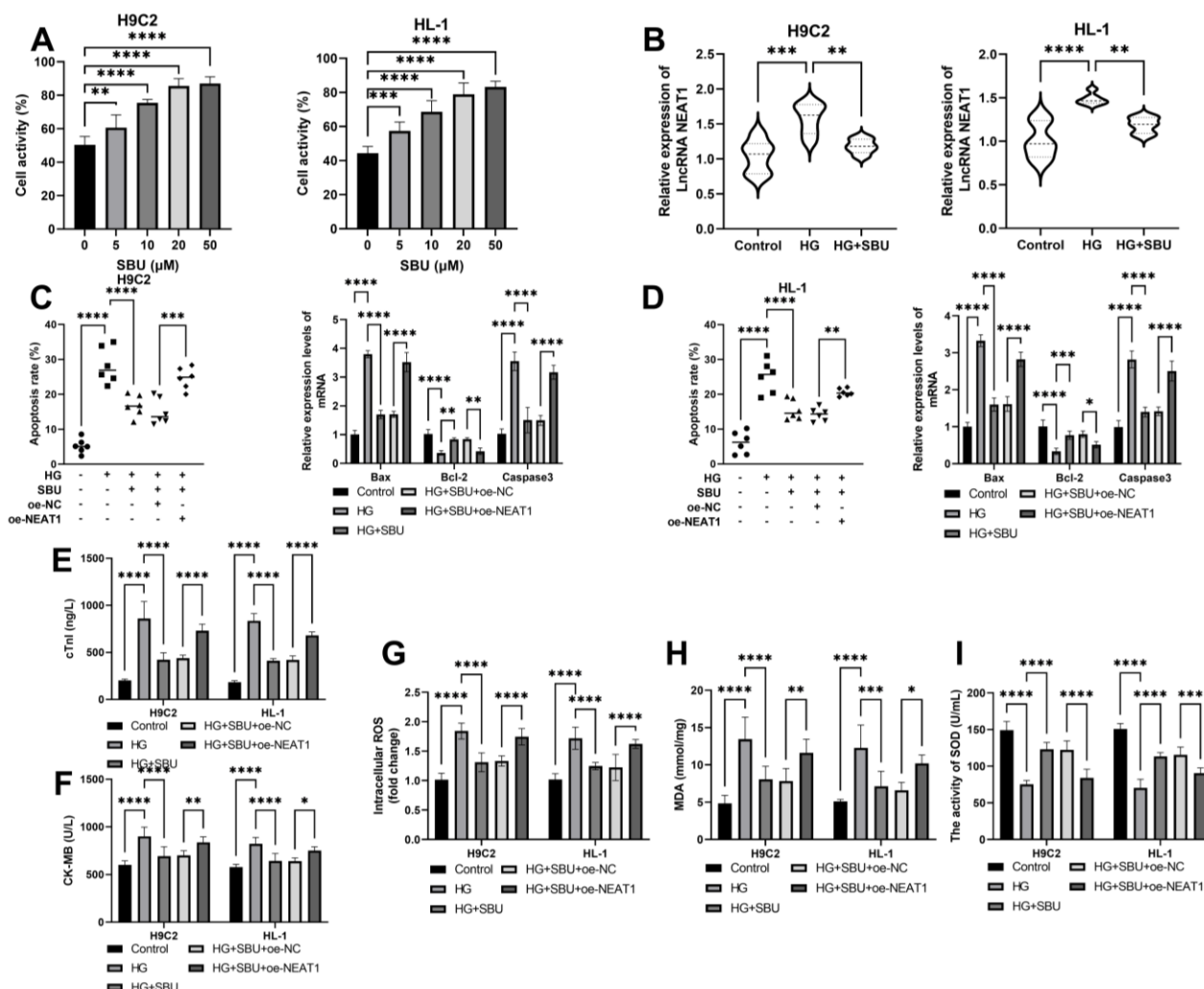


Fig. 2: Effects of HG, SBU and oe-NEAT1 treatments on cardiomyocyte function and oxidative stress levels. (A) Optimal concentration screening of SBU pretreatment in H9C2 and HL-1 cardiomyocytes. The 0 μ M control represents cell viability after high glucose treatment without SBU pretreatment; (B) Effects of different treatments on LncRNA NEAT1 expression in cardiomyocytes; (C-D) Apoptosis status and apoptosis-related gene expression in H9C2 (C) and HL-1 (D) cardiomyocytes after different treatments; (E-F) Changes in cardiomyocyte injury markers cTnI (E) and CK-MB (F) levels after different treatments; (G-I) Oxidative stress levels in cardiomyocytes after different treatments; (G) Reactive oxygen species (ROS) content; (H) Malondialdehyde (MDA) production; (I) Superoxide dismutase (SOD) expression. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

This was manifested by upregulation of the proapoptotic genes Bax and Caspase3 and downregulation of the anti-apoptotic gene Bcl-2 (Figs. 2C and 2D). In the HG group, cardiomyocytes indicated markedly increased expression of myocardial injury markers cTnI and CK-MB, but these levels decreased following SBU pretreatment ($P < 0.05$, Figs. 2E and 2F). Moreover, HG treatment increased the expression levels of ROS and MDA in cardiomyocytes while decreasing SOD expression ($P < 0.05$, Figs. 2G-2I). However, SBU pretreatment counteracted HG-induced oxidative stress damage. To further investigate LncRNA NEAT1's function, its expression was successfully knocked down in HG-treated cardiomyocytes via si-NEAT1 transfection ($P < 0.0001$, Fig. S4A). Results revealed that

LncRNA NEAT1 deficiency inhibited HG-induced cardiomyocyte apoptosis ($P < 0.0001$, Supplementary Fig. 3B). It also reduced expression of myocardial injury markers (cTnI, CK-MB) and decreased oxidative stress levels ($P < 0.0001$, Figs. S4C-S4F).

miR-147a may be a downstream target gene of LncRNA NEAT1

Using the LncRNASNP2 database, it was predicted that miR-147a has target binding sites with LncRNA NEAT1 (Fig. 3A). Nucleocytoplasmic separation experiments indicated that LncRNA NEAT1 is mainly distributed in the cytoplasm (Fig. 3B).

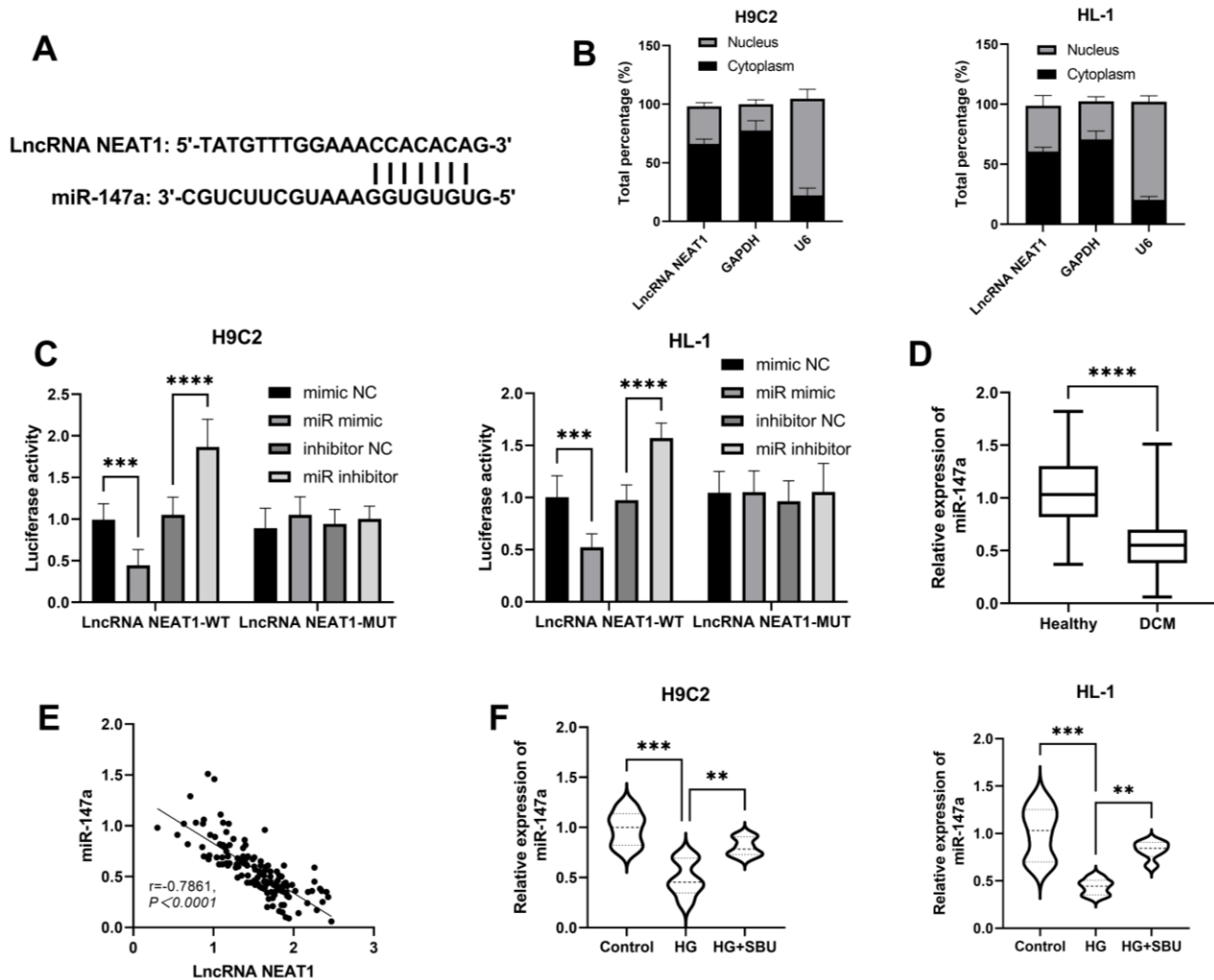


Fig. 3: Expression of miR-147a in DCM patients and identification of its interaction with LncRNA NEAT1. (A) Target site information for the binding of LncRNA NEAT1 with miR-147a; (B) Subcellular localization analysis of LncRNA NEAT1; (C) Dual-luciferase reporter assay to validate the targeting relationship between LncRNA NEAT1 and miR-147a; (D) Expression of miR-147a in DCM patients; (E) Correlation analysis of LncRNA NEAT1 and miR-147a expression in the serum of DCM patients; (F) Effect of SBU pretreatment on miR-147a expression in HG-treated cardiomyocytes. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

When wild-type plasmids coexisted with a miR mimic, the fluorescence activity in cell lysates was significantly inhibited. However, when they coexisted with a miR inhibitor, the fluorescence activity increased markedly, whereas no significant change in fluorescence activity was observed in cells containing mutant plasmids ($P < 0.001$, Fig. 3C). Furthermore, the results showed that the expression level of miR-147a in the serum of DCM patients was markedly downregulated ($P < 0.0001$, Cohen's $d = 1.62$, $R^2 = 0.3973$, Fig. 3D). The results also found a distinct negative correlation between the expression of LncRNA NEAT1 and miR-147a in DCM patients ($r = -0.7861$, $R^2 = 0.6180$, $P < 0.0001$, Fig. 3E). Additionally, the present study observed that in cardiomyocytes treated with HG, miR-147a expression was also obviously

downregulated, but SBU pretreatment increased miR-147a expression in damaged cardiomyocytes ($P < 0.01$, Fig. 3F).

LncRNA NEAT1 participates in the protective effect of SBU on HG-induced cardiomyocytes via miR-147a

Based on the above results, the present study investigated its role in HG-induced cardiomyocytes by transfecting miR mimic. The results revealed that in HG cardiomyocytes pretreated with SBU, transfection with oe-LncRNA NEAT1 obviously downregulated the expression of miR-147a ($P < 0.001$, Fig. 4A), whereas transfection with miR mimic reversed this effect. Secondly, compared with the LncRNA NEAT1 overexpression group, miR mimic transfection reduced the rate of apoptosis in damaged cardiomyocytes and the expression of pro-apoptotic genes ($P < 0.05$; Figs. 4B and 4C).

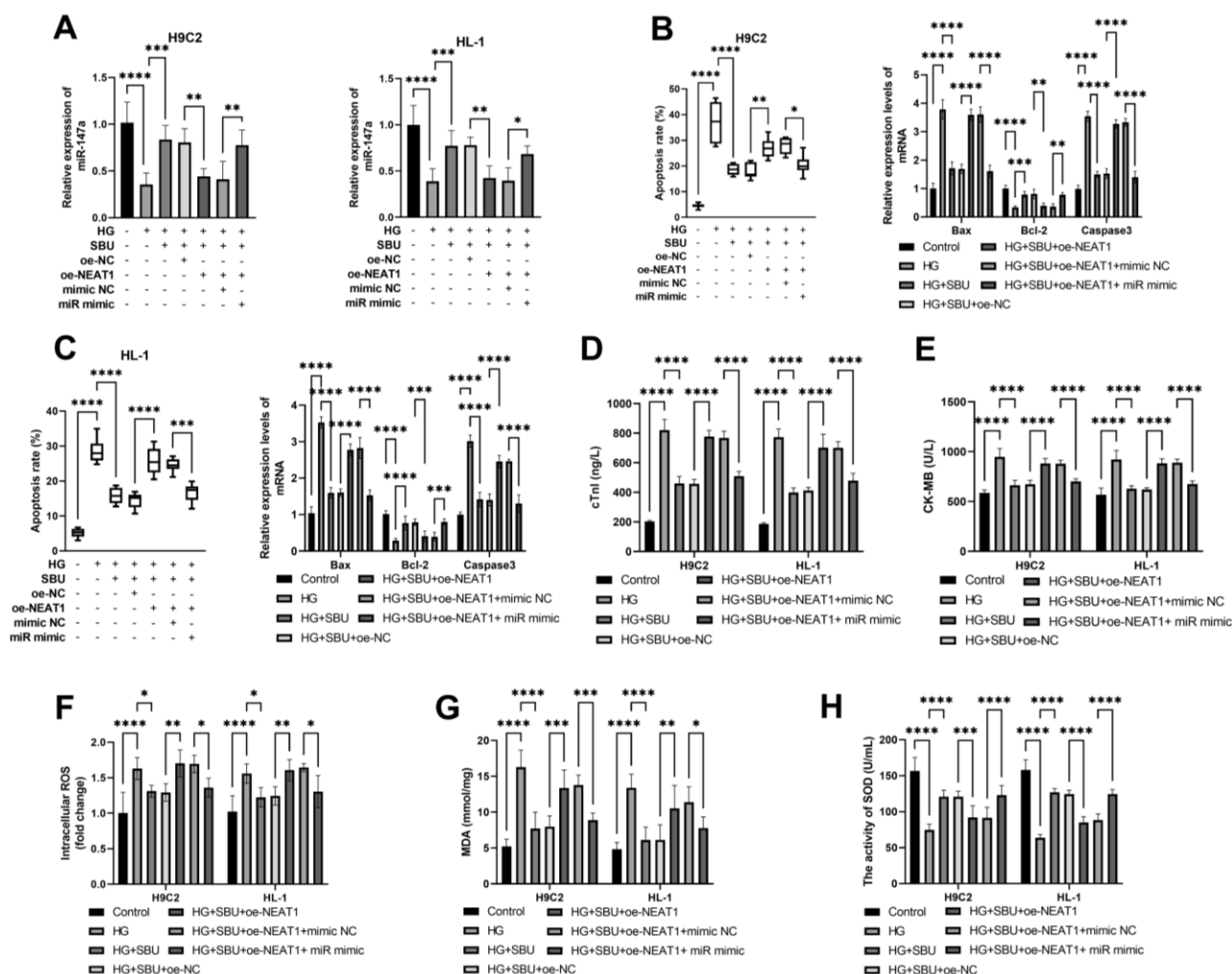


Fig. 4: Effects of miR-147a mimic on cardiac myocyte function and oxidative stress levels following HG and SBU treatment. (A) miR-147a expression levels in cardiac myocytes after miR mimic transfection; (B-C) Effects of miR mimic transfection on apoptosis levels and apoptosis-related gene expression in H9C2; (B) and HL-1; (C) cardiomyocytes; (D-E) Changes in cardiomyocyte injury markers cTnI; (D) and CK-MB; (E) expression after miR mimic transfection; (F-H) Changes in oxidative stress levels in cardiomyocytes after miR-147a overexpression. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Additionally, miR mimic transfection counteracted the upregulation of myocardial injury markers induced by LncRNA NEAT1 overexpression ($P < 0.0001$, Figs. 4D and 4E). Moreover, ROS and MDA expressions in damaged cardiomyocytes were significantly suppressed, while SOD levels markedly increased following miR mimic transfection ($P < 0.05$, Figs. 4F-H). To further elucidate miR-147a's function, the present study supplemented miR-147a inhibition experiments. Results showed that transfection with a miR-147a inhibitor significantly counteracted SBU-induced miR-147a upregulation in cardiomyocytes ($P < 0.0001$, Fig. S5A). Furthermore, after miR-147a inhibition, the proportion of apoptotic cardiomyocytes markedly increased, the expression of myocardial injury markers rose and oxidative stress levels were significantly elevated ($P < 0.05$, Figs.

S5B-S5F). These findings suggest that inhibiting miR-147a completely abolishes the protective effect of SBU on cardiomyocytes.

Prediction and analysis of potential downstream target genes of miR-147a involved in DCM

Integrating results from five databases, the present research identified 82 common genes converging simultaneously (Fig. 5A). These overlapping genes formed a protein interaction network comprising 81 nodes and 59 edges ($P < 0.0001$, Fig. 5B). Among them, target genes involved in multiple events included NR3C1, HSPA8, MDM2, MECP2, NF1, SGK1, etc. Notably, the present research observed significant upregulation of HSPA8, MDM2 and MECP2 in HG-induced cardiomyocytes (Figs. S6A and S6B).

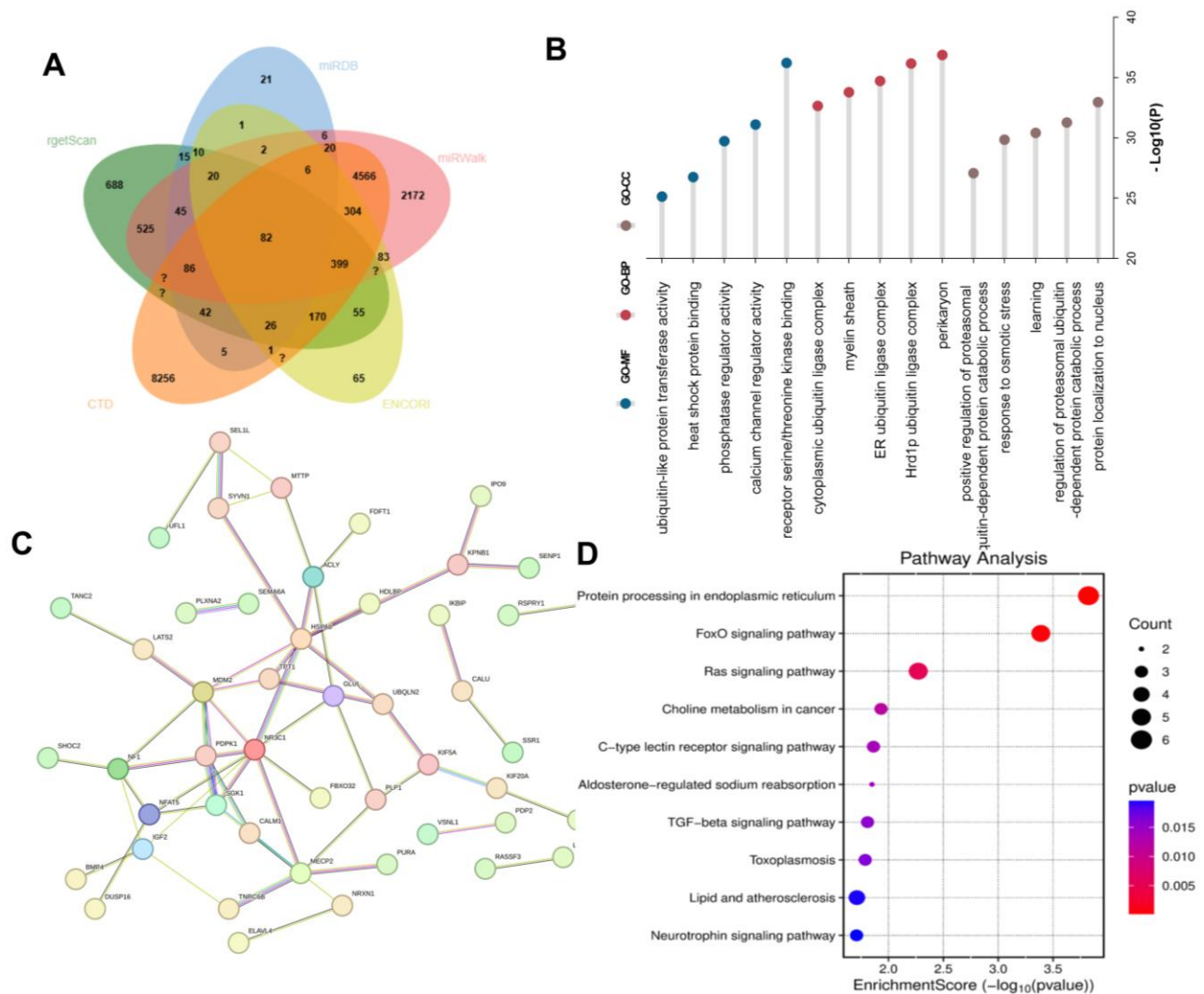


Fig. 5: Prediction and enrichment analysis of miR-147a downstream target genes. (A) TargetScan, miRDB, miRWalk, ENCORI and CTD databases for miR-147a; (B) Construction of the protein network for overlapping genes; (C-D) GO; (C) and KEGG (D) enrichment analysis of overlapping genes.

Furthermore, GO analysis (Fig. 5C) revealed that overlapping genes predominantly functioned in structures such as the Hrd1p ubiquitin ligase complex, myelin sheath and microtubules, exhibiting activities including receptor serine/threonine kinase binding, calcium channel regulation and heat shock protein activity. These may be involved in processes such as protein localization to the nucleus, proteasome-mediated ubiquitinylation regulation and transmembrane transport. KEGG enrichment analysis (Fig. 5D) revealed that downstream signaling pathways regulated by the LncRNA NEAT1/miR-147a axis primarily included endoplasmic reticulum protein processing, FOXO signaling and Ras signaling.

DISCUSSION

Structural changes and myocardial fibrosis in DCM

patients often lead to arrhythmia and heart failure (Tuleta and Frangogiannis 2021) and, in severe cases, death (Wong *et al.* 2014). Hyperglycemia can cause insulin resistance (Ng *et al.* 2018) and dyslipidemia (Xu *et al.* 2020), which in turn lead to myocardial cell dysfunction by inducing myocardial fibrosis (Frangogiannis 2019) and inflammatory signaling (Zhang *et al.* 2019). Additionally, as more drugs are developed and explored for treating DCM, an increasing number of studies have reported the significant value of *Hippophae rhamnoides* and its derivatives in DCM treatment (Zhang *et al.* 2025).

This study found that the level of LncRNA NEAT1 was significantly higher, while miR-147a expression was notably lower in DCM patients and HG myocardial cells. SBU may upregulate miR-147a levels by inhibiting LncRNA NEAT1 expression, thereby reducing myocardial cell apoptosis, decreasing the expression of myocardial

injury markers and oxidative stress levels and alleviating DCM-induced damage to myocardial cells.

The regulatory role of ncRNA in cardiovascular diseases is worth discussing. LncRNA NEAT1 is a regulatory gene present in cardiomyocytes and its abnormal expression is often associated with heart diseases. It has been reported that LncRNA NEAT1 is involved in the phenotypic switch of vascular smooth muscle (Ahmed *et al.* 2018). It has been found that LncRNA NEAT1/MALAT1 deficiency leads to atherosclerosis in ApoE^{-/-} mice (Gast *et al.* 2019). Furthermore, it has been discovered that NEAT1 induces myocardial infarction in mice via the miR-22-3p/LTB4R1 signaling pathway (Wang, *et al.* 2021). It has been found that LncRNA NEAT1 is upregulated in the serum of patients with diabetic kidney disease (DKD) and the expression level of this gene is associated with adverse cardiovascular events in patient prognosis (Liu *et al.* 2024). Furthermore, miRNA-147a is an ncRNA that has been found to be abnormally expressed in diabetes-related diseases. Studies have shown abnormal expression of miR-147a in DKD patients (Ji *et al.* 2020) and the miR-147a/SOX6 axis mediating inflammation and oxidative stress in HGMC cells may serve as a therapeutic target for DKD (Xu and Zhan 2022). In the context of cardiovascular diseases, downregulation of miR-147a has been detected in atherosclerotic cell models (Chen *et al.* 2023). Furthermore, miR-147a acts as a regulatory factor in the development of hypertrophic cardiomyopathy (HCM) by interacting with IGFBP3 and JAK2 (Cai *et al.* 2025). This research revealed that LncRNA NEAT1 predominantly localizes in the cytoplasm and exhibits a target relationship with miR-147a. Overexpression of LncRNA NEAT1 promotes cardiomyocyte apoptosis and elevates oxidative stress levels. However, transfection with miR-147a mimics partially suppressed apoptosis in damaged cardiomyocytes while reducing myocardial injury and oxidative stress. Based on prior research (Tay, Rinn and Pandolfi 2014), cytoplasmic LncRNA NEAT1 may participate in regulating downstream mechanisms of DCM through a competitive endogenous RNA (ceRNA) network with its target gene miR-147a. This interaction suggests additional possibilities for co-regulation by multiple modulators and signaling pathways, consistent with the complex regulatory mechanisms observed *in-vivo*.

Although the cardioprotective effects of *Hippophae rhamnoides* and its derivatives have been identified and its derivatives alleviate DCM remain largely unknown. This research found that the LncRNA NEAT1/miR-147a regulatory axis may be one of the mechanisms through which SBU protects damaged cardiomyocytes. However, *Hippophae rhamnoides* and its derivatives have many other protective and therapeutic effects on cardiovascular diseases. Previous reports indicated that the total rhamnose flavonoids in *Hippophae rhamnoides* are involved in cAMP, NF- κ B and PI3K/AKT signaling pathways in rat

cardiomyocytes (Liu, Li and Shi 1988; Xiao *et al.* 2003; Gao *et al.* 2017; Al Malki *et al.* 2018; Al-Malki and Abdel-Raheem 2019). It has been found that alkaloids in *Hippophae rhamnoides* can counteract the toxicity of doxorubicin on H9C2 cells (Zhou *et al.* 2021). It has been reported that *Hippophae rhamnoides* could alleviate isoproterenol-induced cardiac dysfunction in rats (Malik *et al.* 2011). Additionally, other studies found that seabuckthorn can improve cardiovascular function in hypertensive stroke rats (Koyama, Taka and Togashi 2009) and reduce nicotine-induced oxidative stress in rat hearts (Gumustekin *et al.* 2010). This study further revealed that miR-147a downstream target genes HSPA8, MDM2 and MECP2 were upregulated in HG-induced cardiomyocytes. This finding aligns with previous research trends. Prior studies indicated that the HSPA8 gene was predicted as a core gene in DCM by weighted gene co-expression network analysis (WGCNA) (Liu *et al.* 2025). In DCM tissues, elevated p53 and its regulatory factor MDM2 participate in apoptosis via polyubiquitin proteins (Birks *et al.* 2008). Meanwhile, MeCP2 serves as a key regulator of DCM and cardiac fibroblast proliferation (Tao *et al.* 2019). Additionally, this study identified endoplasmic reticulum protein processing, FOXO and Ras as potential signaling pathways involving the LncRNA NEAT1/miR-147a axis in DCM. Studies indicate that endoplasmic reticulum stress serves as an initial protective mechanism against protein folding disorders, but its prolonged or excessive activation promotes DCM progression (Chen *et al.* 2024). For instance, it has been reported that endoplasmic reticulum stress plays a crucial role in myocardial cell apoptosis in DCM mice (Tian *et al.* 2021). It has been found that overexpression of LncRNA AC061961.2 suppresses endoplasmic reticulum stress-induced apoptosis in DCM rat cardiomyocytes by activating the Wnt/ β pathway (Qiu *et al.* 2021). Furthermore, the FOXO signaling pathway also plays a crucial role in insulin signaling. Researchers have reported that GYY4137 protects against type 2 diabetes-associated myocardial autophagy by inhibiting the FOXO1 signaling pathway (Han *et al.* 2022; Zhu *et al.* 2025). Furthermore, Ras system activation exhibits multifaceted roles in myocardial cell function (Li and Qu 2025). Studies have demonstrated that the Ras signaling pathway is upregulated in cardiovascular diseases and its sustained activation contributes to structural and functional alterations in the hearts of DCM patients. Studies have also reported that overexpression of the RAS pathway's renin receptor (PRR) exacerbates inflammatory responses and reactive oxygen species production in cardiomyocytes (Zamora and Villena 2019; Dong *et al.* 2019). Therefore, subsequent studies will employ techniques such as single-cell sequencing to more precisely map the relationships between target genes and signaling pathways. This will further validate the primary molecular mechanisms through which the LncRNA NEAT1/miR-147a regulatory axis contributes to DCM.

Currently, certain limitations in this research warrant thorough discussion. First, this clinical cohort is a single-center study, which may restrict the generalizability of the findings. In subsequent research, multicenter, large-sample studies were conducted and external validation was performed to enhance the extrapolation of experimental results. Second, there is a lack of analysis on the specific components of SBU. Due to resource constraints, SBU as a flavonoid compound via UV spectrophotometry were only preliminarily identified. Moving forward, the extraction process was optimized. Advanced analytical techniques such as high-performance liquid chromatography (HPLC), mass spectrometry (MS) and nuclear magnetic resonance (NMR) were employed to comprehensively characterize the chemical properties of SBU (Gao *et al.* 2021). Additionally, to minimize batch effects, subsequent analyses incorporated one-way analysis of variance (ANOVA) and orthogonal experiments to comprehensively validate the impact of experimental conditions on SBU extraction. Third, although cell lines (H9C2, HL-1) have accumulated extensive experience in cardiomyocyte research and are stable in origin (Wang *et al.* 2023, Dong *et al.* 2021), however, they may not fully replicate the unique physiological and pathological characteristics of adult ventricular cardiomyocytes (including mature myocardial contractile function, complex ion channel regulatory mechanisms and specific metabolic patterns).

Therefore, in future studies, it has been planned to introduce primary adult ventricular cardiomyocytes or differentiate pluripotent stem cells into mature ventricular cardiomyocytes for validation. Second, while the study demonstrated SBU's protective effect on cardiomyocytes via the LncRNA NEAT1/miR-147a axis, other researches indicated SBU also exerts protection through the PI3K/Akt pathway (Gao *et al.* 2017), which may confound results. Future studies may incorporate dual pathway intervention experiments (e.g., concurrent inhibition of the RNA axis and PI3K/Akt pathway) to assess SBU's cardioprotective effects. Additionally, gene editing techniques (e.g., CRISPR-Cas9 knockout) may be employed to further confirm the necessity of the RNA axis. Last but not least: considering practical constraints, short-term hyperglycemic exposure and chronic metabolic stress in DCM may differ in pathological changes, metabolic adaptation mechanisms and signaling pathway activation. However, in preliminary experiments using glucose at varying concentrations, this research observed irreversible toxicity even at the optimal treatment concentration following prolonged glucose exposure. Therefore, subsequent studies established a DCM mouse model using streptozotocin (STZ) to enable long-term (1- or 6-month) observation of mouse behavior and myocardial status. This research will further examine the functional effects of SBU and LncRNA NEAT1 on myocardial cells in DCM mice at the *in-vivo* level.

CONCLUSION

In summary, the study found that LncRNA NEAT1 is abnormally upregulated in patients with DCM, while SBU pretreatment can significantly inhibit its expression in HG cardiomyocytes. SBU may promote the expression of miR-147a by reducing NEAT1 expression, thereby reducing the apoptosis of damaged cardiomyocytes and lowering the expression of markers of myocardial injury and oxidative stress, thereby alleviating the progression of DCM.

Acknowledgments

None.

Authors' contributions

All authors designed this study. WBL, CWK, QYX, DZ, YYJ and CYG: Conducted the experiment and analyzed the data; WBL and CWK: Wrote the manuscript. CYG: Revised the manuscript. All authors reviewed and approved for publication.

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Data availability statement

All data generated or analysed during this study are included in this published article

Ethical approval

The study protocol was approved by the Ethics Committee of PLA Navy Characteristic Medical Center (Date:2021.09.2/No.2021011). All procedures performed in studies involving human participants were in accordance with the 1964 Declaration of Helsinki and subsequent versions. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved. This study was performed in adherence with the STROBE guidelines. See supplementary file for the STROBE checklist.

Conflict of interest

The authors declare no conflict of interest.

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