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Original Article

Identification of dysregulated microRNAs involved in arachidonic acid metabolism regulation in dilated cardiomyopathy-mediated heart failure patients

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Abstract: Heart failure (HF), a clinical syndrome with high morbidity and mortality, is becoming a growing public health problem. Dilated cardiomyopathy (DCM) is one of the major causes of HF, yet the molecular mechanisms underlying DCM-mediated HF are not completely understood. Previous studies have shown that dysregulation of arachidonic acid (AA) metabolism could contribute to the development of HF. To explore the roles of microRNAs (miRNAs) in regulating AA metabolism in HF, we used two public datasets to analyze the expression changes of miRNAs in the patients of DCM-mediated HF. A total of 101 and 88 miRNAs with significant abundance alterations in the two dataset were obtained, respectively. Around 1/3 of these miRNAs were predicted to target AA metabolic pathway genes. We also investigated the distribution of known single nucleotide polymorphisms (SNPs) within the sequences of miRNAs dysregulated in DCM-mediated HF patients, and identified miRNAs harboring high number of SNPs in either the seed regions or the entire sequences. These information could provide clues for further functional studies of miRNAs in the pathogeny of DCM-mediated HF.

Key words: dilated cardiomyopathy; heart failure; microRNAs; arachidonic acid; single nucleotide polymorphisms

扩张型心肌病并发心力衰竭患者中参与花生四烯酸代谢调控的异常表达microRNAs的鉴定

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摘 要：心力衰竭作为一种具有较高发病率和死亡率的临床综合征，正成为日益严重的公共卫生问题。扩张型心肌病是心力衰竭的一种主要诱因，但其分子机制还有很多未知。之前的研究发现花生四烯酸(arachidonic acid, AA)代谢紊乱可导致心力衰竭的发生。为了探究调控花生四烯酸代谢的microRNAs (miRNAs)在扩张型心肌病并发心力衰竭过程中的作用，我们利用两个公共数据集中的miRNAs测序数据，分析了由扩张型心肌病引发的心力衰竭患者心肌组织中的miRNAs表达情况。我们分别在上述数据集中鉴定到101个和88个具有明显表达变化的miRNAs。在这些miRNAs里，大约有1/3的miRNAs被预测可以靶向调控AA代谢通路相关的基因。我们也研究了已知单核苷酸多态性(single nucleotide polymorphisms, SNPs)在扩张型心肌病并发心力衰竭患者中差异表达的miRNAs序列中的分布，发现在种子区域或整个序列上具有较多SNPs的miRNAs。以上这些结果可为未来研究miRNAs在扩张型心肌病并发心力衰竭发病机理中的功能提供线索。

关键词：扩张型心肌病；心力衰竭；microRNAs；花生四烯酸；单核苷酸多态性
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Heart failure (HF), characterized by structural or functional cardiac impairment, is a clinical syndrome with high morbidity and mortality [1–4]. Many cardiovascular diseases, including myocardial infarction, hypertension, valvulopathy, will lead to HF [1, 5]. In China, HF has become an emerging epidemic which has affected more than 13.7 million individuals. Over the past 15 years, the incidence rate of HF among Chinese people has increased by around 44% [6, 7]. Dilated cardiomyopathy (DCM), the most common form of cardiomyopathy, is one of the major causes of HF [4, 8]. In comparison with HF induced by other cardiomyopathy, DCM-mediated HF has distinct clinical features, such as fatigue, chest pain and dyspnea on exertion, *etc* [9, 10]. Regretfully, currently available drugs or treatments for DCM-mediated HF can only alleviate the pathological symptoms rather than cure the disease. Further investigations on the pathogenic factors and pathologic processes contributing to DCM-mediated HF may provide clues for better disease treatment method or drug development.

Arachidonic acid (AA) is one of the most important polyunsaturated fatty acids (PUFAs) present in the plasma membrane of cells. AA can be obtained from the animal products in diet, such as fish, meat, eggs, *etc* [11]. AA is metabolized via three pathways, namely cyclooxygenases (COXs), lypoxygenases (LOXs), and cytochrome P450 (CYPs), to form multiple subfamilies of eicosanoids. These eicosanoids play important roles in regulating blood pressure, inflammation, reproduction, as well as many other physiological and pathological processes [12–14]. COXs catalyze the generation of prostaglandins and thromboxane from AA, which can regulate blood flow by vasodilation or vasoconstriction [15–17]. LOXs catalyze the formation of leukotrienes from AA. Leukotrienes are potential mediators of cardiovascular diseases, as they can trigger the contraction of smooth muscles and regulate innate immune responses [18, 19]. Products of CYPs pathway are epoxyeicosatrienoic acids, which can inhibit inflammation and blood clot formation, and also repress platelet activation, therefore are important risk factors for HF [20].

MicroRNAs (miRNAs) are a class of ~22 nt long single strand noncoding RNAs which mainly function by pairing with mRNAs to repress their translation or induce degradation [21]. The 5′ 2–8 nt of miRNAs are also called the seed regions of miRNAs, which are considered to be more essential in mediating miRNA-target pairing [21]. Several dozens of miRNAs have been identified to be associated with HF pathophysiology, such as miR-1 and miR-133 in regulating cardiac remodeling, miR-15, miR-29 and miR-214 in promoting apoptosis of cardiomyocytes, miR-24 and miR-92a in inhibiting angiogenesis, and miR-126 in enhancing angiogenesis [22–24]. Previous studies have demonstrated that multiple miRNAs are involved in AA metabolism [25–32]. For example, hsa-miR-144, hsa-miR-26b, and hsa-miR-558 directly target COX2 mRNA to down-regulate its expression and lead to a decrease of prostaglandins in several tissues [14, 25–32]. Hsa-miR-19a-3p and hsa-miR-203 affect 5-LOX and 15-LOX expression to regulate the synthesis of leukotrienes [13, 14, 26, 28]. MiRNA let-7b reduces CYP2J2 expression and the production of epoxyeicosatrienoic acids [20, 32]. As abnormal AA metabolism has been linked to many types of cardiovascular diseases, including chronic HF, systematical identification of miRNAs involved in AA metabolism during HF would help us to understand the roles of AA and its metabolites in HF.

In this work, we compared the expression of miRNAs from the heart tissues of DCM-mediated HF patients with those of healthy donors using two public datasets, identified differentially expressed (DE) miRNAs putatively targeting genes in the AA metabolic pathways, and analyzed the distribution of known single nucleotide polymorphisms (SNPs) within the sequences of DE miRNAs. Our study would provide clues for further functional studies of miRNAs in DCM-mediated HF.

1 MATERIALS AND METHODS

1.1 *Data source*

The miRNA-seq datasets used in this study (GSE53081 and GSE135055) were downloaded from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). The GSE53081 dataset was generated from the left ventricle heart tissues of 34 HF patients (21 patients with DCM-mediated HF) and 8 healthy donors [33]. The GSE135055 dataset was generated from the left ventricle heart tissues of 21 HF patients (18 patients with DCM-mediated HF) and 9 healthy donors [34]. MiRNA-seq data of all DCM-mediated HF patients and the control individuals in these two datasets were collected and used in this analysis.

1.2 *Data preprocessing and known miRNA identification*

Raw sequencing data of each miRNA dataset were first cleaned by low quality bases ($Q < 20$) trimming and

removal of 3′ adaptor using the Cutadapt program (version 2.10) [35]. Clean reads were mapped against the Rfam (Release 14.2) [36] database to filter out other types of small RNAs (sRNAs) (Blastn, version 2.10) [37]. The remaining reads were compared with known human miRNAs collected in the miRbase database (Release 22) [38], then aligned to the human genome (GRCh38.p13) using Bowtie (version 1.0.0) [39]. For quantification of known miRNAs, precursor and mature miRNAs of human were downloaded from the miRbase database (Release 22) [38] and compared with the mapped reads. The Quantifier module of miRDeep2 package was applied to calculate the expression of known miRNAs [40].

1.3 *Identification and functional enrichment analysis of DE miRNAs*

The DE miRNAs between samples from the HF patients and healthy donors were identified using R package DESeq2 with fold change ≥ 1.5 and adjusted P value < 0.05 (Benjamini-Hochberg adjustment) as the thresholds.

Functional enrichment analysis of DE miRNAs was performed using online tool TAM 2.0 (http://www.lirmed.com/tam2/) [41] with the default miRNA dataset as the background miRNA set. Bonferroni adjusted P value < 0.05 was applied to select diseases associated with DE miRNAs, and the results were plotted using R package ggplot2.

1.4 *Target prediction for DE miRNAs*

The target genes of DE miRNAs were predicted by miRDB (http://mirdb.org/) [42] and mirDIP (http://ophid.utoronto.ca/mirDIP/) [43] with default parameter settings, and validated by miRTarBase (http://mirtarbase.cuhk.edu.cn/php/index.php) [44], which is a database collecting experimentally validated miRNA-target interactions. Target gene and miRNA pairs predicted by miRDB or miRDIP and also present in miRTarBase were collected.

1.5 *SNP analysis in DE miRNAs*

SNPs in DE miRNAs were obtained from the miRNASNP-v3 database (http://bioinfo.life.hust.edu.cn/miRNASNP/#!/) [45], which is a database collecting known SNPs in miRNAs from dbSNP v151 [46], GWAS Catalog [47], ClinVar [48] and COSMIC v88 [49]. We browsed and searched SNPs sites for each DE miRNA targeting AA metabolic pathway genes in the miRNASNP-v3 database. To better reflect the functional changes of miRNAs, SNP located in the seed regions of miRNAs were collected and counted separately.

2 RESULTS

2.1 Identification of dysregulated miRNAs in DCM-mediated HF patients

To identify miRNAs with dysregulated expression in DCM-mediated HF patients, we first collected the small RNA sequencing data of 39 DCM-mediated HF patients and 17 healthy controls from two public datasets (GSE53081 and GSE135055) in the GEO database. The basic information of samples in these datasets was listed in Table 1. All samples were taken from the left ventricle heart tissues of either the HF patients or the controls. After removing low quality reads and quantifying the expression of known human miRNAs, a total

Table 1. Basic information of datasets used in this work

	GSE53081		GSE135055	
Diagnosis	DCM		DCM	
NYHA class	II: 0		II: 2	
	III: 0		III: 6	
	IV: 21		IV: 10	
Sample source	Human LV hearts		Human LV hearts	
Sample group	HF	Healthy	HF	Healthy
Sample number	22	10	18	9
Donor number	21	8	18	9
Donor age	57.1 ± 12.3	47.6 ± 26.1	33.3 ± 16.3	41.7 ± 4.0
Gender (Male + Female)	20 + 1	5 males, 3 NA	10 + 8	9 + 0
Type	miRNA-seq (Illumina HiSeq 2000)		miRNA-seq (Illumina HiSeq 2500)	

DCM, dilated cardiomyopathy; NYHA class, New York Heart Association class; HF, heart failure; Human LV hearts, human left ventricle hearts; NA, not available. Donor age was presented as the mean ± SD.

of 101 and 88 DE miRNAs were identified to be up- or down-regulated (≥ 1.5 fold change, Benjamini-Hochberg adjusted $P < 0.05$) in DCM-mediated HF patients from datasets GSE53081 and GSE135055, respectively (Fig. 1). Among these DE miRNAs, 39 were up-regulated and 62 were down-regulated in the GSE53081 dataset, whereas 55 were up-regulated and 33 were down-regulated in the GSE135055 dataset (Fig. 2*A* and *B*).

Using fold change ≥ 1.5 as the threshold, a total of 12 DE miRNAs were identified to be up- or down-regulated in both datasets (Fig. 2*C*, Table 2). In addition, majority of DE miRNAs from the two datasets showed the same expression change trends (fold change ≥ 1.1) in the other dataset (Fig. 2*D*), indicating the consistent functions of these miRNAs in regulating DCM-mediated HF in both patient groups. Functional enrichment analysis of the DE miRNAs also revealed overlapping roles of the DE miRNAs in both datasets. Specifically, DE miRNAs in the two datasets were both enriched with functions related to cardiovascular-associated diseases, especially hypertrophic cardiomyopathy (Fig. 2*E* and *F*). Among the 12 DE miRNAs shared by both datasets, 7 have been reported to be associated with HF (Table 2). The enriched functions of DE miRNAs from dataset GSE53081 also included heart failure.

2.2 Analysis of DE miRNAs involved in AA metabolic pathways

To better understand the roles of miRNA-mediated AA metabolism in regulating DCM-mediated HF, we focused on DE miRNAs targeting genes of the AA metabolic pathways. We first collected genes involved in the AA metabolic pathways or DCM-mediated HFs from the KEGG database (http://www.genome.ad.jp/kegg/) [71]. Target analysis results showed that there were 30 and 28 DE miRNAs targeting AA metabolic pathway genes in dataset GSE53081, and 22 and 24 in databset GSE135055, respectively, which represented more than 30% of DE miRNAs in each dataset (Fig. 3*A*–*C*). Members of the cytoplasmic phospholipase A2 (cPLA2) family, which catalyze the release of AA from cell membranes, were the major targets of the identified DE miRNAs (Fig. 3*A*, *B*, and Table 3) [11]. All three AA metabolic pathways were targeted by multiple DE miRNAs (Fig. 3*A* and *B*).

Among the AA metabolic pathway genes targeted by DE miRNAs, over 1/3 were targeted by more than one DE miRNA in either dataset (Fig. 3*D*). There were 13 AA gene targets shared by both datasets (Fig. 3*E*), of which, PLA2G12A (a member of the cPLA2 family) and CYP2U1 (a member of the CYP2 family) were

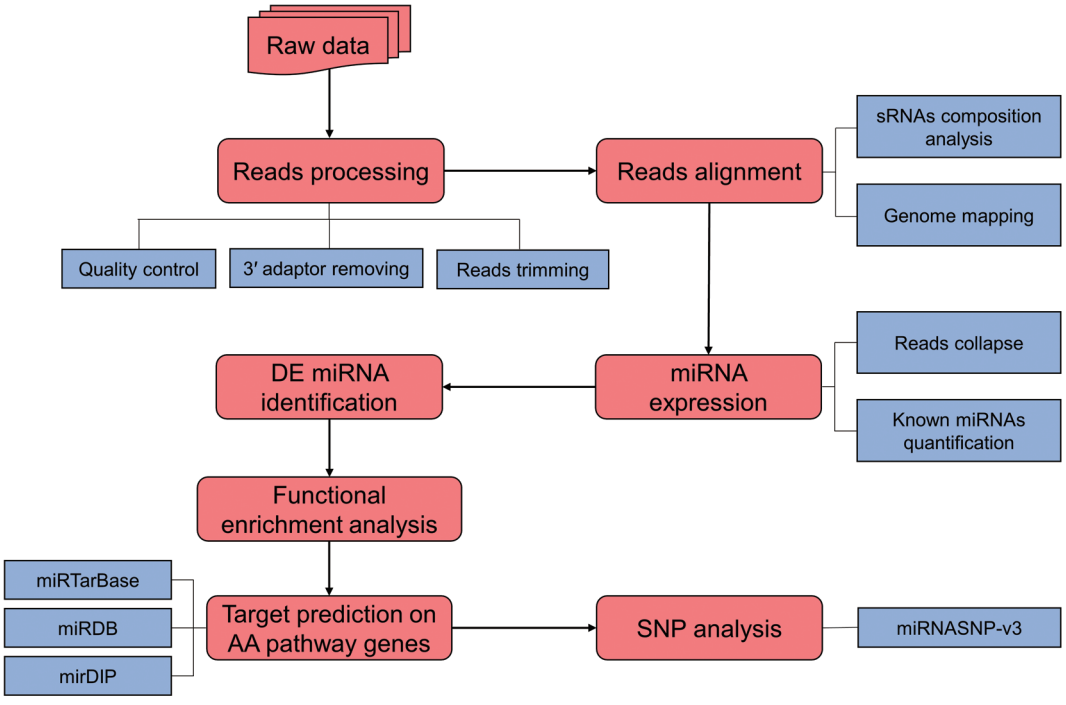

Fig. 1. Overview of the miRNA-seq data analysis pipeline. sRNAs, small RNAs; DE miRNAs, differentially expressed miRNAs; AA, arachidonic acid; SNP, single nucleotide polymorphism.

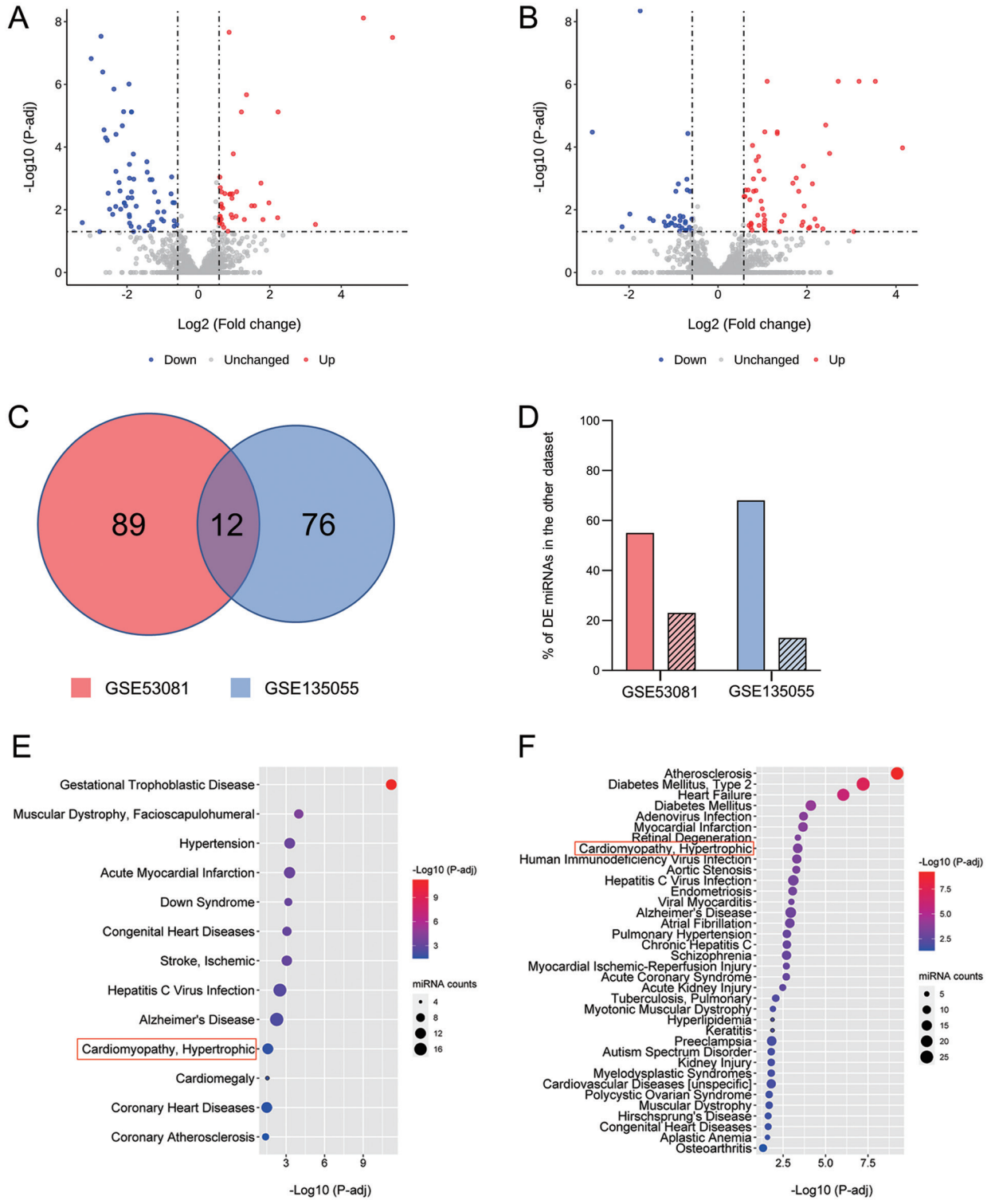

Fig. 2. Distribution and functional analysis of differentially expressed (DE) miRNAs identified from datasets GSE53081 and GSE135055. *A* and *B*: Volcano plots showing DE miRNAs in datasets GSE53081 (*A*) and GSE135055 (*B*). Up- or down-regulated miRNAs in heart failure samples were identified with ≥ 1.5 fold change and adjusted *P* value < 0.05 (Benjamini-Hochberg adjustment) as thresholds. *C*: Number of shared DE miRNAs between the two datasets. *D*: Cross comparison of DE miRNAs between the two datasets. Shown are percentages of DE miRNAs of one dataset with the similar expression change trends (fold change ≥ 1.1) (red and blue bars) or without differential expression (fold change < 1.1) (shaded light red and light blue bars) in the other dataset. *E* and *F*: Bubble plots displaying the functional enrichment of DE miRNAs in datasets GSE53081 (*E*) and GSE135055 (*F*). X-axis represents the log10 transformed Bonferroni adjusted *P*-value of each enriched GO term (Bonferroni adjusted *P* value < 0.05).

Table 2. Summary of known cardiovascular diseases related to the 12 common DE miRNAs

miRNA	Expression change in HF	Related cardiovascular diseases
hsa-miR-1-3p	Down	HF [22, 24], hypertrophic cardiomyopathy [50]
hsa-miR-100-5p	Up	HF [51], cardiac hypertrophy [52]
hsa-miR-130b-5p	Up	HF [53], coronary artery disease [54]
hsa-miR-155-5p	Up	HF [55, 56], cardiac amyloidosis [57], rheumatic heart disease [58], rheumatic carditis [59], atherosclerosis [60]
hsa-miR-195-5p	Up	HF [60, 61]
hsa-miR-204-5p	Up	HF [62], myocardial ischemia [62], cardiac hypertrophy [63], hypertension [64]
hsa-miR-216a-5p	Up	HF [65], severe acute pancreatitis [66]
hsa-miR-216b-5p	Up	Diabetic angiopathy [67]
hsa-miR-217-5p	Up	Arrhythmogenic cardiomyopathy [68]
hsa-miR-34c-5p	Up	Atrial fibrillation [69]
hsa-miR-493-5p	Up	Coronary microembolization [70]
hsa-miR-493-3p	Up	NA

DE miRNAs, differentially expressed miRNAs; HF, heart failure; NA, not available.

predicted to be targeted by more than 10 DE miRNAs (Table 3). For the 12 DE miRNAs shared by both datasets, 5 of them were predicted to target AA metabolic pathway genes, namely PTGIS, PLA2G12A, ALOX12, CYP2U1, LTA4H (Table 4). In addition, most of these shared DE miRNAs could also target HF-related genes, including TPM1, TPM2 and SLC8A1, *etc* (Table 4).

2.3 Analysis of SNPs in DE miRNAs involved in AA metabolic pathways

SNPs are the most common source of genetic variations that impair the function of miRNAs. To investigate whether the functions of miRNAs in this study could be affected by SNPs, we collected known SNPs within the sequences of DE miRNAs using the miRNASNP-v3 database, which curates previously reported SNPs in the precursor and mature sequences of human miRNAs. A total of 216 and 204 SNP sites (nucleotide position with known SNPs) were identified in the sequences of DE miRNAs targeting AA metabolic pathway genes in datasets GSE53081 and GSE135055, respectively (Fig. 4*A*). Among them, approximately 25% of total SNP sites in each dataset located in the seed regions of the DE miRNAs (Fig. 4*B*). In particular, hsa-miR-486-3p and hsa-miR-486-5p were both found to have more than 15 SNP sites in their sequences, of which, 6 SNP sites of hsa-miR-486-3p and 4 SNP sites of hsa-miR-486-5p were located in the seed regions (Fig. 4*C* and *D*). The predicted targets of hsa-miR-486-3p included PLA2G6, which is an important member of the cPLA2 family and functions to promote the production of free AA [11]. Hsa-miR-486-5p, together with hsa-miR-232-3p and hsa-miR-1827, were predicted to target GPX8, a member of GPXs family and affect the generation of pro-angiogenic factor 15-HETE [72]. Hsa-miR-34c-5p (predicted to target PTGIS), a miRNA reported to be involved in regulation of atrial fibrillation, had 5 SNP sites in its seed region (Fig. 4*D* and *E*) [69]. DE miRNAs with more than 3 SNPs in their seed regions also included hsa-miR-195-5p, hsa-miR-512-3p, and hsa-miR-302d-3p, and the putative targets of these miRNAs were ALOX12, PLA2G12A, PTGIS, CYP2U1, HPGDS, which are all key regulators in different AA metabolic pathways (Fig. 4*D*).

3 DISCUSSION

As the final outcome of all types of cardiomyopathies, HF is a chronic and currently incurable disease. Although the development of HF can be attributed to many genetic or non-genetic factors, the progression of HF is regulated by multiple metabolites of the AA pathways, from both the beneficial and deleterious aspects [15–20]. To investigate the functions of miRNAs in regulating AA metabolism and their potential links with DCM-mediated HF, in this work, by employing two public available datasets, we characterized miRNAs dysregulated in DCM-mediated HF patients and potentially targeting AA metabolic pathway genes. Moreover, we identified 101 and 88 DE miRNAs to be up- or down-regulated in DCM-mediated HF patients from two public datasets, and found that about 1/3 DE

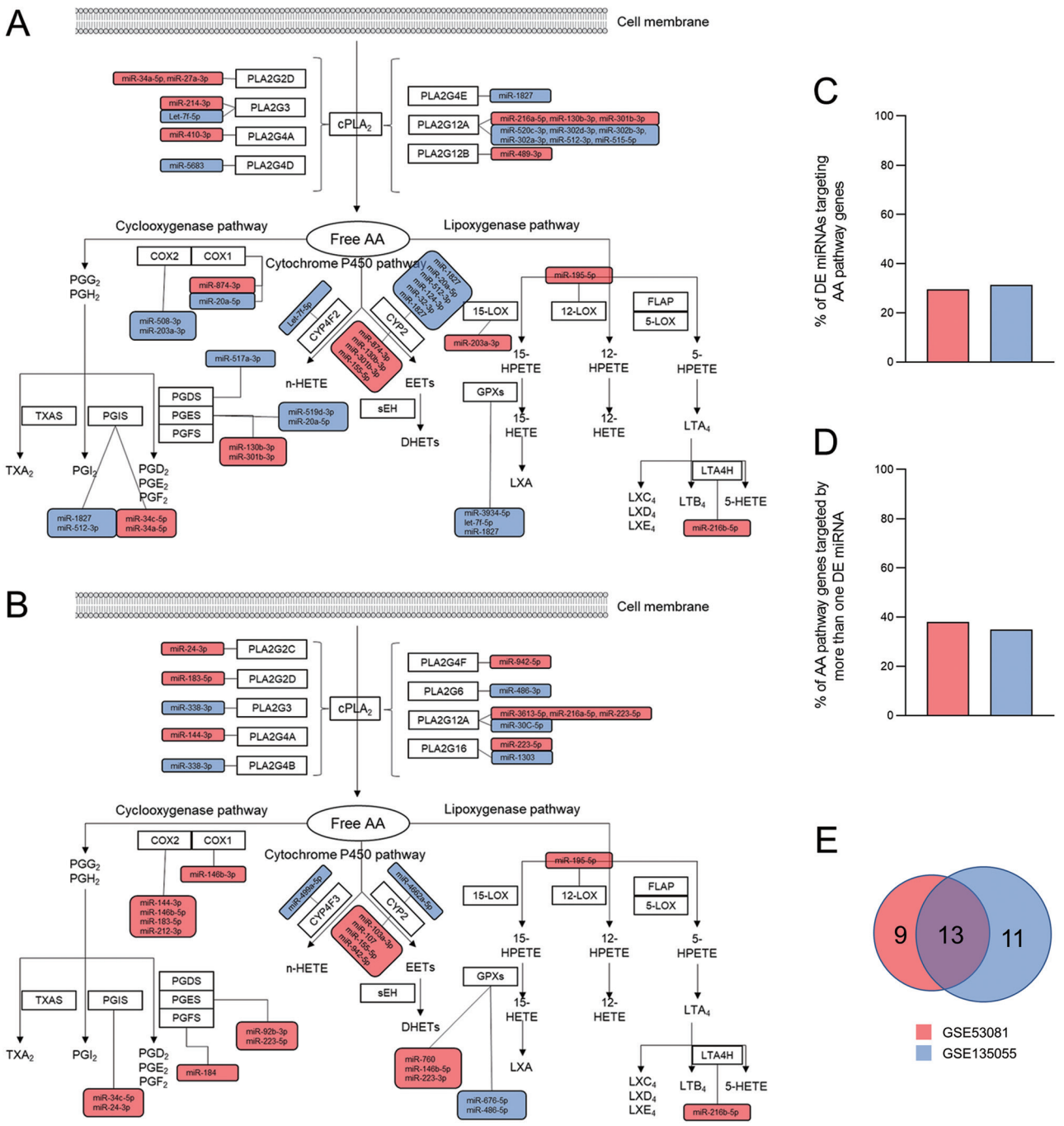

Fig. 3. Analysis of differentially expressed (DE) miRNAs targeting arachidonic acid (AA) metabolic pathway genes. *A* and *B*: Summary of DE miRNAs involved in AA metabolic pathways in datasets GSE53081 (*A*) and GSE135055 (*B*). Red and blue rounded rectangles represent significantly up- and down-regulated miRNAs, respectively. Uncolored rectangles present proteins or protein families involved in AA metabolism. Others are metabolites of AA. *C*: Statistical analysis of DE miRNAs with AA pathway target genes. *D*: Statistical analysis of AA pathway genes targeted by more than one DE miRNA. *E*: Venn diagram showing shared AA pathway genes targeted by DE miRNAs from either dataset.

miRNAs putatively targeted AA metabolic pathway genes. We also analyzed known SNPs among this DE miRNAs and their potential effects on AA pathways genes.

The two datasets used in this study (GSE53081 and GSE135055) were published in 2014 and 2020, respectively [33, 34]. In the original work, the GSE53081 dataset mainly analyzed and compared the expression changes

Table 3. Summary of common AA pathway genes targeted by DE miRNAs from either dataset

Genes	Pathway	miRNAs
PLA2G2D	AA release	miR-183-5p, miR-34a-5p, miR-27a-3p
PLA2G3	AA release	miR-338-3p, miR-214-3p, Let-7f-5p
PLA2G4A	AA release	miR-144-3p, miR-410-3p
PLA2G12A	AA release	miR-3613-5p, miR-216a-5p, miR-223-5p, miR-130b-3p, miR-301b-3p, miR-30C-5p, miR-520c-3p, miR-302d-3p, miR-302b-3p, miR-302a-3p, miR-512-3p, miR-515-5p
PTGS1 (COX1)	Cyclooxygenase	miR-146b-3p, miR-874-3p, miR-20a-5p
PTGS2 (COX2)	Cyclooxygenase	miR-144-3p, miR-146b-5p, miR-183-5p, miR-212-3p, hsa-508-3p, miR-203a-3p
PTGES3	Cyclooxygenase	miR-223-5p, miR-519d-3p, miR-20a-5p
PTGIS	Cyclooxygenase	miR-34c-5p, miR-24-3p, miR-34a-5p, miR-1827, miR-512-3p
CYP2B6	Cytochrome P450	miR-4662a-5p, miR-1827
CYP2U1	Cytochrome P450	miR-155-5p, miR-942-5p, miR-130b-3p, miR-301b-3p, miR-155-5p, miR-20a-5p, miR-512-3p, miR-124-3p, miR-32-3p, miR-1827
ALOX12	Lipoxygenase	miR-195-5p
GPX8	Lipoxygenase	miR-223-3p, miR-486-5p, miR-1827
LTA4H	Lipoxygenase	miR-216b-5p

The gene list in this table is from KEGG (Kyoto Encyclopedia of Genes and Genomes). DE miRNAs, differentially expressed miRNAs; AA release means the release of arachidonic acid (AA) from cell membranes.

Table 4. Summary of HF and AA metabolic pathway genes targeted by the 12 shared DE miRNAs of the two datasets

miRNA	Expression change in HF	HF related genes	AA pathway genes
hsa-miR-1-3p	Down	TPM3, TPM2, TPM1, TPM4, ITGA6, IGF1, ACTB, SLC8A1, PRKACB, SLC8A2, ADCY1	NA
hsa-miR-100-5p	Up	ADCY1	NA
hsa-miR-130b-5p	Up	NA	NA
hsa-miR-155-5p	Up	GNAS, DMD	CYP2U1
hsa-miR-195-5p	Up	TPM3, TPM2, ITGA2, ITGA10, ADCY5, SGCA, CACNB4, CACNA2D1	ALOX12
hsa-miR-204-5p	Up	SGCD, CACNA1C, ADCY1, CACNG2, ADCY6, ITGB3	NA
hsa-miR-216a-5p	Up	SGCD, ACTB	PLA2G12A
hsa-miR-216b-5p	Up	TPM3	LTA4H
hsa-miR-217-5p	Up	NA	NA
hsa-miR-34c-5p	Up	CACNB3, ITGA11	PTGIS
hsa-miR-493-5p	Up	ATP2A2, ITGB1, ADCY1, CACNB2, DMD	NA
hsa-miR-493-3p	Up	NA	NA

The gene list in this table is from KEGG (Kyoto Encyclopedia of Genes and Genomes). HF, heart failure; AA, arachidonic acid; DE miRNAs, differentially expressed miRNAs; NA, not available.

of myocardial and circulating miRNAs from HF patients and the controls, with the aim to investigate the feasibility of using circulating miRNAs as biomarkers for HF [33]. Similarly, the original report of the GSE135055 dataset also focused on the characterization of biomarkers associated with the progression of HF, and identified COL1A1, a fibrosis-associated gene, as a potential plasma biomarker [34]. Thus, although both studies performed miRNA target analysis, they did not pay specific attention to the roles of miRNAs in regulating AA metabolism.

Although our analysis almost recapitulated the DE miRNAs reported in the above mentioned works, the number of overlapping DE miRNAs between the two datasets was limited. One possible explanation for this inconsistency could be that, although both studies extracted miRNAs from the left ventricular heart tissues of DCM-mediated HF patients, the average age of HF patients of the GSE53081 dataset was about 24 years elder than that of the GSE135055 dataset (Table 1).

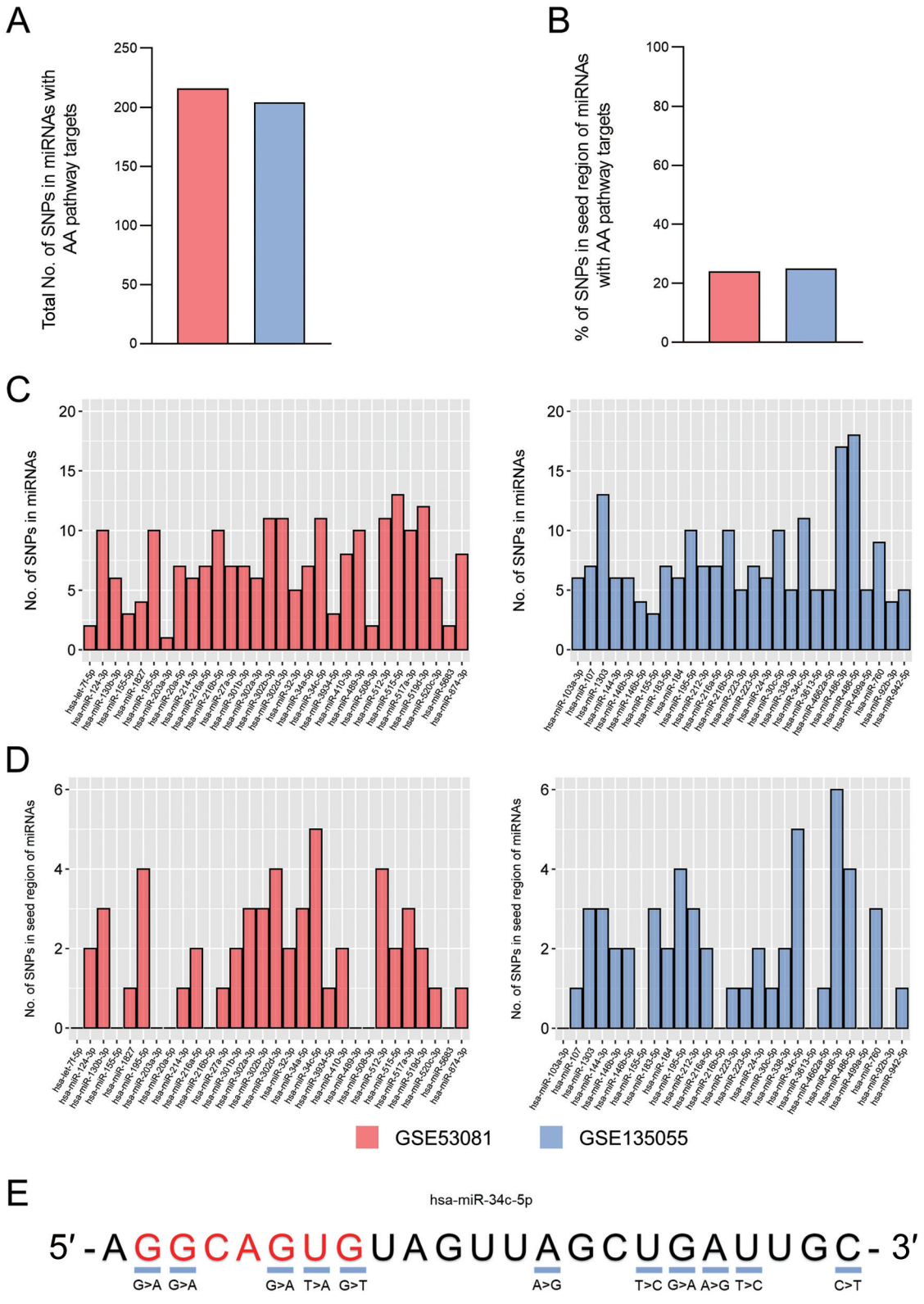

Fig. 4. Statistics of known SNP sites in differentially expressed (DE) miRNAs targeting arachidonic acid (AA) pathway genes. *A*: Total number of SNP sites identified in the whole sequences of DE miRNAs targeting AA pathway genes. *B*: Percent of SNP sites in the seed region sequences of DE miRNAs targeting AA pathway genes. *C* and *D*: Number of SNP sites in the whole sequences of DE miRNAs (*C*) or the seed region sequences of DE miRNAs (*D*) targeting AA pathway genes. *E*: Example of SNP sites distribution in hsa-miR-34c-5p sequence. Nucleotides in the seed region are shown in red. Known SNPs are shown under each corresponding nucleotide.

And the gender distribution of the HF patients in the two datasets was also different. In addition, although only 12 DE miRNAs with statistical significance were identified to present in both datasets, around 80% DE miRNAs in each dataset showed the same expression change trend or remained non-changed in the other dataset, indicating the consistency of miRNA functions in both datasets.

Studies have shown that SNPs are commonly observed within miRNA sequences or miRNA target sites, thus impair miRNA functions [45, 73, 74]. Using known human SNPs in the public database, we analyzed potential SNPs in miRNAs targeting AA metabolic pathway genes. Some miRNAs with high frequency of SNPs, eg. hsa-miR-486-3p and hsa-miR-486-5p, have been shown to play important roles in regulating HF-related processes [75, 76]. Examining the presence of SNPs in these miRNAs using blood samples could be a potential approach for the pathogenesis diagnosis and corresponding therapeutic strategy design of HF in the future.

In summary, by comparing the miRNA expression changes in DCM-mediated HF patients, we have identified a series of dysregulated miRNAs which might alter AA metabolism in left ventricular heart and thus contribute to the development of HF in human. We also analyzed the functional preference and SNP frequency of these miRNAs. These results may shed light on the pathogeny studies of DCM-mediated HF and provide targets for new drug/therapeutic method development against DCM-mediated HF.

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