

Expression Profile of Circular RNAs in Epicardial Adipose Tissue in Heart Failure

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Abstract

Recent studies have reported circular RNA (circRNA) expression profiles in various tissue types; specifically, a recent work showed a detailed circRNA expression landscape in the heart. However, circRNA expression profile in human epicardial adipose tissue (EAT) remains undefined. RNA-sequencing was carried out to compare circRNA expression patterns in EAT specimens from coronary artery disease (CAD) cases between the heart failure (HF) and non-HF groups. The top highly expressed EAT circRNAs corresponded to genes involved in cell proliferation and inflammatory response, including KIAA0182, RHOBTB3, HIPK3, UBXN7, PCMTD1, N4BP2L2, CFLAR, EPB41L2, FCHO2, FNDC3B and SPECC1. Among the 141 circRNAs substantially different between the HF and non-HF groups ($P < 0.05$; fold change > 2), hsa_circ_0005565 stood out, and was mostly associated with positive regulation of metabolic processes and insulin resistance in GO and KEGG pathway analyses, respectively. These data indicate EAT circRNAs contribute to the pathogenesis of metabolic disorders causing HF.

Introduction

Circular RNAs (circRNAs) constitute a new group of non-coding RNAs with a cyclic structure¹, and contribute to gene regulation. A recent study² carried out deep sequencing of ribosome-free RNAs from 12 human and 25 mouse hearts, as well as human embryonic stem cell-derived cardiomyocytes during differentiation for 28 days, and showed cardiac circRNA expression profile in detail, which provides a valuable reference for the further analysis of circRNAs. Not only have circRNAs been considered intracellular effectors in the pathophysiological alterations of cardiovascular tissues as well as cardiovascular disease markers³, they are also involved in multiple cardiovascular ailments^{2,4}.

However, as a key cardio-metabolic factor, epicardial adipose tissue (EAT) is scarcely included in heart tissue specimens in previous studies identifying circRNAs. EAT produces multiple bioactive molecules, including adipokines and cytokines^{5,6}, as well as microparticles carrying proteins, lipids and ribonucleic acids (RNAs)⁷. In addition, EAT is correlated with heart failure (HF), without regard to metabolic status or coronary artery disease (CAD)⁸⁻¹⁰. Meanwhile, EAT releases molecules with vasocrine and paracrine impacts on the myocardium^{11,12}. Therefore, EAT secretome alterations could regulate heart function.

This work aimed to compare circRNA expression patterns in EAT in CAD cases between the HF and non-HF groups. Our findings would supplement circRNA expression patterns in EAT for circRNA detection in cardiac specimens, also providing plausible HF markers.

Materials and Methods

Study Participants

The participants assessed in the present study have been described in our previous report¹³. EAT specimens were obtained from 10 CAD cases undergoing coronary artery bypass grafting in the Department of Heart Center, Beijing Chao-yang Hospital, Capital Medical University. They were assigned to the HF and non-HF groups (n=5/group). HF was defined by brain natriuretic peptide (BNP) >500ng/L and abnormal echocardiograms (left ventricular end diastolic diameter [LVEDD] >50mm

and >55mm in females and males, respectively; left ventricular ejection fraction [LVEF]<50%). The non-HF group comprised individuals showing BNP<100 ng/L and normal echocardiograms. This study had approval from the Ethics Committee of Beijing Chao-yang Hospital, Capital Medical University. Each patient provided written informed consent.

RNA-sequencing Procedure

Figure 1 shows the detailed RNA-sequencing procedure, which was described in our previous study¹³.

Statistical Analysis.

Data are mean±SD for continuous variables. Categorical variables were presented in percentages and numbers. Unpaired Student's t-test and the chi-squared test were employed to compare continuous and categorical variables, respectively. Fisher's exact test was utilized for assessing GO enrichment and pathways, with p<0.05 considered of significance. R was employed for determining FPKM and performing other statistical tests such as hierarchical clustering of genes showing differential expression levels.

Results

Patient Characteristics

The major characteristics of the HF and non-HF groups showed no significant differences (**Table 1**). However, the HF group had elevated BNP amounts and LVEDD, and reduced LVEF, in comparison with the non-HF group.

RNA Sequencing Findings

We performed RNA-seq analysis of ribosomal-depleted total RNAs extracted from EAT in CAD patients of the HF and non-HF groups (n=5/group). In total, 2278 EAT circRNAs were detected in the 10 CAD patients. The lengths of the EAT circRNAs detected were mainly within 2000nt (**Figure 2**). The numbers of exons per circRNA averaged 3 (ranging between 1 and 21). Single-exon circRNAs accounted for 9% (211/2278), while 2.7% (63/2278) of all circRNAs putatively had 10 exons or more. The circRNA hsa_circ_0087255 was the longest (21 exons). A total of 90% (2051/2278) circRNAs had average read counts of less than 10, while 0.5% (11/2278)

had values exceeding 50. This indicated the latter group had highest levels in human EAT; these circRNAs corresponded to genes such as KIAA0182, RHOBTB3, HIPK3, UBXN7, PCMTD1, N4BP2L2, CFLAR, EPB41L2, FCHO2, FNDC3B and SPECC1 (**Table 2**).

Hierarchical clustering was carried out for grouping differently expressed circRNAs in CAD cases of the HF and non-HF groups based on expression, and circRNA expression patterns in the HF group showed marked differences in comparison with the non-HF group. A total of 1240 circRNAs with significant level changes ($p < 0.05$) were detected, with 561 upregulated and 679 downregulated (**Figure 3**).

To explore the possible roles of circRNAs in EAT, we selected the circRNAs with substantially different amounts between the HF and non-HF groups ($P < 0.05$; fold change > 2) as potential novel biomarkers of HF; in total, they were 141, including 56 and 85 showing upregulation and downregulation, respectively (**Supplementary material, Table S1 and Table S2**). Among them, hsa_circ_0005565 had the highest fold change (27.4), and was highly expressed in all the 5 CAD patients with HF and lowly expressed in the non-HF group (**Figure 4**).

Protein-coding genes associated with differentially expressed circRNAs

The majority of circRNAs have unknown functions. Therefore, GO and KEGG pathway analyses were carried out of circRNAs with differential expression between the HF and non-HF groups to determine the associated protein-coding genes. The 3 first enriched GO terms in order were positive regulation of metabolic process (GO:0009893), positive regulation of cellular metabolic process (GO:0031325), and macromolecule metabolic process (GO:0043170). The first 3 KEGG pathways in order included insulin resistance (hsa04931), transcriptional misregulation in cancer (hsa05202), and platinum drug resistance (hsa01524).

Discussion

Here, circRNA expression patterns in EAT of CAD cases were compared between the HF and non-HF groups. A total of 11 circRNAs were highly expressed in human EAT, including hsa_circ_0000722, hsa_circ_0007444, hsa_circ_0000284,

hsa_circ_0001380, hsa_circ_0001801, hsa_circ_0000471, hsa_circ_0001092, hsa_circ_0001640, hsa_circ_0002490, hsa_circ_0006156 and hsa_circ_0000745). The circRNAs with differential expression in EAT among HF cases (including 56 upregulated and 85 downregulated circRNAs), as well as GO terms and KEGG pathways were also determined.

Expression profiling of circRNAs has been performed in various tissue types¹⁴. *In vivo*, circRNAs have been reported in cardiovascular tissues (right atrium, vena cava and heart) based on mouse or human GWAS data from blood cells^{15,16}. *In vitro*, circRNAs have been described in cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells, endothelial cells, macrophages, and blood monocytes¹⁷⁻¹⁹. A recent study² assessed ribosome-free RNAs from human and mouse cardiac specimens as well as human embryonic stem cell-derived cardiomyocytes by RNA-seq, detailedly revealing cardiac circRNA expression patterns, which provides a valuable reference for further circRNA analysis. However, circRNA expression profiles in human EAT remain largely unclear. Meanwhile, it is known that circRNAs are associated with different types of cardiovascular diseases, such as HF¹⁶.

EAT is a link between metabolic disorders and HF. Its thickness, independently of BMI, is positively associated with left ventricular mass²⁰. Epicardial fat amounts, without regard to metabolic status or CAD, correlates with impaired left ventricular function²¹ and myocardial fibrosis²². EAT is considered to be involved in the pro-inflammatory polarization and fibrotic transformation occurring in HF^{12, 22-24}. Similarly to other visceral adipose tissues, EAT secretes many molecules with exocrine and paracrine impacts on adjacent organs. These factors, released from EAT, might affect cardiac cell metabolism, as well as endothelial, arterial smooth muscle and inflammatory cell functions, causing HF.

In summary, the expression patterns of EAT circRNAs in CAD cases were identified, with special attention to those involved in HF. These findings also reveal potential HF biomarkers, which should be confirmed in further investigations.

Conflict of interest:

None declared

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Author contribution statement

Meili Zheng and Lei Zhao carried out data analysis and manuscript writing. Xinchun Yang performed study design.

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Table 1. Participants Characteristics

	CAD with HF (N=5)	CAD without HF (N=5)	P value
Male, n(%)	2(40)	3(60)	0.999
Age, years	67.8±5.0	60.8±8.1	0.139
BMI, kg/m ²	23.8±4.6	25.0±5.5	0.718
Diabetes, n(%)	2(40)	2(40)	1.000
Hypertension, n(%)	4(80)	5(100)	1.000
HbA1c, %	6.85±0.87	7.00±1.81	0.871
Total cholesterol, mmol/L	3.67±3.07	3.60±1.52	0.965
LDL-C, mmol/L	2.34±2.52	2.24±1.46	0.941
HDL-C, mmol/L	0.84±0.53	0.70±0.12	0.580
Triglyceride, mmol/L	0.89±0.38	1.86±0.89	0.055
Creatinine, µmol/L	88.2±25.2	76.8±16.8	0.424
Uric acid, µmol/L	363.2±82.4	357.0±69.3	0.901
BNP, pg/ml	1795.4±1053.3	76.4±22.6	0.006
LVEDD, mm	60.0±5.9	47.5±5.2	0.007
LVEF, %	44.2±10.2	62.0±4.8	0.008

Notes: BMI, body mass index; HbA1c, glycosylated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BNP, brain natriuretic peptide; LVEDD, left ventricular end diastolic diameter; LVEF, left ventricular ejection fraction.

Table 2. Top highest expressed circRNA in human epicardial adipose tissue

CircRNA	Locus	Gene	Length	Read Counts
				293
hsa_circ_0000722	chr16:85667519-85667738:+	KIAA0182	219	(236, 412, 306, 202, 294, 438, 151, 250, 376, 263)
				264
hsa_circ_0007444	chr5:95091099-95099324:+	RHOBTB3	479	(149, 332, 142, 203, 196, 234, 404, 403, 348, 232)
				258
hsa_circ_0000284	chr11:33307958-33309057:+	HIPK3	1099	(183, 430, 205, 214, 224, 192, 288, 266, 276, 305)
				72
hsa_circ_0001380	chr3:196118683-196129890:-	UBXN7	247	(75, 84, 46, 82, 63, 81, 73, 62, 80, 76)
				68
hsa_circ_0001801	chr8:52773404-52773806:-	PCMTD1	402	(56, 63, 47, 31, 48, 63, 126, 111, 76, 59)
				61
hsa_circ_0000471	chr13:33091993-33101669:-	N4BP2L2	388	(86, 50, 50, 54, 45, 57, 67, 63, 56, 77)
				57
hsa_circ_0001092	chr2:202010100-202014558:+	CFLAR	187	(43, 53, 50, 32, 47, 79, 80, 63, 49, 76)
				56
hsa_circ_0001640	chr6:131276244-131277639:-	EPB41L2	719	(41, 62, 36, 94, 41, 54, 71, 62, 50, 45)
				53
hsa_circ_0002490	chr5:72370568-72373320:+	FCHO2	268	(66, 43, 79, 30, 38, 35, 55, 66, 48, 66)
				51
hsa_circ_0006156	chr3:171965322-171969331:+	FNDC3B	526	(34, 81, 48, 50, 36, 52, 75, 40, 57, 41)
				51
hsa_circ_0000745	chr17:20107645-20109225:+	SPECC1	1580	(32, 53, 28, 37, 34, 57, 96, 66, 44, 61)

Notes: Locus, the position of circRNA in reference genome; Gene, the gene name of circRNA; Length, the sequence length of circRNA; Read Counts, the average read counts (the read counts of circRNA of all samples).

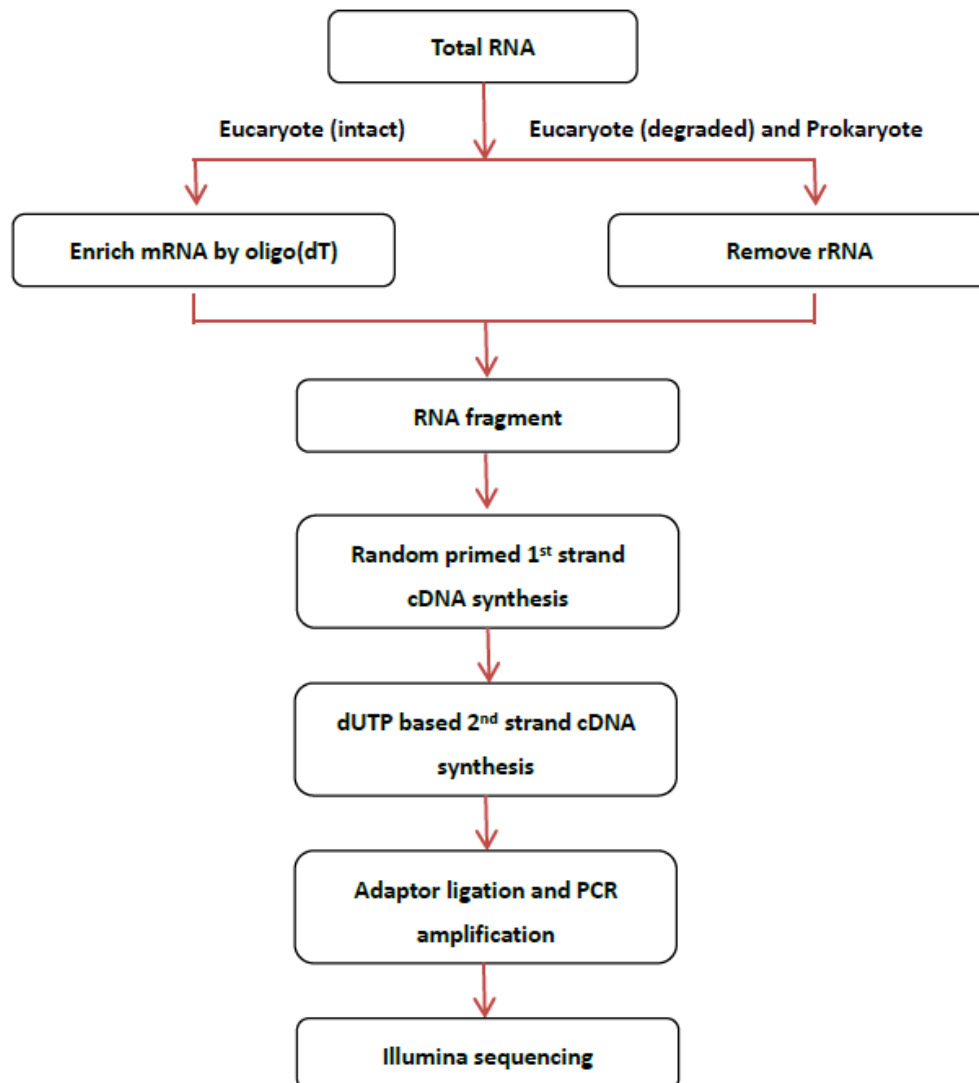


Figure 1. Flowchart of the RNA-sequencing Procedure.

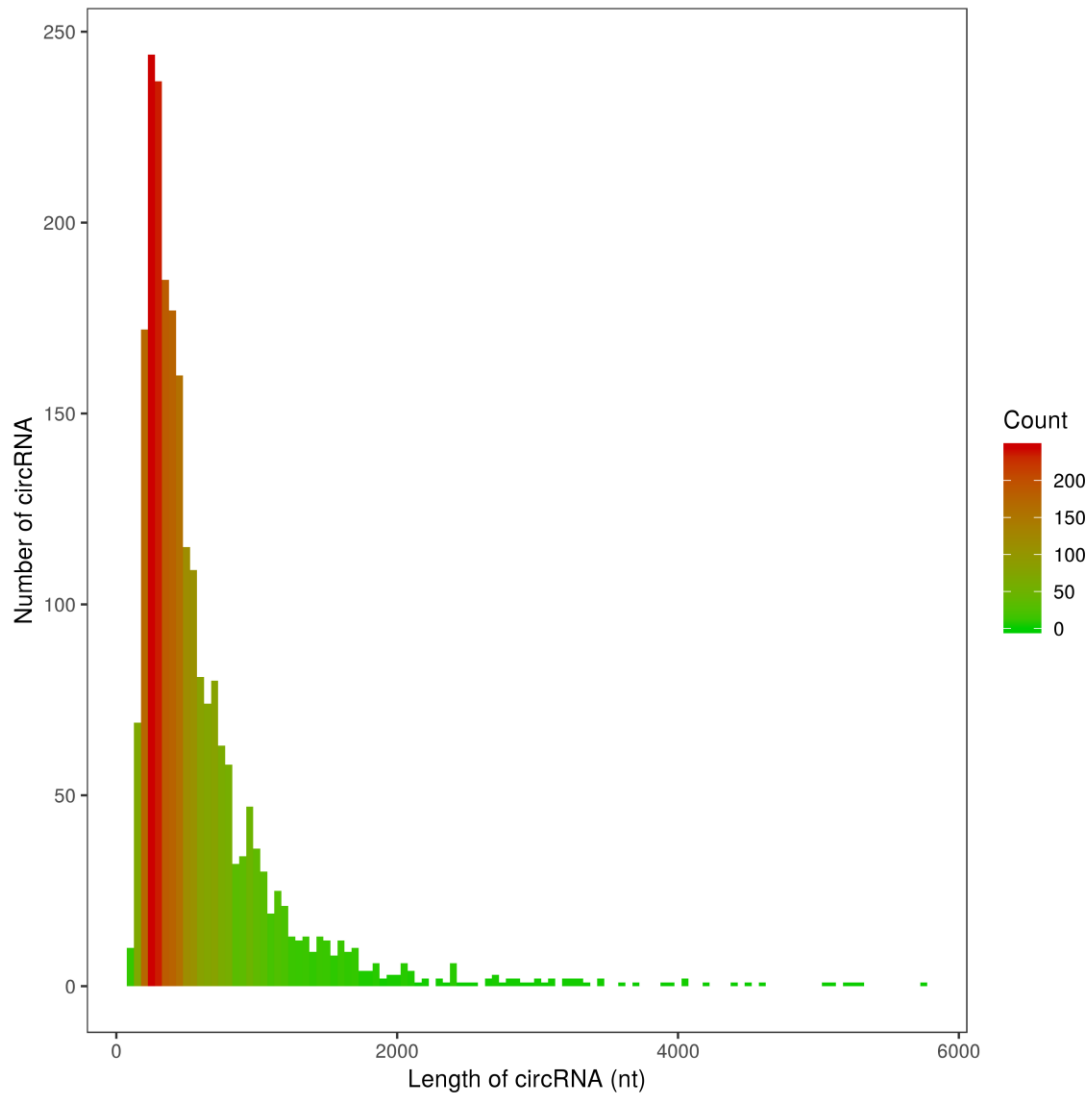


Figure 2. Length range of circRNAs in human epicardial adipose tissue.

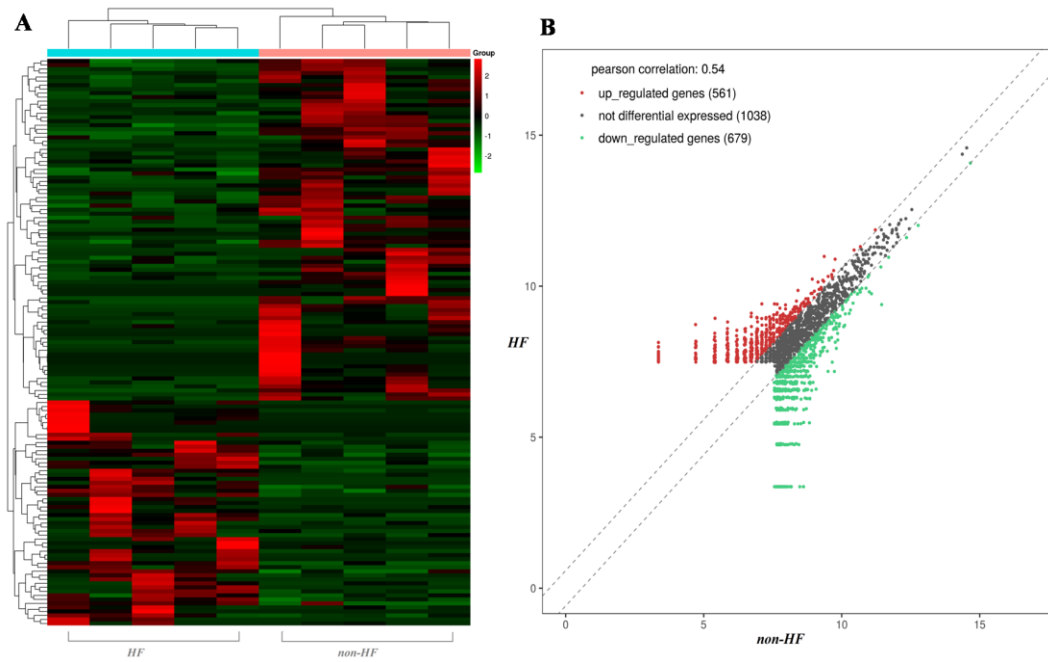


Figure 3. Hierarchical clustering (A) and scatter plot (B) of circRNAs with differential expression ($p < 0.05$) in the heart failure (HF) and non-HF groups (red and green denote upregulation and downregulation, respectively). A total of 1240 circRNAs were found (561 upregulated and 679 downregulated).

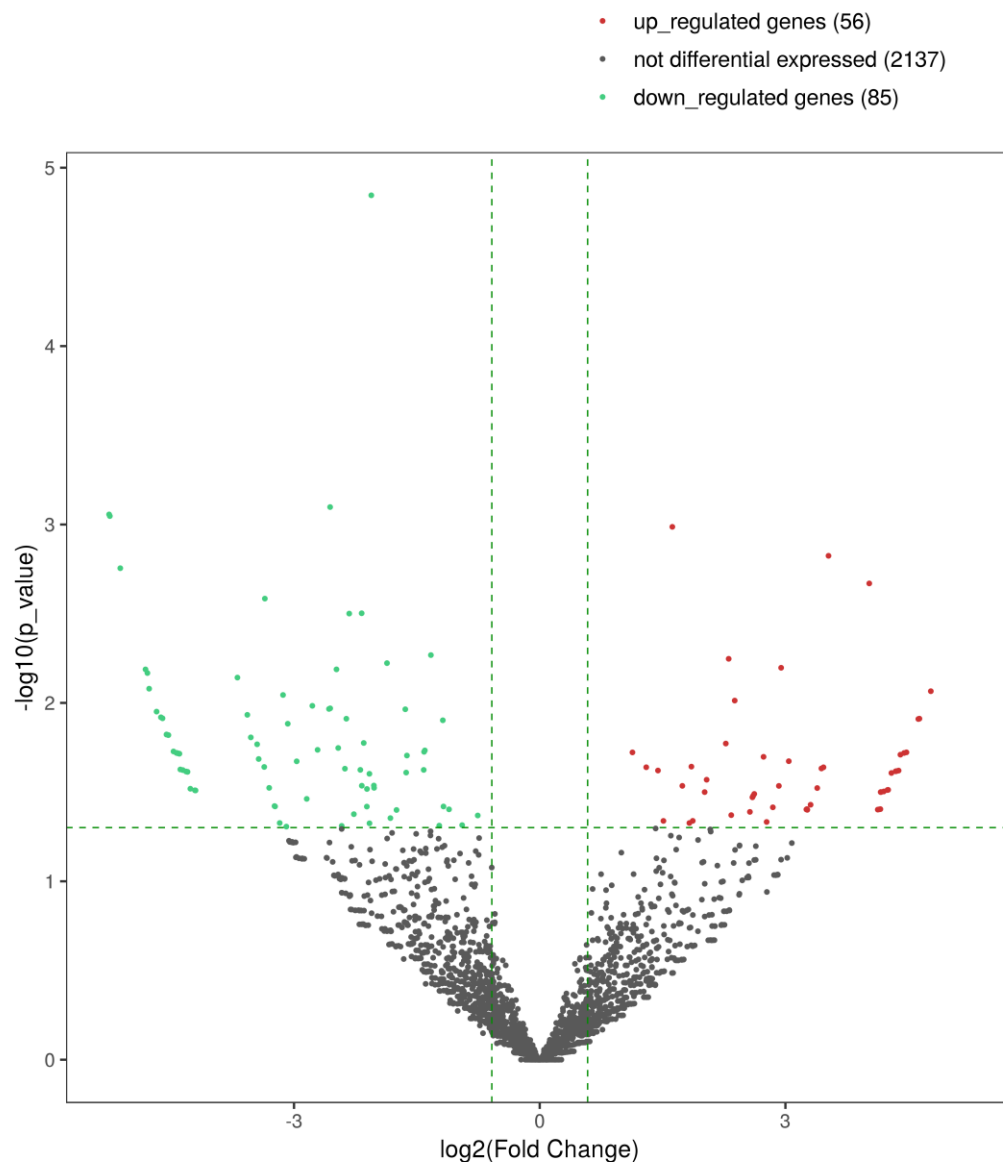


Figure 4. Volcano plot of circRNAs with differential expression ($P < 0.05$; fold change > 2) in the heart failure (HF) and non-HF groups (red and green denote upregulation and downregulation, respectively). A total of 141 circRNAs were found (56 upregulated and 85 downregulated).

