Analysis of the Molecular Mechanism of Acute Coronary Syndrome Based on circRNA-miRNA Network Regulation

Fei Lin, YaMing Yang, Quan Guo, Mingzhang Xie, Siyu Sun, Xiulong Wang, Dongxu Li, Guhao Zhang, Meng Li, Jie Wang, Guoan Zhao

The First Hospital of Xinxiang Medical University, Xinxiang, Henan 453100, China
Heart Center of Xinxiang Medical University, Xinxiang, China
Henan Engineering Research Center for Clinical Data and Biobank of Cardiovascular Diseases, Zhengzhou, Henan, China
Guang’anmen Hospital, China Academy of Chinese Medical Sciences, Beijing 100053, China

Correspondence should be addressed to Jie Wang; wangjie0103@126.com and Guoan Zhao; guoanzhao@xxmu.edu.cn

Received 15 December 2019; Revised 12 March 2020; Accepted 10 April 2020; Published 29 April 2020

Academic Editor: Ghee T. Tan

Copyright © 2020 Fei Lin et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. With the development of biological technology, biomarkers for the prevention and diagnosis of acute coronary syndrome (ACS) have become increasingly evident. However, the study of novel circular RNAs (circRNAs) in ACS is still in progress. This study aimed to investigate whether the regulation of circRNA-miRNA networks is involved in ACS pathogenesis.

Methods. We used microarray analysis to detect significantly expressed circRNAs and miRNAs in the peripheral blood of patients in the control group (CG) and ACS groups, including an unstable angina pectoris (UAP) group and an acute myocardial infarction (AMI) group. A circRNA-miRNA interaction network analysis was carried out with open-source bioinformatics. The gene ontology (GO), pathway, and disease enrichment analyses for differentially expressed circRNAs were further analysed with hierarchical clustering.

Results. A total of 266 circRNAs (121 upregulated and 145 downregulated, \( P < 0.05, \text{fold change } FC \geq 2 \)) and 3 miRNAs (1 upregulated and 2 downregulated, \( P < 0.05, \text{FC} \geq 1.2 \)) were differentially expressed in the ACS groups compared with those in the CG. In addition, among these expressed circRNAs and miRNAs, a single circRNA could bind to more than 1–100 miRNAs, and vice versa. Next, an AMI-UAP network, an AMI-CG network, a UAP-CG network, and an AMI-CG-UAP network were constructed. The top 30 enriched GO terms among the three groups were emphasized as differentially expressed. Disease enrichment analysis showed that these differentially expressed circRNAs are involved in the pathogenesis of cardiovascular diseases. KEGG pathway analysis was performed to identify pathways associated with circRNAs targeting mRNAs.

Conclusion. circRNAs are closely related to the pathological process of ACS via a mechanism that may be related to the up- or down-regulation of circRNAs and miRNAs and circRNA-miRNA coexpression. The metabolic pathways, signalling pathways, and diseases affected by these circRNAs can be predicted by enrichment analysis.

1. Introduction

Circular RNAs (circRNAs), which contain a covalently closed continuous loop, are an abundant class of endogenous RNAs that are formed during the maturation of precursor mRNA. circRNAs are widely expressed in eukaryotes, are evolutionarily conserved, and can be specific to certain cell types or developmental stages. In addition, circRNAs have been found in the nucleus and mitochondria. Unlike linear RNA, circRNAs have no 5’ cap or 3’ tail structure and is not easily degraded by the exonuclease RNase R, which is stable in cells [1–3]. The formation mechanism of circRNAs also determines its diverse and complex biological functions [4–7]. Among them, its characteristics of transcription, translation, protein interaction, and signal transduction regulation have been confirmed by a growing number of studies [3, 8]. In particular, circRNAs can specifically change the biological behaviour of cells in tissues and in diseases [3]. Moreover, many studies have shown that circRNAs are widely and
specifically expressed in tumours, ageing, diabetes, cardiovascular and cerebrovascular diseases, and skin diseases [1, 7, 9–11]. These results offer a new perspective on biomolecular science. Acute coronary syndrome (ACS) can lead to a series of acute cardiovascular events, such as arrhythmia, heart failure, and even sudden death. Its main pathogenesis is closely related to plaque rupture, vasospasm, platelet aggregation, and thrombosis. However, the mechanism remains unclear. With the rapid development of next-generation gene sequencing technology, an increasing number of reports have indicated that noncoding RNAs (ncRNAs), such as circRNAs, microRNAs (miRNAs), and long noncoding RNAs (lncRNAs), have a significant influence on cardiovascular diseases [12]. CircRNAs, as regulators of gene expression, may be an important genetic mechanism underlying the pathogenesis of multifactorial complex diseases [10, 13–16]. Thus, elucidating the process by which miRNAs regulate the gene expression and the specificity of the regulated targets is highly important for probing the mechanism underlying ACS [17]. However, few studies have investigated whether circRNAs and miRNAs are involved in the occurrence and development of ACS. The results of our previous work have indicated that circRNAs are significantly expressed in the blood of patients with coronary heart disease (CHD) [18]. Thus, in this work, we screened the characteristic circRNA and miRNA expression profiles of ACS using a microarray gene chip and predicted the possible circRNA-miRNA interaction. We aimed to provide critical information for investigations into the complex regulatory mechanisms of ACS.

## 2. Materials and Methods

### 2.1. Study Subjects

We included inpatients diagnosed with ACS (I24.901), including those diagnosed with acute myocardial infarction (AMI) (I21) and unstable angina pectoris (UAP) (I20.001) according to the criteria of the International Classification of Diseases–10th edition, who were treated at the Department of Cardiology of the First Affiliated Hospital of Xinxiang Medical College between November 2016 and February 2017. The diagnostic criteria for AMI and UAP were based on the globally harmonized definition of AMI and on the 2012 American College of Cardiology Foundation (ACCF)/American Heart Association (AHA) Focused Update of the Guidelines for the Management of Patients with Unstable Angina/Non-ST-elevation Myocardial Infarction [19–21]. All participants underwent coronary angiography (CAG), and Gensini scores (GS) were calculated. Patients with the following characteristics were excluded: (1) severe congestive heart failure, malignant hypertension, severe arrhythmia, or severe lung dysfunction; (2) severe neurosis, hyperthyroidism, cervical spondylosis, hepatobiliary disease, gastric and oesophageal reflux, or chest pain caused by nonangina pectoris; (3) AMI/UAP complicated by severe primary diseases such as those of the liver, kidney, or haematopoietic system; (4) mental illness; (5) current pregnancy or lactation; (6) allergies to iodine or contrast agents or the allergic physique; and (7) various infectious diseases. Ultimately, 15 inpatients were selected and subsequently divided into the CG (control group), UAP group, and AMI group (5 patients per group). The CG was filtered according to baseline data such as the clinical CAG score (GS < 2). Then, we collected the participants’ baseline data.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical College (approval number: 2016039).

### 2.2. Plasma Sample Collection and CircRNA-miRNA Microarray Analysis

Five millilitres of whole blood was collected into an anticoagulant tube containing ethylenediaminetetraacetic acid (EDTA). The blood samples were stored in an ice box at 4°C and transported to the...
Figure 1: Heatmap of circRNAs with differential expression between groups. The columns represent patients, and the rows represent the degree to which a gene was expressed at different copy numbers. The colour key in the top left indicates the expression level (red indicates upregulation; green indicates downregulation). The expression of circRNAs is hierarchically clustered on the $y$-axis; the corresponding miRNAs are shown at the top. The left-most bar on the $y$-axis indicates the group assignment. The genes in 15 clusters, denoted as CG (a9, a14, H2, H3, H4), AMI (G2, G3, G5, G7, G8) and UAP (a10, a15, Z8, Z10, Z11), included 121 upregulated and 145 downregulated genes.
2.3. Statistical Analysis. Image data of the hybridized microarray (Agilent Human CircRNA Array V2.0) in tiff format were analysed by Agilent Feature Extraction (V10.7) software, and the data were extracted. Then, the circRNA array data used threshold fold change (FC) values of ≥2 and ≤−2 and a t test P value of 0.05, and miRNA array data FC ≥ 1.2 and ≤−1.2 and a t test P value of 0.05, and circRNAs and miRNAs were analysed for data summarization, normalization, and quality control by using GeneSpring GX software (Agilent). To select the differentially expressed genes, data normalization and quality control analysis were performed for each sample. CLUSTER 3.0 software was used for data analysis and graphical display. MiRanda-3.3 software was used to predict the circRNAs that may bind miRNAs and to construct a network diagram though the open-source bioinformatics software Cytoscape. In a network analysis, a degree of centrality is defined as the number of linkages one node has to another. A degree is the simplest and most important measure of gene centrality within a network for determining the relative importance. Gene ontology (GO), pathway, and disease enrichment analyses were conducted for differentially expressed circRNAs with Kyoto Encyclopedia of Genes and Genomes (KEGG) Orthology-Based Annotation System (KOBAS) software. The processing and sorting of circRNA and miRNA expression profile chip data were performed in whole or in part with the CapitalBio Technology Expression Spectrum Chip Analysis System V1.0 (computer software copyright registration number: 2014SR122558) [15, 22, 23].

Table 2: Expression profiles of 121 circRNAs that were upregulated (P < 0.05, FC ≥ 2).

| No. | ProbeName | P     | FC (abs) | geneSymbol | circStart | circEnd | Strand | miRNA number | miRNA number more than 1 |
|-----|-----------|-------|----------|------------|-----------|---------|--------|--------------|----------------------------|
| 1   | hsa-circ16316-10 | 0.00183 | 156.1532 | UTY        | 15447442  | 15481229 | −      | 100          | 17                          |
| 2   | hsa_circ040759   | 0.00078 | 145.55   | UTY        | 15466682  | 15481229 | −      | 95           | 6                           |
| 3   | hsa_circ040760   | 0.00082 | 93.2578  | UTY        | 15476172  | 15471765 | −      | 100          | 66                          |
| 4   | hsa-circ16316-13 | 0.0041  | 88.45529 | UTY        | 15447442  | 15478273 | −      | 100          | 17                          |
| 5   | hsa_circ040758   | 0.00147 | 80.8945  | UTY        | 1547442   | 15448215 | −      | 100          | 3                           |
| 6   | hsa-circ16316-4  | 0.00199 | 61.61567 | UTY        | 15466682  | 15472408 | −      | 85           | 5                           |
| 7   | hsa-circ16316-11 | 0.00116 | 44.99736 | UTY        | 15471646  | 15471866 | −      | 11           | −                           |
| 8   | hsa_circ040781   | 0.00129 | 26.7885  | KDM5D      | 21901413  | 21903743 | −      | 49           | 1                           |
| 9   | hsa_circ040736   | 0.00149 | 24.01664 | USP9Y      | 14821320  | 14885859 | +      | 100          | 7                           |
| 10  | hsa_circ0003368  | 0.00068 | 22.6279  | UTY        | 15478146  | 15481229 | −      | 9            | −                           |
| 11  | hsa-circ16316-9  | 0.00064 | 21.13425 | UTY        | 15435434  | 15438230 | −      | 43           | 4                           |
| 12  | hsa_circ0009224  | 0.00047 | 17.24446 | −          | 21749095  | 21749393 | +      | 25           | −                           |
| 13  | hsa_circ040746   | 0.00106 | 16.81741 | USP9Y      | 14870435  | 14885859 | +      | 42           | −                           |
| 14  | hsa-circ16316-2  | 0.00316 | 10.52771 | UTY        | 15435434  | 15481229 | −      | 100          | 24                          |
| 15  | hsa-circ16316-12 | 0.00040 | 15.87258 | UTY        | 15435434  | 1548215  | −      | 100          | 7                           |
| 16  | hsa_circ040783   | 0.00160 | 5.52771  | −          | 22669237  | 22683186 | −      | 100          | 100                         |
| 17  | hsa-circ16316-1  | 0.00156 | 4.98803  | UTY        | 15478146  | 15508852 | −      | 12           | −                           |
| 18  | hsa_circ0007907  | 0.00053 | 13.54894 | ZFY        | 2829114  | 2829667 | +      | 25           | −                           |
| 19  | hsa_circ040780   | 0.00062 | 12.87783 | KDM5D      | 21901413  | 21903743 | −      | 12           | −                           |
| 20  | hsa-circ16316-7  | 0.00914 | 12.75820 | UTY        | 15522872  | 15526673 | −      | 15           | −                           |

ProbeName = probe address; FC = fold change; geneSymbol = abbreviated gene name; circStart = gene initiation site; circEnd = gene termination position; strand = circRNA in chain; miRNA number = number of miRNAs that the circRNA can bind to (sorted by the number of binding sites—if greater than 100, only the top 100 binding sites are selected); miRNA number more than 1 = the circRNA can bind to 2 or more miRNAs.
Table 4: MiRNAs expression profiling ($P < 0.05$, $FC \geq 1.2$).

| No. | ProbeName | $P$  | FC (abs) | geneSymbol | circStrat | circEnd | Strand | miRNA number | miRNA number more than 1 |
|-----|-----------|------|----------|------------|-----------|---------|--------|--------------|-------------------------|
| 1   | hsa-miR-4299  | 0.016 | 9.07     | ABCA5      | 67270099  | 67305564 | +       |              |                         |
| 2   | hsa-miR-20b-5p | 0.033 | 1.29     | ABCA5      | 67270099  | 67305564 | +       |              |                         |
| 3   | hsa-miR-363-3p | 0.046 | 1.21     | ABCA5      | 67270099  | 67305564 | +       |              |                         |

ProbeName = probe address; FC = fold change; There shows miRNAs that were differentially expressed between the groups.

Figure 2: Comparison of circRNA-miRNA prediction network maps between the AMI and UAP group. Prediction of miRNAs that may be bound by circRNA and construction of a circRNA-miRNA network. According to the relationship between circRNAs and target miRNAs, the top circRNAs with the most FGs were selected to construct the circRNA-miRNA network map. The squares represent miRNAs and the pentagrams represent circRNAs, where green indicates downregulation and purple indicates upregulation.
Statistical analyses of baseline data were performed using SPSS 22.0, and all data are presented as the means ± standard deviations (\( \bar{x} \pm s \)).

3. Results

3.1. Baseline Data. Table 1 outlines the patient characteristics. Patients with AMI had lower blood pressure values and higher levels of blood glucose and myocardial enzymes than patients with UAP (\( P < 0.05 \)). There was no significant change in blood lipid biochemistry. Patients with UAP had a calculated GS of >15 and those with AMI had a GS of >40, as evidenced by CAG. The GSs in the UAP and AMI groups were higher than those in the CG.

3.2. CircRNA and miRNA Expression Profiling. To identify whether circRNAs and miRNAs are differentially expressed in ACS, we extracted total RNA from the peripheral blood of 5 AMI patients, 5 UAP patients, and 5 CG with matched clinical and statistical characteristics and analysed the samples using a microarray gene chip. The detected circRNAs were differentially expressed among the groups (Figure 1). A total of 266 circRNAs (121 upregulated and 145 downregulated) were predicted in the AMI and UAP groups (\( P < 0.05, \text{FC} \geq 2 \)) (Tables 2 and 3). The data were log2-transformed and median centred by gene using the Adjust Data function in CLUSTER 3.0 software and then further analysed with hierarchical clustering with average linkage criterion (Tables 2 and 3).

Three miRNAs were differentially expressed in the AMI and UAP groups compared with those in the CG: hsa-miR-4299 was upregulated, and hsa-miR-20b-5p and hsa-miR-363-3p were downregulated (\( P < 0.05, \text{FC} \geq 1.2 \)) (Table 4).

3.3. Correlation Analysis of the circRNA-miRNA Network. CircRNAs are significant influencing factors in miRNA function and transcriptional control by acting as sponges,
competing endogenous RNAs, or positive regulators of their parent coding genes. In this study, we utilized miRanda software to construct the circRNA-miRNA network, and these circRNA-miRNA pairs were screened though the open-source bioinformatics software Cytoscape. We constructed a network that matched the differentially expressed circRNAs by cross comparing the biological information among the AMI-UAP network, the AMI-CG network, the UAP-CG network, and the AMI-CG-UAP network. A large number of circRNAs that were predicted to bind to miRNAs with a combined weight score of 1 were screened as differentially expressed genes.

Specifically, of the many circRNAs predicted to bind to miRNAs, not only did a single circRNA bind more than 1–100 miRNAs, but more than 1–100 circRNAs also bound a single miRNA, such as hsa_circ_8316-4, hsa_circ_0097809, hsa_circ_0097810, hsa_circ_0139861, and hsa_circ_0140538, with significantly decreased expression, and hsa-circ_0140759,
hsa_circ_0140758, hsa_circ_16316-13, hsa_circ_0140760, and as well as other circRNAs with significantly increased expression. Multiple miRNAs had a common locus for binding to the same circRNA, and vice versa. These results suggested that circRNAs exhibited coexpression correlations with miRNAs and that circRNAs could act as source genes to interact with miRNAs to regulate the occurrence and development of ACS (Figures 2–5). All differentially expressed circRNAs are presented in Table 5. Simultaneously, miRNAs could act as target genes to interact with copious circRNAs (Table 6, Figures 6–9). However, how these novel genes participate in the process of disease development is still unclear. Further cells culture or animal experiments are needed to verify these findings.

3.4. CircRNA Enrichment Analyses. To better understand the functions of the genes associated with the differentially expressed circRNAs, GO (Figure 10), disease (Figure 11), and pathway (Figure 12) enrichment analyses were performed with KOBAS software. Pathway and disease terms were selected from the first 30 significantly enriched terms. GO analysis was used to select the first 30 significantly enriched biological process (BP), molecular function (MF), and cellular component (CC) terms, and a histogram was

Figure 5: Comparison of circRNA-miRNA prediction network maps in the CG and ACS (AMI and UAP) groups. Prediction of miRNAs that may be bound by circRNA and construction of a circRNA-miRNA network. According to the relationship between circRNAs and target miRNAs, the top circRNAs with the most FCs were selected to construct the circRNA-miRNA network map. The squares represent miRNAs and the pentagrams represent circRNAs, where green indicates downregulation and purple indicates upregulation.
drawn according to the $P$ values, which directly reflected the significantly enriched terms.

3.5. GO Enrichment Analysis. We carried out KEGG pathway mapping based on the encyclopaedia’s orthology terms to assess related pathways correlating with differentially expressed circRNAs from the AMI and UAP groups compared with those from the CG. A total of 53 MF terms, 241 BP terms, and 35 CC terms were significantly enriched ($P < 0.05$). These three major categories define and describe various aspects of a gene’s function. We selected the first 30 significantly enriched terms from the three categories and plotted a histogram according to the $P$ values, which directly reflect the significantly enriched terms ($P < 0.05$; Figure 10). The top-ranking GO terms involved in ACS included the metabolic process (GO:0008152), cellular process (GO:0009987), single organism process (GO:0044699), organelle (GO:0043226), cell (GO:0005623), cell part (GO:0044464), and binding (GO:0005488).

3.6. Disease Enrichment Analysis. Disease enrichment analysis was performed using the NHGRI GWAS Catalog. A total of 19 terms were significantly enriched ($P < 0.05$). The diseases predicted to be associated with ACS included cardiovascular disease risk factors and high-density lipoprotein cholesterol (HDL-C).

The differentially expressed circRNAs were found to be involved in terms related to cardiovascular diseases, such as bone mineral density (hsa123803), phosphorus levels (hsa221496), metabolite levels (5−HIAA) (hsa4023), tourette syndrome (hsa5251), and anger (hsa56776) ($P < 0.05$; Figure 11).

3.7. Pathway Enrichment Analysis. In the KEGG pathway database, 266 circRNAs with different expression levels were enriched, and the first 30 significantly enriched terms were selected ($P < 0.05$; Figure 12).

4. Discussion

Accumulating evidence suggests that ncRNA participates in diseases such as cardiovascular diseases, diabetes mellitus, and hypertension. Recent progress in the ncRNA research field has uncovered central aspects for the regulation and
Figure 6: Comparison of circRNA-miRNA (red-yellow) coexpression maps between the AMI and UAP groups. These images depict the number of circRNAs that can bind to the bound target gene miRNA. The circRNAs have the same trend as miRNA changes, or the correlation is relatively close.
functions of biogenesis and biology in the pathophysiology of the cardiovascular system [11, 12]. More importantly, the circRNA-miRNA-mRNA regulatory network plays an important role in the occurrence and development of cardiovascular disease [11].

The research results reported in this paper offer many important findings and imply that circRNA and miRNA are differentially expressed between the ACS groups and the CG. Differential expression was detected for a total of 266 circRNAs, of which 121 were upregulated and 145 were downregulated in the ACS groups (\( P < 0.05, \text{FC} \geq 2 \)), and the circRNAs were found to be related to UTY, KDM5D, USP9Y, MRPL39, ABCA5, and CCT8 and as well as other genes (Tables 2 and 3). In previous studies, a total of 1670 circRNAs were identified in the AMI group (859 upregulated and 811 downregulated) (\( P < 0.05, \text{FC} \geq 2.0 \)). Furthermore, hsa_circ_16316-13 was found to be significantly increased in CHD patients [18, 24]; similarly, hsa_circ_16316-13 was found to be upregulated in this paper.

To date, many outcomes have confirmed the view that circRNAs can be used as sponges for miRNA to regulate the gene expression. Recent evidence points to a pivotal role for circRNAs in the regulation of miRNA function as miRNA sponges; in addition, they may play a significant role in pathophysiology of cardiovascular diseases [25]. Strikingly, genetic network bioinformatics analysis for ACS revealed not only that a single circRNA could bind to more than 1–100 miRNAs but also more than 1–100 circRNAs bound to a single miRNA (Figures 2–9). The results provide evidence to show that circRNA is bound with miRNA in the occurrence and development of ACS. Another study showed that circRNA_101237 acts as a sponge for let-7a-5p,
regulating cardiomyocyte death and autophagy; additionally, the circRNA-101237/let-7a-5p/IGF2BP3 (insulin-like growth factor 2 mRNA-binding protein 3) axis serves as a regulator of cardiomyocyte death [26]. CircNCX1 was increased in response to reactive oxygen species and promotes cardiomyocyte apoptosis by competitive binding to miR-133a-3p, suppressing the activity of CDIP1 (a proapoptotic gene cell death-inducing protein) by acting as an endogenous miR-133a-3p sponge [27]. Furthermore, circRNA-miRNA axes are involved in a series of disease pathways such as myocardial infarction, myocardial hypertrophy, cardiac regeneration, cardiac fibroblasts, and heart failure [2, 25, 28]. Moreover, targeted localization of circRNA-miRNA-mRNA may be a potential target for cardiovascular disease treatment [16].

The results of the KEGG pathway enrichment analysis revealed that differentially expressed genes are involved in the ACS signalling pathway, such as dilated cardiomyopathy, hypertrophic cardiomyopathy, and arrhythmogenic right-ventricular cardiomyopathy (Figure 12). The dilated cardiomyopathy (DCM) pathway involves proteins such as Desmin, DMD, Titin, Tnt, ACTA1, TPM, Laminac, SGCD, TNF-α, IGF-1, TGF-β, and Ang-II. Additionally, more valuable in current clinical practice, cTnT is the preferred biochemical marker for myocardial cell necrosis, and alleviated cTnT levels are detected only 3–6 hours after the onset of ischaemic symptoms. Currently, a positive troponin result is associated with clinically important increases in mortality, regardless of age, even if the level is only slightly above normal [29]. In addition, the
hsa-miR-4299
hsa_circ_0093021_CBC1
hsa_circ_0139598_CBC1
hsa_circ_0094614_CBC1
hsa_circ_0114458_CBC1
hsa_circ_0140697_CBC1
hsa_circ_0044097_CBC1
hsa_circRNA16239-2_CBC1
hsa_circ_0091105_CBC1
hsa_circ_0091343_CBC1
hsa_circ_0067624_CBC1
hsa_circ_0140581_CBC1
hsa_circRNA5268-2_CBC1
hsa_circ_00440891_CBC1
hsa_circ_0099400_CBC1
hsa_circRNA5268-2_CBC1

Figure 9: Comparison of circRNA-miRNA (blue-red) coexpression maps between the CG, AMI, and UAP groups. These images depict the number of circRNAs that can bind to the bound target gene miRNA. The circRNAs have the same trend as miRNA changes, or the correlation is relatively close.

Figure 10: Functional annotation of differentially expressed genes (DEGs). It shows the enrichment of DEGs compared with the background genes.
pathway is related to arrhythmogenic right-ventricular cardiomyopathy in ACS (Figure 12).

Among the results of the enrichment analysis of 155 diseases, 19 diseases were predicted to be associated with ACS (Figure 11), such as cardiovascular disease risk factors and HDL-C. Risk factors for ACS are known to include hypertension, hyperlipidaemia, triglycerides, smoking, alcoholism, diabetes, lack of exercise, anger, overweight, and genetic factors. In recent years, many basic science and clinical studies have reported that glycaemic variability [30], impaired spontaneous/endogenous fibrinolytic status [31], low-density lipoprotein (LDL), nonadherence [32], socioeconomic and psychosocial factors, grip strength, household environment, ambient pollution, and sodium intake [33] are highly significant risk factors for cardiovascular disease.

Encouragingly, some of these differentially expressed genes have been validated in cardiovascular diseases. Clinical research conducted by Vilade et al. revealed that hsa_circ_0001445 exists stably in plasma and can serve as a new biomarker for coronary artery disease, as it was associated with a higher extent of coronary atherosclerosis [34]. Recent reports have also suggested that circRNA is another type of large noncoding RNA with translation potential [35–37]. Sebastiaan van Heesch et al. focused on protein translation in the heart for the first time using a method combining ribosomal imprinting and provided information on protein translation regulation during DCM [38]. Experiments on mice carried out by Garikipati VNS et al. revealed that overexpression of circFndc3b contributed to reducing myocardial and endothelial cell apoptosis and improving myocardial function. Furthermore, circFndc3b interacted
with the RNA-binding protein FUS to positively regulate the expression of VEGF-A, thereby improving the function and reconstruction of the myocardium after infarction [39].

In conclusion, circRNAs are involved in the occurrence and development of ACS through multiple points of network correlation for miRNA regulation. We speculate that circRNAs may serve as a potential therapeutic avenue for a pathophysiological mechanism of ACS and may even become diagnostic and therapeutic biomarkers for ACS. In the future, we will perform in vitro and in vivo tests to further validate the involvement of circRNAs in the atherosclerotic process of ACS.

Data Availability

The datasets used and/or analysed during the current study are available from the corresponding author upon reasonable request.

Additional Points

Highlights. (a) CircRNA and miRNA may be closely related to the pathogenesis of acute coronary syndrome. (b) Based on gene chip analysis, 266 circRNAs and 3 miRNAs were differentially expressed in acute coronary syndrome. (c) A single circRNA can bind to more than 1–100 miRNAs, and vice versa. (d) The metabolism pathways, signalling pathways, and involved diseases affected by these circRNAs were predicted by enrichment analysis.

Ethical Approval

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xinxiang Medical University.
Consent
The patients in this research provided written consent to participate and had completed clinical data. Signed written informed consent forms were obtained from the patients and/or guardians.

Disclosure
Fei Lin and Guoan Zhao take responsibility for all aspects of the reliability and freedom from bias.

Conflicts of Interest
The authors declare that they have no conflicts of interest.

Authors’ Contributions
Guoan Zhao conceived the study. Fei Lin and Yaming Yang drafted the manuscript. Guhao Zhang and Meng Li acquired the data. Quan Guo and Siyu Sun analysed the data. Xiulong Wang, Dongxu Li, and Mingzhang Xie revised the manuscript. All authors read and approved the final manuscript.

Acknowledgments
Financial support for this work was provided by the Key Scientific Research Projects of Higher Education Institutions in Henan Province (No. 18A320005 and No. 19A360032) and the Scientific and Technological Research Projects of Henan Province (No. 182102310182).

References
[1] X. Li, L. Yang, and L.-L. Chen, “The biogenesis, functions, and challenges of circular RNAs,” *Molecular Cell*, vol. 71, no. 3, pp. 428–442, 2018.
[2] S. Aufiero, Y. J. Reckman, Y. M. Pinto, and E. E. Creemers, “Circular RNAs open a new chapter in cardiovascular biology,” *Nature Reviews Cardiology*, vol. 16, no. 8, pp. 503–514, 2019.
[3] L. S. Kristensen, M. S. Andersen, L. V. W. Stagsted, K. K. Ebbesen, T. B. Hansen, and J. Kjems, “The biogenesis, biology and characterization of circular RNAs,” *Nature Reviews Genetics*, vol. 20, no. 11, pp. 675–691, 2019.
[4] W. R. Jeck, J. A. Sorrentino, K. Wang et al., “Circular RNAs are abundant, conserved, and associated with ALU repeats,” *RNA*, vol. 19, no. 2, pp. 141–157, 2013.
[5] Y. Zhang, X.-O. Zhang, T. Chen et al., “Circular intronic long noncoding RNAs,” *Molecular Cell*, vol. 51, no. 6, pp. 792–806, 2013.
[6] Z. Li, C. Huang, C. Bao et al., “Exon-intron circular RNAs regulate transcription in the nucleus,” *Nature Structural & Molecular Biology*, vol. 22, no. 3, pp. 256–264, 2015.
[7] C. Braicu, A.-A. Zimta, D. Gulei, A. Olariu, and I. Berindan-Neagoe, “Comprehensive analysis of circular RNAs in pathological states: biogenesis, cellular regulation, and therapeutic relevance,” *Cellular and Molecular Life Sciences*, vol. 76, no. 8, pp. 1559–1577, 2019.
[8] M. Lei, G. Zheng, Q. Ning, J. Zheng, and D. Dong, “Translation and functional roles of circular RNAs in human cancer,” *Molecular Cancer*, vol. 19, no. 1, p. 30, 2020.

Evidence-Based Complementary and Alternative Medicine
[9] S. L. Mehta, R. J. Dempsey, and R. Vemuganti, “Role of circular RNAs in brain development and CNS diseases,” *Progress in Neurobiology*, vol. 186, p. 101746, 2020.
[10] M.-y. Zhou, J.-M. Yang, and X.-d. Xiong, “The emerging landscape of circular RNA in cardiovascular diseases,” *Journal of Molecular and Cellular Cardiology*, vol. 122, pp. 134–139, 2018.
[11] D. Lu and T. Thum, “RNA-based diagnostic and therapeutic strategies for cardiovascular disease,” *Nature Reviews Cardiology*, vol. 16, no. 11, pp. 661–674, 2019.
[12] J. Beermann, M.-T. Piccoli, J. Viereck, and T. Thum, “Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches,” *Physiological Reviews*, vol. 96, no. 4, pp. 1297–1325, 2016.
[13] J. Salzman, “Circular RNA expression: its potential regulation and function,” *Trends in Genetics*, vol. 32, no. 5, pp. 309–316, 2016.
[14] Y. Chen, C. Li, C. Tan, and X. Liu, “Circular RNAs: a new frontier in the study of human disease,” *Journal of Medical Genetics*, vol. 53, no. 6, pp. 359–365, 2016.
[15] S. Memczak, M. Jens, A. Elefsinioti et al., “Circular RNAs are a large class of animal RNAs with regulatory potency,” *Nature*, vol. 495, no. 7441, pp. 333–338, 2013.
[16] G. Zhao, “Significance of non-coding circular RNAs and micro RNAs in the pathogenesis of cardiovascular diseases,” *Journal of Medical Genetics*, vol. 55, no. 11, pp. 713–720, 2018.
[17] P. Jakob, T. Kacprowski, S. Briand-Schumacher et al., “Profiling and validation of circulating microRNAs for cardiovascular events in patients presenting with ST-segment elevation myocardial infarction,” *European Heart Journal*, vol. 38, no. 7, pp. 511–515, 2017.
[18] F. Lin, G. Zhao, Z. Chen et al., “circRNAmiRNA association for coronary heart disease,” *Molecular Medicine Reports*, vol. 19, no. 4, pp. 2527–2536, 2019.
[19] K. Thygensen, J. S. Alpert, A. S. Jaffe et al., “Fourth universal definition of myocardial infarction (2018),” *European Heart Journal*, vol. 40, no. 3, pp. 237–269, 2019.
[20] M. Writing Committee, H. Jneid, J. L. Anderson et al., “ACCF/AHA focused update of the guideline for the management of patients with unstable angina/Non-ST-elevation myocardial infarction (updating the 2007 guideline and replacing the 2011 focused update): a report of the American College of Cardiology Foundation/American Heart Association task force on practice guidelines,” *Circulation*, vol. 126, no. 7, pp. 875–910, 2012.
[21] S. D. Fihn, J. C. Blankenship, K. P. Alexander et al., “2014 ACC/AHA focused update of the guideline for the diagnosis and management of patients with stable ischemic heart disease,” *Circulation*, vol. 130, no. 19, pp. 1749–1767, 2014.
[22] X. You, I. Vlatkovic, A. Babic et al., “Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity,” *Nature Neuroscience*, vol. 18, no. 4, pp. 603–610, 2015.
[23] T. A. Patterson, E. K. Lobenhofer, S. B. Fulmer-Smentek et al., “Performance comparison of one-color and two-color platforms within the MicroArray Quality Control (MAQC) Project,” *Nature Biotechnology*, vol. 24, no. 9, pp. 1140–1150, 2006.
[24] Z. G. Lin F, Z. G. Chen, X. H Wang et al., “Network correlation of circRNA-miRNA and the possible regulatory mechanism in acute myocardial infarction,” *Zhonghua Yi Xue Za Zhi*, vol. 98, no. 11, pp. 851–854, 2018.
[25] F. Lin, H.-W. Chen, G.-A. Zhao et al., "Advances in research on the circRNA-miRNA-mRNA network in coronary heart disease treated with traditional Chinese medicine," Evidence-Based Complementary and Alternative Medicine, vol. 2020, pp. 1–10, 2020.

[26] J. Gan, J. Yuan, Y. Liu et al., "Circular RNA_101237 mediates anoxia/reoxygenation injury by targeting let7a5p/IGF2BP3 in cardiomyocytes," International Journal of Molecular Medicine, vol. 45, no. 2, pp. 451–460, 2020.

[27] M. Li, W. Ding, M. A. Tariq et al., "A circular transcript of ncX1 gene mediates ischemic myocardial injury by targeting miR-133a-3p," Theranostics, vol. 8, no. 21, pp. 5855–5869, 2018.

[28] C. P. d. C. Gomes, B. Schroen, G. M. Kuster et al., "Regulatory RNAs in heart failure," Circulation, vol. 141, no. 4, pp. 313–328, 2020.

[29] A. Kaura, V. Panoulas, B. Glampson et al., "Association of troponin level and age with mortality in 250,000 patients: cohort study across five UK acute care centres," BMJ, vol. 367, p. 16055, 2019.

[30] E. Gerbaud, R. Darier, M. Montaudon et al., "Glycemic variability is a powerful independent predictive factor of midterm major adverse cardiac events in patients with diabetes with acute coronary syndrome," Diabetes Care, vol. 42, no. 4, pp. 674–681, 2019.

[31] D. A. Gorog and G. Y. H. Lip, "Impaired spontaneous/endogenous fibrinolytic status as new cardiovascular risk factor?" Journal of the American College of Cardiology, vol. 74, no. 10, pp. 1366–1375, 2019.

[32] R. Kones, U. Rumana, and A. Morales-Salinas, "Confronting the most challenging risk factor: non-adherence," The Lancet, vol. 393, no. 10167, pp. 105–106, 2019.

[33] S. Yusuf, P. Joseph, S. Rangarajan et al., "Modifiable risk factors, cardiovascular disease, and mortality in 155,722 individuals from 21 high-income, middle-income, and low-income countries (PURE): a prospective cohort study," The Lancet, vol. 395, no. 10226, pp. 795–808, 2019.

[34] D. Vilades, P. Martinez-Camblor, A. Ferrero-Gregori et al., "Plasma circular RNA hsa_circ_0001445 and coronary artery disease: performance as a biomarker," The FASEB Journal, vol. 34, no. 3, pp. 4403–4414, 2020.

[35] I. Legnini, G. Di Timoteo, F. Rossi et al., "Circ-ZNF609 is a circular RNA that can be translated and functions in myogenesis," Molecular Cell, vol. 66, no. 1, pp. 22–37.e29, 2017.

[36] Y. Yang, X. Fan, M. Mao et al., "Extensive translation of circular RNAs driven by N6-methyladenosine," Cell Research, vol. 27, no. 5, pp. 626–641, 2017.

[37] K. Abdelmohsen, A. C. Panda, R. Munk et al., "Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by CircPABPN1," RNA Biology, vol. 14, no. 3, pp. 361–369, 2017.

[38] S. Van Heesch, F. Witte, V. Schneider-Lunitz et al., "The translational landscape of the human heart," Cell, vol. 178, no. 1, pp. 242–260.e229, 2019.

[39] V. N. S. Garikipati, S. K. Verma, Z. Cheng et al., "Circular RNA CircFndc3b modulates cardiac repair after myocardial infarction via FUS/VEGF-A axis," Nature Communications, vol. 10, no. 1, p. 4317, 2019.