Circulating Long Noncoding RNA LIPCAR Acts as a Novel Biomarker in Patients with ST-Segment Elevation Myocardial Infarction

Background:
Long noncoding RNAs (lncRNAs) recently have been implicated in the pathological processes of cardiovascular diseases. In this study, LncRNADisease database and PubMed database were used to screen myocardial infarction (MI)-related lncRNAs and to investigate the diagnostic role of lncRNAs in ST-segment elevation myocardial infarction (STEMI).

Material/Methods:
Forty-six patients with STEMI and 40 healthy controls were included in the study. Venous blood samples acquired at different time points and the expression levels of lncRNAs in plasma were measured by qRT-PCR. In addition, other blood samples were collected before and after percutaneous coronary intervention (PCI). Correlation analysis and receiver operating characteristic (ROC) curve were used to assess the diagnosis value of the markers. All included patients were followed up for 12±1 months.

Results:
Nine MI-related lncRNAs were selected from the database. The qRT-PCR results showed that the expression of hypoxia inducible factor 1A antisense RNA 2 (aHIF), member 1 opposite strand/antisense transcript 1 (KCNQ1OT1), and mitochondrial long noncoding RNA uc022bqs.1 (LIPCAR) were significantly increased in patients with STEMI compared to the control patients. The ROC curve showed that LIPCAR (AUC=0.782, 95% CI: 0.707–0.894) had better diagnostic accuracy. Moreover, correlation analysis indicated that LIPCAR were positively correlated with myocardial enzymes and negatively correlated with left ventricular ejection fraction. The level of LIPCAR in STEMI patients after PCI was lower (P<0.05). Multivariate regression analysis indicated that higher levels of LIPCAR were independent predictors of major adverse cardiovascular events in patients with STEMI (HR=5.93; 95% CI, 1.46–9.77; P=0.001).

Conclusions:
Highly expressed LIPCAR in plasma may serve as a warning sign for the diagnosis of STEMI.

MeSH Keywords: Biological Markers • Diagnosis • Myocardial Infarction • RNA, Long Noncoding

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**Background**

Acute myocardial infarction (AMI) is one of the most common types of coronary heart disease (CHD) clinically, characterized with multiple complications, high morbidity and high mortality [1]. The diagnosis of ST-segment elevation myocardial infarction (STEMI) mainly depends on chest pain symptoms, electrocardiogram (ECG) and myocardial enzyme. However, the survey showed that STEMI accounted for only 5% of patients with persistent chest pain [2]. ECG is susceptible to interference, such as early repolarization patterns, acute pericarditis and Brugada syndrome [3,4]. Although cardiac troponin (cTn) is the main force in the diagnosis of STEMI, it still faces the sensitivity and specificity deficit in the first few hours of STEMI due to a delayed increase of circulating levels [5,6]. In view of this, it is of great importance to explore highly sensitive and specific biomarkers for the early diagnosis of STEMI.

Long noncoding RNAs (lncRNAs) are defined as non-protein coding transcripts longer than 200 nucleotides and are closely related to various biological processes including cell growth, differentiation, cell proliferation, and apoptosis [7,8]. Recently, many studies have shown that lncRNAs can regulate the pathological process of a variety of cardiovascular diseases, including AMI [9], heart failure (HF) [10], and congenital heart disease [11]. Interestingly, plasma and serum were used as samples for gene chip screening, and some lncRNAs were described as candidate biomarkers [9,12,13]. However, studies about circulating noncoding RNA as a clinical biomarker of AMI is still in its infancy, and its stability and reliability in diagnosing AMI still requires further exploration.

In this study, we selected 9 candidate MI-related lncRNAs from the LncRNA Disease database and PubMed database which integrated published lncRNAs, and tested their expression levels in plasma by RT-PCR. Then we evaluated the diagnostic value of hypoxia inducible factor 1A antisense RNA 2 (aHIF), membrane 1 opposite strand/antisense transcript 1 (KCNQ1OT1), and mitochondrial long noncoding RNA uc0022bg.1 (LIPCAR) for STEMI patients and finally determined LIPCAR as the potential biomarker. The expression pattern of LIPCAR in the process of AMI and predictive value were also evaluated.

**Material and Methods**

**Participants**

From July 2015 to October 2016, 50 patients diagnosed with STEMI due to typical chest pain were admitted to the Heart Center of Beijing Chaoyang Hospital. In the same period, another 40 non-AMI patients in the same hospital were selected as the control group. Before registration, data on patients’ age, height, weight, smoking history, and cardiovascular risk factors were collected. The diagnosis of STEMI was based on the criteria recommended by the European Society of Cardiology guidelines in 2012 [14], combining several parameters: ischemic symptoms, significantly elevated myocardial enzymes (cTnI and CK-MB), elevated ST-segment of ECG, pathological Q wave and narrowing ≥50% in the left main coronary artery and ≥70% in one or several of the major coronary arteries. All patients who were diagnosed with AMI were given echocardiography and immediate percutaneous coronary intervention (PCI) as soon as possible. Exclusion criteria included heart failure (HF) primarily due to severe valve disease and dilated cardiomyopathy, combined acute and chronic infection, serious liver and kidney dysfunction, malignancies and immune system diseases, and cardiac complications affecting prognosis. The study was approved by the ethics committee of Beijing Chaoyang Hospital. All participants signed an informed consent form.

**Samples collection and detection of myocardial enzymes**

The blood samples were taken at the following time points (4 hours, 6 hours, 12 hours, 24 hours, 3 days and 7 days) after chest pain symptoms occurred. The rest of the participants had fasting venous blood collected on the next morning. Specimens were placed in heparin anticoagulant tubes and centrifuged at 3000 rpm for 10 min to separate the plasma. The supernatant was dispensed and stored at −80°C. The concentration of cTnI was detected by enzyme linked immunosorbent assay (No. ABIN1979499, Elabscience Biotechnology Co, Wuhan, China) with a measuring range of 100–8000 pg/mL. The concentration of CK-MB was detected by ELISA (No. ABIN5067597, Elabscience Biotechnology Co, Wuhan, China) with a measuring range of 0.3–80 U/L. The absorbance was measured at 450 nm with a SpectraMax M5e multi-mode microplate reader (Molecular Devices, USA).

**Quantitative real-time PCR**

The total RNA was isolated from plasma using TRIzol reagent (Invitrogen, CA, USA). After quantification, cDNA was synthesized from 1 µg RNA using a TransScript Uni cDNA Synthesis SuperMix kit (AU311-03, Transgen, Cat. Beijing, China). Reaction was performed in a GeneAmp PCR System 9700 HT Fast (Applied Biosystems, USA) for 60 min at 37°C. Real-time PCR was performed using a LightCycler 480 II Real-time PCR instrument (Roche, Switzerland) with TransStart Top Green qPCR SuperMix (AQ131-03, Transgen, Cat. Beijing, China). The reaction conditions are as follows: 95°C for 15 min, followed by 40 cycles of 95°C for 5 sec and 60°C for 32 sec. At the end of the amplification, the melt curve was performed to verify the product quality. Samples were analyzed in triplicate and included no-template controls. In this study, U6 was selected as an internal reference. Primers sequences for lncRNAs and U6 were
synthesized by Generay Biotech (Shanghai, China). The specific primer sequences are shown in Supplementary Table 1. The relative gene expression is analyzed by the method of $2^{-\Delta\Delta Ct}$.

Follow-up and major adverse cardiac events

All patients were followed for 12±1 months. During the follow-up period, professionals were responsible for continuous observation the occurrence of HF, angina pectoris, recurrent infarction, readmission, and death. Follow-up mainly included outpatient visits, telephone calls, and follow-up visits. The recurrence of MI and cardiac death was defined as major adverse cardiovascular events (MACE).

Statistical analysis

The data was analyzed using SPSS version 19.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA: IBM Corp) software and GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA; www.graphpad.com), the normal distribution measurement data was calculated as the mean ±SD and skewed distribution data was indicated by medians and interquartile range (IQR). The Student’s t-test was used to compare 2 groups of continuous variables, and the chi-square test was performed to compare categorical data. The receiver operating characteristic (ROC) curves were performed to evaluate the diagnostic efficacy of lncRNAs.

Multivariable Cox regression analysis was carried out to evaluate the predictive value of lncRNA to MACE. $P<0.05$ was determined to be statistically significant.

Results

Study populations

Baseline clinical characteristics of the study population are show in Table 1. Forty-six STEMI patients and 40 controls were researched the correlation of circulating lncRNAs with STEMI. Patients with STEMI showed more obesity ($P=0.031$), higher smoking rates ($P=0.04$), lower ejection fraction ($P=0.024$), and significantly elevated myocardial enzymes (both cTnI and CK-MB, $P<0.01$). However, there were no differences in age, low-density lipoprotein cholesterol (LDL-C), hypertension, and diabetes history between the STEMI group and the control group.

Identify the STEMI-related lncRNA in plasma

We screened a total of 9 MI-related lncRNAs from the LncRNADisease database [15] (http://cmbi.bjmu.edu.cn/lncrnadisease) and PubMed database (https://www.ncbi.nlm.nih.gov/pmc/) detected their expression levels in patient’s plasma, respectively. The results of qRT-PCR showed that the 3 lncRNAs with elevated expression were aHIF, KCNQ1OT1, and...
LIPCAR; and decreased expressions were HOX antisense intergenic RNA (HOTAIR), urothelial carcinoma-associated 1 (UCA1), myocardial infarction-associated transcript 1 (MIAT), metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), aclin-dependent kinase inhibitor 2B antisense RNA 1 (ANRIL), and Gene COPINE III (CPNE3). In order to determine which lncRNA had a high diagnostic value, ROC curve was performed; LIPCAR provided the greatest predictive ability, with an AUC of 0.782 (95% CI: 0.707–0.894, sensitivity 82% and specificity 75%) (Figure 1).

**Expression pattern of plasma LIPCAR levels in the patients with STEMI**

After confirming that LIPCAR was clearly associated with STEMI, we further explored the dynamic changes of LIPCAR in the circulation after MI. As show in Figure 2A, the expression of LIPCAR in STEMI was significantly increased within 4 hours of onset of symptoms compared with controls, reached a peak at 12–24 hours and gradually returned to base line levels at 7 days. Moreover, correlation analysis indicated that LIPCAR was positively correlated with cTnI (r=0.74, P<0.001) and CK-MB (r=0.67, P<0.001) and negatively related to LVEF (r=0.8, P<0.001) in patients with STEMI (Figure 2B–2D).

**Relationship between LIPCAR and the severity of coronary occlusion**

Of the 46 patients with STEMI, 7 patients had 1-branch lesions, 28 patients had 2-branch lesions, and 11 patients had 3-branch lesions. As shown in Figure 3A, LIPCAR level was significantly decreased after the occluded vessels recanalization. Subgroup analysis found that plasma LIPCAR level was obviously higher in STEMI patients with 2- and 3-branches lesions (P<0.05). Moreover, correlation analysis showed a positive relation between LIPCAR and Gensini scores (r=0.65, P<0.001), suggesting that the extent of lncRNA elevation reflects the severity of coronary stenosis (Figure 3B, 3C).

**Association between LIPCAR levels and MACE**

In Cox regression analyses, both Gensini score and circulating LIPCAR were associated with increased risk of 1-year MACE in STEMI patients (all P values <0.05). According to the median,
LIPCAR was divided into high and low levels. High LIPCAR expression level significantly increased the risk of MACE compared to low LIPCAR expression level (HR=5.93, 95% CI: 1.46–9.77, \(P=0.001\)) (Table 2).

**Discussion**

AMI is associated with progressive cardiomyocytes loss and leads to the continuous release of myocardial enzymes.
Currently used markers (including troponin, CK-MB) are found to be limited by the heterogeneity of some other diseases, genetic backgrounds, and lifestyles. Accumulating evidence has shown that aberrantly regulated IncRNA is correlated with the progression of various diseases, like cardiovascular disease [16–18]. Although little is known about the origin and function of IncRNAs in circulation, their sensitive and stable differential expression in the blood of patients with cardiovascular diseases and healthy people makes them a potential biomarker [19]. The mechanism of action is likely that cardiac tissue damage leads to an additional release of IncRNAs, similar to the release of proteins.

The LncRNADisease database is a website containing about 480 IncRNA entries of experimentally supported IncRNA-disease associations, including 160 diseases, 478 entries of IncRNA interacting partners at various molecular levels. In total, 166 diseases were included, and cardiovascular diseases accounted for 10.8% [15]. Some of these IncRNAs have been identified as having significant differences in the blood of patients with AMI compared to healthy individuals [6,9,12,20].

In this study, we focused on the expression of IncRNAs in peripheral blood of STEMI patients. Nine MI-related IncRNAs were screened from the database and tested for expression by RT-PCR, of which 3 IncRNAs (aHIF, KCNQ1OT1, and LIPCAR) were significantly elevated. By modulating the stability of HIF1α messenger RNA, upregulation of aHIF has the capacity to regulate angiogenesis and anti-apoptosis [21]. KCNQ1OT1 is highly expressed in the peripheral blood of patients with AMI and is associated with ischemia-reperfusion injury [22]. However, in our study, only LIPCAR showed the optimal sensitivity and specificity for the diagnosis of AMI in ROC curve analysis. Therefore, we choose LIPCAR for further study.

Accumulated evidence supports many noncoding RNAs origin is mitochondrial. The contribution of mitochondrial IncRNA to total IncRNA pools in human left ventricle is approximately 70%, indicating that a large proportion of circulating mitochondrial IncRNAs might come from the heart [23,24]. Although it is still controversial whether LIPCAR is derived from the nucleus or mitochondria, it does not affect it as a biomarker of ventricular remodeling after MI [10,25].

Our results showed that IncRNA rapidly increased in the short term after the onset of symptoms, and its expression pattern in plasma was close to that of CK-MB. Plasma LIPCAR expression in patients with STEMI was obviously decreased after PCI, indicating that the dynamic changes of plasma LIPCAR reflects the situation of myocardial ischemia and coronary lesions. Higher expression of LIPCAR gene may increase the risk of AMI by promoting formation of coronary artery lesions. Multivariate Cox regression analysis revealed that Genisi score and LIPCAR levels were independent predictors of MACE after AMI. This result was consistent with Kumarswamy et al. findings [10]. Although we found that LIPCAR was differentially expressed between STEMI and control groups, additional studies are needed to determine the consistency of LIPCAR expression in tissues and plasma. Larger sample sizes are needed to further confirm the potential applications of LIPCAR as a novel biomarker to diagnosis AMI.

### Table 2. Multivariate Cox regression analysis of MACE.

| Variable                        | HR (95% CI)       | p Value |
|---------------------------------|-------------------|---------|
| Gender, Male vs. Female         | 1.21 (0.74–2.98)  | 0.831   |
| BMI                             | 1.19 (0.87–4.75)  | 0.062   |
| Smoking, %                      | 0.84 (0.44–2.13)  | 0.449   |
| Hypertension, %                 | 1.01 (0.74–1.05)  | 0.542   |
| Diabetes mellitus, %            | 0.56 (1.04–3.31)  | 0.150   |
| LVEF, %                         | 1.94 (0.81–2.57)  | 0.459   |
| LDL-C, mmol/L                   | 1.35 (0.97–1.42)  | 0.731   |
| Gensini score                   | 3.88 (0.52–7.66)  | 0.002   |
| LIPCAR, according to the median | –                 | 0.026   |
| Low LIPCAR expression level     | 2.14 (0.59–4.16)  | 0.15    |
| High LIPCAR expression level    | 5.93 (1.46–9.77)  | 0.001   |

BMI – body mass index; LVEF – left ventricular ejection fraction; LDL-c – low-density lipoprotein cholesterol; LIPCAR – mitochondrial long noncoding RNA uc022bqs.1. P<0.05 means a statistically significant difference between groups.

### Conclusions

This study found that LIPCAR may serve as a potential biomarker of AMI, especially for STEMI, and could predict the severity and progression of CHD. However, the clinical application value and expression mechanism of LIPCAR still need further study.

### Conflict of interest

None.
**Supplementary Table**

**Supplementary Table 1.** Primers used for qRT-PCR.

| Name  | Sequence | Name  | Sequence |
|-------|----------|-------|----------|
| aHIF  | Forward   | MIAT  | Forward   |
|       | TCTAGCCCATCTTATATTCT | Reverse | TTACTTAAACAGACCAGAAA |
| KCNQ1OT1 | Forward   | MALAT1 | Reverse   |
|       | ATATGATTGAGACTCA | Reverse | TTTAAGATGACATTAGTGG |
| HOTAIR | Forward   | ANRIL  | Reverse   |
|       | ACGTAAACTGCACTTGGTTT | Reverse | CTCCTTGAAGAGTATT |
| UCA1  | Forward   | CPNE3  | Forward   |
|       | TGGGAAATTCTGGGTAGGG  | Reverse | CTCTAGCATTATAGTCACAA |
| LIPCAR| Forward   | U6     | Reverse   |
|       | TTAAGGATTCTGAGGGATTTG | Reverse | CGCTTCAGGACTTGGGTGAT |

KCNQ1OT1—member 1 opposite strand/antisense transcript 1; aHIF—hypoxia inducible factor 1A antisense RNA 2; HOTAIR—H3K4 methyltransferase-associated RNA; UCA1—urothelial carcinoma-associated 1; LIPCAR—mitochondrial long noncoding RNA uc022bqs.1; MALAT1—metastasis-associated lung adenocarcinoma transcript 1; MIAT—myocardial infarction-associated transcript; ANRIL—cyclin-dependent kinase inhibitor 2B antisense RNA 1; CPE3—gene COPINE III.

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