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Downregulated long noncoding RNA LUCAT1 inhibited proliferation and promoted apoptosis of cardiomyocyte *via* miR-612/HOXA13 pathway in chronic heart failure

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Abstract. – OBJECTIVE: Long non-coding RNAs (lncRNAs) have been reported to play important roles in numerous kinds of cardiovascular disease, including chronic heart failure (CHF). In this study, we mainly focused on investigating the potential roles of lncRNA LUCAT1 patients with CHF.

PATIENTS AND METHODS: RT-PCR was used to detect the expressions of LUCAT1 and miR-612 in serum samples of CHF patients (n=60) and healthy volunteers. Relationships between the expressions of LUCAT1 and miR-612, LUCAT1 and overall survival (OS) were analyzed using the Kaplan-Meier method. Si-LUCAT1 and miR-612 mimic were constructed and respectively transfected into AC16 cells to explore the functions of LUCAT1 and miR-612. Cell proliferation abilities were detected by CCK-8 assay AC16 cells. Cell apoptotic rates were measured by flow cytometry (FACS) analysis. Western blot (WB) was performed to detect the protein levels of HOXA13, Bcl-2, Bax, Bad and Cleaved Caspase3. In addition, luciferase gene reporter assay was used to prove the relationships between LUCAT1 and miR-612, miR-612 and HOXA13.

RESULTS: Firstly, we found that LUCAT1 was decreased for 1.7 folds in CHF patients, which was correlated with poor prognosis patients. LUCAT1 repression inhibited cell proliferation and promoted cell apoptosis in human cardiomyocyte cell line AC16 cells. Furthermore, we found that miR-612 was increased for 2.0 folds in CHF patients, which was negatively interacted with LUCAT1 expression. Luciferase gene reporter assay demonstrated that LUCAT1 could directly bind with miR-612 in AC16 cells. Moreover, miR-612 overexpression also inhibited cell proliferation and promoted cell apoptosis in AC16 cells. Luciferase reporter assay indicated that miR-612 could directly target at HOXA13 in AC16 cells, which was associated with cell proliferation and apoptosis. Finally, miR-612 inhibitor was transfected into AC16 cells with si-LUCAT1. The results showed that the inhibited cell proliferation and promoted cell apoptosis were reversed, which confirmed that LUCAT1 repression inhibited cell proliferation and promoted apoptosis via miR-612/HOXA13 axis in CHF patients.

CONCLUSIONS: According to the above results, our study revealed that LUCAT1 was decreased in CHF patients, which was correlated with poor prognosis of CHF patients. Furthermore, the downregulation of LUCAT1 inhibited cell proliferation and promoted cell apoptosis via targeting miR-612/HOXA13 axis. Our results elucidated a potential mechanism underlying cardiomyocyte apoptosis, which might be used as a promising prognostic marker and a potential target for CHF patients.

Key Words:
LncRNA- LUCAT1, MiR-612, Cardiomyocyte apoptosis, Chronic heart failure.

Introduction

Chronic heart failure (CHF) is a kind of heart disease, which is the leading cause of hospital admission in the world[1,2]. It has been reported that more than five million Americans are suffering CHF, which is responsible for 1 out of 9 deaths[1,3,4]. With the growth of age, the incidence of CHF is increasing all over the world[5,6]. It has been reported that cardiomyocyte apoptosis plays a critical role in the development progression of CHF[7-9]. Therefore, it is important to make a better understanding of cardiomyocyte apoptosis in CHF and develop novel therapeutic targets to improve the efficacy of CHF patients.

Long non-coding RNAs (lncRNAs) are kinds of RNAs longer than 200 nucleotides (nt). Although these lncRNAs can't be translated into proteins,

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they also play some roles in different biological processes of many different diseases[10-15]. Studies have found that lncRNAs are involved in the development of CHF[16-18]. LncRNA GASL1 was found to be downregulated in CHF, which participated in regulating cardiomyocyte apoptosis[16]. Long et al[17] revealed that FTX could promote cardiomyocyte apoptosis via interacting with miR-29b-1-5p and Bcl2l2. Yu et al[18] found that serum UCA1 was increased in CHF patients, which was associated with the poor outcomes of CHF. Lung cancer associated transcript 1 (LUCAT1) was located on chromosome 5, which was first found to regulate cell proliferation in non-small lung cancer[19]. Further, it had been found to be involved in couples of cancers[20-24], such as ovarian cancer[22], esophageal squamous cell carcinoma[24], glioma[20], breast cancer[23], cervical cancer[21], etc. Previous studies have revealed the functions of LUCAT1 in cancers; however, whether it can be involved in the development of CHF remains unknown.

MicroRNAs (MiRNAs) are classes of RNAs about 22 nucleotides (nt), which have been proved to be involved in biological functions of various diseases[25-29]. For the first time, Salmena et al[30] and Tay et al[31] presented that lncRNAs could interact with miRNAs as letters of a new language and this process was called "competing endogenous RNA " (ceRNA), which plays important roles in pathological conditions. MiR-612 had been proved to play important roles in tumorigenesis and progression of various cancers[32-34]. Liu et al[34] found that miR-612 suppressed stem cell-like property of hepatocellular carcinoma cells (HCC) by regulating Sp1/Nanog axis. Cai et al[32] demonstrated that miR-612 was involved in regulating the malignancy of glioblastoma; Sheng et al[33] revealed that miR-612 negatively regulated tumor growth and metastasis by targeting AKT2 in colorectal cancer. However, the functions of miR-612 in CHF are still unclear.

In this study, we aimed at investigating the different expressions and functions of LUCAT1 in CHF. We observed significant LUCAT1 repression in CHF patients. Therefore, we intended to investigate the roles and underlying mechanism of LUCAT1 in CHF patients.

Patients and Methods

Patient Samples

60 serum samples from CHF patients and 60 serum samples from healthy volunteers were collected in our hospital from June 2014 to June 2016. The patients were excluded if they were suffered from other clinical disorders and received any therapies within 90 days. 5 ml fasting blood was collected from each CHF patients and healthy volunteers, the blood samples were centrifuged at 1200 g for 15 mins and all samples were frozen at -80°C until use. This study was approved by the Ethics Committee of our hospital, in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all CHF patients and healthy volunteers.

Cell Culture

Human cardiomyocyte cell line AC16 (EMD Millipore, Billerica, MA, USA) was used and AC16 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) as well as 12% fetal bovine serum (FBS; Gibco, Invitrogen, Carlsbad, CA, USA), 1% penicillin and 1% streptomycin. Then cells were cultured in the incubator at 37°C and 5% CO_2.

Construction of siRNA and Cell Transfection

For small interfering RNAs (siRNAs) analysis, LUCAT1 siRNA (si-LUCAT1) and negative control siRNA (si-NC) were synthesized and provided by Invitrogen, resulting with LUCAT1 inhibition. AC16 cells were pre-incubated to about 50% confluence on a six-well plate and then transfected by incubation with si-LUCAT1 or si-NC with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) as the manufacturer's protocols. AC16 cells were seeded in 6-well plates until growing up to about 50%; the transfection reagent Lipofectamine 2000, serum-free DMEM and miR-612 mimic or miR-612 inhibitor were mixed and incubated at room temperature for 30 mins, and then added into the prepared 6-well plates with complete medium with 10% FBS. After that, cells were harvested for further analysis.

CCK8 Assay

Cell proliferation abilities were detected by Cell Counting Kit 8 (CCK8, Dojindo Molecular Technology, Kumamoto, Japan) according to its protocols. AC16 cells (2×10^3/well) were respectively seeded in 96-well plates and transfected with indicated si-LUCAT1 or miR-612 mimic or miR-612 inhibitor at 37°C with 5% CO_2. Three replicate wells were performed for each group. For each well, 10 µl CCK8 was added at 0 d, 1 d, 3 d and 7 d and incubated in darkness for 2 h at room temperature. The absorbance (OD) value was measured at 450 nm with a microplate reader

and cell proliferation curves were plotted using each absorbance value.

RNA Extraction and Quantitative Real-Time PCR

Total RNAs of serum samples were extracted by using TRIzol LS (Invitrogen, Carlsbad, CA, USA) and total RNAs of AC16 cells were extracted by using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the protocols. PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) was used to perform reverse transcription, and PCR primers were synthesized by Gene Pharma (ShangHai Gene Pharma, ShangHai, China), and listed in Table I. mRNA expressions were detected by SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan). The relative mRNA expressions were normalized to β-actin or U6, which was calculated using $2^{-\Delta\Delta CT}$ method.

Protein Extraction and Western Blot

Total protein was extracted from treated AC16 cells using ReadyPrep™ Protein Extraction Kit (Bio-Rad, Hercules, CA, USA) and protein concentration was measured by BCA protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the protocols. Firstly, 40 ug proteins were mixed and added to 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), then the separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes. Secondly, these PVDF membranes were blocked at room temperature by 5% non-fat milk for 1 h. Thirdly, these membranes were incubated with primary antibodies overnight at 4°C, all primary antibodies were purchased from Abcam (Abcam, Cambridge, MA, USA), including anti-Bcl-2 (1:1000, 26 kDa), anti-Bax (1:1000, 21 kDa), anti-Bad (1:2000, 23 kDa), anti-Cleaved Caspase-3 (1:500, 17 kDa), anti-HOXA13 (1:1000, 40 kDa), β-actin (1:5000, 42 kDa). Subsequently, membranes were incubated with matched secondary antibodies for 1 h. Protein bands were detected by Pierce ECL Western blot substrate (Sigma-Aldrich, St. Louis, MO, USA) with ECL detection system (Sigma-Aldrich).

Flow Cytometric Analysis of the Cell Apoptosis

The treated AC16 cells were washed twice, treated with trypsin, and harvested. Cells of each pellet were stained with FITC-Annexin V (Dojindo) and Propidium iodide (PI), and flow cytometry (FACS) was conducted within 5 mins. Finally, the apoptotic cells were obtained using a FACSCalibur system (BD Biosciences, Franklin Lakes, NJ, USA), and the data were analyzed using the FlowJo software (Tree Star Corp, Ashland, OR, USA).

Luciferase Gene Reporter Assay

The potential binding site of wt-LUCAT1, mutant sequence mut-LUCAT1, wt-HOXA13 and mutant sequence mut-HOXA13 were synthesized and constructed into pmiR-GLO (Promega, Madison, WI, USA). AC16 cells were seeded and cultured in a 48-well plate for 12 h, then transfected with indicated plasmids and miR-612 mimic for 24 h. 200 ng plasmids were mixed with Lipofectamine 2000 and DMEM medium for 30 mins, which were added into the prepared AC16 cells for 24 h. Finally, cells were lysed and the activ-

Table I. Sequences of primers for RT-PCR.

Gene names	Primer sequences
LUCAT1	Forward: 5'- GCTCGGATTGCCTTAGACAG -3'
	Reverse: 5'- GGGTGAGCTTCTTGTGAGGA-3'
miR-612	Forward: 5'- GCTGGGCAGGGCTTCT -3'
	Reverse: 5'- CAGTGCGTGTCGTGGAGT -3'
Bad	Forward:5'- TGAAGGGACTTCCTCGCCCGT-3'
	Reverse: 5'- GGCTTGGTCCCATCGGAAG -3'
Bax	Forward:5'- GCGACTGATGTCCCTGTCTC-3'
	Reverse: 5'- AAAGATGGTCACGGTCTGCC-3'
Bcl-2	Forward:5'- CTCCCACAGACTCTGTAAG-3'
	Reverse: 5'- GCATTACCTGGGGCTGTAATT-3'
Caspase3	Forward:5'-ATTTGGAACCAAAGATCATACA-3'
	Reverse: 5'- CTGAGGTTTGCTGCATCGAC-3'
β-actin	Forward: 5'- CTGGCCGGGACCTGACT -3'
	Forward: 5'- TCCTTAATGTCACGCACG-3'
U6	Forward: 5'-GCTTCGGCAGCACATATACTA-3'
	Forward: 5'-CGCTTCACGAATTTGCGTGTC-3'

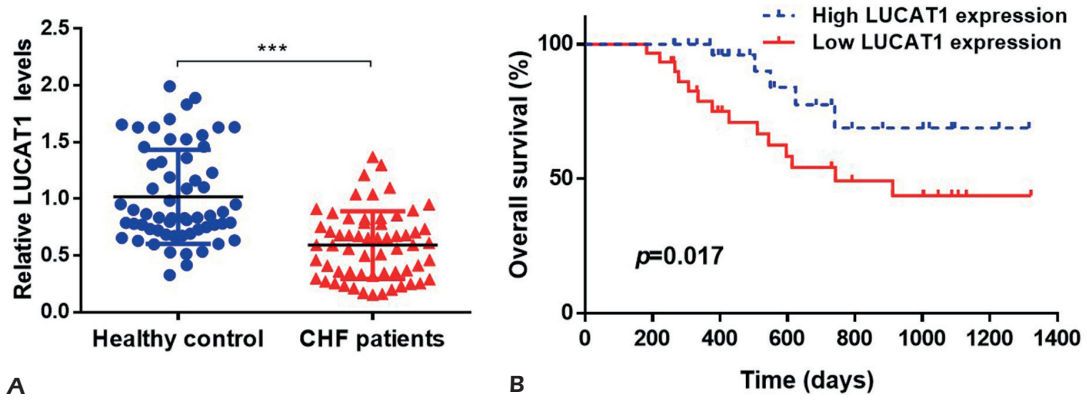

Figure 1. LUCAT1 was downregulated in CHF patients than in healthy controls. **(A)** The mRNA levels of LUCAT1 in serum samples of CHF patients (n=60) and healthy volunteer (n=60) were detected by RT-PCR. **(B)** Kaplan-Meier survival analysis demonstrated that overall survival was lower in patients with LUCAT1 low expression group (n=30) compared with those with LUCAT1 high expression group (n=30). $^{*}p<0.05$; $^{***}p<0.001$.

ities of firefly luciferase and Renilla luciferase were measured by using a Promega luciferase assay (Promega, Madison, WI, USA) according to the protocol. Data were normalized against the activity of the Renilla luciferase gene.

Statistical Analysis

All data were expressed as the mean±SD and analyzed by SPSS 19.0 (SPSS Inc., Armonk, NY, USA) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). The significance between groups was analyzed by Student's *t*-test and multiple comparison between the groups was performed by SNK method after ANOVA analysis. *p*-value <0.05 was considered statistically significant.

Results

LUCAT1 was Downregulated in CHF Patients than in Healthy Controls

To explore the roles and functions of LUCAT1 in patients with CHF, we first used qRT-PCR to detect the expressions of LUCAT1 in serum samples from 60 patients and 60 healthy volunteers. Results showed that LUCAT1 was significantly decreased for about 1.7 folds in patients with CHD (Figure 1A) ($p<0.05$), compared with healthy control. Furthermore, based on serum levels of LUCAT1, 60 CHF patients were divided into LUCAT1 high expression group (n=30) and LUCAT1 low expression group (n=30). Kaplan-Meier survival analysis was performed to plot and compare the survival curves in CHF patients. Results revealed that patients with low serum levels of LUCAT1 had a significantly lower overall survival rate than patients with high serum levels of LUCAT1 (Figure 1B) ($p<0.05$). These data suggested that LUCAT1 was decreased in CHF patients, which was correlated with poor future prognosis of CHF patients. However, the roles and detailed mechanisms of LUCAT1 in CHF remained unknown.

Downregulation of LUCAT1 Inhibited Cell Proliferation and Promoted Apoptosis in AC16 Human Cardiomyocyte Cell Line

To further explore the functions of LUCAT1 in CHF, the si-LUCAT1 was constructed, resulting in LUCAT1 downregulation. After si-LUCAT1 transfection to AC16 cells, the LUCAT1 level was significantly reduced (Figure 2A) ($p<0.01$). Furthermore, the CCK8 assay revealed that LUCAT1 downregulation significantly inhibited cell proliferation after 3 d and 7 d, compared with si-control group (Figure 2B) ($p<0.01$). Moreover, flow cytometry (FACS) results indicated that LUCAT1 silence significantly increased the apoptotic rate of AC16 cells (Figure 2C) ($p<0.01$). Additionally, the mRNA and protein levels of Bcl-2 were decreased, while levels of Bax, Bad and cleaved caspase-3 were significantly increased in si-LUCAT1 group (Figure 2D-F) ($p<0.05$), compared to si-control. Collectively, these results indicated that LUCAT1 repression inhibited cell proliferation and promoted apoptosis in AC16 cells.

LUCAT1 Directly Targeted at miR-612 in AC16 Cells

To explore the underlying mechanisms of LUCAT1 that regulated cell proliferation and apoptosis

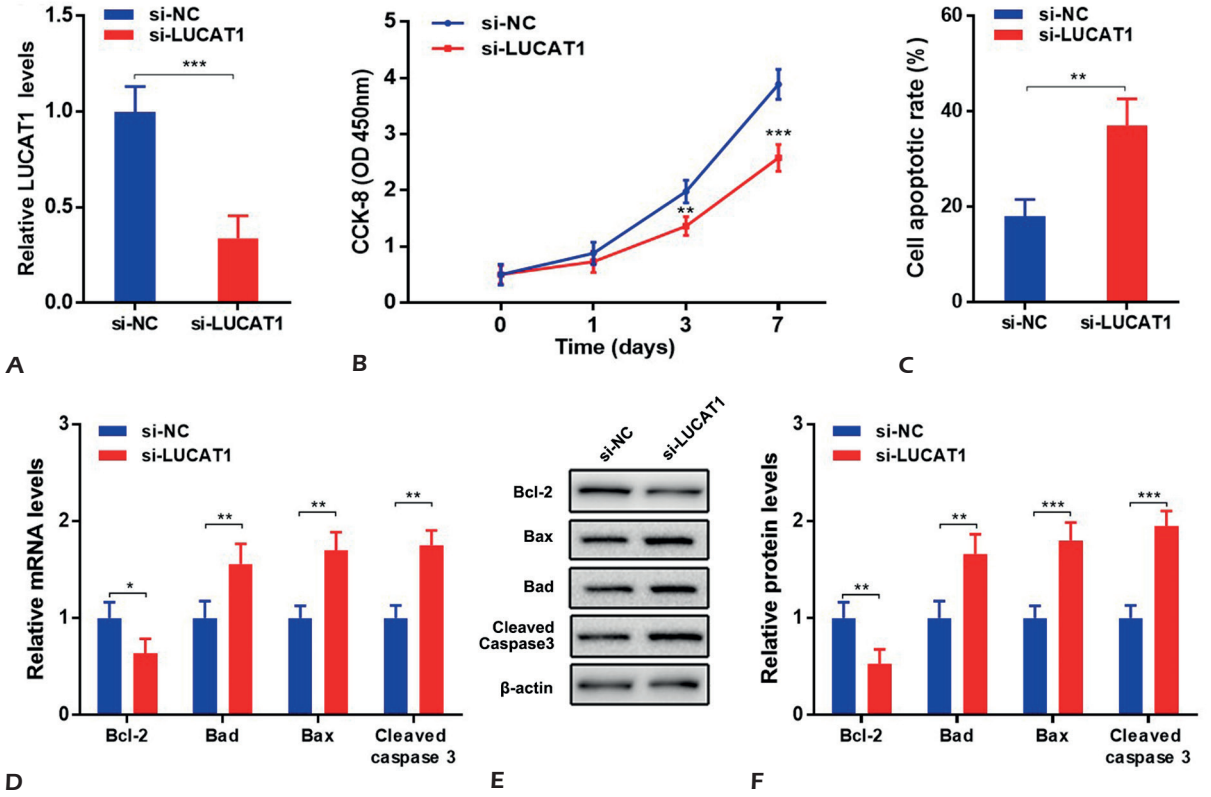

Figure 2. Downregulation of LUCAT1 inhibited cell proliferation and promoted apoptosis in AC16 human cardiomyocyte cell line. **A,** The LUCAT1 expression was detected by RT-PCR after si-LUCAT1 or si-control transfection into AC16 cells. **B,** The proliferation abilities of AC16 cells were measured by CCK8 assay. **C,** The apoptotic rates of AC16 cells were measured by FACS. **D-F,** The mRNA and protein levels of apoptotic and anti-apoptotic genes were detected by RT-PCR and WB (magnifications x 1.5). Data are shown as mean ± SD based on at least three independent experiments, *p<0.05; **p<0.01, ***p<0.001.

in human cardiomyocyte, bioinformatics analysis was performed and miR-612 was identified as a potential targeting miRNA by using starBase v2.0 database. Then expressions of miR-612 in CHF patients and healthy control were detected by RT-PCR. Results showed that miR-612 was significantly increased for about 2.1 folds in CHF patients (n=60) (Figure 3A) (p<0.001). Furthermore, miR-612 was negatively correlated with LUCAT1 in CHF patients (Figure 3B) (p<0.01), while no correlation had been found in healthy control (Figure 3C) (p>0.05). Moreover, miR-612 expressions in AC16 cells infected with si-LUCAT1 were significantly increased following with the repression of LUCAT1 (Figure 3D) (p<0.01). These results indicated that LUCAT1 negatively interacted with miR-612, which was predicted to be a target of LUCAT1. To confirm whether LUCAT1 could directly bind with miR-612, WT-LUCAT1 and MUT-LUCAT1 sequences were constructed to explore further the association between LUCAT1 and miR-612 in AC16 cells and the luciferase gene reporter assay was performed (Figure 3E). Results showed that the relative luciferase activity in AC16 cells co-transfected with WT-LUCAT1 and miR-612 mimic was significantly repressed compared with that in cells transfected with miR-NC. However, the relative luciferase activity was reversed in cells co-transfected with MUT-PVT1 and miR-612 mimic (Figure 3F) (p<0.01). These findings indicated that LUCAT1 could function as a ceRNA to competitively bind with miR-612 in AC16 cells.

MiR-612 Inhibited Cell Proliferation and Promoted Apoptosis in AC16 Cells

To further investigate the roles of miR-612 in the regulation of cell proliferation and apoptosis in AC16 cells, miR-612 mimic or miR-NC was respectively transfected into AC16 cells. Results showed that miR-612 was increased after miR-612 mimic transfection into AC16 cells (Figure 4A) (p<0.01), and the cell proliferation ability

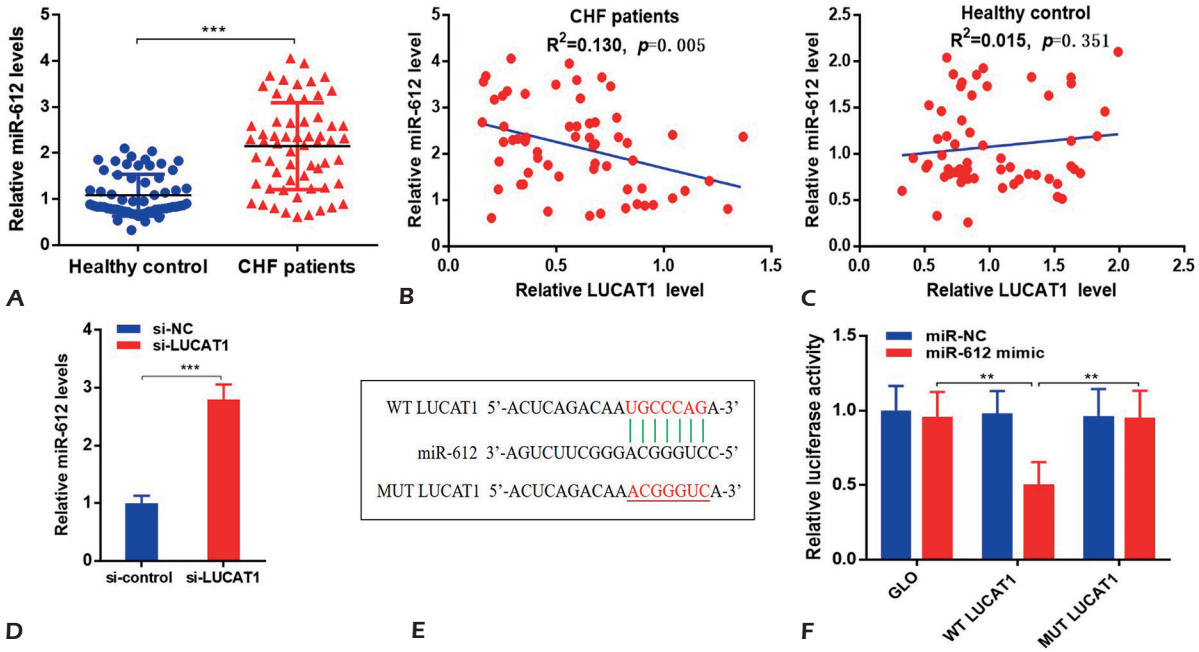

Figure 3. LUCAT1 directly targeted at miR-612 in AC16 cells. **A,** The mRNA expressions of miR-612 were detected by RT-PCR in CHF patients (n=60) and healthy volunteers (n=60). **B-C**, The relationship between LUCAT1 and miR-612 was analyzed by correlation analysis in CHF patients and healthy volunteers. **D,** The levels of miR-612 were detected by RT-PCR in AC16 cells transfected with si-LUCAT1 and si-control. **E,** Potential binding sites between LUCAT1 and miR-612 were predicted by starBase v2.0 database. **F,** The luciferase reporter assay was performed to determine the binding site. Data are shown as mean ± SD based on at least three independent experiments, **p<0.01, ***p<0.001.

was significantly repressed (Figure 4B) (p>0.01). Furthermore, FACS results revealed that miR-612 overexpression significantly increased the apoptotic rate of AC16 cells (Figure 4C) (p<0.01). In addition, the mRNA and protein levels of Bcl-2 were decreased, while the levels of Bax, Bad and cleaved caspase-3 were significantly increased in miR-612 mimic (Figure 4D-F) (p<0.05), compared to the control. Collectively, these results demonstrated that miR-612 overexpression inhibited cell proliferation and promoted apoptosis in AC16 cells. As we know, miRNAs could regulate biological functions in many diseases through targeting at the 3′-UTR of target genes; however, the underlying mechanism of miR-612 in inhibiting cell proliferation and promoting cell apoptosis in CHF still remained unclear.

MiR-612 Could Directly Bind with HOXA13 in AC16 Cells

To further explore how miR-612 inhibited cell proliferation and promoted apoptosis in AC16 cells, TargetScan database was performed to analyze the target genes of miR-612. As a result, HOXA13, which was associated with cell proliferation in some cancers[35-38], was predicted to be a target gene of miR-612. We detected HOXA13 protein expression in AC16 cells transfected with miR-612 mimic or si-LUCAT1. Results showed that the protein level of HOXA13 was repressed in miR-612 mimic group, compared with miR-NC (Figure 5A,B) (p<0.01). Furthermore, protein level of HOXA13 was increased in si-LUCAT1 group, compared with si-control (Figure 5C,D) (p<0.01). These results indicated that LUCAT1 positively interacted with HOXA13 and miR-612 was negatively interacted with HOXA13, which was predicted to be a target of miR-612. To detect whether miR-612 could target at HIG2 in AC16 cells, WT-HOXA13 and MUT-HOXA13 sequences were constructed into GLO vectors and the luciferase gene reporter assay was performed (Figure 5E). Luciferase gene reporter assay revealed that miR-612 overexpression could repress the luciferase activity of WT-HOXA13 but not in MUT-HOXA13 reporter in AC16 cells (Figure 5F) (p<0.01). Above all, these results suggested that miR-612 directly binds with HOXA13 in AC16 cells. Collectively, we assumed that LUCAT1 could directly bind with miR-612, targeting at repressing HOXA13 expression, thereby promoting cell proliferation and cell apoptosis in CHF patients.

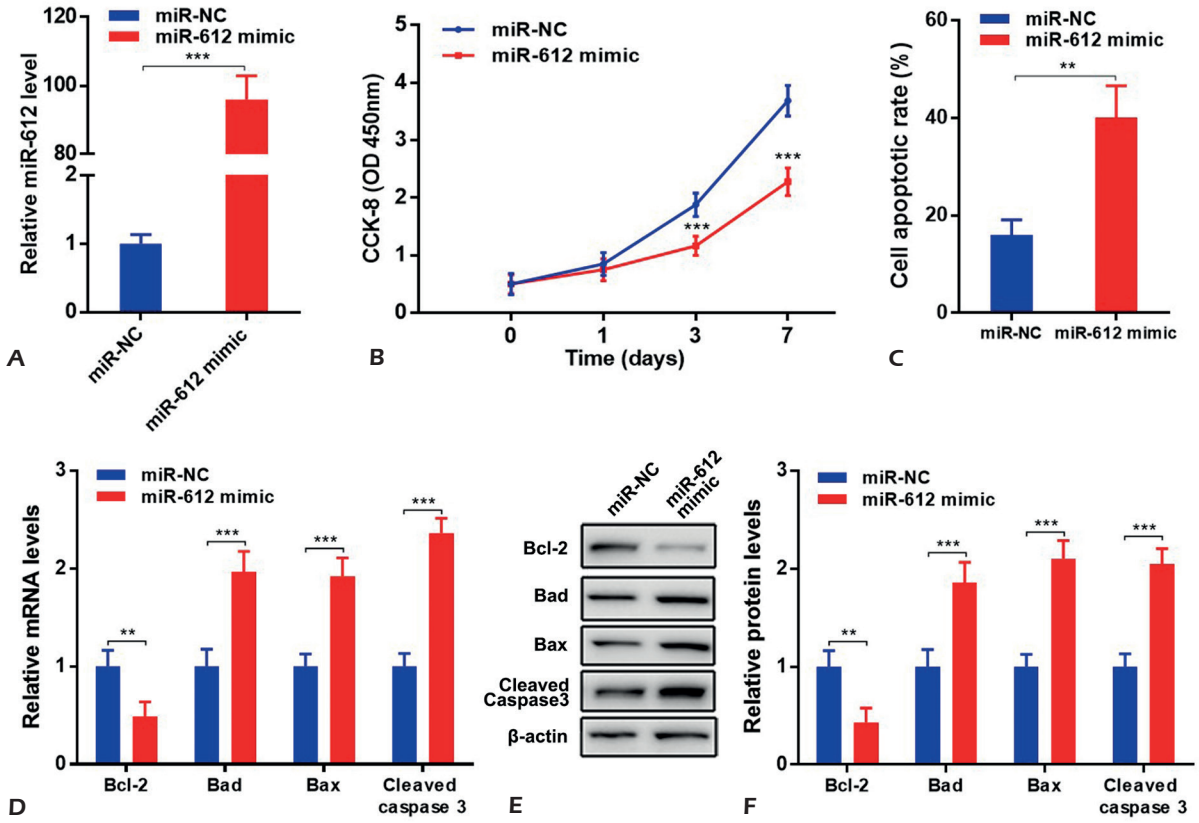

Figure 4. MiR-612 inhibited cell proliferation and promoted apoptosis in AC16 cells. **A,** The miR-612 expression was detected by RT-PCR after miR-612 mimic or miR-NC transfection into AC16 cells. **B,** The proliferation abilities of AC16 cells were measured by CCK8 assay. **C,** The apoptotic rates of AC16 cells were measured by FACS. **D-F,** The mRNA and protein levels of apoptotic and anti-apoptotic genes were detected by RT-PCR and WB (magnifications x 1.5). Data are shown as mean ± SD based on at least three independent experiments, **p<0.01, ***p<0.001.

LUCAT1 promoted cell proliferation and inhibited apoptosis via miR-612/HOXA13 axis in CHF

To confirm the assumption, miR-612 inhibitor or miR-NC was respectively added into AC16 cells with si-LUCAT1, and the cell proliferation ability and apoptotic rate were evaluated. Results showed that LUCAT1 was decreased and miR-612 was increased in cells with si-LUCAT1, while LUCAT1 was reversed and miR-612 was decreased after miR-612 inhibitor transfection into AC16 cells (Figure 6A) (p<0.01). Furthermore, CCK8 assays showed that the cell proliferation abilities of AC16 cells with si-LUCAT1 were repressed, while they were reversed after adding miR-612 inhibitor (Figure 6B) (p<0.01). Moreover, FACS results revealed that the cell apoptotic rate was increased in AC16 cells with si-LUCAT1, while it was reversed after adding miR-612 inhibitor (Figure 6C) (p<0.01). In addition, WB results showed that Bcl-2 protein level was decreased; however, the levels of HOXA13, Bax, Bad and cleaved caspase-3 were increased in si-LUCAT1 group, while they were all reversed after miR-612 inhibitor transfection (Figure 6D,E) (p<0.01). Collectively, these data revealed that LUCAT1 promoted cell proliferation and inhibited apoptosis via miR-612/HOXA13 axis in CHF.

Discussion

Abnormally expressions of lncRNAs have been demonstrated to be involved in different biological processes of many different diseases[10-15] and increasing evidence showed that lncRNAs are involved in the development of CHF[16-18]. LUCAT1 was found to regulate tumor progression and development in couples of cancers[20-24]. However, the roles of LUCAT1 in CHF remained unclear. In

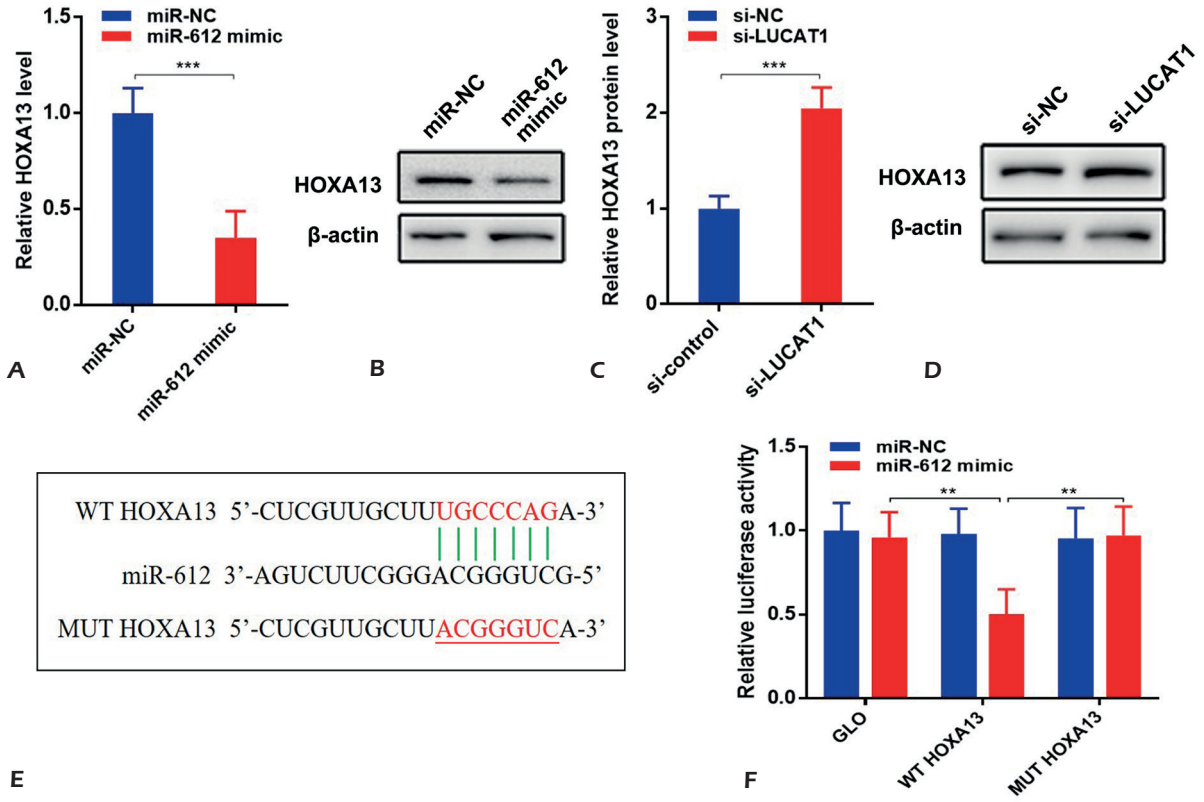

Figure 5. MiR-612 could directly bind with HOXA13 in AC16 cells. **A-B** The levels of HOXA13 were detected by WB in AC16 cells transfected with miR-612 mimic or miR-NC. **C-D** The levels of HOXA13 were detected by WB in AC16 cells transfected with si-LUCAT1 or si-control. **E,** Potential binding sites between miR-612 and HOXA13 were predicted by TargetScan starBase. **F,** The luciferase reporter assay was performed to determine the binding site. Data are shown as mean ± SD based on at least three independent experiments, $^{**}p<0.01$, $^{***}p<0.001$.

this study, we wanted to investigate the functions of LUCAT1 in CHF and we intended to explore the detailed mechanism.

We firstly found that LUCAT1 was decreased in serum samples of CHF patients, compared to the healthy control, which was associated with poor overall survival of CHF patients. To explore the functions of LUCAT1 in CHF, si-LUCAT1 was constructed and transfected into AC16 cells. Results revealed that the downregulation of LUCAT1 inhibited cell proliferation as well as promoted cell apoptosis, compared with si-control group, indicating that LUCAT1 was associated with cardiomyocyte apoptosis in CHF. To further investigate the mechanism of LUCAT1 in CHF, starBase v2.0 database was performed and miR-612 was predicted to be a potential target of LUCAT1. Then, we detected the expressions of miR-612 in serum samples. The results showed that miR-612 was significantly increased in CHF patients, which was negatively correlated with LUCAT1 in CHF patients. Luciferase gene reporter assay demonstrated that LUCAT1 could function as a ceRNA to competitively bind with miR-612 in AC16 cells. However, the underlying mechanism remained unknown.

To further investigate the functions of miR-612 in CHF, miR-612 mimic or miR-NC was respectively transfected into AC16 cells. MiR-612 overexpression inhibited cell proliferation and promoted the apoptotic rate of AC16 cells, as well as upregulating the expressions of apoptotic genes and repressing anti-apoptotic gene expressions. As we know, miRNAs could regulate the biological progress through targeting at the 3′-UTR of target genes; however, the underlying mechanism of miR-612 in inhibiting cell proliferation and promoting cell apoptosis in CHF remained unclear. Then, we used TargetScan database to analyze the target genes of miR-612. As a

result, HOXA13, which was associated with cell proliferation in some cancers[35-38], was predicted to be a target gene of miR-612. We found that the protein levels of HOXA13 were repressed in miR-612 mimic group, while it was increased in si-LUCAT1 group. Luciferase gene reporter assay revealed that miR-612 overexpression could repress the luciferase activity of WT-HOXA13 but not in MUT-HOXA13 reporter in AC16 cells. Collectively, we assumed that LUCAT1 could directly bind with miR-612, targeting at repressing HOXA13 expression, thereby promoting cell proliferation and cell apoptosis in CHF patients.

To further verify our assumption, miR-612 inhibitor or miR-NC was respectively co-transfected into AC16 cells with si-LUCAT1 or si-NC. Results revealed that LUCAT1 was decreased and miR-612 was increased in cells with si-LUCAT1, while LUCAT1 was increased and miR-612 was decreased after transfection with miR-612 inhibitor. Furthermore, the repressed cell proliferation abilities and promoted cell apoptosis in si-LUCAT1 were reversed after miR-612 inhibitor transfection. Moreover, WB results showed that the increased HOXA13 level was reduced after co-transfecting with miR-612 inhibitor. Collectively, these results confirmed that LUCAT1 promoted cell proliferation and inhibited apoptosis via miR-612/HOXA13 axis in CHF patients.

Conclusions

Above all, our study revealed that LUCAT1 was decreased in CHF patients, which was correlated with poor prognosis of CHF patients. Fur-

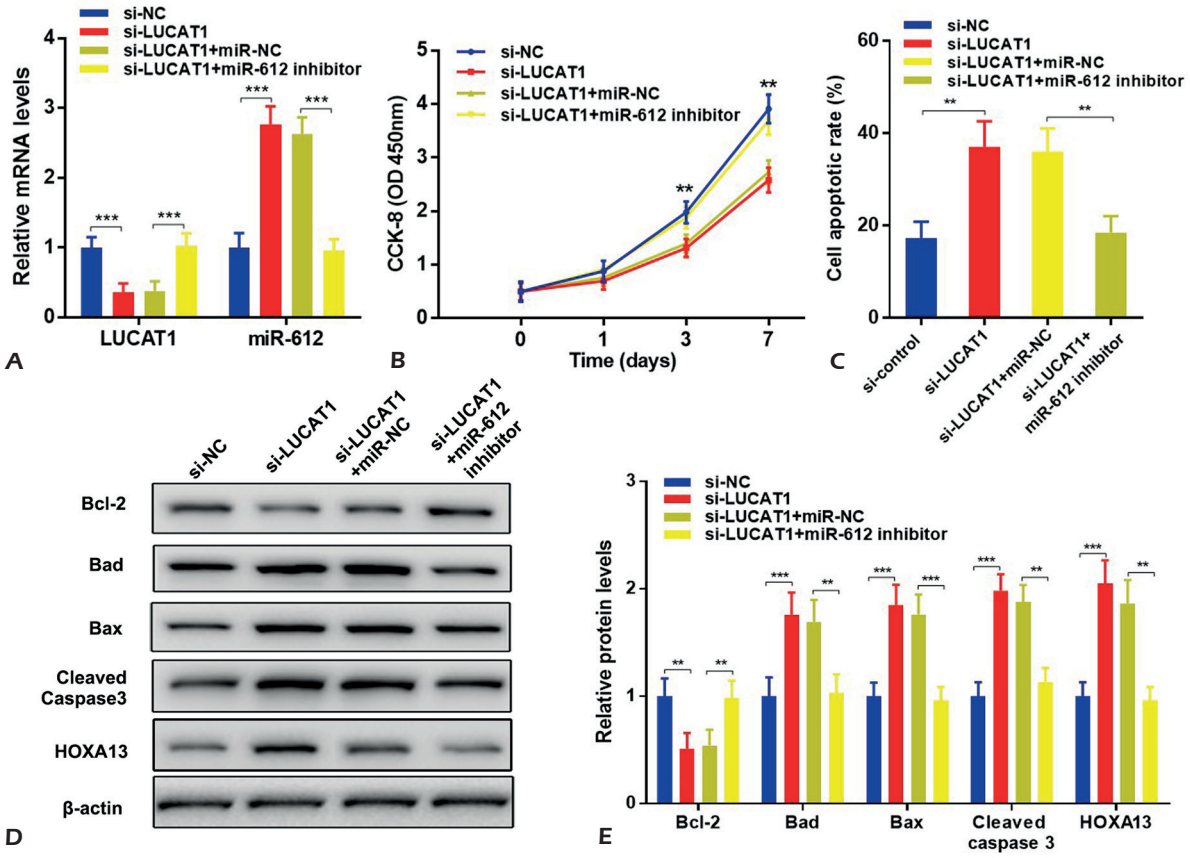

Figure 6. LUCAT1 promoted cell proliferation and inhibited apoptosis via miR-612/HOXA13 axis in CHF. **A,** The expressions LUCAT1 and miR-612 were detected by RT-PCR in AC16 cells with si-LUCAT1 or co-transfected with miR-612 inhibitor or miR-NC. **B,** The proliferation abilities of AC16 cells were measured by CCK8 assay. **C**, The apoptotic rates of AC16 cells were measured by FACS. **D-E**, The protein levels of HOXA13, Bcl-2, Bax, Bad and cleaved caspase-3 were detected by WB (magnifications × 1.5). Data are shown as mean ± SD based on at least three independent experiments, *p<0.05; **p<0.01, ***p<0.001.

thermore, we found that the downregulation of LUCAT1 inhibited cell proliferation and promoted cell apoptosis via targeting miR-612/HOXA13 axis. Our results elucidated a potential mechanism underlying cardiomyocyte apoptosis, which might be used as a promising prognostic marker and a potential target for CHF patients.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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