Research on the circular RNA bioinformatics in patients with acute myocardial infarction

Lianli Yin1 | Yinghua Tang2 | Minghe Jiang3

Abstract

Objective: Through the detection of circular RNA (circRNA) using expression profiling chips, we searched for circRNAs related to acute myocardial infarction (AMI) and explored their relationship and possible mechanisms with AMI.

Method: The study subjects included 3 AMI patients and 3 controls, and circRNA expression profiling analysis was performed using a microarray gene chip to identify circRNAs with large differences in expression between groups and to construct a circRNA-miRNA network.

Results: Compared with the control group, there were 650 differentially expressed circRNAs found in AMI patients (P < .05, fold change > 2), including 535 up-regulated circRNAs, such as hsa_circ_0050908, hsa_circRNA4010-22, hsa_circ_0081241, hsa_circ_0010551, hsa_circRNA4010-20, hsa_circRNA14702, hsa_circ_0115392, has_circRNA1825-44, has_circRNA8493-7, and hsa_circ_0025097. Furthermore, there were 115 down-regulated circRNAs, such as hsa_circ_0066439, hsa_circ_0054211, hsa_circ_0095920, hsa_circ_0122984, hsa_circ_0113067, hsa_circ_0039155, hsa_circRNA4014-45, hsa_circ_0122979, hsa_circ_0059665, and hsa_circ_0009319. The circRNAs hsa_circ_0066439, hsa_circ_0095920, and hsa_circ_0122984 can regulate multiple signal pathways to participate in the AMI process through hsa-miR-1254, hsa-miR-328-5p, and other miRNAs. In addition, the expression of circRNA-miRNA in peripheral blood is related to the network. Differentially expressed circRNAs are involved in chromatin organization, chromatin-modifying enzymes, signal transduction, lysine degradation, the mitogen-activated protein kinase (MAPK) signaling pathway, focal adhesion, and a variety of other pathways, such as myocardial infarction, coronary heart disease, hypertension, and other diseases. The gene ontology analysis results show that molecular function mainly involves binding and molecular structural activity, whereas the biological process mainly involves a single biological process, a cellular component for organization, and a cellular process, and the cellular component mainly involves a protein complex, an extracellular matrix, and a membrane.

Conclusion: circRNA and microRNA interact to participate in the development of AMI. circRNA may be involved in the pathogenesis of AMI.

Keywords
acute myocardial infarction, bioinformatics, circRNA
1 | INTRODUCTION

In recent years, the incidence, disability, and mortality in cardiovascular diseases (CVDs) have increased dramatically, and the main cause of death in CVDs is acute myocardial infarction (AMI). Although significant progress has been made in the diagnosis and treatment of AMI, effective therapeutic targets for the protection of myocardial cells from apoptosis remain limited. Therefore, there is an urgent need to understand the pathogenesis of AMI at the molecular level. Most important is the need for the discovery of novel molecular entities for AMI-related apoptosis. Recently, non-coding RNA (ncRNA) has been suggested as a biomarker for AMI. Circular RNA (circRNA) is a type of ncRNA that exists in the form of a covalently closed continuous loop and is stably expressed in many types of cells, and its ability to regulate gene expression comes mainly from its binding or inhibiting of microRNA (miRNA). Several studies have demonstrated the key role of circRNA in heart development and physiology. The abnormal expression of circRNA has been linked to CVDs such as heart failure, myocardial infarction, and atherosclerosis, suggesting the potential importance of circRNA in these pathological conditions. However, there have been few studies on the correlation between circRNA and AMI. In this study, a microarray gene chip was used to analyze the circRNA expression profile of patients with AMI, and the circRNA with a large expression difference from the control group was analyzed. By searching for candidate circRNA related to AMI, a new promising breakthrough point was provided for AMI diagnostic markers or targeted therapy, and key information was provided for revealing the complex regulatory mechanism of A-MI.

2 | MATERIALS AND METHODS

2.1 | Subject selection and sample collection

Selection of study subjects: The study selected 3 patients diagnosed with AMI and 3 healthy subjects.

Inclusion and exclusion criteria: The diagnostic criteria for AMI were as follows 

1. Typical severe sternum pain with a duration of more than 30 minutes: The clinical manifestations are dull pain, squeezing pain in the posterior sternum or precordial area, and radioactive pain lasting more than 30 minutes in the neck, shoulder, and back, accompanied by sweating and dying. Furthermore, there may be clinical manifestations of heart failure or cardiogenic shock.

2. Typical dynamic changes of the patient’s electrocardiogram: The patient’s relevant signs were dynamically monitored by electrocardiogram, and the results of the electrocardiogram showed pathological abnormal Q waves and ST segment elevation. Although the electrocardiogram did not have pathological Q waves or ST segment elevation, it showed that the T wave and ST segment had an ischemic and dynamically changing performance.

3. Increased levels of creatinine kinase isoenzyme MB, myoglobin, and cardiac troponin I.

4. Emergency coronary angiography or percutaneous coronary intervention (PCI), confirming that at least one of the three main coronary arteries had a luminal stenosis greater than 50%.

By meeting 2 of the 4 criteria above, the diagnosis of AMI can be made.

Exclusion criteria were as follows: previous old myocardial infarction or PCI; acute or chronic infection; hematological or systemic immune diseases; severe heart, kidney, liver, and lung dysfunction; combined stroke; combined thyroid disease; previous various organ transplants; known or treated malignant tumors; no family history of other cardiovascular-related diseases or hypertension, diabetes, etc. The definition of hypertension is based on the presence of a systolic blood pressure of ≥140 mmHg and a diastolic blood pressure of ≥90 mmHg measured on different days or currently using anti-hypertensive drugs. The definition of diabetes is based on clinical characteristics and the requirements of dietary treatment or drug treatment to control blood sugar. Hyperlipidemia is defined as the total serum cholesterol level of ≥5.2 mmol/L, a triglyceride level of ≥1.7 mmol/L, or low-density lipoprotein of ≥2.6 mmol/L, or taking statins.

The control group was a healthy population. All selected persons signed an informed consent form. All subjects had blood samples taken and combined with ethylenediaminetetraacetic acid (EDTA) anticoagulant within 3 h of admission, which were centrifuged at 3000 r/min for 10 min within 4 h, and collected plasma and centrifuged at 2000 r/min for 10 min. The separated supernatant was stored at −80°C. The study was carried out in accordance with the ethics committee of the Institute and Ethical Committees.

2.2 | RNA extraction

The total RNA from the each patient’s plasma was extracted using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions. In accordance with the manufacturer’s procedures, a mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) was used for purification. The purity and concentration of the RNA were determined from a 260/280 reading by using a spectrophotometer (NanoDrop Nd-1000, Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was determined with a lab-on-a-chip kit using an RNA 6000 nano chip and Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) to assess RNA quality comprehensively.

2.3 | RNA amplification, labeling, and hybridization

Fluorescent dye Cy3 dCTP-labeled cDNA was prepared using Eberwine linear RNA amplification and enzymatic reaction. In addition, we used a labeling kit (CapitalBio, Beijing, China) and
CapitalBio-cRNA amplification to produce higher yields of labeled cDNA according to the manufacturer’s instructions.

2.4 | Microarray imaging and data analysis

Data normalization and difference analysis were performed on the circRNA array data using GeneSpring software V13.0 (Agilent Technologies, Santa Clara, CA, USA). The data results were analyzed for data summarization, normalization, and quality control. These circRNA target sequences were obtained from Circbase, Deepbase, and Rybak-Wolf (2015).\(^{11}\) In order to select differentially expressed genes, thresholds of > 2 and < -2-fold changes were used in this study, and the P-value corrected by the Benjamini-Hochberg procedure was 0.05. Using the adjust data function of Cluster 3.0 (Stanford University, Stanford, CA, USA) software, the data were log2-transformed, and the median location was centered on the gene, and then, hierarchical clustering with average linkage was used for further analysis. Finally, Java TreeView (Stanford University School of Medicine, Stanford, CA, USA) was used for tree visualization.

2.5 | Construction of the circRNA-miRNA network

After the differentially expressed circRNAs were screened, StarBase (Sun Yat-sen University, China) software was used to predict the target miRNAs of the circRNAs screened in the AMI group to obtain a list of miRNAs. circRNA plays an important role in miRNA function and transcriptional control by acting as a competitive endogenous RNA or positive regulator on its parent-encoded gene. A circRNA-miRNA network was constructed based on miRanda database prediction (http://mirdb.org/). These circRNA-miRNA pairs were selected to construct the network using the open source bioinformatics software Cytoscape (National Institute of General Medical Sciences, USA). In network analysis, degree centrality is defined as the link number between one node and another node. Degree is the simplest and most important measure of gene centrality in a network of relative importance.\(^{12}\)

2.6 | Enrichment analysis

The NHGRI GWAS Catalog (http://www.genome.gov/gwastudiey/), KEGG DISEASE (http://www.genome.jp/kegg/disease/), and OMIM (http://www.ncbi.nlm.nih.gov/omim) bioinformatics databases were used to search for the disease enrichment analysis of the genes that were significantly expressed in the whole blood samples of the patients with AMI, and \(P < .05\) was considered significant. The differentially expressed circRNAs in the whole blood of subjects were analyzed by pathway enrichment analysis using the Reactome, KEGG PATHWAY, BioCyc, and PANTHER databases, and \(P < .05\) was considered as a meaningful analysis. Similarly, gene ontology analysis was performed on the linear mRNA transcripts corresponding to the 650 differentially expressed circRNAs selected from the specimens of the AMI group. The analysis included mainly molecular functions, biological processes, and cellular components.

2.7 | Statistical analysis

Basic data were gathered regarding the age, weight, height, blood pressure, and blood lipids of the patients in the control and AMI groups. Statistical calculations were performed using SPSS (version 23.0, IBM, USA) software, and continuous data were expressed as the mean + standard deviation (\(\bar{x} \pm s\)). The t test was used to compare the continuous variables between the two groups, and the categorical variables were expressed as counts, while the chi-square test was used to compare the categorical variables between the two groups. \(P < .05\) was considered statistically significant.

3 | RESULTS

3.1 | Population parameters of the subjects

The population parameters of the subjects are shown in Table 1. It can be seen from this table that there were no statistically significant differences for age, body mass index, systolic blood pressure, diastolic blood pressure, or blood lipid biochemistry between the AMI group and control group (\(P > .05\)).

3.2 | Microarray gene chip analysis identified significantly different circRNAs

In order to understand the molecular mechanisms involved and to search for AMI biomarkers, a microarray gene chip was used to screen and analyze the circRNA expression profiles of our study. A total of 13,804 circRNAs were identified through the gene chip analysis of circRNA expression profiles (Figure 1A, Figure 1B). As compared to the control group, 650 circRNAs were screened for differential expression in the AMI group (Figure 1B), among which 535 circRNAs were up-regulated (the top ten up-regulated circRNAs were as follows: hsa_circ_0050908, hsa_circRNA4010-22, hsa_circ_0081241, hsa_circ_0010551, hsa_circRNA4010-20, hsa_circRNA14702, hsa_circ_0115392, hsa_circRNA1825-44, hsa_circRNA8493-7, and hsa_circ_0025097) and 115 circRNAs were down-regulated (the top ten down-regulated circRNAs were as follows: hsa_circ_0066439, hsa_circ_0054211, hsa_circ_0095920, hsa_circ_0122984, hsa_circ_0113067, hsa_circ_0039155, hsa_circRNA4014-45, hsa_circ_0122979, hsa_circ_0059665, and hsa_circ_0009319). It can be seen that circRNA may play a certain role in the occurrence and progression of AMI (Table 2, Figure 1C).
TABLE 1 Subject baseline data

| Groups | n | Age (years) | BMI (kg/m2) | SBP (mmHg) | DBP (mmHg) | CHO (mmol/l) | TG (mmol/l) |
|--------|---|-------------|-------------|-----------|-----------|-------------|-------------|
| Control | 3 | 47 ± 10.54 | 24.42 ± 1.73 | 86.23 ± 3.42 | 135.77 ± 5.57 | 4.14 ± 0.4 | 1.55 ± 0.06 |
| AMI    | 3 | 45.67 ± 9.29 | 25.74 ± 1.21 | 84.90 ± 4.56 | 132.9 ± 3.96 | 3.98 ± 0.23 | 1.66 ± 0.06 |

AMI, acute myocardial infarction; APOA1, apolipoprotein A1; APOB, apolipoprotein B; BMI, body mass index; CHO, cholesterol; CK-MB, creatine kinase isoenzyme MB; CTnI, cardiac troponin I; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MYO, myoglobin; SBP, systolic blood pressure; TG, triglyceride.

*P < .05.

FIGURE 1 Profiling of circular RNAs in the plasmas from AMI patients and normal controls. (a) Cluster analysis of gene expression between AMI patients and control samples. Each column represents the expression profile of a tissue sample, and each row corresponds to a circRNA. High expression level is indicated by red and lower levels by green. (b) Volcano plot shows the up-regulated and down-regulated circRNAs in AMI patients vs control group. Higher expression levels are indicated by red, lower expression levels are indicated by green, and no significant difference is indicated by black. (c) Circos diagram of the difference circRNA between the control group and the AMI group. The length of the bar indicates the multiple of the differential gene, the red bar in the inner circle indicates that the differential gene is up-regulated, and the green bar indicates that the differential gene is down-regulated.
| Number | ProbeName   | P     | FC (abs)  | Regulation | Chromosome | miRNA number more than 2 |
|--------|-------------|-------|----------|------------|------------|-------------------------|
| 1      | hsa_circ_0050908 | 0.028 | 7.9732   | up         | chr19      | -                       |
| 2      | hsa_circRNA4010-22 | 0.0102 | 7.7393   | up         | chr19      | 2                       |
| 3      | hsa_circ_0081241  | 0.038 | 7.5621   | up         | chr7       | 8                       |
| 4      | hsa_circ_0010551  | 0.0283 | 7.4077   | up         | chr1       | 100                     |
| 5      | hsa_circRNA4010-20 | 0.0182 | 6.8538   | up         | chr19      | -                       |
| 6      | hsa_circRNA14702  | 0.015 | 6.8083   | up         | chr6       | -                       |
| 7      | hsa_circ_0115392  | 0.0001 | 6.6244   | up         | chr20      | -                       |
| 8      | hsa_circRNA1825-44 | 0.0383 | 6.5754   | up         | chr12      | 1                       |
| 9      | hsa_circRNA8493-7 | 0.058 | 6.5741   | up         | chr1       | -                       |
| 10     | hsa_circ_0025097  | 0.0361 | 6.1700   | up         | chr12      | 100                     |
| 11     | hsa_circ_0097682  | 0.0013 | 6.1357   | up         | chr12      | -                       |
| 12     | hsa_circ_0003531  | 0.0352 | 5.9584   | up         | chr3       | -                       |
| 13     | hsa_circ_0049121  | 0.0474 | 5.8384   | up         | chr19      | 3                       |
| 14     | hsa_circ_0060784  | 0.0069 | 5.8181   | up         | chr20      | 17                      |
| 15     | hsa_circ_0044537  | 0.0334 | 5.7443   | up         | chr17      | 86                      |
| 16     | hsa_circ_0016320  | 0.0488 | 5.7141   | up         | chr1       | 100                     |
| 17     | hsa_circ_0015063  | 0.0009 | 5.6756   | up         | chr1       | 10                      |
| 18     | hsa_circ_0050906  | 0.0191 | 5.6475   | up         | chr19      | -                       |
| 19     | hsa_circ_0135204  | 0.0151 | 5.6078   | up         | chr7       | 8                       |
| 20     | hsa_circ_0020887  | 0.0000 | 5.5918   | up         | chr11      | 26                      |
| 21     | hsa_circ_0126393  | 0.0468 | 5.4984   | up         | chr4       | -                       |
| 22     | hsa_circ_0000946  | 0.0333 | 5.4939   | up         | chr19      | 100                     |
| 23     | hsa_circ_0080478  | 0.0025 | 5.4834   | up         | chr7       | 75                      |
| 24     | hsa_circ_0075752  | 0.0378 | 5.4398   | up         | chr6       | 10                      |
| 25     | hsa_circ_0118836  | 0.0048 | 5.4305   | up         | chr2       | -                       |
| 26     | hsa_circ_0074348  | 0.0478 | 5.4190   | up         | chr5       | -                       |
| 27     | hsa_circ_0114870  | 0.0424 | 5.4090   | up         | chr20      | 1                       |
| 28     | hsa_circ_0043563  | 0.0357 | 5.4019   | up         | chr17      | 54                      |
| 29     | hsa_circ_0081150  | 0.0458 | 5.3873   | up         | chr7       | 16                      |
| 30     | hsa_circ_0114865  | 0.0432 | 5.3303   | up         | chr20      | 2                       |
| 31     | hsa_circ_0096364  | 0.0486 | 5.3301   | up         | chr11      | 16                      |
| 32     | hsa_circRNA12871-14 | 0.0008 | 5.2871   | up         | chr2       | -                       |
| 33     | hsa_circ_0007326  | 0.0098 | 5.2759   | up         | chr14      | 2                       |
| 34     | hsa_circ_0115190  | 0.0438 | 5.2530   | up         | chr20      | 17                      |
| 35     | hsa_circ_0041218  | 0.0487 | 5.2061   | up         | chr17      | 90                      |

(Continues)
| Number | ProbeName            | P     | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|----------------------|-------|----------|------------|------------|--------------------------|
| 36     | hsa_circRNA5200-8   | .0372 | 5.1911   | up         | chr21      | 4                        |
| 37     | hsa_circRNA5199-28  | .0303 | 5.1872   | up         | chr21      | 2                        |
| 38     | hsa_circ_0026362    | .0161 | 5.1638   | up         | chr12      | -                        |
| 39     | hsa_circ_0000542    | .0329 | 5.1468   | up         | chr14      | 2                        |
| 40     | hsa_circ_0004867    | .0293 | 5.1261   | up         | chr15      | 91                       |
| 41     | hsa_circ_0041635    | .0398 | 5.1003   | up         | chr17      | 40                       |
| 42     | hsa_circ_0026432    | .0047 | 5.0848   | up         | chr12      | 1                        |
| 43     | hsa_circ_0081616    | .0375 | 5.0751   | up         | chr7       | 100                      |
| 44     | hsa_circRNA12789-2  | .0468 | 4.9916   | up         | chr2        | -                        |
| 45     | hsa_circ_0016846    | .0150 | 4.9841   | up         | chr1        | 6                        |
| 46     | hsa_circ_0058306    | .0385 | 4.9675   | up         | chr2        | 33                       |
| 47     | hsa_circRNA7287-4   | .0460 | 4.9535   | up         | chr7        | 1                        |
| 48     | hsa_circ_0017921    | .0045 | 4.9350   | up         | chr10       | 9                        |
| 49     | hsa_circRNA12245-5  | .0005 | 4.9070   | up         | chr19       | 9                        |
| 50     | hsa_circ_0110254    | .0002 | 4.8930   | up         | chr1        | 2                        |
| 51     | hsa_circRNA12245-25 | .0001 | 4.8891   | up         | chr19       | 6                        |
| 52     | hsa_circ_0061943    | .0402 | 4.8853   | up         | chr21       | 35                       |
| 53     | hsa_circ_0060832    | .0449 | 4.8002   | up         | chr20       | 11                       |
| 54     | hsa_circRNA5599-5   | .0197 | 4.7854   | up         | chr3        | -                        |
| 55     | hsa_circ_0028650    | .0000 | 4.7764   | up         | chr12       | 11                       |
| 56     | hsa_circ_0029375    | .0467 | 4.7582   | up         | chr12       | 5                        |
| 57     | hsa_circRNA9376-69  | .0021 | 4.7539   | up         | chr11       | 9                        |
| 58     | hsa_circRNA5060-14  | .0049 | 4.6888   | up         | chr20       | -                        |
| 59     | hsa_circ_0009590    | .0005 | 4.6848   | up         | chr1        | -                        |
| 60     | hsa_circ_0117770    | .0391 | 4.6756   | up         | chr2        | 100                      |
| 61     | hsa_circ_0054098    | .0250 | 4.6417   | up         | chr2        | 11                       |
| 62     | hsa_circ_0007897    | .0312 | 4.6374   | up         | chr3        | 6                        |
| 63     | hsa_circ_0119137    | .0308 | 4.6289   | up         | chr2        | 100                      |
| 64     | hsa_circ_0081635    | .0247 | 4.5833   | up         | chr7        | -                        |
| 65     | hsa_circ_0122013    | .0274 | 4.5272   | up         | chr3        | 4                        |
| 66     | hsa_circ_0044563    | .0137 | 4.4890   | up         | chr17       | 69                       |
| 67     | hsa_circ_0115393    | .0065 | 4.4798   | up         | chr20       | 6                        |
| 68     | hsa_circ_0053188    | .0253 | 4.4770   | up         | chr2        | 100                      |
| 69     | hsa_circ_0050905    | .0134 | 4.4564   | up         | chr19       | -                        |
| 70     | hsa_circ_0063356    | .0092 | 4.4373   | up         | chr22       | 16                       |
| 71     | hsa_circ_0079065    | .0373 | 4.4277   | up         | chr7        | 3                        |
| 72     | hsa_circRNA8426-8   | .0404 | 4.4039   | up         | chr1        | 12                       |
| 73     | hsa_circ_0124205    | .0010 | 4.4011   | up         | chr3        | -                        |
| 74     | hsa_circ_0044521    | .0237 | 4.3930   | up         | chr17       | 100                      |
| 75     | hsa_circ_0074574    | .0010 | 4.3928   | up         | chr5        | 90                       |
| 76     | hsa_circ_0026092    | .0002 | 4.3773   | up         | chr12       | 100                      |
| 77     | hsa_circRNA11596-26 | .0095 | 4.3764   | up         | chr17       | 2                        |
| 78     | hsa_circ_0109687    | .0410 | 4.3675   | up         | chr19       | 100                      |
| 79     | hsa_circ_0050237    | .0357 | 4.3469   | up         | chr19       | 21                       |
| Number | ProbeName               | P       | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|-------------------------|---------|----------|------------|------------|-------------------------|
| 80     | hsa_circRNA2312-10      | .0044   | 4.3465   | up         | chr14      | 2                       |
| 81     | hsa_circ_0102224        | .0046   | 4.3441   | up         | chr14      | 5                       |
| 82     | hsa_circ_0067036        | .0154   | 4.3368   | up         | chr3       | -                       |
| 83     | hsa_circRNA3597-4       | .0113   | 4.3000   | up         | chr18      | 1                       |
| 84     | hsa_circ_0050900        | .0010   | 4.2987   | up         | chr19      | 4                       |
| 85     | hsa_circ_0080676        | .0342   | 4.2818   | up         | chr7       | -                       |
| 86     | hsa_circ_0007935        | .0175   | 4.2791   | up         | chr2       | -                       |
| 87     | hsa_circ_0088235        | .0071   | 4.2782   | up         | chr9       | 12                      |
| 88     | hsa_circ_0004847        | .0482   | 4.2735   | up         | chr12      | 9                       |
| 89     | hsa_circ_0045961        | .0475   | 4.2424   | up         | chr17      | 36                      |
| 90     | hsa_circRNA12308-1      | .0142   | 4.2413   | up         | chr19      | 1                       |
| 91     | hsa_circRNA4720-6       | .0468   | 4.2333   | up         | chr2       | 1                       |
| 92     | hsa_circ_0021922        | .0384   | 4.2236   | up         | chr11      | 8                       |
| 93     | hsa_circ_0056766        | .0490   | 4.2095   | up         | chr2       | -                       |
| 94     | hsa_circRNA3313-7       | .0193   | 4.2026   | up         | chr17      | 11                      |
| 95     | hsa_circ_0068491        | .0193   | 4.2000   | up         | chr3       | 8                       |
| 96     | hsa_circ_0035515        | .0067   | 4.1976   | up         | chr15      | 27                      |
| 97     | hsa_circ_0063873        | .0057   | 4.1935   | up         | chr22      | -                       |
| 98     | hsa_circ_0115275        | .0136   | 4.1572   | up         | chr20      | 100                     |
| 99     | hsa_circ_0031357        | .0210   | 4.1505   | up         | chr14      | 5                       |
| 100    | hsa_circ_0116655        | .0377   | 4.1444   | up         | chr22      | 36                      |
| 101    | hsa_circ_0132805        | .0498   | 4.1399   | up         | chr7       | -                       |
| 102    | hsa_circRNA8620-24      | .0324   | 4.1352   | up         | chr1       | -                       |
| 103    | hsa_circ_0114892        | .0021   | 4.1320   | up         | chr20      | 100                     |
| 104    | hsa_circ_0031252        | .0235   | 4.1315   | up         | chr14      | -                       |
| 105    | hsa_circ_0040794        | .0008   | 4.1162   | up         | chr16      | 100                     |
| 106    | hsa_circ_0081548        | .0341   | 4.1045   | up         | chr7       | 9                       |
| 107    | hsa_circ_0102223        | .0194   | 4.1004   | up         | chr14      | -                       |
| 108    | hsa_circ_0091005        | .0228   | 4.0970   | up         | chrX       | 51                      |
| 109    | hsa_circ_0078460        | .0415   | 4.0936   | up         | chr6       | 3                       |
| 110    | hsa_circRNA11596-28     | .0119   | 4.0882   | up         | chr17      | 18                      |
| 111    | hsa_circ_0106756        | .0202   | 4.0860   | up         | chr17      | -                       |
| 112    | hsa_circ_0034918        | .0431   | 4.0830   | up         | chr15      | 6                       |
| 113    | hsa_circ_0035020        | .0347   | 4.0708   | up         | chr15      | 7                       |
| 114    | hsa_circ_0106884        | .0364   | 4.0636   | up         | chr17      | 5                       |
| 115    | hsa_circ_0010572        | .0342   | 4.0474   | up         | chr1       | 100                     |
| 116    | hsa_circ_0093278        | .0407   | 4.0439   | up         | chr10      | -                       |
| 117    | hsa_circ_0093277        | .0396   | 4.0366   | up         | chr10      | 2                       |
| 118    | hsa_circ_0027083        | .0424   | 4.0295   | up         | chr12      | 1                       |
| 119    | hsa_circ_0088229        | .0001   | 4.0238   | up         | chr9       | 38                      |
| 120    | hsa_circRNA1309-3       | .0005   | 4.0215   | up         | chr11      | -                       |
| 121    | hsa_circRNA2312-5       | .0109   | 4.0103   | up         | chr14      | -                       |
| 122    | hsa_circ_0039003        | .0495   | 3.9968   | up         | chr16      | 14                      |
| 123    | hsa_circ_0043159        | .0358   | 3.9846   | up         | chr17      | 22                      |

(Continues)
| Number | ProbeName       | P      | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|-----------------|--------|----------|------------|------------|--------------------------|
| 124    | hsa_circ_0065957| .0203  | 3.9833   | up         | chr3       | 4                        |
| 125    | hsa_circ_0046774| .0461  | 3.9730   | up         | chr18      | 100                      |
| 126    | hsa_circ_0037814| .0360  | 3.9721   | up         | chr16      | -                        |
| 127    | hsa_circ_0032221| .0346  | 3.9681   | up         | chr14      | 100                      |
| 128    | hsa_circRNA797-8| .0257  | 3.9445   | up         | chr1       | 7                        |
| 129    | hsa_circ_0051640| .0412  | 3.9368   | up         | chr19      | 10                      |
| 130    | hsa_circ_0062530| .0491  | 3.9361   | up         | chr22      | 25                      |
| 131    | hsa_circ_0093276| .0311  | 3.9357   | up         | chr10      | 3                        |
| 132    | hsa_circ_0104623| .0098  | 3.9353   | up         | chr15      | 17                      |
| 133    | hsa_circ_0133736| .0000  | 3.9342   | up         | chr7       | -                        |
| 134    | hsa_circ_0137340| .0239  | 3.9308   | up         | chr8       | 100                      |
| 135    | hsa_circRNA16090-8| .0462  | 3.9278   | up         | chrX       | -                        |
| 136    | hsa_circ_0024527| .0452  | 3.9168   | up         | chr11      | 16                      |
| 137    | hsa_circRNA11985-8| .0061 | 3.9155   | up         | chr19      | 4                        |
| 138    | hsa_circ_0050588| .0455  | 3.9151   | up         | chr19      | -                        |
| 139    | hsa_circ_0089115| .0349  | 3.9131   | up         | chr9       | 14                      |
| 140    | hsa_circ_0060538| .0355  | 3.9022   | up         | chr20      | 2                        |
| 141    | hsa_circ_0045044| .0135  | 3.8987   | up         | chr17      | 10                      |
| 142    | hsa_circ_0044532| .0437  | 3.8951   | up         | chr17      | 100                     |
| 143    | hsa_circ_0052364| .0069  | 3.8833   | up         | chr19      | -                        |
| 144    | hsa_circ_0049107| .0343  | 3.8821   | up         | chr19      | 100                     |
| 145    | hsa_circRNA3264-5| .0033 | 3.8722   | up         | chr17      | -                        |
| 146    | hsa_circ_0082921| .0297  | 3.8617   | up         | chr7       | 30                      |
| 147    | hsa_circ_0135667| .0228  | 3.8598   | up         | chr8       | 1                        |
| 148    | hsa_circ_0091310| .0389  | 3.8561   | up         | chrX       | 100                     |
| 149    | hsa_circ_0029569| .0460  | 3.8559   | up         | chr12      | 53                      |
| 150    | hsa_circ_0002849| .0134  | 3.8403   | up         | chr14      | 28                      |
| 151    | hsa_circ_0028516| .0473  | 3.8380   | up         | chr12      | 100                     |
| 152    | hsa_circRNA11054-14| .0208 | 3.8377   | up         | chr16      | 1                        |
| 153    | hsa_circRNA13583-5| .0000 | 3.8372   | up         | chr3       | -                        |
| 154    | hsa_circ_0059527| .0451  | 3.8360   | up         | chr20      | 6                        |
| 155    | hsa_circ_0054140| .0287  | 3.8258   | up         | chr2       | -                        |
| 156    | hsa_circ_0109852| .0128  | 3.8251   | up         | chr19      | 100                     |
| 157    | hsa_circ_0125974| .0069  | 3.8101   | up         | chr4       | -                        |
| 158    | hsa_circ_0036023| .0375  | 3.8092   | up         | chr15      | 8                        |
| 159    | hsa_circ_0090862| .0414  | 3.8054   | up         | chrX       | 2                        |
| 160    | hsa_circRNA9907-9| .0364 | 3.7986   | up         | chr12      | 100                     |
| 161    | hsa_circ_0008101| .0304  | 3.7936   | up         | chr2       | 5                        |
| 162    | hsa_circ_0009253| .0359  | 3.7920   | up         | chr1       | -                        |
| 163    | hsa_circRNA5242-25| .0423 | 3.7915   | up         | chr22      | 56                      |
| 164    | hsa_circRNA1670-5| .0386 | 3.7898   | up         | chr12      | -                        |
| 165    | hsa_circ_0134796| .0119  | 3.7869   | up         | chr7       | 1                        |
| 166    | hsa_circ_0051230| .0179  | 3.7867   | up         | chr19      | 1                        |
| 167    | hsa_circ_0065947| .0473  | 3.7845   | up         | chr3       | 47                      |
### TABLE 2 (Continued)

| Number | ProbeName       | P       | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|-----------------|---------|----------|------------|------------|--------------------------|
| 168    | hsa_circ_0132049| .0290   | 3.7667   | up         | chr6       | -                        |
| 169    | hsa_circ_0068342| .0314   | 3.7577   | up         | chr3       | 100                      |
| 170    | hsa_circ_0005237| .0376   | 3.7485   | up         | chr12      | 3                        |
| 171    | hsa_circ_0089852| .0388   | 3.7423   | up         | chrX       | -                        |
| 172    | hsa_circRNA3967-8| .0132  | 3.7356   | up         | chr19      | 4                        |
| 173    | hsa_circ_0033552| .0394   | 3.7291   | up         | chr14      | 80                       |
| 174    | hsa_circ_0032609| .0382   | 3.7151   | up         | chr14      | 28                       |
| 175    | hsa_circ_0078198| .0339   | 3.7122   | up         | chr6       | 41                       |
| 176    | hsa_circ_0022296| .0262   | 3.7103   | up         | chr11      | 23                       |
| 177    | hsa_circRNA3864-4| .0471  | 3.6980   | up         | chr19      | 25                       |
| 178    | hsa_circRNA2644-16| .0062  | 3.6964   | up         | chr15      | 28                       |
| 179    | hsa_circ_0132343| .0095   | 3.6932   | up         | chr6       | 100                      |
| 180    | hsa_circ_0041099| .0158   | 3.6809   | up         | chr16      | 100                      |
| 181    | hsa_circ_0047459| .0241   | 3.6796   | up         | chr18      | 100                      |
| 182    | hsa_circ_0105164| .0324   | 3.6692   | up         | chr16      | 18                       |
| 183    | hsa_circ_0079068| .0303   | 3.6599   | up         | chr7       | 3                        |
| 184    | hsa_circ_0126390| .0264   | 3.6444   | up         | chr4       | -                        |
| 185    | hsa_circ_0014206| .0433   | 3.6420   | up         | chr1       | 28                       |
| 186    | hsa_circRNA12408-1| .0383  | 3.6188   | up         | chr2       | 4                        |
| 187    | hsa_circ_0011244| .0159   | 3.6126   | up         | chr1       | -                        |
| 188    | hsa_circRNA13829-4| .0012  | 3.6085   | up         | chr3       | -                        |
| 189    | hsa_circ_0038498| .0367   | 3.6079   | up         | chr16      | -                        |
| 190    | hsa_circ_0057189| .0454   | 3.6013   | up         | chr2       | -                        |
| 191    | hsa_circ_0109756| .0306   | 3.5994   | up         | chr19      | 100                      |
| 192    | hsa_circ_0035705| .0441   | 3.5992   | up         | chr15      | 100                      |
| 193    | hsa_circRNA12566-10| .0408 | 3.5987   | up         | chr2       | 5                        |
| 194    | hsa_circ_0139927| .0067   | 3.5914   | up         | chrX       | 11                       |
| 195    | hsa_circ_0081593| .0136   | 3.5912   | up         | chr7       | 1                        |
| 196    | hsa_circRNA12696-2| .0405  | 3.5862   | up         | chr2       | 24                       |
| 197    | hsa_circ_0051173| .0392   | 3.5726   | up         | chr19      | 56                       |
| 198    | hsa_circ_0032642| .0487   | 3.5663   | up         | chr14      | 1                        |
| 199    | hsa_circRNA2921-20| .0151  | 3.5602   | up         | chr16      | 1                        |
| 200    | hsa_circ_0010191| .0090   | 3.5588   | up         | chr1       | 20                       |
| 201    | hsa_circ_0113430| .0011   | 3.5507   | up         | chr1       | 10                       |
| 202    | hsa_circ_0034090| .0040   | 3.5492   | up         | chr15      | -                        |
| 203    | hsa_circRNA7202-21| .0484  | 3.5251   | up         | chr7       | 100                      |
| 204    | hsa_circ_0063931| .0476   | 3.5249   | up         | chr22      | 100                      |
| 205    | hsa_circ_0008450| .0443   | 3.5224   | up         | chr16      | 5                        |
| 206    | hsa_circ_0058447| .0410   | 3.5211   | up         | chr2       | 1                        |
| 207    | hsa_circRNA13061-2| .0176  | 3.5168   | up         | chr20      | 25                       |
| 208    | hsa_circ_0075645| .0353   | 3.5130   | up         | chr6       | 3                        |
| 209    | hsa_circ_0002720| .0464   | 3.5129   | up         | chr1       | 6                        |
| 210    | hsa_circ_0113486| .0140   | 3.5110   | up         | chr1       | 4                        |
| 211    | hsa_circ_0124829| .0479   | 3.5061   | up         | chr3       | 13                       |
| Number | ProbeName         | P     | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|-------------------|-------|----------|------------|------------|-------------------------|
| 212    | hsa_circ_0036904  | .0449 | 3.5045   | up         | chr15      | 3                       |
| 213    | hsa_circ_0016796  | .0488 | 3.5024   | up         | chr1       | 7                       |
| 214    | hsa_circ_0051639  | .0003 | 3.4975   | up         | chr19      | 10                      |
| 215    | hsa_circ_0057402  | .0314 | 3.4962   | up         | chr2       | 15                      |
| 216    | hsa_circRNA9086-5 | .0122 | 3.4923   | up         | chr10      | -                       |
| 217    | hsa_circ_0104381  | .0266 | 3.4913   | up         | chr15      | -                       |
| 218    | hsa_circRNA11981-18 | .0077 | 3.4870   | up         | chr19      | 52                      |
| 219    | hsa_circ_0081161  | .0433 | 3.4850   | up         | chr7       | 37                      |
| 220    | hsa_circ_0052788  | .0251 | 3.4820   | up         | chr2       | 2                       |
| 221    | hsa_circ_0112353  | .0232 | 3.4723   | up         | chr1       | -                       |
| 222    | hsa_circ_0037423  | .0018 | 3.4716   | up         | chr16      | 75                      |
| 223    | hsa_circRNA8046-14 | .0137 | 3.4681   | up         | chrX       | 51                      |
| 224    | hsa_circ_0031707  | .0154 | 3.4672   | up         | chr14      | 19                      |
| 225    | hsa_circ_0060455  | .0025 | 3.4601   | up         | chr20      | 2                       |
| 226    | hsa_circRNA3930-2 | .0414 | 3.4389   | up         | chr19      | 7                       |
| 227    | hsa_circRNA6462   | .0394 | 3.4387   | up         | chr5       | 1                       |
| 228    | hsa_circ_0026618  | .0213 | 3.4371   | up         | chr12      | 29                      |
| 229    | hsa_circ_0050367  | .0472 | 3.4357   | up         | chr19      | 8                       |
| 230    | hsa_circ_0049457  | .0438 | 3.4170   | up         | chr19      | 18                      |
| 231    | hsa_circ_0080798  | .0029 | 3.4043   | up         | chr7       | 2                       |
| 232    | hsa_circ_0048975  | .0454 | 3.3981   | up         | chr19      | 54                      |
| 233    | hsa_circRNA7061-6 | .0004 | 3.3933   | up         | chr7       | -                       |
| 234    | hsa_circ_0050183  | .0482 | 3.3794   | up         | chr19      | 15                      |
| 235    | hsa_circRNA8934-1 | .0472 | 3.3781   | up         | chr1       | 2                       |
| 236    | hsa_circ_0099199  | .0477 | 3.3731   | up         | chr12      | 100                     |
| 237    | hsa_circ_0070111  | .0447 | 3.3692   | up         | chr4       | 7                       |
| 238    | hsa_circ_0020753  | .0483 | 3.3629   | up         | chr11      | 43                      |
| 239    | hsa_circ_0094123  | .0395 | 3.3626   | up         | chr10      | -                       |
| 240    | hsa_circ_0007436  | .0012 | 3.3565   | up         | chr16      | 8                       |
| 241    | hsa_circ_0013819  | .0114 | 3.3526   | up         | chr1       | 100                     |
| 242    | hsa_circ_0039006  | .0434 | 3.3509   | up         | chr16      | -                       |
| 243    | hsa_circRNA11853-7| .0497 | 3.3415   | up         | chr19      | 2                       |
| 244    | hsa_circRNA1868-3 | .0265 | 3.3342   | up         | chr12      | -                       |
| 245    | hsa_circ_0046208  | .0261 | 3.3328   | up         | chr17      | -                       |
| 246    | hsa_circ_0079357  | .0108 | 3.3212   | up         | chr7       | 7                       |
| 247    | hsa_circ_0049030  | .0405 | 3.3192   | up         | chr19      | 28                      |
| 248    | hsa_circ_0063241  | .0356 | 3.3151   | up         | chr22      | 16                      |
| 249    | hsa_circ_0001463  | .0284 | 3.3150   | up         | chr5       | 3                       |
| 250    | hsa_circ_0050824  | .0054 | 3.3146   | up         | chr19      | 17                      |
| 251    | hsa_circRNA12060-3| .0489 | 3.2955   | up         | chr19      | 6                       |
| 252    | hsa_circ_0024576  | .0195 | 3.2948   | up         | chr11      | 7                       |
| 253    | hsa_circ_0000775  | .0467 | 3.2901   | up         | chr17      | 4                       |
| 254    | hsa_circ_0124075  | .0001 | 3.2883   | up         | chr3       | 1                       |
| 255    | hsa_circ_0078647  | .0403 | 3.2858   | up         | chr6       | 5                       |
| Number | ProbeName          | P     | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|--------------------|-------|----------|------------|------------|--------------------------|
| 256    | hsa_circ_0010395   | .0403 | 3.2858   | up         | chr1       | 68                       |
| 257    | hsa_circRNA6943-7  | .0154 | 3.2839   | up         | chr6       | 1                        |
| 258    | hsa_circ_0024308   | .0034 | 3.2758   | up         | chr11      | 100                      |
| 259    | hsa_circRNA4853-8  | .0266 | 3.2729   | up         | chr2       | 100                      |
| 260    | hsa_circ_0021075   | .0260 | 3.2701   | up         | chr11      | 21                       |
| 261    | hsa_circ_0064717   | .0333 | 3.2648   | up         | chr3       | 3                        |
| 262    | hsa_circ_0102265   | .0437 | 3.2622   | up         | chr14      | 39                       |
| 263    | hsa_circ_0116219   | .0039 | 3.2562   | up         | chr22      | 4                        |
| 264    | hsa_circ_0099410   | .0308 | 3.2540   | up         | chr1       | 70                       |
| 265    | hsa_circ_0011115   | .0106 | 3.2510   | up         | chr1       | -                        |
| 266    | hsa_circ_0090418   | .0418 | 3.2438   | up         | chrX       | 48                       |
| 267    | hsa_circ_0044107   | .0328 | 3.2404   | up         | chr17      | -                        |
| 268    | hsa_circRNA15745-14| .0417 | 3.2342   | up         | chr9       | 100                      |
| 269    | hsa_circ_0066317   | .0440 | 3.2315   | up         | chr3       | 46                       |
| 270    | hsa_circRNA482-153 | .0470 | 3.2287   | up         | chr1       | 2                        |
| 271    | hsa_circRNA7202-26 | .0115 | 3.2260   | up         | chr7       | 100                      |
| 272    | hsa_circ_0032569   | .0468 | 3.2199   | up         | chr14      | 7                        |
| 273    | hsa_circ_0023203   | .0116 | 3.2154   | up         | chr11      | 100                      |
| 274    | hsa_circ_0113354   | .0480 | 3.2153   | up         | chr1       | 11                       |
| 275    | hsa_circ_0044525   | .0075 | 3.2145   | up         | chr17      | 74                       |
| 276    | hsa_circ_0049587   | .0402 | 3.2077   | up         | chr19      | 3                        |
| 277    | hsa_circ_0009768   | .0457 | 3.1982   | up         | chr1       | 4                        |
| 278    | hsa_circ_0137665   | .0454 | 3.1963   | up         | chr9       | 18                       |
| 279    | hsa_circ_0021214   | .0265 | 3.1851   | up         | chr11      | 100                      |
| 280    | hsa_circRNA1075-40 | .0014 | 3.1817   | up         | chr10      | 11                       |
| 281    | hsa_circ_0085201   | .0063 | 3.1787   | up         | chr8       | 100                      |
| 282    | hsa_circ_0032592   | .0315 | 3.1777   | up         | chr14      | 23                       |
| 283    | hsa_circ_0080327   | .0213 | 3.1748   | up         | chr7       | 30                       |
| 284    | hsa_circ_0049973   | .0071 | 3.1691   | up         | chr19      | 100                      |
| 285    | hsa_circRNA1828-19 | .0370 | 3.1664   | up         | chr12      | -                        |
| 286    | hsa_circ_0138057   | .0428 | 3.1594   | up         | chr9       | 1                        |
| 287    | hsa_circ_0009823   | .0323 | 3.1594   | up         | chr1       | 92                       |
| 288    | hsa_circRNA3136-8  | .0266 | 3.1546   | up         | chr16      | 20                       |
| 289    | hsa_circ_0039874   | .0129 | 3.1510   | up         | chr16      | 100                      |
| 290    | hsa_circRNA9236-9  | .0455 | 3.1500   | up         | chr10      | 3                        |
| 291    | hsa_circRNA1456-53 | .0383 | 3.1446   | up         | chr11      | 35                       |
| 292    | hsa_circ_0089150   | .0383 | 3.1376   | up         | chr9       | -                        |
| 293    | hsa_circ_0119201   | .0090 | 3.1290   | up         | chr2       | 3                        |
| 294    | hsa_circ_0048322   | .0315 | 3.1262   | up         | chr19      | 74                       |
| 295    | hsa_circ_0089419   | .0303 | 3.1240   | up         | chr9       | 50                       |
| 296    | hsa_circRNA15570-43| .0232 | 3.1226   | up         | chr8       | 1                        |
| 297    | hsa_circ_0082601   | .0468 | 3.1221   | up         | chr7       | 14                       |
| 298    | hsa_circ_0012345   | .0378 | 3.1087   | up         | chr1       | 13                       |
| 299    | hsa_circRNA204-11  | .0179 | 3.1038   | up         | chr1       | 3                        |

(Continues)
| Number | ProbeName          | P   | FC (log2) | Regulation | Chromosome | miRNA number | more than 2 |
|--------|--------------------|-----|----------|------------|------------|--------------|-------------|
| 29157  | has_circ_0064603   | 0.0325 | 2.9929 | up         | chr3        | 341          | 2           |
| 29227  | has_circ_0064603   | 0.0325 | 2.9927 | up         | chr3        | 341          | 2           |
| 29251  | has_circ_0064603   | 0.0325 | 2.9926 | up         | chr3        | 341          | 2           |
| 29282  | has_circ_0064603   | 0.0325 | 2.9923 | up         | chr3        | 341          | 2           |
| 29510  | has_circ_0064603   | 0.0325 | 2.9918 | up         | chr3        | 341          | 2           |
| 29525  | has_circ_0064603   | 0.0325 | 2.9917 | up         | chr3        | 341          | 2           |
| 29547  | has_circ_0064603   | 0.0325 | 2.9916 | up         | chr3        | 341          | 2           |
| 29659  | has_circ_0064603   | 0.0325 | 2.9914 | up         | chr3        | 341          | 2           |
| 29757  | has_circ_0064603   | 0.0325 | 2.9913 | up         | chr3        | 341          | 2           |
| 29794  | has_circ_0064603   | 0.0325 | 2.9912 | up         | chr3        | 341          | 2           |
| 29823  | has_circ_0064603   | 0.0325 | 2.9911 | up         | chr3        | 341          | 2           |
| 29838  | has_circ_0064603   | 0.0325 | 2.9910 | up         | chr3        | 341          | 2           |
| 29843  | has_circ_0064603   | 0.0325 | 2.9909 | up         | chr3        | 341          | 2           |
| 29922  | has_circ_0064603   | 0.0325 | 2.9908 | up         | chr3        | 341          | 2           |

(Continued)
| Number | ProbeName                  | P    | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|----------------------------|------|----------|------------|------------|--------------------------|
| 344    | hsa_circRNA4024-3          | .0107| 2.9113   | up         | chr19      | 1                        |
| 345    | hsa_circ_0085882           | .0444| 2.9109   | up         | chr8       | 47                       |
| 346    | hsa_circRNA2312-13         | .0500| 2.9028   | up         | chr14      | -                        |
| 347    | hsa_circ_0048054           | .0358| 2.9028   | up         | chr19      | 7                        |
| 348    | hsa_circ_0051507           | .0274| 2.8966   | up         | chr19      | 3                        |
| 349    | hsa_circ_0109795           | .0417| 2.8938   | up         | chr19      | 13                       |
| 350    | hsa_circ_0089417           | .0147| 2.8914   | up         | chr9       | 1                        |
| 351    | hsa_circRNA1246            | .0482| 2.8892   | up         | chr11      | -                        |
| 352    | hsa_circ_0106385           | .0023| 2.8866   | up         | chr17      | 29                       |
| 353    | hsa_circ_0050895           | .0226| 2.8860   | up         | chr19      | -                        |
| 354    | hsa_circ_0126795           | .0424| 2.8852   | up         | chr4       | 7                        |
| 355    | hsa_circRNA11889-2         | .0326| 2.8845   | up         | chr19      | 10                       |
| 356    | hsa_circ_0017009           | .0167| 2.8702   | up         | chr1       | 14                       |
| 357    | hsa_circRNA1152-14         | .0305| 2.8674   | up         | chr10      | 7                        |
| 358    | hsa_circRNA3717-2          | .0199| 2.8625   | up         | chr18      | -                        |
| 359    | hsa_circ_0037270           | .0323| 2.8606   | up         | chr16      | -                        |
| 360    | hsa_circRNA3016-41         | .0476| 2.8596   | up         | chr16      | 3                        |
| 361    | hsa_circ_0014801           | .0292| 2.8555   | up         | chr1       | 63                       |
| 362    | hsa_circ_0045705           | .0385| 2.8514   | up         | chr17      | 6                        |
| 363    | hsa_circ_0063527           | .0132| 2.8504   | up         | chr22      | 9                        |
| 364    | hsa_circ_0049724           | .0292| 2.8487   | up         | chr19      | 16                       |
| 365    | hsa_circ_0034423           | .0458| 2.8454   | up         | chr15      | 100                      |
| 366    | hsa_circRNA9471-8          | .0335| 2.8437   | up         | chr11      | 87                       |
| 367    | hsa_circRNA15152-25        | .0493| 2.8436   | up         | chr7       | 6                        |
| 368    | hsa_circRNA10499-12        | .0121| 2.8419   | up         | chr14      | 17                       |
| 369    | hsa_circ_0055871           | .0272| 2.8411   | up         | chr2       | -                        |
| 370    | hsa_circRNA3521-29         | .0429| 2.8394   | up         | chr17      | 26                       |
| 371    | hsa_circ_0074315           | .0494| 2.8392   | up         | chr5       | 100                      |
| 372    | hsa_circRNA2786-7          | .0456| 2.8366   | up         | chr16      | 8                        |
| 373    | hsa_circ_0001223           | .0264| 2.8360   | up         | chr22      | 2                        |
| 374    | hsa_circ_0052235           | .0460| 2.8341   | up         | chr19      | 100                      |
| 375    | hsa_circ_0048074           | .0205| 2.8282   | up         | chr19      | 100                      |
| 376    | hsa_circ_0095664           | .0381| 2.8270   | up         | chr11      | 4                        |
| 377    | hsa_circ_0096835           | .0017| 2.8165   | up         | chr11      | -                        |
| 378    | hsa_circ_0108880           | .0289| 2.8109   | up         | chr18      | 100                      |
| 379    | hsa_circ_00600072          | .0087| 2.8067   | up         | chr20      | 19                       |
| 380    | hsa_circRNA15751-7         | .0380| 2.8061   | up         | chr9       | 5                        |
| 381    | hsa_circRNA657-6           | .0351| 2.8049   | up         | chr1       | 7                        |
| 382    | hsa_circ_0016316           | .0247| 2.8004   | up         | chr1       | 13                       |
| 383    | hsa_circ_0030010           | .0273| 2.7993   | up         | chr13      | 9                        |
| 384    | hsa_circ_0078702           | .0035| 2.7986   | up         | chr6       | 41                       |
| 385    | hsa_circ_0010754           | .0387| 2.7984   | up         | chr1       | 1                        |
| 386    | hsa_circ_0020615           | .0369| 2.7888   | up         | chr11      | 3                        |
| 387    | hsa_circ_0032237           | .0496| 2.7866   | up         | chr14      | 2                        |
| Number | ProbeName       | P     | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|-----------------|-------|----------|------------|------------|--------------------------|
| 388    | hsa_circRNA15950-4 | 0.0136 | 2.7659   | up         | chr9       | 14                       |
| 389    | hsa_circ_0067300  | 0.0320 | 2.7637   | up         | chr3       | 14                       |
| 390    | hsa_circRNA13222-1 | 0.0452 | 2.7622   | up         | chr21      | -                        |
| 391    | hsa_circ_0016063  | 0.0374 | 2.7604   | up         | chr1       | 7                        |
| 392    | hsa_circRNA11054-15 | 0.0149 | 2.7584   | up         | chr16      | 3                        |
| 393    | hsa_circRNA4223-6  | 0.0041 | 2.7582   | up         | chr19      | 1                        |
| 394    | hsa_circ_0087060  | 0.0417 | 2.7564   | up         | chr9       | 18                       |
| 395    | hsa_circ_0059325  | 0.0217 | 2.7513   | up         | chr20      | 1                        |
| 396    | hsa_circRNA1377   | 0.0130 | 2.7473   | up         | chr11      | 40                       |
| 397    | hsa_circ_0083940  | 0.0441 | 2.7468   | up         | chr8       | 100                      |
| 398    | hsa_circRNA5357-12 | 0.0387 | 2.7467   | up         | chr22      | 100                      |
| 399    | hsa_circ_0090721  | 0.0458 | 2.7458   | up         | chrX       | 62                       |
| 400    | hsa_circ_0010714  | 0.0490 | 2.7342   | up         | chr1       | 60                       |
| 401    | hsa_circ_0018975  | 0.0001 | 2.7339   | up         | chr10      | 31                       |
| 402    | hsa_circ_0017743  | 0.0226 | 2.7294   | up         | chr10      | -                        |
| 403    | hsa_circ_0135200  | 0.0447 | 2.7221   | up         | chr7       | 7                        |
| 404    | hsa_circ_0057366  | 0.0299 | 2.7191   | up         | chr2       | 100                      |
| 405    | hsa_circ_0086865  | 0.0169 | 2.7160   | up         | chr9       | 100                      |
| 406    | hsa_circ_0027204  | 0.0211 | 2.7136   | up         | chr12      | 100                      |
| 407    | hsa_circ_0042741  | 0.0488 | 2.7118   | up         | chr17      | 10                       |
| 408    | hsa_circRNA11231-9 | 0.0314 | 2.7094   | up         | chr16      | 2                        |
| 409    | hsa_circ_0139402  | 0.0302 | 2.7013   | up         | chr9       | 17                       |
| 410    | hsa_circRNA14685  | 0.0482 | 2.6995   | up         | chr6       | 6                        |
| 411    | hsa_circRNA10909-25 | 0.0045 | 2.6989   | up         | chr16      | 9                        |
| 412    | hsa_circ_0001281  | 0.0001 | 2.6967   | up         | chr3       | 2                        |
| 413    | hsa_circ_0076072  | 0.0041 | 2.6910   | up         | chr6       | 10                       |
| 414    | hsa_circ_0124246  | 0.0454 | 2.6868   | up         | chr3       | 3                        |
| 415    | hsa_circ_0065729  | 0.0444 | 2.6759   | up         | chr3       | -                        |
| 416    | hsa_circRNA13812-3 | 0.0361 | 2.6685   | up         | chr3       | -                        |
| 417    | hsa_circ_0074496  | 0.0282 | 2.6658   | up         | chr5       | 100                      |
| 418    | hsa_circ_0064827  | 0.0080 | 2.6642   | up         | chr3       | 15                       |
| 419    | hsa_circ_0129443  | 0.0199 | 2.6564   | up         | chr5       | 100                      |
| 420    | hsa_circ_0044163  | 0.0451 | 2.6486   | up         | chr17      | 100                      |
| 421    | hsa_circ_0040983  | 0.0316 | 2.6461   | up         | chr16      | 1                        |
| 422    | hsa_circ_0097672  | 0.0258 | 2.6430   | up         | chr12      | -                        |
| 423    | hsa_circRNA5250-15 | 0.0444 | 2.6296   | up         | chr22      | 66                       |
| 424    | hsa_circ_0134538  | 0.0384 | 2.6269   | up         | chr7       | 100                      |
| 425    | hsa_circ_0067163  | 0.0491 | 2.6204   | up         | chr3       | 9                        |
| 426    | hsa_circ_0076768  | 0.0488 | 2.6173   | up         | chr6       | -                        |
| 427    | hsa_circ_0037342  | 0.0484 | 2.6162   | up         | chr16      | 50                       |
| 428    | hsa_circ_0053992  | 0.0398 | 2.6155   | up         | chr2       | 26                       |
| 429    | hsa_circ_0109585  | 0.0193 | 2.5950   | up         | chr19      | 1                        |
| 430    | hsa_circ_0016973  | 0.0386 | 2.5878   | up         | chr1       | 5                        |
| 431    | hsa_circ_0046114  | 0.0464 | 2.5858   | up         | chr17      | 84                       |
| Number | ProbeName             | P    | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|-----------------------|------|----------|------------|------------|-------------------------|
| 432    | hsa_circ_0065709     | .0124| 2.5807   | up         | chr3       | 39                      |
| 433    | hsa_circ_0059966     | .0431| 2.5687   | up         | chr20      | 23                      |
| 434    | hsa_circRNA14235-17  | .0454| 2.5685   | up         | chr5       | 21                      |
| 435    | hsa_circ_0137320     | .0483| 2.5601   | up         | chr8       | 2                       |
| 436    | hsa_circRNA12330-1   | .0326| 2.5564   | up         | chr19      | 3                       |
| 437    | hsa_circ_0046066     | .0395| 2.5556   | up         | chr17      | -                       |
| 438    | hsa_circRNA10194-72  | .0314| 2.5552   | up         | chr12      | 81                      |
| 439    | hsa_circ_0122981     | .0398| 2.5538   | up         | chr3       | 6                       |
| 440    | hsa_circRNA2731-1    | .0374| 2.5514   | up         | chr15      | 1                       |
| 441    | hsa_circ_0082648     | .0413| 2.5424   | up         | chr7       | -                       |
| 442    | hsa_circ_0116177     | .0352| 2.5420   | up         | chr22      | 2                       |
| 443    | hsa_circ_0116532     | .0049| 2.5396   | up         | chr22      | 23                      |
| 444    | hsa_circ_0051082     | .0450| 2.5327   | up         | chr19      | 1                       |
| 445    | hsa_circ_0063612     | .0455| 2.5325   | up         | chr22      | 100                     |
| 446    | hsa_circ_0069044     | .0475| 2.5302   | up         | chr4       | 2                       |
| 447    | hsa_circ_0081609     | .0362| 2.5222   | up         | chr7       | 100                     |
| 448    | hsa_circ_0127589     | .0335| 2.5174   | up         | chr5       | -                       |
| 449    | hsa_circ_0081039     | .0218| 2.5172   | up         | chr7       | -                       |
| 450    | hsa_circ_0051014     | .0264| 2.5148   | up         | chr19      | 100                     |
| 451    | hsa_circ_0040729     | .0441| 2.5125   | up         | chr16      | 100                     |
| 452    | hsa_circ_00300190    | .0177| 2.5062   | up         | chr13      | 28                      |
| 453    | hsa_circ_0026401     | .0360| 2.5058   | up         | chr12      | 10                      |
| 454    | hsa_circ_0132336     | .0435| 2.5026   | up         | chr6       | 4                       |
| 455    | hsa_circ_0058137     | .0491| 2.4929   | up         | chr2       | 21                      |
| 456    | hsa_circ_0054585     | .0443| 2.4891   | up         | chr2       | 1                       |
| 457    | hsa_circRNA3328-4    | .0418| 2.4882   | up         | chr17      | -                       |
| 458    | hsa_circ_0049956     | .0323| 2.4881   | up         | chr19      | 2                       |
| 459    | hsa_circRNA11112-4   | .0485| 2.4861   | up         | chr16      | -                       |
| 460    | hsa_circRNA5224      | .0392| 2.4771   | up         | chr22      | 15                      |
| 461    | hsa_circ_0051608     | .0280| 2.4755   | up         | chr19      | 6                       |
| 462    | hsa_circ_0034836     | .0032| 2.4749   | up         | chr15      | 100                     |
| 463    | hsa_circ_0069882     | .0483| 2.4733   | up         | chr4       | 3                       |
| 464    | hsa_circ_0061063     | .0019| 2.4665   | up         | chr20      | 25                      |
| 465    | hsa_circ_0076915     | .0315| 2.4563   | up         | chr6       | -                       |
| 466    | hsa_circ_0019676     | .0392| 2.4555   | up         | chr10      | 3                       |
| 467    | hsa_circRNA1461-11   | .0320| 2.4550   | up         | chr11      | 6                       |
| 468    | hsa_circ_0080817     | .0462| 2.4404   | up         | chr7       | 32                      |
| 469    | hsa_circ_0128040     | .0442| 2.4404   | up         | chr5       | 8                       |
| 470    | hsa_circ_0074132     | .0416| 2.4372   | up         | chr5       | 4                       |
| 471    | hsa_circ_0019666     | .0250| 2.4312   | up         | chr10      | 5                       |
| 472    | hsa_circ_0110775     | .0338| 2.4309   | up         | chr1       | 84                      |
| 473    | hsa_circ_0020770     | .0403| 2.4298   | up         | chr11      | 81                      |
| 474    | hsa_circRNA783-1     | .0375| 2.4056   | up         | chr1       | 1                       |
| 475    | hsa_circRNA13081-7   | .0365| 2.4009   | up         | chr20      | 24                      |

(Continues)
| Number | ProbeName       | P     | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|-----------------|-------|----------|------------|------------|--------------------------|
| 476    | hsa_circ_0063732| .0380 | 2.3995   | up         | chr22      | 6                        |
| 477    | hsa_circ_0049471| .0372 | 2.3872   | up         | chr19      | 59                       |
| 478    | hsa_circ_0001553| .0389 | 2.3792   | up         | chr5       | -                        |
| 479    | hsa_circ_0139150| .0403 | 2.3791   | up         | chr9       | 4                        |
| 480    | hsa_circ_0046515| .0420 | 2.3777   | up         | chr17      | 5                        |
| 481    | hsa_circ_0007065| .0343 | 2.3772   | up         | chr17      | -                        |
| 482    | hsa_circRNA1883-3| .0457| 2.3743   | up         | chr12      | 2                        |
| 483    | hsa_circ_0003779| .0214 | 2.3703   | up         | chr10      | 9                        |
| 484    | hsa_circ_0046387| .0372 | 2.3686   | up         | chr17      | 98                       |
| 485    | hsa_circRNA693-8| .0396 | 2.3671   | up         | chr1        | 1                        |
| 486    | hsa_circ_0119500| .0075 | 2.3637   | up         | chr2        | 2                        |
| 487    | hsa_circRNA499-6| .0454 | 2.3560   | up         | chr1        | 4                        |
| 488    | hsa_circRNA11657-4| .0220| 2.3390   | up         | chr17      | -                        |
| 489    | hsa_circ_0085879| .0438 | 2.3288   | up         | chr8        | 7                        |
| 490    | hsa_circ_0020549| .0366 | 2.3279   | up         | chr10       | 2                        |
| 491    | hsa_circ_0004525| .0229 | 2.3249   | up         | chr20       | 4                        |
| 492    | hsa_circRNA4125-2| .0221| 2.3202   | up         | chr19       | 2                        |
| 493    | hsa_circ_0128024| .0302 | 2.3087   | up         | chr5        | 2                        |
| 494    | hsa_circ_0113803| .0304 | 2.3059   | up         | chr1        | -                        |
| 495    | hsa_circ_0019561| .0330 | 2.3036   | up         | chr10       | 3                        |
| 496    | hsa_circ_0048441| .0493 | 2.2988   | up         | chr19       | 69                       |
| 497    | hsa_circ_0104540| .0395 | 2.2893   | up         | chr15       | 14                       |
| 498    | hsa_circRNA11147-16| .0288| 2.2709   | up         | chr16       | 1                        |
| 499    | hsa_circ_0132953| .0432 | 2.2581   | up         | chr7        | 1                        |
| 500    | hsa_circ_0063099| .0444 | 2.2539   | up         | chr22       | 100                      |
| 501    | hsa_circ_0058314| .0439 | 2.2498   | up         | chr2        | 100                      |
| 502    | hsa_circRNA6675-2| .0373| 2.2444   | up         | chr6        | 3                        |
| 503    | hsa_circRNA2866-179| .0226| 2.2403   | up         | chr16       | 37                       |
| 504    | hsa_circ_0096997| .0426 | 2.2366   | up         | chr12       | 1                        |
| 505    | hsa_circ_0127068| .0343 | 2.2301   | up         | chr4        | 100                      |
| 506    | hsa_circ_0058139| .0303 | 2.2159   | up         | chr2        | 29                       |
| 507    | hsa_circ_0013816| .0420 | 2.1921   | up         | chr1        | 100                      |
| 508    | hsa_circ_0043736| .0472 | 2.1741   | up         | chr17       | -                        |
| 509    | hsa_circ_0079708| .0492 | 2.1714   | up         | chr7        | 20                       |
| 510    | hsa_circ_0028752| .0453 | 2.1518   | up         | chr12       | 100                      |
| 511    | hsa_circ_0076091| .0395 | 2.1488   | up         | chr6        | -                        |
| 512    | hsa_circRNA12187-14| .0437| 2.1445   | up         | chr19       | 3                        |
| 513    | hsa_circ_0058943| .0467 | 2.1413   | up         | chr2        | 100                      |
| 514    | hsa_circ_0098884| .0280 | 2.1348   | up         | chr12       | 1                        |
| 515    | hsa_circ_0037728| .0334 | 2.1331   | up         | chr16       | 19                       |
| 516    | hsa_circ_0081211| .0447 | 2.1331   | up         | chr7        | 16                       |
| 517    | hsa_circ_0026694| .0405 | 2.1295   | up         | chr12       | 4                        |
| 518    | hsa_circRNA13636-3| .0026| 2.1137   | up         | chr3        | 1                        |
| 519    | hsa_circRNA8528-1| .0442| 2.1105   | up         | chr1        | 1                        |
| 520    | hsa_circ_0056856| .0095 | 2.1067   | up         | chr2        | 2                        |
| Number | ProbeName           | P     | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|---------------------|-------|----------|------------|------------|--------------------------|
| 521    | hsa_circ_0001605    | 0.0316| 2.1065   | up         | chr6       | 44                       |
| 522    | hsa_circ_0098702    | 0.0232| 2.0930   | up         | chr3       | 28                       |
| 523    | hsa_circ_00563247   | 0.0470| 2.0878   | up         | chr11      | 100                      |
| 524    | hsa_circ_0018930    | 0.0463| 2.0803   | up         | chr10      | 100                      |
| 525    | hsa_circRNA5174-30  | 0.0383| 2.0728   | up         | chr21      | -                        |
| 526    | hsa_circ_0032414    | 0.0366| 2.0701   | up         | chr14      | 49                       |
| 527    | hsa_circRNA7795-18  | 0.0485| 2.0676   | up         | chr9       | 18                       |
| 528    | hsa_circ_0039734    | 0.0499| 2.0625   | up         | chr16      | 2                        |
| 529    | hsa_circRNA2382-11  | 0.0326| 2.0545   | up         | chr16      | 2                        |
| 530    | hsa_circ_0051355    | 0.0473| 2.0096   | up         | chr19      | 33                       |
| 531    | hsa_circ_0031019    | 0.0278| 2.0069   | up         | chr13      | 29                       |
| 532    | hsa_circ_0094149    | 0.0487| 2.0263   | up         | chr10      | -                        |
| 533    | hsa_circ_0038829    | 0.0419| 2.0523   | up         | chr12      | -                        |
| 534    | hsa_circRNA2382-11  | 0.0008| 2.0123   | up         | chr12      | -                        |
| 535    | hsa_circ_0050576    | 0.0162| 2.0343   | up         | chr11      | -                        |
| 1      | hsa_circ_0066439    | 0.0311| 8.1982   | down       | chr3       | 100                      |
| 2      | hsa_circ_0054211    | 0.0380| 7.7496   | down       | chr2       | -                        |
| 3      | hsa_circ_0095920    | 0.0204| 7.7266   | down       | chr11      | 7                        |
| 4      | hsa_circ_0112984    | 0.0215| 7.0786   | down       | chr3       | 100                      |
| 5      | hsa_circ_01113067   | 0.0389| 6.9119   | down       | chr1       | -                        |
| 6      | hsa_circ_0039155    | 0.0433| 6.7674   | down       | chr16      | 9                        |
| 7      | hsa_circRNA4014-45  | 0.0295| 6.4383   | down       | chr19      | 1                        |
| 8      | hsa_circ_0112979    | 0.0200| 6.1909   | down       | chr3       | 37                       |
| 9      | hsa_circ_0059665    | 0.0208| 5.8536   | down       | chr20      | 3                        |
| 10     | hsa_circ_009319     | 0.0119| 4.4715   | down       | chr1       | 14                       |
| 11     | hsa_circ_0108269    | 0.0466| 4.0740   | down       | chr18      | 100                      |
| 12     | hsa_circRNA15520-3  | 0.0178| 4.0661   | down       | chr8       | 4                        |
| 13     | hsa_circRNA7795-20  | 0.0323| 3.8447   | down       | chrX       | 3                        |
| 14     | hsa_circ_0111673    | 0.0198| 3.5157   | down       | chr22      | -                        |
| 15     | hsa_circ_0072126    | 0.0030| 3.4456   | down       | chr5       | 3                        |
| 16     | hsa_circ_0138278    | 0.0013| 3.1260   | down       | chr9       | 24                       |
| 17     | hsa_circRNA4269-5   | 0.0028| 2.9634   | down       | chr19      | -                        |
| 18     | hsa_circ_0024093    | 0.0109| 2.8327   | down       | chr11      | 100                      |
| 19     | hsa_circ_0072127    | 0.0060| 2.7938   | down       | chr5       | -                        |
| 20     | hsa_circRNA14838-129| 0.0031| 2.7874   | down       | chr6       | 100                      |
| 21     | hsa_circ_0048276    | 0.0048| 2.7553   | down       | chr19      | 11                       |
| 22     | hsa_circ_0087798    | 0.0305| 2.7281   | down       | chr9       | 100                      |
| 23     | hsa_circ_0072124    | 0.0135| 2.7164   | down       | chr5       | 43                       |
| 24     | hsa_circ_0051911    | 0.0433| 2.7030   | down       | chr19      | 2                        |
| 25     | hsa_circ_0015835    | 0.0034| 2.6614   | down       | chr1       | 13                       |
| 26     | hsa_circ_0083577    | 0.0162| 2.6553   | down       | chr8       | 100                      |
| 27     | hsa_circ_0109563    | 0.0094| 2.6242   | down       | chr19      | 8                        |
| 28     | hsa_circRNA9277-12  | 0.0033| 2.5709   | down       | chr10      | 21                       |
| 29     | hsa_circ_0083773    | 0.0054| 2.5485   | down       | chr8       | 100                      |

(Continues)
| Number | ProbeName          | P     | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|--------------------|-------|----------|------------|------------|--------------------------|
| 30     | hsa_circ_0072695   | .0109 | 2.5287   | down       | chr5       | 1                        |
| 31     | hsa_circ_0054909   | .0109 | 2.5037   | down       | chr2       | 7                        |
| 32     | hsa_circ_0055714   | .0487 | 2.4975   | down       | chr2       | 100                      |
| 33     | hsa_circ_0072118   | .0178 | 2.4935   | down       | chr5       | 62                       |
| 34     | hsa_circ_0019871   | .0011 | 2.4815   | down       | chr10      | 3                        |
| 35     | hsa_circ_0040298   | .0024 | 2.4803   | down       | chr16      | 3                        |
| 36     | hsa_circ_0031515   | .0001 | 2.4737   | down       | chr14      | 100                      |
| 37     | hsa_circRNA1322    | .0425 | 2.4663   | down       | chr11      | 6                        |
| 38     | hsa_circ_0037397   | .0003 | 2.4556   | down       | chr16      | 21                       |
| 39     | hsa_circ_0079644   | .0205 | 2.4410   | down       | chr7       | 24                       |
| 40     | hsa_circ_0048377   | .0057 | 2.4364   | down       | chr19      | 3                        |
| 41     | hsa_circ_0072696   | .0073 | 2.4173   | down       | chr5       | 2                        |
| 42     | hsa_circ_0010232   | .0021 | 2.3984   | down       | chr1       | 6                        |
| 43     | hsa_circ_0031872   | .0023 | 2.3965   | down       | chr14      | 28                       |
| 44     | hsa_circRNA2454-1  | .0111 | 2.3875   | down       | chr14      | 1                        |
| 45     | hsa_circ_0098827   | .0355 | 2.3841   | down       | chr12      | 6                        |
| 46     | hsa_circ_0021365   | .0040 | 2.3776   | down       | chr11      | 100                      |
| 47     | hsa_circ_0129820   | .0063 | 2.3762   | down       | chr5       | 1                        |
| 48     | hsa_circ_0077341   | .0487 | 2.3622   | down       | chr6       | 2                        |
| 49     | hsa_circ_0043578   | .0035 | 2.3564   | down       | chr17      | 2                        |
| 50     | hsa_circ_0052184   | .0048 | 2.3548   | down       | chr19      | -                        |
| 51     | hsa_circRNA10194-34| .0012 | 2.3485   | down       | chr12      | 1                        |
| 52     | hsa_circRNA3816-16 | .0002 | 2.3475   | down       | chr19      | -                        |
| 53     | hsa_circ_0073199   | .0358 | 2.3331   | down       | chr5       | 9                        |
| 54     | hsa_circ_0077874   | .0025 | 2.3325   | down       | chr6       | -                        |
| 55     | hsa_circ_0072702   | .0246 | 2.3260   | down       | chr5       | -                        |
| 56     | hsa_circ_0079655   | .0343 | 2.3179   | down       | chr7       | -                        |
| 57     | hsa_circ_0073196   | .0172 | 2.3099   | down       | chr5       | 23                       |
| 58     | hsa_circ_0077873   | .0190 | 2.2875   | down       | chr6       | -                        |
| 59     | hsa_circ_0073193   | .0062 | 2.2837   | down       | chr5       | 23                       |
| 60     | hsa_circ_0077799   | .0009 | 2.2833   | down       | chr6       | -                        |
| 61     | hsa_circ_0073640   | .0453 | 2.2724   | down       | chr5       | 3                        |
| 62     | hsa_circ_0055072   | .0035 | 2.2633   | down       | chr2       | 100                      |
| 63     | hsa_circ_0048764   | .0032 | 2.2609   | down       | chr19      | 13                       |
| 64     | hsa_circ_0009272   | .0021 | 2.2603   | down       | chr1       | 100                      |
| 65     | hsa_circ_0020796   | .0029 | 2.2403   | down       | chr11      | 50                       |
| 66     | hsa_circ_0077317   | .0312 | 2.2343   | down       | chr6       | 100                      |
| 67     | hsa_circRNA92277-14| .0059 | 2.2323   | down       | chr10      | 1                        |
| 68     | hsa_circ_0043573   | .0015 | 2.2293   | down       | chr17      | 16                       |
| 69     | hsa_circ_0041351   | .0071 | 2.2214   | down       | chr17      | -                        |
| 70     | hsa_circRNA7333-72 | .0232 | 2.2150   | down       | chr7       | 1                        |
| 71     | hsa_circ_0050049   | .0061 | 2.2079   | down       | chr19      | 3                        |
| 72     | hsa_circ_0106804   | .0082 | 2.2010   | down       | chr17      | 100                      |
| 73     | hsa_circ_0023050   | .0005 | 2.1980   | down       | chr11      | 67                       |
| 74     | hsa_circRNA14568-1 | .0050 | 2.1942   | down       | chr5       | 1                        |
| 75     | hsa_circ_0019876   | .0204 | 2.1892   | down       | chr10      | -                        |
3.3 | Screening of target miRNAs for the differentially expressed circRNAs

A total of 475 out of 650 circRNAs with different expressions could bind more than two miRNAs. Among the top 10 differentially expressed circRNAs, six had more than two target miRNAs, and their circRNA-miRNA network relationship is shown in Figure 2A. The results show that there is a complex network relationship between circRNA and miRNA. Among them, RNA hsa_circ_0066439, hsa_circ_0081241, and hsa_circ_0122984 can regulate multiple signal

| Number | ProbeName         | P      | FC (abs) | Regulation | Chromosome | miRNA number more than 2 |
|--------|-------------------|--------|----------|------------|------------|-------------------------|
| 76     | hsa_circ_0001583  | .0028  | 2.1863   | down       | chr6       | -                       |
| 77     | hsa_circ_0048576  | .0092  | 2.1799   | down       | chr19      | -                       |
| 78     | hsa_circ_0118577  | .0395  | 2.1789   | down       | chr2       | 100                     |
| 79     | hsa_circ_0015380  | .0004  | 2.1688   | down       | chr1       | 47                      |
| 80     | hsa_circ_0049004  | .0065  | 2.1611   | down       | chr19      | 14                      |
| 81     | hsa_circ_0085214  | .0044  | 2.1552   | down       | chr8       | 100                     |
| 82     | hsa_circ_0050165  | .0170  | 2.1461   | down       | chr19      | 100                     |
| 83     | hsa_circ_0018598  | .0067  | 2.1300   | down       | chr10      | 27                      |
| 84     | hsa_circ_0076015  | .0023  | 2.1297   | down       | chr6       | 100                     |
| 85     | hsa_circ_0050223  | .0109  | 2.1242   | down       | chr19      | 44                      |
| 86     | hsa_circ_0083745  | .0004  | 2.1192   | down       | chr8       | 58                      |
| 87     | hsa_circ_0018976  | .0095  | 2.1189   | down       | chr10      | 100                     |
| 88     | hsa_circ_0030659  | .0125  | 2.1185   | down       | chr13      | 3                       |
| 89     | hsa_circ_0086493  | .0147  | 2.1158   | down       | chr9       | 61                      |
| 90     | hsa_circ_0068338  | .0061  | 2.1138   | down       | chr3       | 4                       |
| 91     | hsa_circ_0107635  | .0198  | 2.1038   | down       | chr17      | -                       |
| 92     | hsa_circ_0067970  | .0063  | 2.0950   | down       | chr3       | 19                      |
| 93     | hsa_circ_0091117  | .0123  | 2.0906   | down       | chrX       | -                       |
| 94     | hsa_circ_0028302  | .0085  | 2.0879   | down       | chr12      | 50                      |
| 95     | hsa_circ_0050003  | .0031  | 2.0856   | down       | chr19      | 100                     |
| 96     | hsa_circRNA3217-22| .0065  | 2.0750   | down       | chr17      | -                       |
| 97     | hsa_circ_0000406  | .0151  | 2.0714   | down       | chr12      | 1                       |
| 98     | hsa_circ_0062102  | .0156  | 2.0675   | down       | chr21      | 66                      |
| 99     | hsa_circ_0009746  | .0492  | 2.0614   | down       | chr1       | 6                       |
|100     | hsa_circ_01249B5  | .0350  | 2.0607   | down       | chr4       | -                       |
|101     | hsa_circ_0092229  | .0027  | 2.0584   | down       | chrY       | 40                      |
|102     | hsa_circ_0079649  | .0182  | 2.0577   | down       | chr7       | 17                      |
|103     | hsa_circ_0123224  | .0135  | 2.0562   | down       | chr3       | -                       |
|104     | hsa_circ_0086256  | .0328  | 2.0482   | down       | chr9       | 14                      |
|105     | hsa_circ_0042272  | .0067  | 2.0476   | down       | chr17      | 6                       |
|106     | hsa_circ_0069858  | .0019  | 2.0442   | down       | chr4       | 100                     |
|107     | hsa_circ_0075406  | .0034  | 2.0400   | down       | chr5       | 1                       |
|108     | hsa_circ_0077876  | .0053  | 2.0356   | down       | chr6       | -                       |
|109     | hsa_circ_0048545  | .0082  | 2.0206   | down       | chr19      | 100                     |
|110     | hsa_circ_0023381  | .0348  | 2.0193   | down       | chr11      | 31                      |
|111     | hsa_circ_0080145  | .0128  | 2.0164   | down       | chr7       | 100                     |
|112     | hsa_circ_0073886  | .0005  | 2.0099   | down       | chr5       | 100                     |
|113     | hsa_circ_0039941  | .0110  | 2.0082   | down       | chr16      | 15                      |
|114     | hsa_circRNA10930-2| .0163  | 2.0068   | down       | chr16      | 1                       |
|115     | hsa_circRNA4796-2 | .0298  | 2.0026   | down       | chr2       | -                       |
pathways through miRNA hsa-miR-1254, hsa-miR-328-5p, and other target miRNAs to participate in the AMI process. By further scrutinizing more stringent parameters, such as processed signal variation between repetitions and referencing to the established circRNA databases and publications, six new circRNA candidates were selected. The miRNA software was used to predict the target miRNAs of the six differentially expressed circRNAs. The results showed that the target miRNAs of the six differentially expressed circRNAs were larger than two. The circRNA-miRNA network of these six circRNAs is shown in Figure 2B. It can be seen from this figure that RNA hsa_circ_0043563, hsa_circ_0119137, hsa_circ_0106804, and hsa_circ_0085214 can regulate multiple signal pathways through miRNA hsa-miR-4763-3p, hsa-miR-328-5p, etc. Thus, it can participate in the occurrence of AMI. Subsequent hsa_circ_0025097 and hsa_circ_0028302 can also regulate multiple signal pathways through hsa-miR-328-5p. Therefore, there are network relationships between circRNA-miRNA that may be the key to their role in the pathogenesis and progression of AMI.

3.4 | Enrichment analysis results for disease

Disease, pathway, and GO analysis suggest that these differentially expressed circRNAs are relevant to several vital biological processes, cellular components, molecular functions, and critical signaling pathways (Figure 3). By searching bioinformatics databases such as KEGG DISEASE, NHGRI GWAS Catalog, and OMIM, the significantly expressed genes in the whole blood samples of the patients with AMI were subject to enrichment analysis, and significance was marked by $P < .05$. The results are shown in Table 3. As can be seen from this table, after using 3 databases to perform a disease enrichment analysis of characteristic genes, 3 diseases closely related to the cardiovascular system were found, which were myocardial infarction, coronary heart disease, and hypertension. From the results, it can be determined that the target genes are closely related to coronary heart disease.

3.5 | Pathway analysis

To study the differentially expressed circRNAs in the whole blood of the patients with AMI, pathway enrichment analysis was performed using the databases of Reactome (http://www.reactome.org/), KEGG PATHWAY (http://www.genome.jp/kegg/), BioCyc (http://biocyc.org/), and PANTHER (http://www.pantherdb.org/). $P < .05$ was considered as a significant analysis, and the results are shown in Table 4.
Based on $P < .05$, no relevant pathways were found in the PANTHER or BioCyc databases, while in the KEGG PATHWAY database, it was suggested that lysine degradation, MAPK signaling pathway, and focal adhesion ($P < .05$) were involved in the pathogenesis of AMI and the process of disease development. The Reactome database prompted multiple pathways, such as signal transduction, chromatin organization, and chromatin-modifying enzymes, as participating in the occurrence and development of AMI.

**TABLE 3** Results from the disease enrichment analysis of 3 databases

| Term                  | Database                        | $P$-value |
|-----------------------|---------------------------------|-----------|
| Myocardial infarction | NHGRI GWAS Catalog              | .003822041|
| Coronary heart disease| NHGRI GWAS Catalog              | .033700966|
| Hypertension          | NHGRI GWAS Catalog              | .015446158|

**TABLE 4** Results from the pathway enrichment analysis of 4 databases

| Term                        | Database                      | $P$-value |
|-----------------------------|-------------------------------|-----------|
| Chromatin organization      | Reactome                      | .001389753|
| Chromatin-modifying enzymes | Reactome                      | .001389753|
| Signal transduction         | Reactome                      | .000009897|
| Lysine degradation           | KEGG PATHWAY                  | .015878021|
| MAPK signaling pathway      | KEGG PATHWAY                  | .022710463|
| Focal adhesion              | KEGG PATHWAY                  | .000000269|

Based on the results of the pathway enrichment analysis performed using the various databases above, it is not difficult to discover that the target genes of these differentially expressed...
circRNAs of the AMI group can participate in the pathogenesis and disease development of AMI through some pathways. Many such pathways exist in the regulation of the development of AMI.

### 3.6 | Gene ontology analysis

Similarly, gene ontology analysis was performed on the linear mRNA transcripts corresponding to 650 differentially expressed circRNAs selected from the specimens of the AMI group. The analysis included mainly molecular functions, biological processes, and cellular components.

The gene ontology analysis demonstrated that the differentially expressed circRNAs play regulatory roles in cells through various biological processes, such as cellular, single-organism, and cellular component organization (Figure 4); cellular components, such as protein complex, extracellular matrix, membrane, and extracellular matrix component (Figure 5); and molecular functions, such as binding and structural molecule activity (Figure 6).

### 4 | DISCUSSION

Based on whether they can be translated, circRNAs were divided into noncoding circRNAs and coding circRNAs that have a closed...
circular structure and are not affected by exonucleases. Its expression is relatively more stable, and it is not easily degraded. In recent years, circRNA has emerged as a new member of the RNA family for that has attracted attention. In the past, studies of miRNAs made a series of advances to the understanding of the mechanisms in the actions of diseases, such as in heart development, cardiac hypertrophy, heart failure, arrhythmia, myocardial injury, apoptosis, and myocardial ischemia. In recent years, there have been reports in the literature that circRNA is related to CVDs. For example, Li et al have found that circRNA hsa_circ_0124644 can be used to diagnose coronary artery disease. Zhang et al discovered that circRNA MFACR inhibited the translation of MTP18 by competitively binding to miR-652-3p, thereby inhibiting mitochondrial division and affecting apoptosis, eventually playing a role in human heart disease. Furthermore, HRCR inhibits cardiac hypertrophy and heart failure, Cdr1as induces myocardial infarction, Circ-Fox03 promotes heart aging, and cANRIL is associated with atherosclerosis. Because the circular structure of circRNA is more stable, it is easier to use as a potential new biomarker, miRNA is also a type of ncRNA, and it has been one of the most studied of these RNAs in recent years. It has been shown to play a very important role in the development of various diseases, and the role of miRNAs in CVDs has also gradually been discovered. Studies have found that circulating miRNA-134, miRNA-22, miRNA-328, and miRNA-499 have abnormal expression levels in the plasma of patients with AMI, suggesting that they may be potential biomarkers of AMI. There are many miRNA response elements on circRNA. The sponge adsorption effect of circRNA on miRNA occurs mainly through response elements binding miRNA to block the inhibition of miRNA on its target gene expression. When circRNA is highly expressed, the target gene expression of miRNA is up-regulated, and when circRNA is lowly expressed, the target gene expression of miRNA is down-regulated. It can be said that the miRNA sponge function performed by circRNA plays an important role in the occurrence of diseases, competitively inhibiting the expression of miRNA and blocking the expression of miRNA on target genes. Therefore, the relationship between circRNA and AMI has great research potential. Since, as compared to linear structures, circRNA does not contain a poly A tail, it is not easily cleaved by exonuclease and exists more stably in an organism. It also has high conservation, expression, and tissue specificity. Therefore, the
regulation efficiency of circRNA is higher than that of linear structure RNA. These prior studies have indicated that circRNA is likely to have an important relationship with the occurrence of CVDs.

In this study, the circRNA expression in patients with AMI was analyzed using a microarray gene chip, and the circRNAs with significantly different expressions from controls were analyzed. The results showed that, as compared to the control group, 650 differentially expressed circRNAs were screened out for the AMI group, of which 535 were up-regulated and 115 were down-regulated (Table 2, Figure 1C). Such a large number of circRNA differential expression results indicate that circRNA is likely to play a positive role in the occurrence and development of AMI. The circRNA-miRNA network was constructed after screening out the differentially expressed circRNAs, and it showed that 475 differentially expressed circRNAs could bind > 2 miRNAs. Among the 10 circRNAs with the largest difference, three of them can bind to miR-328-5p-related cardiovascular diseases, including one up-regulated hsa_circ_0081241 and two down-regulated hsa_circ_0066439 and hsa_circ_0122984 (Figure 2A). In addition, the 6 novel circRNA candidates were able to bind to miR-328-5p related to myocardial infarction (Figure 2B), including 3 up-regulated (hsa_circ_0043563, hsa_circ_0119137, and hsa_circ_0025097) and 3 down-regulated (hsa_circ_0106804, hsa_circ_0085214, and hsa_circ_0028302). In a previous study, He et al found that miR-328 and miR-134 have important diagnostic value for AMI, and levels of miR-328 and miR-134 are related to mortality or an increased risk of developing heart failure. Wang et al reported
that levels of miR-328 in the plasma and whole blood of AMI patients were significantly increased: 10.9 times and 16.1 times as compared to a control group, respectively. The level of miR-133 increased 4.4 times, suggesting that miR-328 and miR-133 may represent novel biomarkers of AMI.

Among the previous studies, Ruan et al.²⁷ have explored the possible regulatory mechanisms of circRNA-miRNA regulatory networks in the pathogenesis and progression of gastric cancer. They discovered the circRNA-miRNA-mRNA pathway and indicated that a possible role for circRNA in the occurrence and development of gastric cancer may be helpful to developing potential tools for the early diagnosis and effective treatment of gastric cancer. It should be noted that the circRNA-miRNA-mRNA axis has become the most studied mechanism for the occurrence and development of gastric cancer. Dudekula et al.²⁸ have constructed a new database for searching the open databases of circRNAs, miRNAs, and RNA-binding proteins to help provide bioinformatics analyses of the binding sites on circRNA. By using this database, we can also search for the binding sites of related circRNA and build a general regulatory network. Recent studies have also shown that identifying the circRNA-miRNA network provides new insights into the prognosis and treatment of breast cancer.²⁹ In addition, some scholars have proposed that the regulatory network of circRNA-miRNA-mRNA also exists in plants and have pointed out that this network is likely to affect the development of flowers; this pathway may also play a potential regulatory role in the response of plants to external environmental stress.²⁸ The results of these many studies fully show that the circRNA-miRNA-mRNA network may be ubiquitous in the occurrence and development of various diseases. This pathway and network can regulate the occurrence and progression of various diseases and disease outcomes. The results of the current study have shown that multiple circRNAs can bind to miR-328-5p, which is associated with myocardial infarction. The possibility of a network correlation between the circRNA and miRNA was first confirmed, and then, it was suggested that this network correlation pathway is likely to have a certain regulatory mechanism during the occurrence and progression of AMI. Thus, this regulatory mechanism will affect the occurrence, development, and prognosis of AMI. This is of great significance for identifying circRNA as a possible biomarker for AMI and revealing the complex regulatory mechanisms in the process of AMI. According to the results of enrichment analysis and gene ontology analysis, it can be found that the circRNA-miRNA pathway is likely to exist in the occurrence and development of AMI, such as lysine degradation, MAPK signaling pathway, and focal adhesion (Table 4). Combined with the screened circRNAs and bound miRNAs, this pathway is likely to participate in the process of regulating the occurrence and development of AMI.

There are some limitations in this study. First, the sample size was small, and the sample was only obtained from six subjects. Second, there is no verification of RT-PCR for the top circRNA candidates expressing differences. In the future, circRNAs, such as hsa_circ_0066439, hsa_circ_0043563, hsa_circ_0119137, and miR-328-5p, should be verified to analyze the levels and interactions of these new circRNAs with miR-328-5p.

5 | CONCLUSION

There were 650 circRNAs that were differentially expressed in AMI disease, and an interaction between circRNA and miRNA is involved in the occurrence and development of AMI. By combining the results of the disease enrichment analysis, pathway enrichment analysis, and gene ontology analysis, it can be found that the circRNA-miRNA interaction pathway very likely participates in regulating the occurrence and development of AMI.

ACKNOWLEDGMENTS

The author thanks all participants for their contributions.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

YH Tang involved in protocol/project development and final approval of the version to be submitted. MH Jiang collected or managed the data. LL Yin analyzed the data, and wrote the study.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

ORCID

Lianli Yin https://orcid.org/0000-0003-4425-1592
Yinghua Tang https://orcid.org/0000-0002-5655-9650

REFERENCES

1. Roth GA, Abate D, Abate KH, et al. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2018;392:1736-1788.
2. Dehghan M, Mente A, Zhang X, et al. Associations of fats and carbohydrate intake with cardiovascular disease and mortality in 18 countries from five continents (PURE): a prospective cohort study. Lancet. 2017;390:2050-2062.
3. Poller W, Dimmelmeier S, Heymans S, et al. Non-coding RNAs in cardiovascular diseases: diagnostic and therapeutic perspectives. Eur Heart J. 2018;39:2704-2716.
4. Hsiao KY, Sun HS, Tsai SJ. Circular RNA - New member of non-coding RNA with novel functions. Exp Biol Med (Maywood). 2017;242:1136-1141.
5. Kulcheski FR, Christoff AP, Margis R. Circular RNAs are miRNA sponges and can be used as a new class of biomarker. J Biotechnol. 2016;238:42-51.
6. Li M, Ding W, Sun T, et al. Biogenesis of circular RNAs and their roles in cardiovascular development and pathology. FEBS J. 2018;285:220-232.
7. Devaux Y, Creemers EE, Boon RA, et al. Circular RNAs in heart failure. Eur J Heart Fail. 2017;19:701-709.
8. Geng H-H, Li R, Su Y-M, et al. The Circular RNA Cdr1as promotes myocardial infarction by mediating the regulation of miR-7a on its target genes expression. *PLoS One*. 2016;11:e0151753.

9. Holdt LM, Stahringer A, Sass K, et al. Circular non-coding RNA ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans. *Nat Commun*. 2016;7:12429.

10. Patterson TA, Lobenhofer EK, Fulmer-Smentek SB, et al. Performance comparison of one-color and two-color platforms within the MicroArray Quality Control (MAQC) project. *Nat Biotechnol*. 2006;24:1140-1150.

11. You X, Vlatkovic I, Babic A, et al. Neural circular RNAs are derived from synaptic genes and regulated by development and plasticity. *Nat Neurosci*. 2015;18:603-610.

12. Barabasi AL, OltvaiZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet*. 2004;5:101-113.

13. Li Z, Ruan Y, Zhang H, et al. Tumor-suppressive circular RNAs: Mechanisms underlying their suppression of tumor occurrence and use as therapeutic targets. *Cancer Sci*. 2019;110:3630-3638.

14. Hou LD, Zhang J. Circular RNAs: An emerging type of RNA in cancer. *Int J Immunopathol Pharmacol*. 2017;30:1-6.

15. Zhao Z, Li X, Gao C, et al. Peripheral blood circular RNA hsa_circ_0124644 can be used as a diagnostic biomarker of coronary artery disease. *Sci Rep*. 2017;7:39918.

16. Wang K, Gan T-Y, Li NA, et al. Circular RNA mediates cardiomyocyte death via miRNA-dependent upregulation of MTP18 expression. *Cell Death Differ*. 2017;24:1111-1120.

17. Fan X, Weng X, Zhao Y, et al. Circular RNAs in cardiovascular disease: an overview. *Biomed Res Int*. 2017;2017:5135781.

18. He F, Lv P, Zhao X, et al. Predictive value of circulating miR-328 and miR-134 for acute myocardial infarction. *Mol Cell Biochem*. 2014;394:137-144.

19. Wang R, Li N, Zhang Y, et al. Circulating microRNAs are promising novel biomarkers of acute myocardial infarction. *Intern Med*. 2011;50:1789-1795.

20. Wang X, Tian L, Sun Q. Diagnostic and prognostic value of circulating miRNA-499 and miRNA-22 in acute myocardial infarction. *J Clin Lab Anal*. 2020;34:2410-2417.

21. Zhong Z, Wu H, Zhong W, et al. Expression profiling and bioinformatics analysis of circulating microRNAs in patients with acute myocardial infarction. *J Clin Lab Anal*. 2020;34:e23099.

22. Wang L, Jin Y. Noncoding RNAs as biomarkers for acute coronary syndrome. *Biomed Res Int*. 2020;2020:3298696.

23. Memczak S, Jens M, Elefsinioti A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature*. 2013;495:333-338.

24. Suzuki H, Tsukahara T. A view of pre-mRNA splicing from RNase R resistant RNAs. *Int J Mol Sci*. 2014;15:9331-9342.

25. Wu J, Liu S, Xiang Y, et al. Bioinformatic analysis of circular RNA-associated cerna network associated with hepatocellular carcinoma. *Biomed Res Int*. 2019;2019:8308694.

26. Thomas LF, Saetrom P. Circular RNAs are depleted of polymorphisms at microRNA binding sites. *Bioinformatics*. 2014;30:2243-2246.

27. Ruan Y, Li Z, Shen Y, et al. Functions of circular RNAs and their potential applications in gastric cancer. *Expert Rev Gastroenterol Hepatol*. 2020;1-8.

28. Dudekula DB, Panda AC, Grammatikakis I, et al. CirCInteractome: A web tool for exploring circular RNAs and their interacting proteins and microRNAs. *RNA Biol*. 2016;13:34-42.

29. Yang S-J, Wang D-D, Zhou S-Y, et al. Identification of circRNA-miRNA networks for exploring an underlying prognosis strategy for breast cancer. *Epigenomics*. 2020;12:101-125.

---

How to cite this article: Yin L, Tang Y, Jiang M. Research on the circular RNA bioinformatics in patients with acute myocardial infarction. *J Clin Lab Anal*. 2020;35:e23621. [https://doi.org/10.1002/jcla.23621](https://doi.org/10.1002/jcla.23621)