

Original Article

Long noncoding RNAs and novel inflammatory genes determined by RNA sequencing in human lymphocytes are up-regulated in permanent atrial fibrillation

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Abstract: Atrial fibrillation (AF) is a common arrhythmia in clinical practice. Currently, approximately 33.5 million individuals are affected by AF globally. AF involves multiple complicated mechanisms which have not been fully investigated yet. RNA sequencing (RNAseq) is an outstanding method for investigation of diseases due to its high-throughput information. Here, RNAseq was applied to determine mRNA and long noncoding RNA (lncRNA) expression profiles in human lymphocytes from 6 permanent atrial fibrillation (pmAF) patients and 6 healthy controls. Quantitative real-time PCR (qRT-PCR) was applied to further validate 3 lncRNAs and 4 inflammatory mRNAs. It was discovered that there were numerous differentially-expressed mRNAs and lncRNAs between these two groups. GO analysis indicated that differentially-expressed mRNAs were mainly involved in native immunity, inflammation, signaling transduction and so forth, and they were also enriched in pathways like TNF signaling pathway, NF-kappa B signaling pathway, Toll-like receptor pathway and NOD-like receptor pathway. Moreover, co-expression network demonstrated that dysregulated mRNAs and lncRNAs in pmAF lymphocytes participated in inflammation, autophagy, mitochondrial functions, oxidative stress, etc. Further validation by qRT-PCR demonstrated mRNAs and lncRNAs were significantly higher in lymphocytes from pmAF patients compared with controls. In conclusion, mRNA and lncRNA expression profiles in lymphocytes are significantly different between pmAF and controls, differentially-expressed mRNAs and lncRNAs are involved in pathways closely associated with inflammation, oxidative stress, autophagy, cell apoptosis and collagen synthesis, suggesting lymphocytes might play indispensable roles in the development of pmAF.

Keywords: Inflammation, long noncoding RNA, lymphocyte, oxidative stress, permanent atrial fibrillation, RNA sequencing

Introduction

Atrial fibrillation (AF) is one of the most common arrhythmias in the global world. It is estimated that about 33.5 million individuals are affected by AF globally, with a prevalence about 2.3-3.4% among adults and 9% in the old over their 80s [1, 2]. It is predicted that prevalence of AF will be doubled in 2050 [2]. AF is characterized by thrombosis, which is the leading cause for death. Current research illustrates that electrical and structural remodeling, reentry and ectopic firing, inflammation and oxidative stress are major mechanisms in AF [3-6]. Despite investigation has been lasted nearly a

century, underlying mechanisms about AF are still poorly understood.

Long non-coding RNAs (lncRNAs) are a group of transcripts longer than 200 bp and seldom encode proteins [7]. lncRNAs participate in multiple biological and physiological processes. Moreover, lncRNAs also regulate a great number of genes involved in cancers, myocardial infarction and heart failure [8-12]. However, relationships between lncRNAs and AF remain enigmas. RNA sequencing (RNAseq) is an outstanding technology for disease excavation due to its high-throughput information. Information of the total RNAs extracted from tissues or cells

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Table 1. Primer list

Gene	Forward primer	Reverse primer
β -actin	TTCCTTCTGGGCATGGA	GAGGAGCAATGATCTTGAT
lncRNA1	CTCACTTCAGGCTCTGTTAT	AAAGATGATGGATTGAAGGC
lncRNA2	AACATAAACTACCTTATCACCTC	GGGTAATGTCCTTTCACAC
lncRNA3	GGTTTTCTGACTGCTACAT	GGGTCTTCTGTTTCACGATC
IFI27	ACATTCTCAGGAAGCTCTCCTTCTTT	ACAAGCTCTCCAATCACAAGCTGTAG
IFIT2	TGGAATGTATGGGAAAAGAAAGTTA	TTTCGCTACAGGAGTAAGCTCTTTA
IFI6	GGTTCCTACTATATTGTCCAGGCTA	TTCTGTTTTACATCTAGGTTGTTG
IDH1	TAGATATCCCCTTTTGTGAGGTAGA	GAGGACAAACCTCCAGGTAGTATT

can be obtained in a short time by RNAseq. At present, RNAseq has already been widely applied in the areas of basic science, clinical diseases, diagnostics and drug exploration.

Lymphocytes usually can be attracted by chemokines and cytokines, and they are infiltrated in the matrix of myocardium during AF. Currently, roles that lymphocytes play in AF are still not completely understood. To our knowledge, there is no research revealing mRNA and lncRNA expression profiles of lymphocytes from AF population. In addition, lymphocytes are easily obtained and their RNA quality meets the standard of RNAseq. Permanent atrial fibrillation (pmAF) is the most typical type of AF which is characterized by significant cardiac remodeling. In order to know mRNA and lncRNA expression profiles in lymphocytes from pmAF, RNAseq was applied to explore more underlying mechanisms in AF.

Materials and methods

Study population

In this study, six pmAF and six matched healthy controls were recruited from our hospital. They were informed and required to sign consents before entering this experiment. The diagnosis of pmAF was mainly based on the criteria listed in *2014 AHA/ACC/HRS Guidelines for the Management of Patients with Atrial Fibrillation* [13]. Participants would be excluded if they were: (i) critically ill; (ii) combined with other severe heart diseases, such as myocardial infarction and heart failure; (iii) other types of arrhythmias; (iv) stroke or surgery within 6 months; (v) cancers; (vi) infection; (vii) chronic hepatic or renal failure; (viii) autoimmune diseases; (ix) endocrine disorders. Healthy controls were the population without pmAF and the diseases mentioned above. The whole

experiment was approved by Ethics Committees of Beijing Hospital and performed according to standards of Declaration of Helsinki.

Lymphocytes collection

About 10 ml of fresh fasting intravenous blood was drawn into ethylene diamine tetra-acetic acid (EDTA)-

anticoagulant tubes from each participant. The whole blood was blended with PBS with a ratio of 2:1. Diluted blood was added onto the surface of lymphocytes separation medium (Cedarlane, Canada) and centrifuged at 800 g for 20 minutes. The lymphocyte layer was transferred and washed with PBS twice. Lymphocytes were obtained by centrifugation with a speed of 800 g for 10 minutes.

RNA preparation, cDNA library establishment and RNAseq

RNAs from lymphocytes were extracted by TRIzol (Invitrogen, USA) following manufacturer's instruction. The average A260/A280 was around 1.8-2.0. Qualities of RNAs were also evaluated by Agilent Bio-analyzer 2100. RIN value over 7.5 was considered to be qualified for RNAseq. rRNAs were removed and mRNAs were fragmented during RNAseq. cDNAs were amplified with random primers and reverse transcriptase. cDNA library was enriched and high-seq 4000 Illumina platform was applied for RNAseq with a model of 2*150 bp.

Further validation of differentially-expressed mRNAs and lncRNAs

In order to testify correctness of RNAseq, four up-regulated mRNAs and three up-regulated lncRNAs in pmAF were further determined by quantitative real time polymerase chain reaction (qRT-PCR) within RNAs from lymphocytes of these 12 participants. Reverse transcription reaction was conducted by reverse transcription kit (Promega, USA) and qRT-PCR was performed by SYBR Green kit (Promega, USA) according to instructions provided by manufacturer. B-actin was applied as an internal control. Primers for mRNAs and lncRNAs were listed in the **Table 1**. Relative expression was analyzed by 2- $\Delta\Delta$ Ct method.

Table 2. Basic information about recruiters

Parameters	Healthy controls (L) n=6	PmAF (A) n=6
Gender, Male/Female (n)	3/3	2/4
Age (years)	61.00 ± 0.5774	62.00 ± 0.7746
Body mass index (kg/m ²)	24.93 ± 1.012	23.38 ± 0.4248
smoking	No	No
Alcohol abuse	No	No
NYHA class I/II/III/IV	6/0/0/0	6/0/0/0
Warfarin	3/3	3/3

The table indicated basic information of recruiters. Values were presented as mean ± SD. There was no significant difference between these two groups. P<0.05 was considered to be statistically significant.

Statistical analysis

FPKM was used to evaluate the expression of each transcript. Fisher's exact test, Bonferroni's method, Holm method, Sidak correction, and false discovery rate were applied to make enriched analysis of GO functions and KEGG pathways. Spearman analysis was performed in cluster analysis of differentially-expressed transcripts within the same sample, whereas Pearson analysis was performed among different genes. Hcluster method with complete calculation was performed to do the cluster analysis of differentially-expressed transcripts. WGCNA method was obtained to analyze co-expression network between mRNAs and lncRNAs. The whole data in this article were presented as mean ± SD. Independent student's t-test was applied to compare quantitative parameters between two groups. P value smaller than 0.05 was considered to be statistically significant.

Results

Basic information about recruiters

Basic information about recruiters was analyzed and listed in the **Table 2**. There were no significant differences between age, gender distribution, body mass index (BMI), smoking and alcohol abuse, New York Heart Association (NYHA) classification and warfarin uptake (P>0.05).

Online accession number of RNAseq data

About 201G of raw data were generated from these 12 samples. Clean data with high quali-

ties were compared to referential genome (hg19). RNAseq data can be accessible at Sequence Read Archive (SRA) with an accession number of SRP093226.

Differentially-expressed mRNAs and lncRNAs

It was shown that there were no significant differences in expressions of 52741 mRNAs between pmAF and controls. There were a total of 250 differentially-expressed mRNAs between pmAF and controls. Among these mRNAs, 148 and 102 mRNAs were up- and down-regulated in pmAF compared with controls respectively (**Figure 1A**). It was illustrated that there were no significant differences in expressions of 3993 lncRNAs between pmAF and controls. Among these lncRNAs, 45 and 49 lncRNAs were decreased and increased in pmAF compared with healthy controls respectively (**Figure 1B**). Heat maps indicated cluster analysis of differentially-expressed mRNAs and lncRNAs between pmAF and controls (**Figure 2**).

Top ten up/down-regulated differentially-expressed mRNAs and lncRNAs

Top ten up/down-regulated mRNAs and lncRNAs were listed in the **Tables 3** and **4**. Among these genes, urokinase-type plasminogen activator (PLAU) and endothelin-1 (EDN1) associated with thrombosis were down-regulated in pmAF. PLAU, which functions as an enzyme specifically cleaving plasminogen to active enzyme plasmin, can degrade thrombus. It also interacts with protein C inhibitor. When PLAU is decreased, the ability of anti-coagulation is impaired in pmAF. EDN1 plays an important role in heart development. It also acts as a negative regulator of blood coagulation and NO synthase biosynthetic process, mediating cardiac hypertrophy. Decrease of EDN1 might be a feedback of hypercoagulability in pmAF. Most mRNAs and lncRNAs listed in the tables had never been investigated in AF before, which offered some new directions for further exploration.

Differentially-expressed mRNAs in inflammation, oxidative stress and autophagy

It was shown that mRNAs involved in inflammation, oxidative stress and autophagy were

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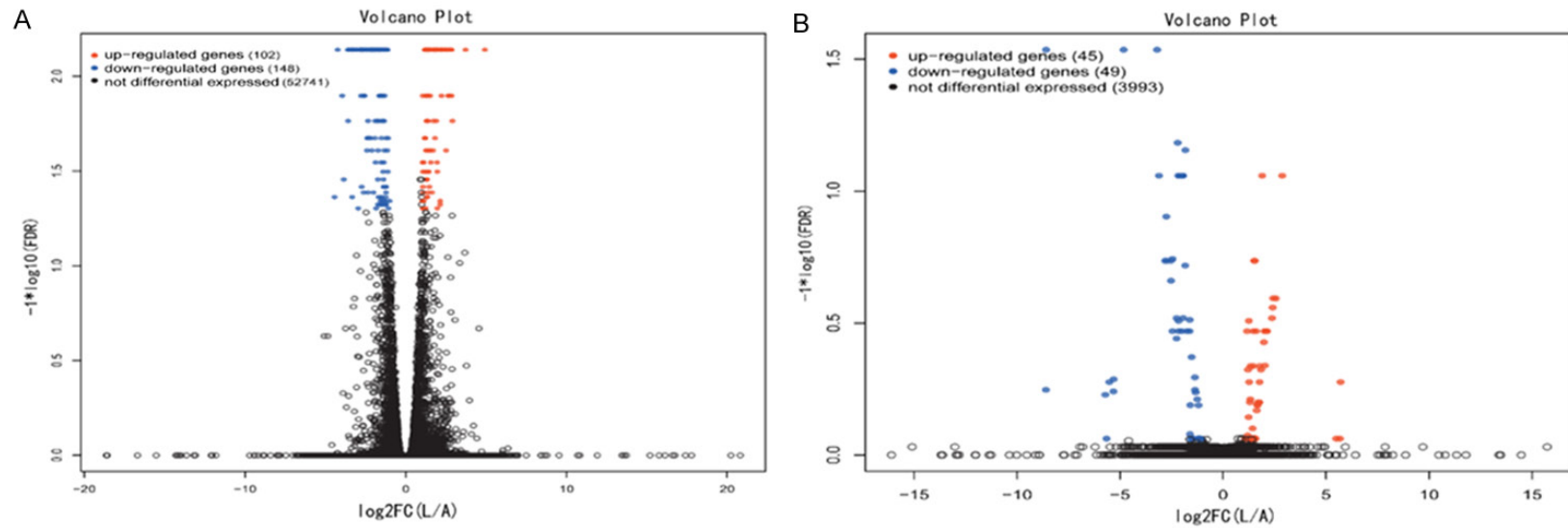


Figure 1. Volcano plots for differentially-expressed mRNAs and lncRNAs in lymphocytes from pmAF and controls. A. Volcano plot indicated differentially-expressed mRNAs in lymphocytes from pmAF and controls. B. Volcano plot indicated differentially-expressed lncRNAs in lymphocytes from pmAF and controls. Each dot indicated each mRNA or lncRNA. The red and blue ones indicated up- or down-regulated mRNAs or lncRNAs in control group compared with pmAF group, respectively. The black ones indicated mRNAs or lncRNAs without different expressions between those two groups. A and L indicated pmAF and controls, respectively. FC indicated the fold change, and $\log_2 \text{FC}(\text{L}/\text{A})$ demonstrated the folds of mRNA or lncRNA expressions in control group compared with pmAF group. FDR (false discovery rate) was the correction for p value. $-1 \cdot \log_{10}(\text{FDR})$ demonstrated calibration of FDR by logarithms, the larger $-1 \cdot \log_{10}(\text{FDR})$ it was, the more significant difference it was between those two groups. $P < 0.05$ was considered to be statistically significant.

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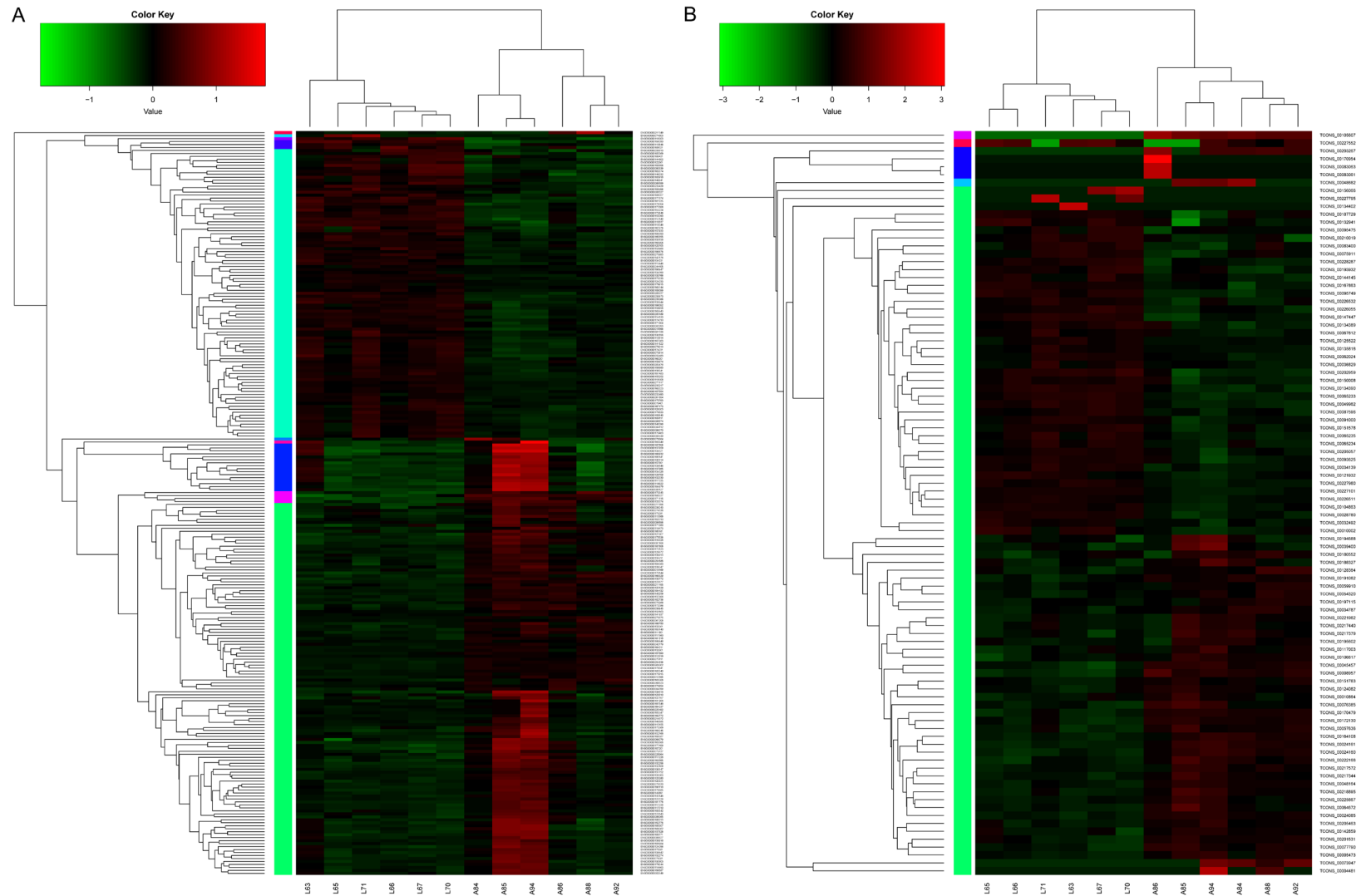


Figure 2. Heat maps of differentially-expressed mRNAs and lncRNAs in pmAF and controls. A. Heat map demonstrated differentially-expressed mRNAs between pmAF and controls. B. Heat map demonstrated differentially-expressed lncRNAs between pmAF and controls. The color bars indicated expression levels of mRNAs or lncRNAs. Red and green indicated up-/down-regulated mRNAs or lncRNAs, respectively. The redder, the higher expression it was. The greener, the lower expression it was. A and L indicated pmAF and controls, respectively. $P < 0.05$ was considered to be statistically significant.

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Table 3. Top ten up/down-regulated mRNAs in pmAF compared with controls

Gene name	A FPKM	L FPKM	Log2FC (A/L)	A/L	P value	Significant
Top ten up-regulated mRNAs						
TMEM252	2.75623	0.127735	4.43147	up	0.00055	yes
USP18	15.8018	0.839798	4.233903	up	5.00E-05	yes
CXCL10	6.54407	0.423444	3.949945	up	1.00E-04	yes
OTOF	1.48988	0.102746	3.858042	up	4.00E-04	yes
IFI27	98.9496	8.21624	3.590144	up	5.00E-05	yes
RSAD2	263.694	22.0013	3.583204	up	0.00015	yes
RTP4	20.8703	1.78538	3.547148	up	5.00E-05	yes
OAS1	159.248	15.0889	3.399716	up	5.00E-05	yes
GIMAP8	15.1104	1.45251	3.378922	up	5.00E-05	yes
ISG15	600.033	57.8741	3.374052	up	5.00E-05	yes
Top ten down-regulated mRNAs						
SLC12A1	0.135164	4.10233	-4.92366	down	5.00E-05	yes
NR4A3	0.105637	1.39382	-3.72186	down	5.00E-05	yes
HIC1	0.528785	3.96389	-2.90616	down	0.00015	yes
GRASP	0.827267	6.00135	-2.85886	down	5.00E-05	yes
LAMB3	0.223647	1.61421	-2.85153	down	1.00E-04	yes
PLAU	0.279901	1.91103	-2.77136	down	5.00E-05	yes
SEMG1	0.132139	0.862969	-2.70725	down	1.00E-04	yes
EDN1	0.154666	0.964286	-2.64031	down	1.00E-04	yes
B3GNT7	0.267646	1.66264	-2.63508	down	5.00E-05	yes
DUSP2	5.56771	32.5693	-2.54836	down	5.00E-05	yes

Top ten up/down-regulated mRNAs in pmAF group compared with controls. A represented pmAF, and L represented controls. FPKM was expression level of mRNA. Log2FC (A/L) was fold change of mRNA expression in pmAF compared with controls. P<0.05 was considered to be statistically significant. B3GNT7, beta-1,3-N-acetylglucosaminyl transferase 7; CXCL10, C-X-C motif chemokine ligand 10; DUSP2, dual specificity phosphatase 2; EDN1, endothelin 1; GIMAP8, GTPase IMAF family member 8; GRASP, general receptor for phosphoinositides 1 associated scaffold protein; HIC1, hyper-methylated in cancer 1; IFI27, interferon alpha inducible protein 27; ISG15, ISG15 ubiquitin-like modifier; LAMB3, laminin subunit beta 3; NR4A3, nuclear receptor subfamily 4 group A member 3; OAS1, 2'-5'-oligoadenylate synthetase 1; OTOF, otoferlin; PLAU, plasminogen activator urokinase; RSAD2, radical s-adenosyl methionine domain containing 2; RTP4, receptor transporter protein 4; SEMG1, semenogelin I; SLC12A1, solute carrier family 12 member 1; TMEM252, transmembrane protein 252; USP18, ubiquitin specific peptidase 18.

increased significantly in pmAF group (**Table 5**). Interferon alpha inducible protein 27 (IFI27), interferon induced protein with tetratricopeptide repeats 2 (IFI27), interferon induced protein 44 (IFI44L) and interferon alpha inducible protein 6 (IFI6) are family members in immune response interferon alpha/beta signaling pathways. They were notably up-regulated in pmAF rather than those in controls (P<0.05). Another gene named isocitrate dehydrogenase NADP(+)-dependent 1 (IDH1) associated with oxidative stress was also increased in pmAF compared with controls (P<0.05), indicating that IDH1-associated oxidative stress might be involved in pmAF as well. Moreover, a gene named lysosomal associated membrane protein 3 (LAMP3), a member of LAMP family participat-

ing in autophagy, was prominently elevated in pmAF compared with controls (P<0.05), suggesting that autophagy might take part in the pathological process of pmAF. Interestingly, there were no significant differences in expressions of microtubule associated protein 1 light chain 3 beta 1 (MAP1LC3B1) and microtubule associated protein 1 light chain 3 beta 2 (MAP1LC3B2), which are two critical genes involved in autophagy (P>0.05).

Gene ontology (GO) annotations and enrichment analysis of differentially-expressed mRNAs

GO analysis indicated that biological processes of differentially-expressed mRNAs were mainly involved in biological adhesion, biological regu-

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Table 4. Top ten up/down-regulated lncRNAs in pmAF group compared with controls

LncRNA name	A FPKM	L FPKM	Log2FC (A/L)	A/L	P value	Significant
The top ten up-regulated lncRNAs						
TCONS_00039400	297.154	32.2735	3.202789	up	5.00E-05	yes
TCONS_00008957	214.207	24.7688	3.11241	up	0.00035	yes
TCONS_00024161	48.4806	6.87962	2.817007	up	0.0017	yes
TCONS_00194688	37.8243	5.59984	2.755856	up	0.001	yes
TCONS_00024160	70.1422	10.6107	2.724763	up	0.0021	yes
TCONS_00045457	92.8827	16.0097	2.536464	up	0.00195	yes
TCONS_00164108	19.8912	3.45415	2.525728	up	0.00275	yes
TCONS_00064672	55.8827	10.1907	2.455149	up	0.00625	yes
TCONS_00076385	53.3023	9.72993	2.453696	up	0.00155	yes
TCONS_00128364	25.8616	5.40935	2.257284	up	0.0089	yes
The top ten down-regulated lncRNAs						
TCONS_00202959	2.75389	20.1453	-2.8709	down	0.00065	yes
TCONS_00065233	4.2231	24.906	-2.56012	down	0.0035	yes
TCONS_00049962	5.66132	30.561	-2.43248	down	0.00335	yes
TCONS_00134390	23.7087	125.827	-2.40795	down	0.00395	yes
TCONS_00095749	114.712	595.568	-2.37625	down	0.00485	yes
TCONS_00147447	40.8992	179.356	-2.13268	down	0.00675	yes
TCONS_00028780	12.4975	53.0548	-2.08584	down	0.0078	yes
TCONS_00065235	5.39614	22.0328	-2.02965	down	0.01205	yes
TCONS_00010002	14.7108	59.3976	-2.01353	down	0.0081	yes
TCONS_00132941	38.8323	153.389	-1.98187	down	0.0094	yes

Top ten up/down-regulated lncRNAs in pmAF group compared with controls. A represented pmAF, and L represented healthy controls. FPKM was expression level of lncRNA. Log2FC (A/L) was fold change of lncRNA expression in pmAF compared with controls. P<0.05 was considered to be statistically significant. (Note: Numbers of TCONS were the numbers used during bioinformatics analysis, corresponding numbers in NONCODE database were listed in the [Supplementary Table 1](#)).

Table 5. mRNAs associated with inflammation, oxidative stress and autophagy in pmAF and controls.

Gene names	A FPKM	L FPKM	log2FC (A/L)	Functions	P values	Significant
IFI27	98.9496	8.21624	3.590144	Inflammation	5.00E-05	Yes
IFIT2	655.64	65.0269	3.333795	Inflammation	0.00055	Yes
IFI44L	196.178	21.1162	3.215741	Inflammation	5.00E-05	Yes
IFI6	439.654	63.8388	2.783863	Inflammation	1.00E-04	Yes
IDH1	11.8463	4.81774	1.298008	Inflammation/Oxidative stress	5.00E-05	Yes
MAP1LC3B1	29.6744	40.6646	0.454555	Autophagy	0.1218	No
MAP1LC3B2	0.695037	1.86503	-1.42404	Autophagy	0.530444	No
LAMP3	3.38359	0.451577	2.905511	Autophagy	5.00E-05	Yes

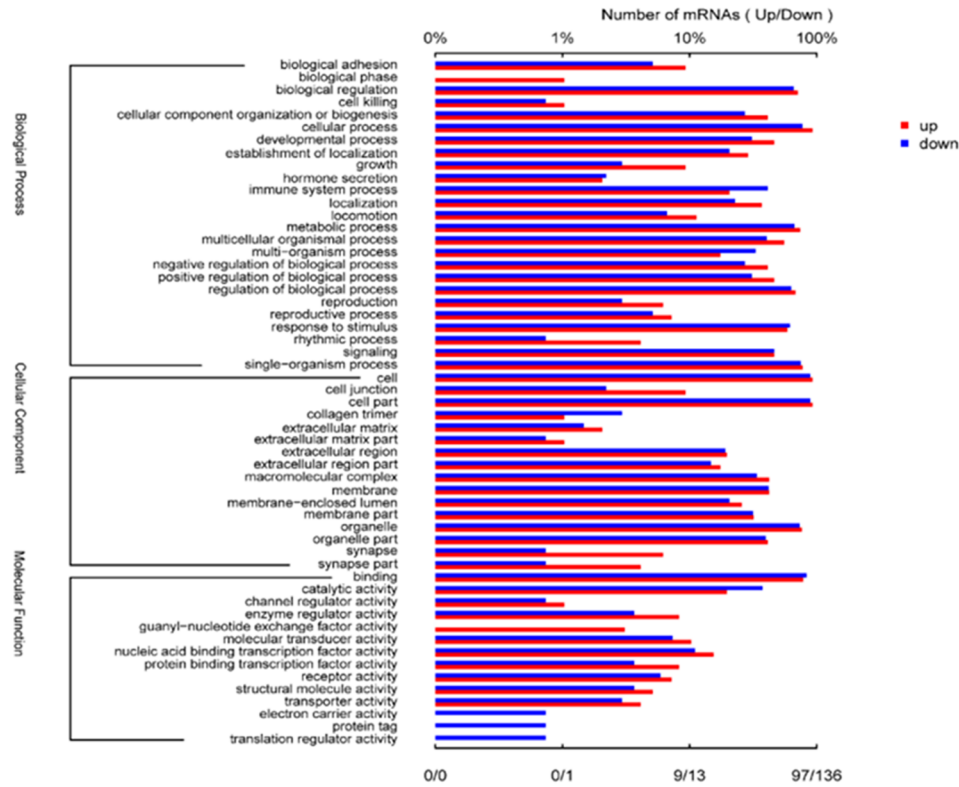
Differentially-expressed mRNAs associated with inflammation, oxidative stress and autophagy in pmAF and controls. A represented pmAF, and L represented controls. FPKM was expression level of mRNA. Log2FC (A/L) was the fold change of mRNA expression in pmAF compared with controls. P<0.05 was considered to be statistically significant. IFI27, interferon alpha inducible protein 27; IFIT2, interferon induced protein with tetratricopeptide repeats 2; IFI44L, interferon induced protein 44 like; IFI6, interferon alpha inducible protein 6; IDH1, isocitrate dehydrogenase NADPb(+); LAMP3, lysosomal associated membrane protein 3; MAP1LC3B1, microtubule associated protein 1 light chain 3 beta 1; MAP1LC3B2, microtubule associated protein 1 light chain 3 beta 2.

lation, developmental process and metabolic process (**Figure 3A**). The main cellular components of these differentially-expressed mRNAs

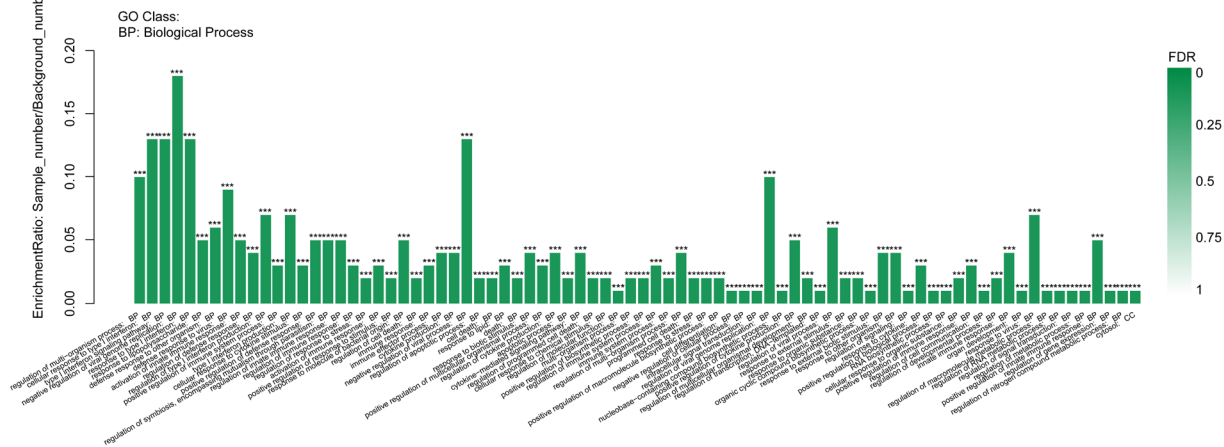
were cellular junction, collagen trimer and extracellular matrix, which were closely associated with structural remodeling of myocardium

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A



B



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Figure 3. GO annotations and enrichment analysis of differentially-expressed mRNAs. A. GO annotations indicated that differentially-expressed mRNAs between pmAF and controls were annotated by biological process, cellular component and molecular function. Red and blue bars indicated up-/down-regulated mRNAs in controls compared with pmAF. Percentages indicated the ratios between the numbers of specifically GO-annotated mRNAs and the total number of differentially-expressed mRNAs. B. Enrichment analysis indicated enrichment of differentially-expressed mRNAs in pmAF and controls. Heights of green bars demonstrated enrichment ratios. The greener the bar was, the more significant the enrichment was. FDR (false discovery rate) was the correction for p value. *indicated $P < 0.05$, **indicated $P < 0.01$, ***indicated $P < 0.001$.

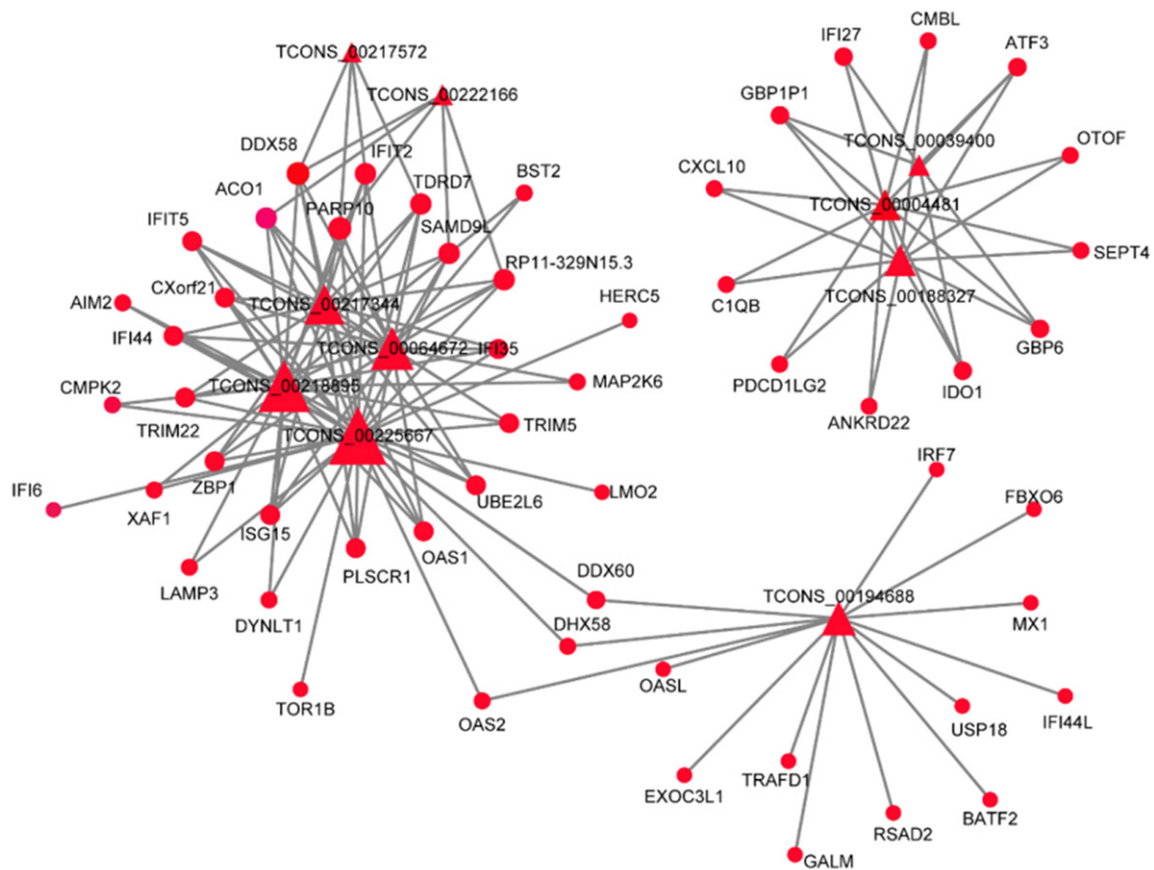


Figure 4. Co-expression network among differentially-expressed mRNAs and lncRNAs. The closer the relationships between lncRNAs and mRNAs were, the bigger the symbols they were. Red triangles indicated lncRNAs and red dots indicated the co-expressed mRNAs.

in AF. Moreover, molecular functions that those differentially-expressed mRNAs enriched in were primarily about channel regulator activity, enzyme regulator activity, protein binding transcription factor activity, translation regulator activity, etc. Enrichment analysis implied that differentially-expressed mRNAs were mainly enriched in GO terms of immune response, programmed cell death, cell differentiation, cell communication and signaling transduction, suggesting that all these processes might participate in the pathological development of pmAF (**Figure 3B**). Furthermore, enriched path-

ways demonstrated that tumor necrosis factor (TNF) signaling pathway, nuclear factor (NF)-kappa B signaling pathway, Toll-like receptor pathway, nucleotide binding oligomerization domain (NOD)-like receptor pathway were significantly activated in pmAF compared with controls ($P < 0.05$) (not listed in the pictures).

Co-expression network among differentially-expressed mRNAs and lncRNAs

It was demonstrated that there were 10 differentially-expressed lncRNAs co-expressed with multiple mRNAs (**Figure 4**). lncRNAs (TCONS_

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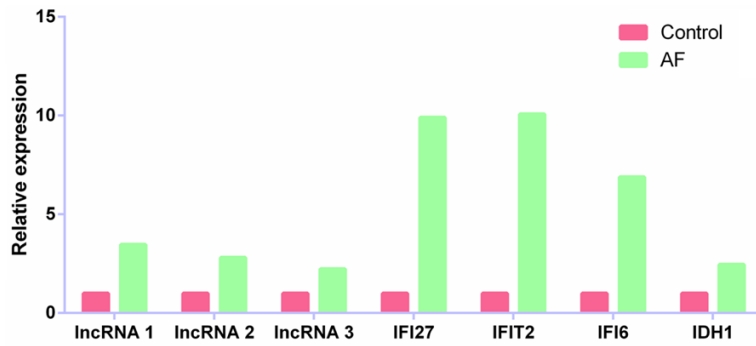


Figure 5. Further validation of lncRNAs and inflammatory genes by qRT-PCR. Results indicated that lncRNA1, lncRNA2, lncRNA3, IFI27, IFIT2, IFI6 and IDH1 were significantly higher in pmAF compared with controls. $P < 0.05$ was considered to be statistically significant.

00217344, TCONS_00064672, TCONS_00218895 and TCONS_00225667) were closely co-expressed with sterile alpha motif domain containing 9 like protein (SAMD9L), interferon induced protein with tetratricopeptide repeats 2 (IFIT2), lysosomal associated membrane protein 3 (LAMP3) and so forth. lncRNAs (TCONS_00039400, TCONS_00004481, and TCONS_00188327) were closely correlated with guanylate binding protein 1 pseudogene 1 (GBP1P1) and septin 4 (SEPT4). lncRNA (TCONS_00194688) was in close relationships with F-box protein 6 (FBXO6), basic leucine zipper ATF-like transcription factor 2 (BATF2), ubiquitin specific peptidase 18 (USP18), etc.

Further validation of differentially-expressed mRNAs and lncRNAs

In order to testify correctness of RNAseq, relative expressions of four mRNAs and three lncRNAs highly-expressed in pmAF were further determined by qRT-PCR. Finally, it was demonstrated that lncRNA1 (TCONS_00076385), lncRNA2 (TCONS_00194688) and lncRNA3 (TCONS_00024161) were indeed up-regulated in pmAF rather than those in controls, $P < 0.05$ (Figure 5). Moreover, mRNA levels of IFI27, IFIT2, IFI6, IDH1 were also much higher in pmAF than those in controls, $P < 0.05$ (Figure 5). (Note: TCONS numbers were used during bioinformatics analysis, corresponding numbers in the NONCODE database were listed in the Supplementary Table 1).

Discussion

Inflammation and immune response are two important mechanisms in AF. Mounting evi-

dence indicates that plasma protein levels of interleukin-1, interleukin-6, C reactive protein, tumor necrosis factor α (TNF- α), P-selectin, fibrinogen, tissue factors are much higher in AF population rather than healthy controls [14-17]. Relevant research from both animals and humans demonstrates that myocardium and endocardium from AF are characterized by infiltration of a great many lymphocytes [18-20]. Moreover, protein levels of adipocytokine, fibroblast growth factor, transforming

growth factor-1, matrix metalloproteinase are all elevated in the plasma and myocardium from AF [21, 22]. Inflammatory genes named IFI27, IFIT2 and IFI6 have never been explored in pmAF before. IFI27 is reported to participate in membranous nephropathy, liver fibrosis and primary myelofibrosis [23-25]. IFIT2 is a protector against virus infection in nervous system [26]. IFI6 can inhibit apoptosis of vascular endothelial cells infected by Dengue virus [27]. They are indispensable in IFN alpha/beta signaling pathways and crucial in regulation of inflammation and immune response. From analysis of RNAseq and qRT-PCR, it was known that they were up-regulated in pmAF, suggesting that IFN alpha/beta signaling pathways must be involved in pmAF. Furthermore, we hypothesized that fibrosis in myocardium might be induced by IFI27 in AF.

Accumulating evidence illustrates that oxidative stress is another important mechanism associated with AF. 8-hydroxy-deoxyguanosine (8-OHdG) is a product generated during the process of DNA damage under oxidative stress. Levels of 8-OHdG in the plasma and urine from AF are significantly higher than healthy controls, indicating that oxidative stress is much intense in AF than controls [28, 29]. Superoxide dismutase (SOD), an important antioxidant, can catalyze products generated during oxidative stress. However, the level of SOD is significantly decreased in AF population than healthy controls, implying that anti-oxidation ability in AF is impaired [30]. IDH1 is a metabolic enzyme, it generates NADPH by metabolism of isocitrate into α -ketoglutarate. Accumulating evidence indicates that IDH1 can decrease reactive oxy-

gen species (ROS) in cell models treated with lipopolysaccharide (LPS) [31]. IDH1 knockout mice present significant increases of inflammatory factors and deaths induced by LPS [31]. Another study points out overexpression of IDH1 is related with enhanced oxidative stress, with elevated expression of manganese superoxide dismutase (MnSOD) and p62; mutation of IDH1 can lead to autophagy as well [32]. These studies indicate that IDH1 plays different roles in oxidative stress, either protective or devastated. In this study, we discovered that mRNA level of IDH1 was notably increased in pmAF compared with controls, suggesting that IDH might be increased as a negative feedback due to high level of oxidative stress, and IDH might decrease ROS level and alleviate oxidative stress in pmAF. However, specific function of IDH1 in AF has never been studied before, which provides a new direction for AF.

Besides, it was demonstrated that differentially-expressed mRNAs and lncRNAs were enriched in signaling pathways like TNF signaling pathway, NF- κ B signaling pathway, Toll-like receptor pathway and NOD-like receptor pathway, which are closely associated with apoptosis, immunity, inflammation, and oxidative stress, suggesting that these signaling pathways might be involved in the initiation and development of AF [33-39]. However, most lncRNAs are newly discovered and their underlying mechanisms in AF still remain elusive.

There also existed some limitations in this study. Firstly, lymphocytes from the whole blood stand for systematic inflammation. Secondly, RNAseq and further validation were performed only on the population with a small scale, a larger population-based study would be required for future verification. Moreover, specific functions of those differentially-expressed mRNAs and lncRNAs in pmAF had not been further excavated in this study. In the next few years, our group will focus on the functions of those genes, lncRNAs and signaling pathways not only in lymphocytes, but also in primary myocytes and AF animal models, in order to know their underlying mechanisms in AF. In addition, we hope to discover some genes, lncRNAs and pathways which could be applied as therapeutic targets for AF.

In conclusion, lncRNA1, lncRNA2, lncRNA3, IFI27, IFIT2, IFI6 and IDH1 are highly-expressed

in lymphocytes from pmAF. Furthermore, lymphocytes might play indispensable roles in inflammation and oxidative stress during the pathological process of pmAF.

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Disclosure of conflict of interest

None.

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