

Research Article

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

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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, $P < 0.05$, fold change $FC \geq 2$) and 3 miRNAs (1 upregulated and 2 downregulated, $P < 0.05$, $FC \geq 1.2$) 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

TABLE 1: Patients characteristics ($\bar{x} \pm s$ group = 5).

	CG	UAP	AMI
Age (years)	55 ± 11	60 ± 11	54 ± 8
HR (min)	66 ± 9	71 ± 13	81 ± 10
SBP (mmHg)	149 ± 10	146 ± 11	130 ± 15
DBP (mmHg)	83 ± 12	79 ± 16	75 ± 6
Glu (3.9–6.1 mmol/L)	5.25 ± 7.82	5.34 ± 0.62	6.46 ± 1.92
CHO (0–5.2 mmol/L)	3.69 ± 0.88	3.99 ± 1.05	3.81 ± 0.37
TG (0.7–1.7 mmol/L)	1.26 ± 0.65	1.21 ± 0.41	1.27 ± 0.45
APOA1 (1–1.76 g/L)	1.13 ± 0.20	1.00 ± 0.10	0.89 ± 0.12
APOB (0.6–1.14 g/L)	0.72 ± 0.20	0.79 ± 1.84	0.71 ± 0.15
HDL (0.8–1.55 mmol/L)	1.11 ± 0.18	0.92 ± 0.20	0.95 ± 0.12
LDL (1.64–3.62 mmol/L)	2.25 ± 0.66	2.54 ± 0.92	2.52 ± 0.44
AST (15–50 U/L)	21.60 ± 4.98	25.60 ± 4.22	96.80 ± 65.06
LDH (313–618 U/L)	149.20 ± 17.20	162.60 ± 29.59	449.00 ± 265.70
CK (55–170 U/L)	48.00 ± 13.21	70.80 ± 35.09	817.60 ± 535.16
CK-MB (0–25 U/L)	15.80 ± 7.22	16.80 ± 4.23	74.40 ± 48.55
Gensini score (%)	1.00 ± 1.41	65.40 ± 49.09	51.90 ± 10.37

APOA1 = apolipoprotein A1; APOB = apolipoprotein B; CHO = total cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein; AST = aspartate transaminase; LDH = lactate dehydrogenase; CK = creatine kinase; CK-MB = creatine kinase isoenzyme MB.

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

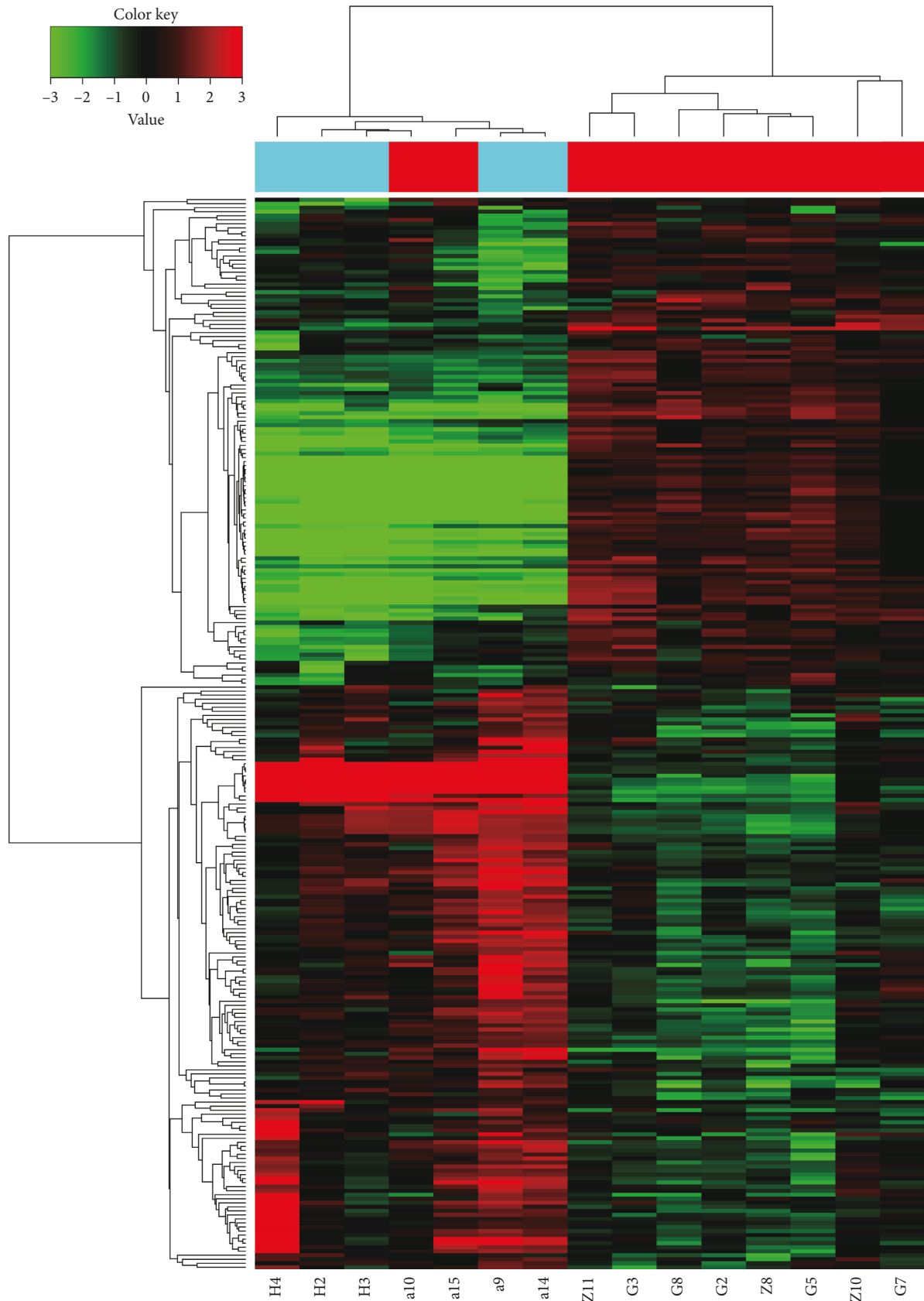


FIGURE 1: Heatmap of circRNAs with differential expression between groups. The columns represent patients, and the rows represent the degree to which a gene was expressed at different copy numbers. The colour key in the top left indicates the expression level (red indicates upregulation; green indicates 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.

TABLE 2: Expression profiles of 121 circRNAs that were upregulated ($P < 0.05$, $FC \geq 2$).

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_circ0140759	0.00078	145.55	UTY	15466882	15481229	-	95	6
3	hsa_circ0140760	0.00139	93.25378	UTY	15467172	15471765	-	100	66
4	hsa-circ16316-13	0.00141	88.45529	UTY	15447442	15478273	-	100	17
5	hsa_circ0140758	0.00147	80.8945	UTY	15447442	15448215	-	100	3
6	hsa-circ16316-4	0.00199	61.61567	UTY	15466882	15472408	-	85	5
7	hsa-circ16316-11	0.00116	44.99736	UTY	15471646	15471866	-	11	—
8	hsa_circ0140781	0.00129	26.7885	KDM5D	21901413	21903743	-	49	1
9	hsa_circ0140736	0.00149	24.01664	USP9Y	14821320	14885859	+	100	7
10	hsa_circ0003368	0.00689	22.6279	UTY	15478146	15481229	-	9	—
11	hsa-circ16316-9	0.00064	21.13425	UTY	15435434	15438230	-	43	4
12	hsa_circ0009024	0.00047	17.24446	—	21749095	21749393	+	25	—
13	hsa_circ0140746	0.00106	16.81741	USP9Y	14870435	14885859	+	42	—
14	hsa-circ16316-2	0.00316	16.45998	UTY	15435434	15481229	-	100	24
15	hsa-circ16316-12	0.00040	15.87258	UTY	15435434	15448215	-	100	7
16	hsa_circ0140783	0.00160	15.52771	—	22669237	22683186	-	100	100
17	hsa-circ16316-1	0.01056	14.98803	UTY	15478146	15508852	-	12	—
18	hsa_circ0007907	0.00053	13.54894	ZFY	2829114	2829687	+	25	—
19	hsa_circ0140780	0.00062	12.87783	KDM5D	21901413	21901548	-	12	—
20	hsa-circ16316-7	0.00914	12.75820	UTY	15522872	15526673	-	15	—
.....
121	hsa_circ0076034	0.00770	2.00799	LEMD2	33744730	33756906	-	100	100

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.

Heart Center of Xinxiang Medical University. Total RNA was extracted from 250 μ l of whole blood with a 750 μ l extraction kit (TRIpure LS Reagent, CapitalBio, Beijing, China) and was cryopreserved at -80°C . Total RNA was extracted and reverse transcribed for the synthesis of first- and second-strand cDNA. *In vitro* transcription and synthesis of cRNA were conducted, and cRNA was transcribed to generate cDNA, which was simultaneously fluorescently labelled using the Ambion WT Expression kit. A Crystal Core[®] CapitalBiotech Human CircRNA Array V2.0 (4 \times 180 K) chip was used to analyse circRNAs. The circRNA target sequences were all from circBase (<http://www.circbase.org/>) and deepBase (<http://rna.sysu.edu.cn/deepBase/browser.php>). Human miRNA Microarray chips (8 \times 60 K) (release 21.0; Agilent Technologies, Inc., Santa Clara, CA, USA) were used for the microarray analysis. The raw data were normalized by the quantile algorithm using GeneSpring Software v12.6 (Agilent Technologies, Inc.) [22, 23].

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 $FC \geq 1.2$ 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 3: Expression profiles of 145 circRNAs that were downregulated ($P < 0.05$, $FC \geq 2$).

No.	ProbeName	P	FC (abs)	geneSymbol	circStrat	circEnd	Strand	miRNA number	miRNA number more than 1
1	hsa_circ0140537	0.001857	66.81375	—	73044457	73044598	—	6	—
2	hsa_circ_0091074	0.001099	62.43283	—	73048902	73051109	—	59	—
3	hsa_circ0140538	0.001115	53.70956	—	73045949	73051109	—	100	100
4	hsa_circ0091073	0.001317	38.93487	—	73040494	73051109	—	100	100
5	hsa_circ0140539	0.001931	27.13039	—	73045949	73057338	—	100	100
6	hsa_circ16166-3	0.001541	23.23273	—	73050900	73053209	—	39	—
7	hsa_circ16166-1	0.001702	20.40522	—	73050900	73057338	—	50	1
8	hsa_circ0140540	0.001844	15.58513	—	73045949	73061308	—	100	100
9	hsa_circ0140536	0.001075	13.07049	—	73044087	73044570	—	16	—
10	hsa_circ0140541	0.00105	6.618035	—	73046801	73046954	—	11	—
11	hsa_circ13156-4	0.029169	5.155549	MRPL39	26966204	26976247	—	39	—
12	hsa_circ0107597	0.005089	4.704399	ABCA5	67270099	67305564	—	100	6
13	hsa_circ0061370	0.028818	4.348159	CCT8	30428647	30434877	—	73	—
14	hsa_circ0058143	0.027599	4.206255	FN1	216279383	216286966	—	92	3
15	hsa_circ0003086	0.024972	4.089472	—	64630738	65139334	+	100	100
16	hsa_circ0140697	0.038202	3.944226	KLHL4	86919763	86924916	+	100	36
17	hsa_circ0052193	0.018474	3.741495	PTPRH	55692614	55699536	—	100	87
18	hsa_circ0140547	0.01636	3.64521	—	73071865	73072197	+	42	5
19	hsa_circ0140553	0.019321	3.624559	—	73071957	73072197	+	38	4
20	hsa_circ0140549	0.018403	3.623656	—	73071909	73072197	+	41	5
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145	hsa_circ_0046285	0.046716	2.004205	PYCR1	79890268	79894968	—	100	100

TABLE 4: MiRNAs expression profiling ($P < 0.05$, $FC \geq 1.2$).

No.	ProbeName	P	FC (abs)	Regulation
1	Hsa-miR-4299	0.016	9.07	Up
2	Hsa-miR-20b-5p	0.033	1.29	Down
3	Hsa-miR-363-3p	0.046	1.21	Down

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

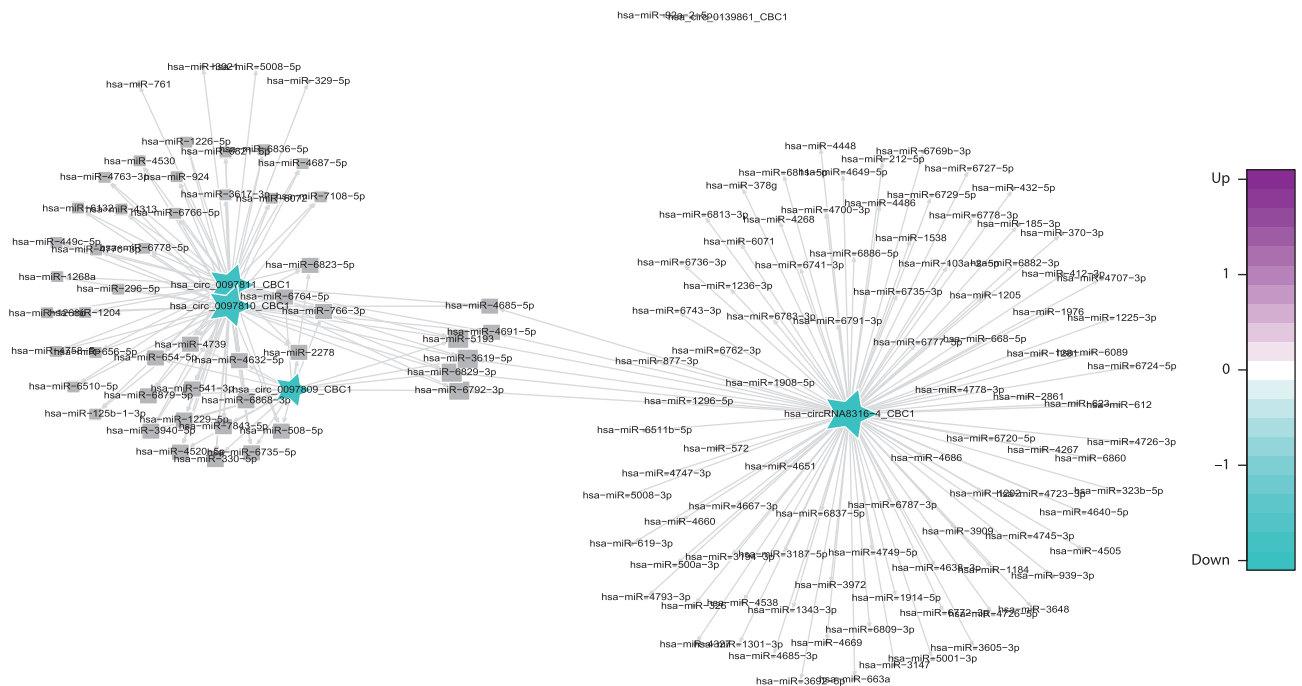


FIGURE 2: Comparison of circRNA-miRNA prediction network maps between the AMI and UAP group. Prediction of miRNAs that may be bound by circRNA and construction of a circRNA-miRNA network. According to the relationship between circRNAs and target miRNAs, the top circRNAs with the most FCs were selected to construct the circRNA-miRNA network map. The squares represent miRNAs and the pentagrams represent circRNAs, where green indicates downregulation and purple indicates upregulation.

TABLE 5: Differential expression of circRNAs in circRNA-miRNA network*.

No.	Gene expression	AMI vs UAP	CG vs AMI	CG vs UAP	CG, AMI, and UAP
1	Upregulated		Hsa_circ_0140758	hsa_circ_0140758	hsa_circ_0140758
2			Hsa_circ_0140759	hsa_circ_0140759	hsa_circ_0140759
3			Hsa_circ_0140760	hsa_circ_0140760	hsa_circ_0140760
4			Hsa_circ_16316-13	hsa_circ_16316-13	hsa_circ_16316-13
5			Hsa_circ_16316-10	hsa_circ_0140736	hsa_circ_16316-10
6				Hsa_circ_16316-4	hsa_circ_0140781
1	Downregulated	hsa_circ_8316-4	Hsa_circ_0140538	hsa_circ_0140538	hsa_circ_0140538
2		hsa_circ_0097809		hsa_circ_0091073	
3		hsa_circ_0097811			
4		hsa_circ_0097810			
		hsa_circ_0139861			

* $P < 0.05$, up $FC \geq 2$, down $FC \geq -2$.

TABLE 6: Analysis of the number of source genes (circRNA) and the number of target gene miRNAs.

No.	AMI and UAP		CG and UAP		AMI and CG		CG, AMI, and UAP	
	SGS (No.)	TGS	SGS (No.)	TGS	SGS (No.)	TGS	SGS (No.)	TGS
1	10	hsa-miR-6832-5p	4	hsa-miR-101-5p	5	hsa-miR-4299	15	hsa-miR-4299
2	19	hsa-miR-1973			4	hsa-miR-6832-5p		
3	13	hsa-miR-4485-3p			8	hsa-miR-1973		
4	2	hsa-miR-664a-5p			1	hsa-miR-4485-3p		
5	3	hsa-miR-3912-5p			4	hsa-miR-3912-5p		
6	1	hsa-miR-8063			6	hsa-miR-8063		
7	1	hsa-miR-3663-3p			4	hsa-miR-6793-5p		
8	4	hsa-miR-1268a			331	hsa-miR-6749-5p		
9	4	hsa-miR-6793-5p			3	hsa-miR-328-5p		
10	1	hsa-miR-34a-5p			6	hsa-miR-6716-3p		
11	1	hsa-miR-6716-3p			320	hsa-miR-1202		

geneSymbol is the gene abbreviation. SGS: source geneSymbol; TGS: target geneSymbol.

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 ($P < 0.05$). We found pathways that may be involved in ACS: dilated cardiomyopathy (hsa05141), transcriptional misregulation in cancer (hsa05202), amoebiasis (hsa05146), Fanconi anaemia pathway (hsa03460), hypertrophic cardiomyopathy (hsa05410), and arrhythmic right-ventricular cardiomyopathy (hsa05412) ($P < 0.05$; 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

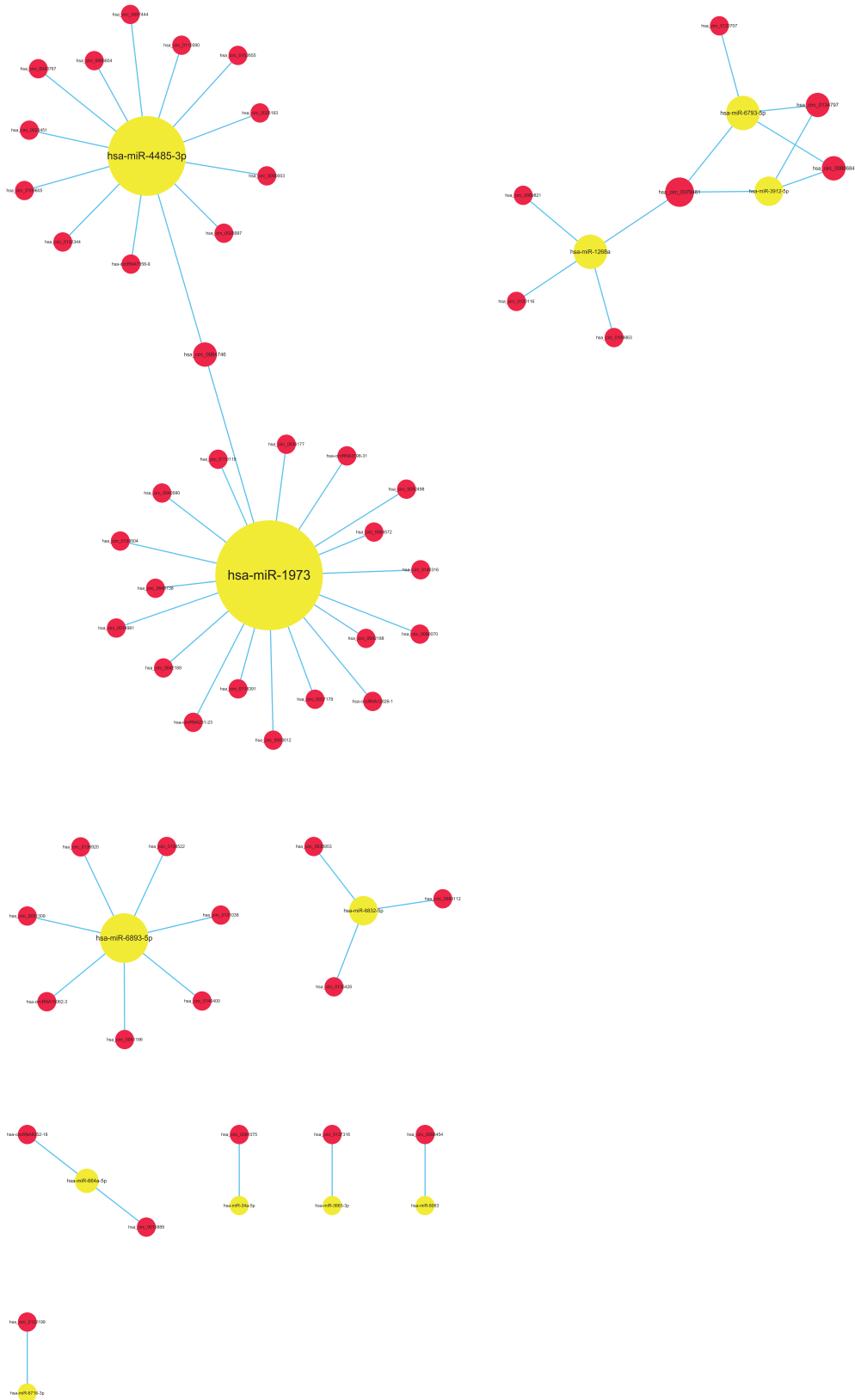


FIGURE 6: Comparison of circRNA-miRNA (red-yellow) coexpression maps between the AMI and UAP groups. These images depict the number of circRNAs that can bind to the bound target gene miRNA. The circRNAs have the same trend as miRNA changes, or the correlation is relatively close.

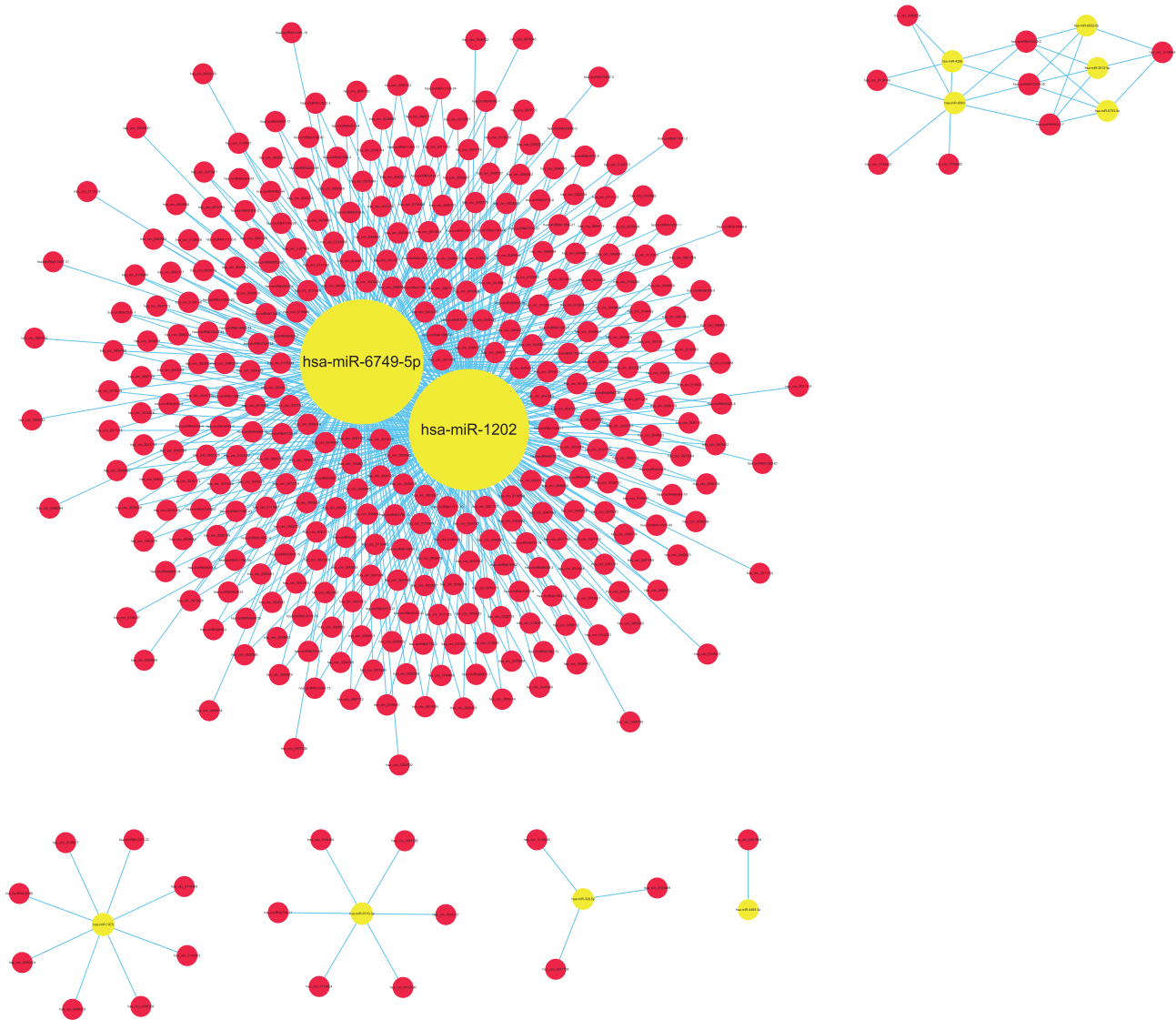


FIGURE 7: Comparison of circRNA-miRNA (red-yellow) coexpression maps between the CG and AMI groups. These images depict the number of circRNAs that can bind to the bound target gene miRNA. The circRNAs have the same trend as miRNA changes, or the correlation is relatively close.

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 ($P < 0.05$, $FC \geq 2$), 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) and a total of 110 circRNAs were identified in the CHD group (73 upregulated and 73

downregulated) ($P < 0.05$, $FC \geq 2.0$). 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,

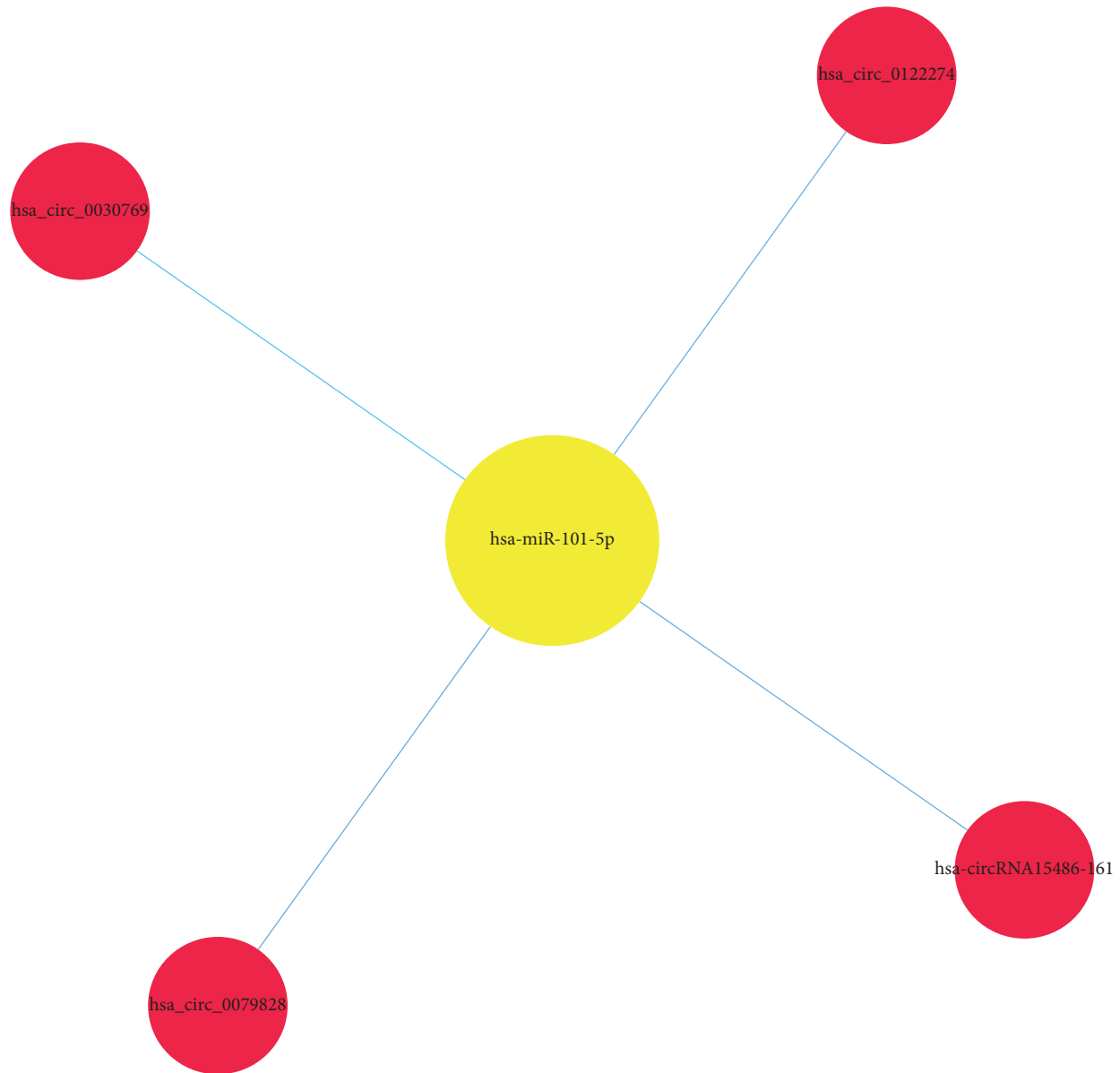


FIGURE 8: Comparison of circRNA-miRNA (red-yellow) coexpression maps between the CG and UAP groups. These images depict the number of circRNAs that can bind to the bound target gene miRNA. The circRNAs have the same trend as miRNA changes, or the correlation is relatively close.

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]. Thus, 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

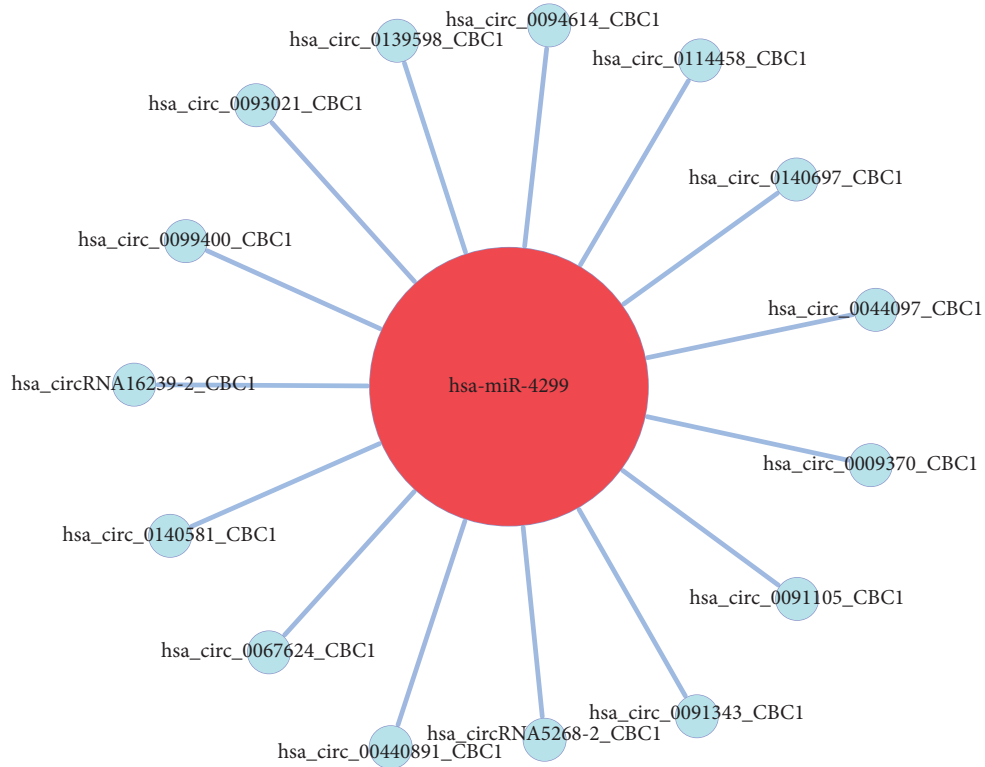


FIGURE 9: Comparison of circRNA-miRNA (blue-red) coexpression maps between the CG, AMI, and UAP groups. These images depict the number of circRNAs that can bind to the bound target gene miRNA. The circRNAs have the same trend as miRNA changes, or the correlation is relatively close.

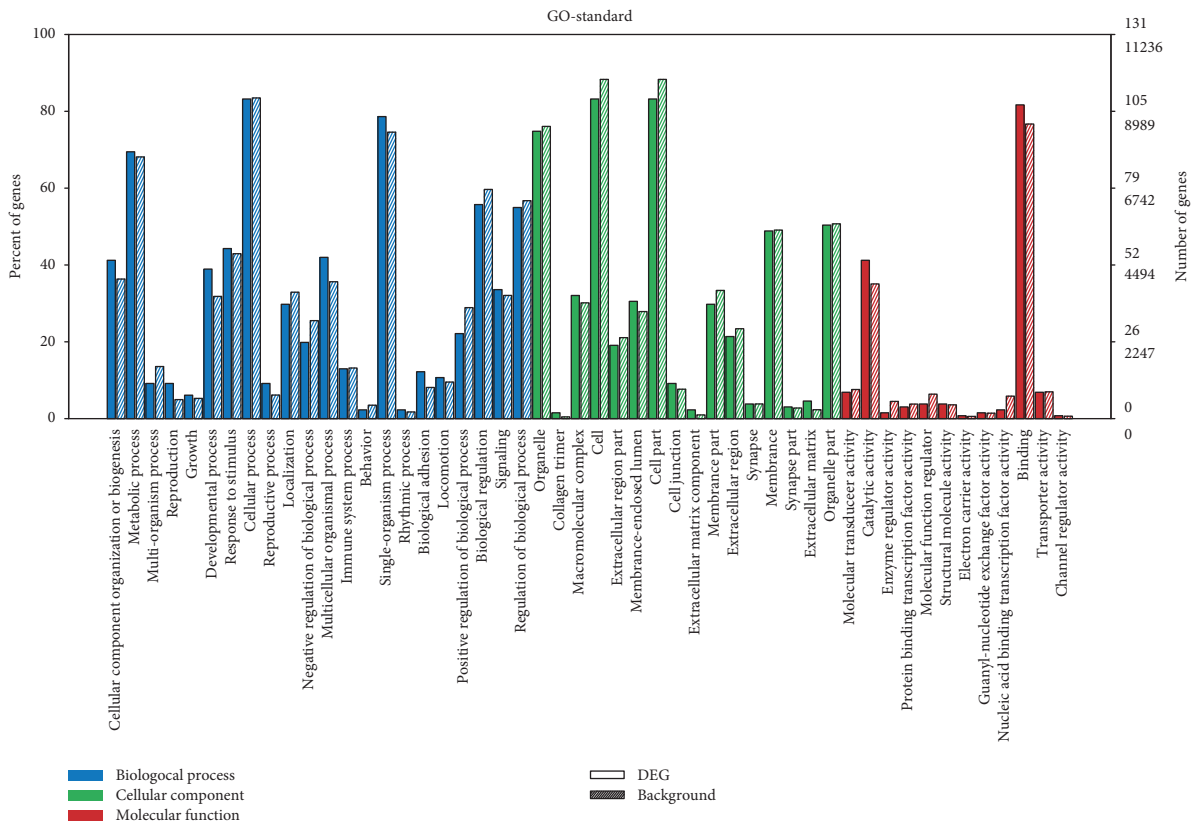


FIGURE 10: Functional annotation of differentially expressed genes (DEGs). It shows the enrichment of DEGs compared with the background genes.

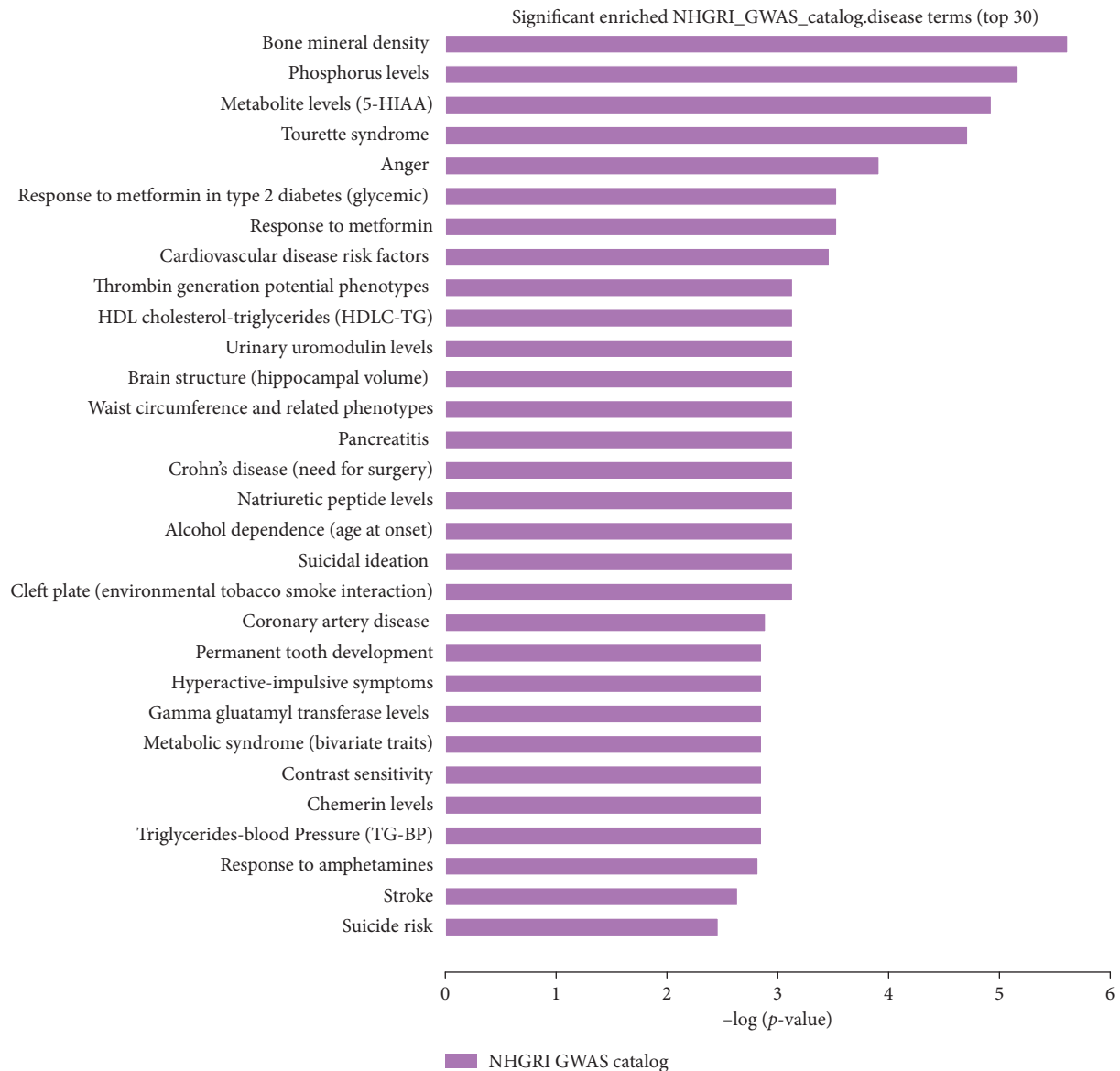


FIGURE 11: Functional annotation of differentially expressed genes (DEGs). Disease ontology analysis was used to select the top 30 terms that were significantly enriched, and the P values were then used to sort the top plots.

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

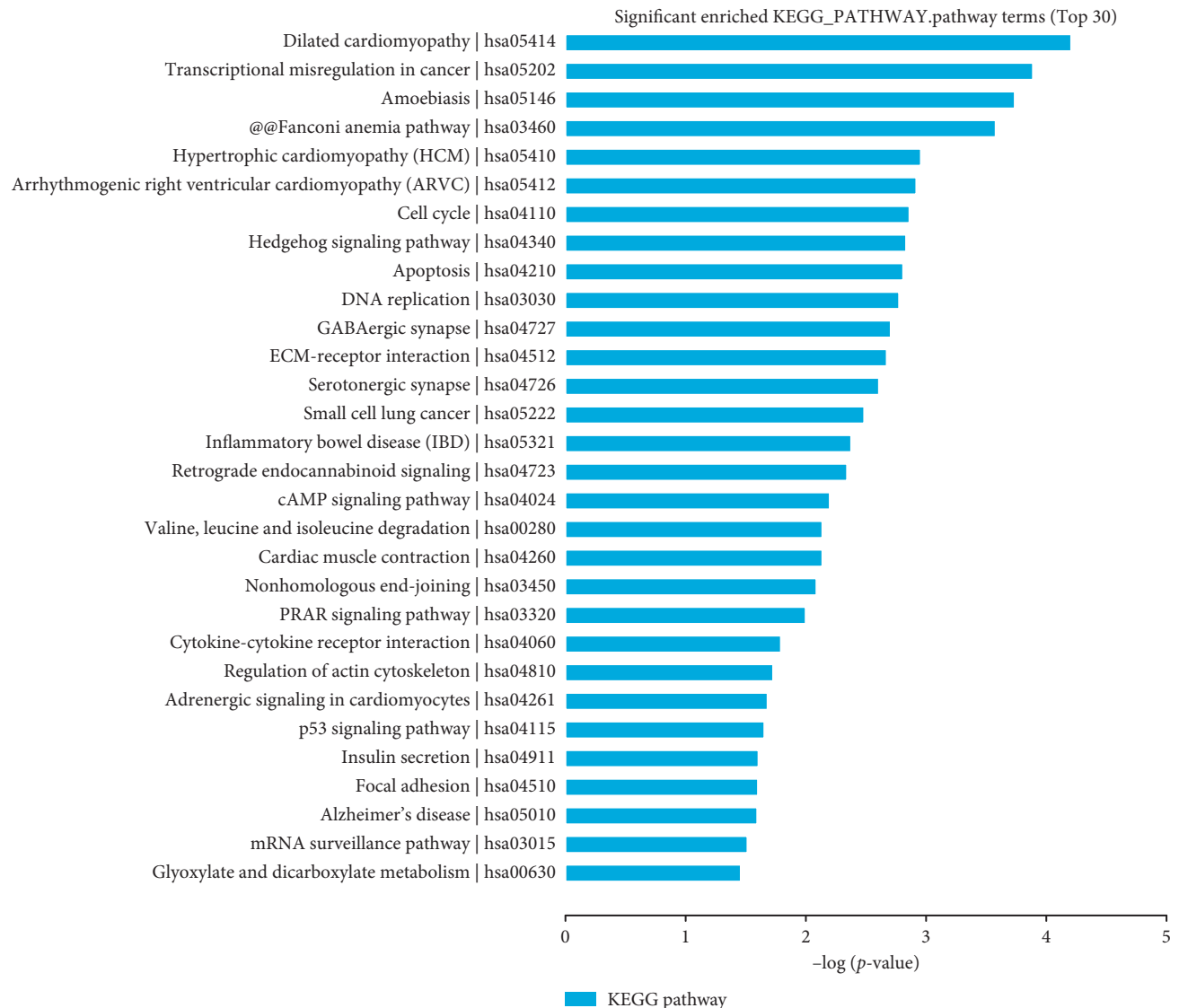


FIGURE 12: Functional annotation of differentially expressed genes (DEGs). The KEGG pathway analysis was used to select the top 30 terms that were significantly enriched, and the P values were then used to sort the top plots.

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.

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