Expression profile of circular RNAs in epicardial adipose tissue in heart failure

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Abstract

Background: Recent studies have reported circular RNA (circRNA) expression profiles in various tissue types; however, circRNA expression profile in human epicardial adipose tissue (EAT) remains undefined. This work aimed to compare circRNA expression patterns in EAT between the heart failure (HF) and non-HF groups.

Methods: RNA-sequencing was carried out to compare circRNA expression patterns in EAT specimens from coronary artery disease cases between the HF and non-HF groups. Quantitative real-time polymerase chain reaction was performed for validation. Comparisons of patient characteristics between the two groups were using *t* test, Mann-Whitney *U* test, and Chi-squared test.

Results: A total of 141 circRNAs substantially different between the HF and non-HF groups ($P < 0.05$; fold change >2) were detected, including 56 up-regulated and 85 down-regulated. Among them, hsa_circ_0005565 stood out, for it had the highest fold change and was significantly increased in HF patients in quantitative real-time polymerase chain reaction validation. The top highly expressed EAT circRNAs corresponded to genes involved in cell proliferation and inflammatory response, including GSE1, RHOBTB3, HIPK3, UBXN7, PCMTD1, N4BP2L2, CFLAR, EPB41L2, FCHO2, FNDC3B, and SPECC1. The top enriched Gene Ontology term and Kyoto Encyclopedia of Genes and Genomes pathway were positive regulation of metabolic processes and insulin resistance, respectively.

Conclusion: These data indicate EAT circRNAs may contribute to the pathogenesis of metabolic disorders causing HF.

Keywords: CircRNA; Epicardial adipose tissue; Heart failure

Introduction

Circular RNAs (circRNAs) constitute a new group of non-coding RNAs with a cyclic structure,[1] and contribute to gene regulation. A recent study[2] 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 pathophysiologic alterations of cardiovascular tissues as well as cardiovascular disease markers,[3] 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 micro-particles 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 study aimed to compare circRNA expression patterns in EAT in patients with CAD 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.

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Methods

Ethical approval

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Ethics Committee of Beijing Chao-yang Hospital, Capital Medical University (No. 2018-Ke-21). Informed written consent was obtained from all patients prior to their enrollment in this study.

Study participants

The participants assessed in the present study have been described in our previous report.[13] EAT specimens were obtained from ten patients with CAD undergoing coronary artery bypass grafting in 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) >500 ng/L and abnormal echocardiograms (left ventricular end diastolic diameter >50 mm and >55 mm in females and males, respectively; left ventricular ejection fraction <50%). The non-HF group comprised individuals showing BNP <100 ng/L and normal echocardiograms.

RNA-sequencing procedure

The detailed RNA-sequencing procedure was described in our previous study[13] [Supplementary Figure 1, http://links.lww.com/CM9/A290].

Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to measure selected hsa_circ_0005565 according to standard methods: the forward primer was 5′-AACCAGCGGCTTCAATAACAA-3′, the reverse primer was 5′-GCTGTTGGTGCAGGTGGTCTA-3′. The house keeping gene used for qRT-PCR analysis was β-actin.

Statistical analysis

Comparisons of patient characteristics between the two groups were performed using SPSS version 24.0 software (IBM, Armonk, NY, USA). Continuous variables with normal distribution were expressed as mean ± standard deviation, and compared by two-sample t test, while those with non-normal distributed were expressed as median (Q1, Q3) and compared by Mann-Whitney U test; categorical variables were expressed as percentages and numbers, and compared using the Chi-squared test. R package was used to calculate the Fragments Per Kilobase of exon model per Million mapped fragments value and differential expression for transcript level and perform hierarchical clustering, Gene Ontology (GO) enrichment, pathway analysis, scatter plots, and volcano plots with the differentially expressed genes. A $P < 0.05$ was considered as statistically significant.

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 (P = 0.006) and left ventricular end diastolic diameter (P = 0.007), and reduced left ventricular ejection fraction (P = 0.008), in comparison with the non-HF group.

RNA sequencing findings

We performed RNA-sequencing analysis of ribosomal-depleted total RNAs extracted from EAT in patients with

Table 1: Clinical characteristics of coronary artery disease patients with and without HF.

Characteristics	HF group (n = 5)	Non-HF group (n = 5)	Statistical values	P
Male	2	3	0*	0.999
Age (years)	67.8 ± 5.0	60.8 ± 8.1	−1.644†	0.139
BMI (kg/m^2)	23.8 ± 4.6	25.0 ± 5.5	0.374†	0.718
Diabetes	2	2	0.417*	1.000
Hypertension	4	5	0*	1.000
HbA1c (%)	6.85 ± 0.87	7.00 ± 1.81	0.167†	0.871
Total cholesterol (mmol/L)	2.68 (1.17, 5.08)	3.13 (2.64,4.63)	−0.046‡	0.965
LDL-C (mmol/L)	1.40 (0.50, 3.10)	1.50 (1.40, 3.20)	−0.077‡	0.941
HDL-C (mmol/L)	0.70 (0.40, 1.30)	0.70 (0.60, 0.80)	−0.576‡	0.580
Triglyceride (mmol/L)	0.78 (0.74, 1.18)	1.39 (1.22, 2.36)	2.241‡	0.055
Creatinine (μmol/L)	88.2 ± 25.2	76.8 ± 16.8	−0.842†	0.424
Uric acid (μmol/L)	363.2 ± 82.4	357.0 ± 69.3	−0.129†	0.901
BNP (pg/mL)	1795.4 ± 1053.3	76.4 ± 22.6	−3.648†	0.006
LVEDD (mm)	60.0 ± 5.9	47.5 ± 5.2	−3.554†	0.007
LVEF (%)	44.2 ± 10.2	62.0 ± 4.8	3.531†	0.008

Data are shown as mean ± standard deviation, median (Q1, Q3), or n. * Chi-squared values. † t values. ‡ U values. BMI: Body mass index; BNP: Brain natriuretic peptide; HbA1c: Glycosylated hemoglobin; HDL-C: High-density lipoprotein cholesterol; HF: Heart failure; LDL-C: Low-density lipoprotein cholesterol; LVEDD: Left ventricular end diastolic diameter; LVEF: Left ventricular ejection fraction.

CAD of the HF and non-HF groups ($n = 5$/group). In total, 2278 EAT circRNAs were detected in the ten patients with CAD. The lengths of the EAT circRNAs detected were mainly within 2000 nt [Figure 1]. On the basis of back-spliced junctions and existing exon-exon splicing annotation (CIRCexplorer2 was used for circRNA analysis according to a previous study[14]), the predicted number of exons per circRNA averaged 3 (ranging between 1 and 21). Single-exon circRNAs accounted for 9.3% (211/2278), while 2.8% (63/2278) of all circRNAs were predicted to span at least ten exons. The circRNA hsa_circ_0087255 was the longest (21 exons). A total of 90.0% (2051/2278) circRNAs had average read counts of less than 10, while 0.5% (11/2278) had values exceeding 50. This indicated that the latter group had highest levels in human EAT; these circRNAs corresponded to genes such as GSE1, RHOBTB3, HIPK3, UBXN7, PCMTD1, N4BP2L2, CFLAR, EPB41L2, FCHO2, FNDC3B, and SPECC1 [Table 2].

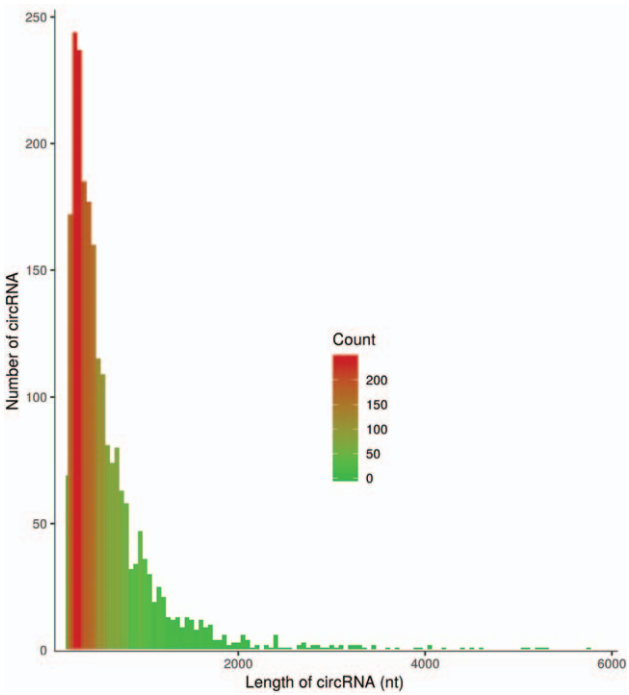

Figure 1: Length range of circular RNAs (circRNAs) in human epicardial adipose tissue. nt: Nucleotide.

Hierarchical clustering was carried out for grouping differently expressed circRNAs in patients with CAD 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 up-regulated and 679 down-regulated [Figure 2].

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 up-regulated circRNAs [Table 3, Supplementary Figure 2, http://links.lww.com/CM9/A291] and 85 down-regulated circRNAs [Table 4, Supplementary Figure 2, http://links.lww.com/CM9/A291], respectively. Among them, hsa_circ_0005565 had the highest fold change (27.4), and was highly expressed in all the five patients with CAD with HF and lowly expressed in the non-HF group. In qRT-PCR validation, hsa_circ_0005565 was significantly increased in HF patients ($P = 0.008$) [Figure 3] and might be a potential biomarker for HF.

Protein-coding genes associated with differentially expressed circRNAs

The majority of circRNAs have unknown functions. Therefore, GO terms [Figure 4] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [Figure 5] analyses were carried out of circRNAs with differential expression between the HF and non-HF groups to determine the associated protein-coding genes. The three 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).

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

CircRNA	Locus	Genes	Length (bp)	Read counts*
hsa_circ_0000722	chr16:85667519-85667738:+	*GSE1*	219	293 (236, 412, 306, 202, 294, 438, 151, 250, 376, 263)
hsa_circ_0007444	chr5:95091099-95099324:+	*RHOBTB3*	479	264 (149, 332, 142, 203, 196, 234, 404, 403, 348, 232)
hsa_circ_0000284	chr11:33307958-33309057:+	*HIPK3*	1099	258 (183, 430, 205, 214, 224, 192, 288, 266, 276, 305)
hsa_circ_0001380	chr3:196118683-196129890:−	*UBXN7*	247	72 (75, 84, 46, 82, 63, 81, 73, 62, 80, 76)
hsa_circ_0001801	chr8:52773404-52773806:−	*PCMTD1*	402	68 (56, 63, 47, 31, 48, 63, 126, 111, 76, 59)
hsa_circ_0000471	chr13:33091993-33101669:−	*N4BP2L2*	388	61 (86, 50, 50, 54, 45, 57, 67, 63, 56, 77)
hsa_circ_0001092	chr2:202010100-202014558:+	*CFLAR*	187	57 (43, 53, 50, 32, 47, 79, 80, 63, 49, 76)
hsa_circ_0001640	chr6:131276244-131277639:−	*EPB41L2*	719	56 (41, 62,36, 94, 41, 54, 71, 62, 50, 45)
hsa_circ_0002490	chr5:72370568-72373320:+	*FCHO2*	268	53 (66, 43, 79, 30, 38, 35, 55, 66, 48, 66)
hsa_circ_0006156	chr3:171965322-171969331:+	*FNDC3B*	526	51 (34, 81, 48, 50, 36, 52, 75, 40, 57, 41)
hsa_circ_0000745	chr17:20107645-20109225:+	*SPECC1*	1580	51 (32, 53, 28, 37, 34, 57, 96, 66, 44, 61)

* The average read counts (the read counts of circRNA of all samples). CircRNA: Circular RNA.

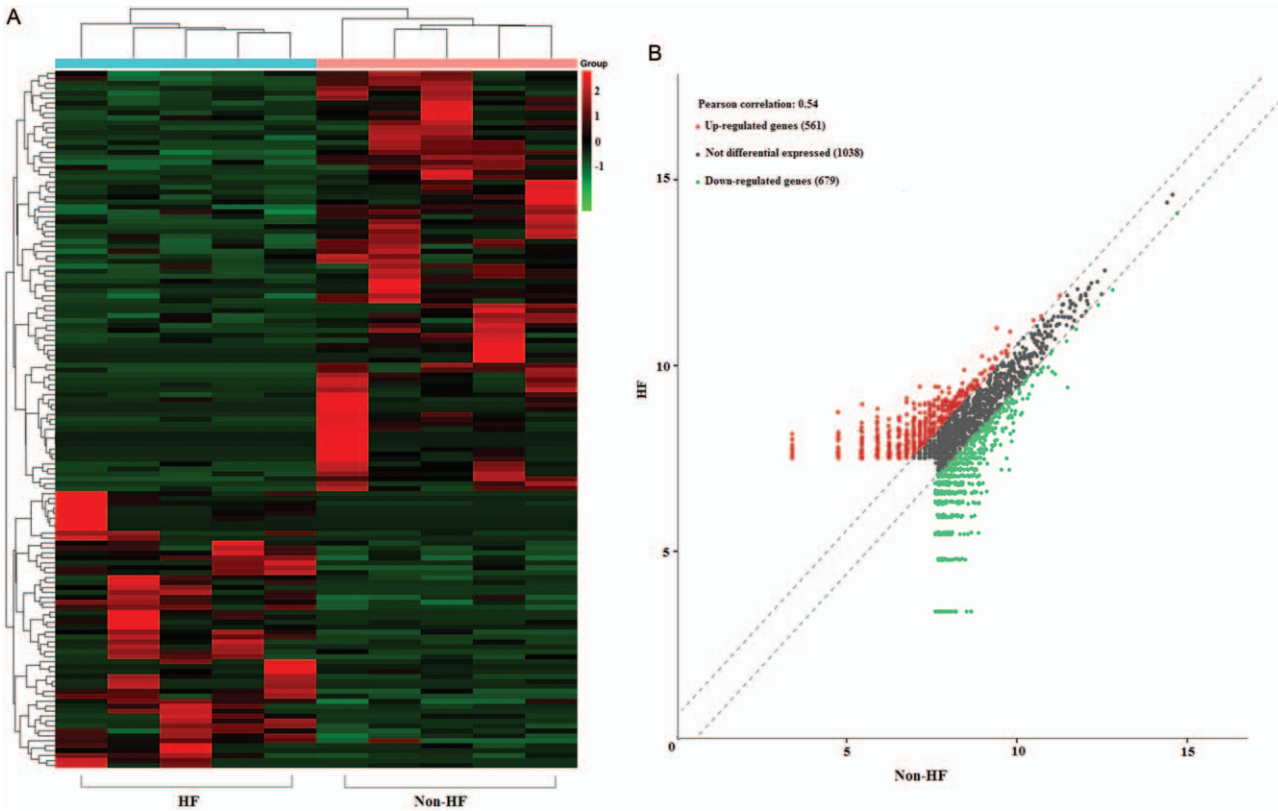

Figure 2: Hierarchical clustering (A) and scatter plot (B) of circRNAs with differential expression ($P < 0.05$) in the HF and non-HF groups (red and green denote up-regulation and down-regulation, respectively). A total of 1240 circRNAs were found (561 up-regulated and 679 down-regulated). HF: Heart failure.

Table 3: Up-regulated circRNA of HF group compared with non-HF group.

CircRNA	Locus	Genes	Length (bp)	Fold change	*P*
hsa_circ_0005565	chr17:30689932-30695033:+	*ZNF207*	565	27.38140408	0.008598851
hsa_circ_0129624	chr5:74675161-74685512:–	*COL4A3BP*	696	24.86264606	0.012255936
hsa_circ_0047021	chr18:13048857-13059311:+	*CEP192*	2421	24.72359702	0.012289967
hsa_circ_0019611	chr10:103558598-103560157:–	*MGEA5*	773	24.63647121	0.012311795
hsa_circ_0000749	chr17:27430616-27434207:–	*MYO18A*	176	22.27650415	0.018929488
hsa_circ_0076125	chr6:35280078-35280549:+	*DEF6*	384	21.84158085	0.019084565
chr6:16486202-16658132:–	chr6:16486202-16658132:–	*ATXN1*	454	21.16825381	0.01950708
hsa_circ_0008263	chr19:46116798-46117958:–	*EML2*	227	20.84579324	0.023950416
hsa_circ_0116462	chr22:32108068-32121596:–	*PRR14L*	5282	20.48584952	0.024105109
hsa_circ_0007203	chr3:196807921-196846401:–	*DLG1*	740	20.32683989	0.024174277
hsa_circ_0118648	chr2:202400743-202446940:–	*ALS2CR11*	990	19.61633381	0.024708032
hsa_circ_0119571	chr2:24787163-24866991:+	*NCOA1*	378	19.0818756	0.03072117
hsa_circ_0057608	chr2:197777605-197786910:–	*PGAP1*	586	19.00729654	0.030762086
hsa_circ_0103215	chr15:25638892-25657118:–	*UBE3A*	339	18.96668046	0.030780743
hsa_circ_0012109	chr1:44363906-44365399:+	*ST3GAL3*	347	18.38544959	0.031360571
hsa_circ_0017099	chr1:236148678-236154358:–	*NID1*	300	17.94901854	0.03159643
chr6:17783876-17790141:–	chr6:17783876-17790141:–	*KIF13A*	322	17.94901854	0.03159643
hsa_circ_0098611	chr12:459786-475270:–	*KDM5A*	942	17.90831862	0.031618867
hsa_circ_0138685	chr9:3488775-3490345:–	*RFX3*	187	17.86765346	0.039392115
hsa_circ_0128726	chr5:179136873-179150780:+	*CANX*	990	17.70686546	0.039494674
hsa_circ_0064654	chr3:30686238-30715738:+	*TGFBR2*	1302	17.47868633	0.039640952
hsa_circ_0008833	chr6:130505247-130505768:–	*SAMD3*	271	16.26295988	0.002139375
hsa_circ_0002290	chr11:77396149-77404656:–	*RSF1*	240	11.51930187	0.001497853
hsa_circ_0023670	chr11:76696686-76709865:+	*ACER3*	177	11.0388378	0.022959611
hsa_circ_0086694	chr9:33932559-33933626:–	*UBAP2*	206	10.84816913	0.023307007
hsa_circ_0007493	chr2:61505299-61508377:–	*USP34*	435	10.46996756	0.030008409
hsa_circ_0025128	chr12:6442234-6443410:–	*TNFRSF1A*	512	9.898405267	0.037227819
hsa_circ_0008271	chr7:102412855-102427916:+	*FAM185A*	273	9.625514855	0.039717893
hsa_circ_0008275	chr1:12359258-12368698:+	*VPS13D*	617	9.62342074	0.03973916
hsa_circ_0031939	chr14:52977957-53011089:–	*TXNDC16*	937	9.593218315	0.039380882
hsa_circ_0008157	chr11:118425173-118430579:–	*IFT46*	518	9.552132968	0.03955805
hsa_circ_0028099	chr12:109577201-109577863:+	*ACACB*	662	8.22971981	0.021220204

(*continued*)

Table 3
(continued).

CircRNA	Locus	Genes	Length (bp)	Fold change	*P*
hsa_circ_0084764	chr8:72211281-72246409:–	*EYA1*	702	7.713988907	0.00635426
hsa_circ_0004538	chr12:51467605-51467865:–	*CSRNP2*	260	7.568205093	0.029190438
hsa_circ_0012425	chr1:51860052-51913807:–	*EPS15*	1156	7.192204797	0.038487019
hsa_circ_0011377	chr1:32689634-32690076:+	*EIF3I*	154	6.823596136	0.046488026
hsa_circ_0008223	chr16:28177828-28181230:–	*XPO6*	238	6.647115397	0.020056305
hsa_circ_0092984	chr10:123298105-123325218:–	*FGFR2*	639	6.145283077	0.032379561
hsa_circ_0066682	chr3:101304280-101309114:+	*PCNP*	131	6.089275279	0.033157013
hsa_circ_0064645	chr3:29910348-29977687:+	*RBMS3*	413	6.049926234	0.033822004
hsa_circ_0027244	chr12:57682791-57690334:–	*R3HDM2*	282	5.922510609	0.040818274
hsa_circ_0083756	chr8:27151596-27151827:–	*TRIM35*	231	5.208018799	0.009705216
hsa_circ_0007717	chrX:13684435-13698717:+	*TCEANC*	964	5.057578474	0.042568327
hsa_circ_0006376	chr8:42812236-42819617:+	*HOOK3*	311	4.948783788	0.005659977
hsa_circ_0006087	chr5:145634505-145638156:+	*RBM27*	295	4.829701417	0.016895556
hsa_circ_0030051	chr13:41515056-41518061:–	*ELF1*	727	4.106755498	0.02695576
hsa_circ_0120233	chr2:48869540-48898819:+	*STON1-GTF2A1L*	1082	4.038002822	0.031619956
hsa_circ_0118701	chr2:203620260-203624076:+	*FAM117B*	491	3.65129251	0.045830218
hsa_circ_0099634	chr12:97886238-97954825:+	*RMST*	1314	3.609560699	0.022760573
hsa_circ_0033144	chr14:99723807-99724176:–	*BCL11B*	369	3.548221774	0.047128109
hsa_circ_0000396	chr12:46622935-46637097:–	*SLC38A1*	522	3.343282539	0.029173369
hsa_circ_0001847	chr9:33953282-33963789:–	*UBAP2*	377	3.071888941	0.001030606
hsa_circ_0007817	chrX:53641494-53642796:–	*HUWE1*	304	2.84867162	0.045858186
hsa_circ_0000053	chr1:36826821-36828257:–	*STK40*	218	2.720299718	0.023934941
hsa_circ_0128780	chr5:21491429-21497305:+	*GUSBP1*	448	2.463027543	0.022946216
hsa_circ_0003426	chr20:32207322-32211102:+	*CBFA2T2*	272	2.191793227	0.018940558

CircRNAs: Circular RNAs; HF: Heart failure.

Table 4: Down-regulated circRNA of HF group compared with non-HF group.

CircRNA	Locus	Genes	Length (bp)	Fold change	*P*
hsa_circ_0005268	chr18:12370847-12371690:–	*AFG3L2*	178	0.026142196	0.000878077
hsa_circ_0002371	chr10:70545892-70546449:+	*CCAR1*	230	0.0263028	0.000896776
chr1:1330773-1330894:–	chr1:1330773-1330894:–	*CCNL2*	121	0.028731724	0.001757764
hsa_circ_0078619	chr6:161469647-161471011:+	*MAP3K4*	1364	0.035567145	0.006488951
hsa_circ_0099494	chr12:93226287-93246801:–	*EEA1*	848	0.036147847	0.006800891
hsa_circ_0004901	chr12:99071202-99080651:+	*APAF1*	511	0.036679856	0.00832476
hsa_circ_0001139	chr20:32659871-32665051:+	*RALY*	837	0.039070179	0.011190314
hsa_circ_0085769	chr8:141856358-141889736:–	*PTK2*	398	0.04052931	0.012041717
hsa_circ_0078617	chr6:161455290-161471011:+	*MAP3K4*	1555	0.041081657	0.012200344
chr1:27697086-27700942:–	chr1:27697086-27700942:–	*FCN3*	567	0.042497835	0.015040601
hsa_circ_0093908	chr10:69726439-69797926:–	*HERC4*	1540	0.042497835	0.015040601
hsa_circ_0006667	chr3:183368083-183390272:+	*KLHL24*	1663	0.043116853	0.01514122
chr5:79974745-80088663:+	chr5:79974745-80088663:+	*MSH3*	1482	0.043171062	0.015150269
hsa_circ_0132295	chr6:76331247-76388643:+	*SENP6*	2002	0.045080967	0.018713783
hsa_circ_0042828	chr17:28593902-28613920:–	*BLMH*	753	0.046198634	0.019062659
hsa_circ_0006248	chr3:185638891-185641772:–	*TRA2B*	389	0.046349754	0.019087833
hsa_circ_0029920	chr13:32768279-32776664:+	*FRY*	427	0.046575201	0.019126662
hsa_circ_0007694	chr11:108137897-108138069:+	*ATM*	172	0.04700422	0.019198614
hsa_circ_0000018	chr1:15860731-15863309:+	*DNAJC16*	407	0.047306387	0.019250139
hsa_circ_0062936	chr22:32108068-32113277:–	*PRR14L*	5209	0.047396027	0.019265095
hsa_circ_0108197	chr18:29218606-29246335:–	*B4GALT6*	473	0.047763942	0.02361012
hsa_circ_0012417	chr1:51829537-51831701:–	*EPS15*	240	0.048772425	0.023796844
hsa_circ_0108003	chr18:18571136-18600200:–	*ROCK1*	871	0.050139593	0.024234392
hsa_circ_0080826	chr7:76990140-77006707:–	*PION*	451	0.050236519	0.024250628
hsa_circ_0083196	chr7:158528193-158531812:–	*ESYT2*	337	0.050738232	0.024340624
hsa_circ_0114230	chr1:7895821-7897209:+	*PER3*	335	0.050751804	0.024342874
hsa_circ_0099310	chr12:81503338-81545875:+	*ACSS3*	787	0.05199158	0.030276693
hsa_circ_0007183	chrX:154736558-154754293:–	*TMLHE*	814	0.054040561	0.030899442
hsa_circ_0006723	chr11:47444124-47444524:–	*PSMC3*	293	0.054206683	0.030930714
hsa_circ_0086743	chr9:34234213-34242106:+	*UBAP1*	1049	0.054314364	0.030950676
hsa_circ_0062938	chr22:32154531-32161046:+	*DEPDC5*	221	0.07743487	0.007212382
hsa_circ_0066536	chr3:71739160-71759635:–	*EIF4E3*	452	0.084201406	0.011664274
hsa_circ_0001512	chr5:94224583-94248681:–	*MCTP1*	583	0.086719534	0.015605756
hsa_circ_0077495	chr6:101246588-101253756:–	*ASCC3*	594	0.091482641	0.017060834
hsa_circ_0084140	chr8:42725146-42729149:–	*RNF170*	185	0.092696407	0.02062798
hsa_circ_0001630	chr6:99860426-99864304:–	*PNISR*	388	0.097155527	0.022856931
hsa_circ_0007503	chr10:28872327-28884970:+	*WAC*	645	0.097663673	0.002602553

(*continued*)

Table 4
(continued).

CircRNA	Locus	Genes	Length (bp)	Fold change	*P*
hsa_circ_0003911	chr16:58608512-58617086:–	*CNOT1*	1173	0.101307578	0.029936354
hsa_circ_0068610	chr3:195785154-195787118:–	*TFRC*	209	0.105989315	0.037863145
hsa_circ_0003570	chr10:126370175-126384781:–	*FAM53B*	828	0.106483482	0.038045427
hsa_circ_0004210	chr7:156758963-156759786:+	*NOM1*	265	0.110755333	0.047127231
hsa_circ_0073706	chr5:122881110-122911657:+	*CSNK1G3*	920	0.113903208	0.009027269
hsa_circ_0056558	chr2:136360069-136362586:+	*R3HDM1*	420	0.117162525	0.049359712
hsa_circ_0001486	chr5:56526672-56527148:+	*GPBP1*	348	0.118562456	0.013083493
hsa_circ_0079673	chr7:28527792-28610155:+	*CREB5*	461	0.12786215	0.021254235
chr17:66898853-66904020:–	chr17:66898853-66904020:–	*ABCA8*	626	0.139198603	0.034514046
hsa_circ_0056248	chr2:120684173-120702816:+	*PTPN4*	514	0.145978995	0.010382494
hsa_circ_0136856	chr8:62531536-62596747:–	*ASPH*	873	0.152604696	0.018328569
hsa_circ_0069718	chr4:52729602-52765544:+	*DCUN1D4*	1156	0.168083179	0.010809857
hsa_circ_0006365	chr7:65751497-65751696:+	*TPST1*	199	0.169678885	0.000798391
hsa_circ_0070562	chr4:106155053-106158508:+	*TET2*	3455	0.169782067	0.010728197
hsa_circ_0022537	chr11:62594626-62595103:–	*STX5*	198	0.179047042	0.006486127
hsa_circ_0021570	chr11:32948702-32956981:+	*QSER1*	3968	0.181703264	0.017892199
hsa_circ_0004717	chr1:154223516-154224129:+	*UBAP2L*	451	0.187111432	0.04890254
hsa_circ_0002910	chr17:63739185-63746842:–	*CEP112*	213	0.192294616	0.023372885
hsa_circ_0003275	chr18:76953182-76974038:+	*ATP9B*	234	0.194625732	0.012264088
hsa_circ_0064335	chr3:12353878-12422990:+	*PPARG*	472	0.199440913	0.003157276
hsa_circ_0007193	chr21:37619814-37623582:+	*DOPEY2*	424	0.207439212	0.04204242
chr19:6697354-6697805:–	chr19:6697354-6697805:–	*C3*	356	0.219123557	0.02373141
hsa_circ_0001947	chrX:147743428-147744289:+	*AFF2*	861	0.221586841	0.003144261
hsa_circ_0004179	chr16:11063017-11076848:+	*CLEC16A*	728	0.221851507	0.029122319
hsa_circ_0001459	chr4:178274461-178274882:+	*NEIL3*	421	0.225498605	0.016772984
hsa_circ_0003164	chr14:89041036-89044484:+	*ZC3H14*	418	0.231510084	0.038124627
hsa_circ_0107487	chr17:64023618-64026141:–	*CEP112*	438	0.2317629	0.030368215
hsa_circ_0125725	chr4:166960490-166999182:+	*TLL1*	1284	0.236598602	0.024965464
hsa_circ_0005362	chr3:169889160-169896726:–	*PHC3*	400	0.236757306	0.047309616
hsa_circ_0073237	chr5:82832825-82838087:+	*VCAN*	5262	0.240401684	1.42787E-05
hsa_circ_0007381	chr2:230701563-230744844:–	*TRIP12*	1193	0.245737648	0.029049766
hsa_circ_0005405	chr9:33351557-33352717:+	*NFX1*	305	0.246129832	0.029932253
hsa_circ_0095427	chr11:14852243-14882912:+	*PDE3B*	1079	0.274572964	0.005984753
hsa_circ_0001932	chrX:76907603-76912143:–	*ATRX*	437	0.28257951	0.044237185
hsa_circ_0002162	chr8:141856358-141874498:–	*PTK2*	231	0.297624737	0.039832629
hsa_circ_0023936	chr11:85718584-85742653:–	*PICALM*	677	0.320591442	0.010857001
hsa_circ_0000347	chr11:85722072-85742653:–	*PICALM*	635	0.322939594	0.024581434
hsa_circ_0046843	chr18:9195548-9221997:+	*ANKRD12*	856	0.325019888	0.019728111
hsa_circ_0130908	chr6:144858717-144864006:+	*UTRN*	362	0.374663002	0.02374435
hsa_circ_0071480	chr4:183522076-183550042:+	*ODZ3*	477	0.375793135	0.018801873
hsa_circ_0006602	chr1:29481207-29481422:–	*SRSF4*	215	0.378252457	0.018468847
hsa_circ_0004658	chr18:2890558-2892484:+	*EMILIN2*	1926	0.398121805	0.005394708
hsa_circ_0086376	chr9:14146687-14179779:–	*NFIB*	363	0.42729236	0.048740491
hsa_circ_0001400	chr4:37633006-37640126:–	*RELL1*	434	0.440418601	0.012514583
hsa_circ_0001346	chr3:149563797-149639014:+	*RNF13*	716	0.442873106	0.038078237
hsa_circ_0001524	chr5:131013395-131044965:–	*FNIP1*	813	0.464163082	0.03950502
hsa_circ_0008732	chr9:16727794-16738483:–	*BNC2*	327	0.518639544	0.048430222
hsa_circ_0001801	chr8:52773404-52773806:–	*PCMTD1*	402	0.591846765	0.042757958

CircRNAs: Circular RNAs; HF: Heart failure.

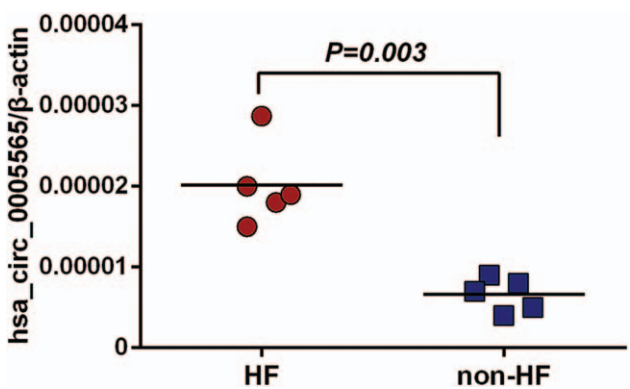

Figure 3: Quantitative real-time polymerase chain reaction analysis of expression of hsa_circ_0005565 in patients with HF and without heart failure (non-HF) ($n = 5$ in each group). HF: Heart failure.

Discussion

Here, circRNA expression patterns in EAT of patients with CAD 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 up-regulated and 85 down-regulated circRNAs), as well as GO terms and KEGG pathways were also determined.

Expression profiling of circRNAs has been performed in various tissue types.[15] *In vivo*, circRNAs have been reported in cardiovascular tissues (right atrium, vena

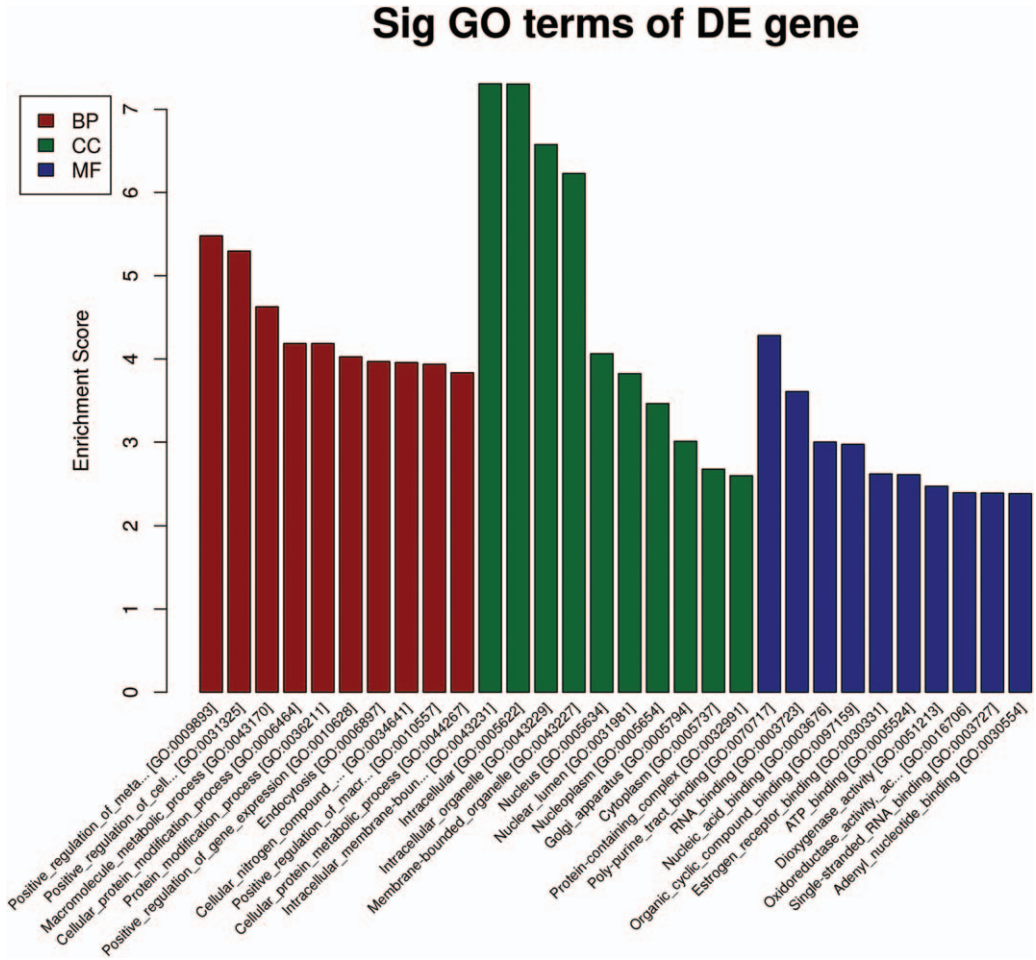

Figure 4: Gene Ontology annotation analysis for circRNAs with differential expression between patients with and without heart failure. BP: Biological process (red pillars); CC: Cellular component (green pillars); DE: Differential expression; GO: Gene Ontology; MF: Molecular function (blue pillars).

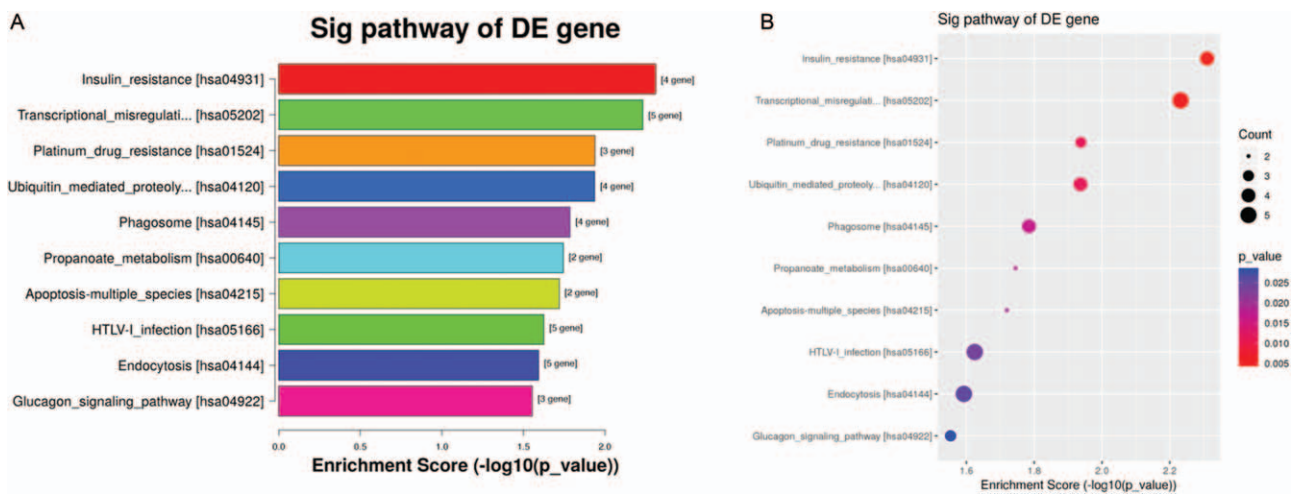

Figure 5: KEGG pathways analysis for circRNAs with differential expression between patients with and without heart failure: (A) Enrichment score; (B) Enrichment score dot plot. DE: Differential expression; KEGG: Kyoto Encyclopedia of Genes and Genomes.

cava, and heart) based on mouse or human Genome-wide association studies (GWAS) data from blood cells.[16,17] *In vitro*, circRNAs have been described in cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells, endothelial cells, macrophages, and blood monocytes.[18-20] A recent study[2] assessed ribosome-free RNAs from human and mousecardiac specimens as well as human embryonic stem cell-derived cardiomyocytes by RNA-seq, detailedly

revealing cardiac circRNA expression patterns, which provided a valuable reference for further circRNA analysis.[2] 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 HF.[17]

EAT is a link between metabolic disorders and HF. Its thickness, independently of BMI, is positively associated with left ventricular mass.[21] Epicardial fat amounts, without regard to metabolic status or CAD, correlates with impaired left ventricular function and myocardial fibrosis.[22,23] ETA is considered to be involved in the pro-inflammatory polarization and fibrotic transformation occurring in HF.[12,23-25] 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 patients with CAD were identified, with special attention to those involved in HF. These findings also reveal potential HF biomarkers, which should be confirmed in further investigations.

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Conflicts of interest

None.

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