Original Article

Bioinformatics and functional experiments reveal that *MRC2* inhibits atrial fibrillation via the PPAR signaling pathway

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Background: Atrial fibrillation (AF) is a prevalent cardiac arrhythmia that requires improved clinical markers to increase diagnostic accuracy and provide insight into its pathogenesis. Although some biomarkers are available, new ones need to be discovered to better capture the complex physiology of AF. However, their limitations are still not fully addressed. Bioinformatics and functional studies can help find new clinical markers and improve the understanding of AF, meeting the need for early diagnosis and individualized treatment.

Methods: To identify AF-related differentially expressed genes (DEGs), We applied the messenger RNA (mRNA) expression profile retrieved in Series Matrix File format from the GSE143924 microarray dataset obtained from the Gene Expression Omnibus (GEO) database, and then used weighted gene co-expression network analysis (WGCNA) to identify the overlapping genes. These genes were analyzed by enrichment analysis, expression analysis and others to obtain the hub gene. Additionally, the potential signaling pathway of hub gene in AF was explored and verified by functional experiments, like quantitative real-time polymerase chain reaction (qRT-PCR), cell counting kit-8 (CCK-8), flow cytometry, and Western blotting (WB) assay.

Results: From the GSE143924 data (410 DEGs) and tan module (57 genes), 10 overlapping genes were identified. A central down-regulated gene in AF, *MRC2*, was identified through bioinformatics analysis. based on these results, it was hypothesized that the PPAR signaling pathway is related to the mechanism of action of *MRC2* in AF. Moreover, over-*MRC2* markedly reduced the growth speed of angiotensin II (Ang II)-induced human cardiac fibroblasts (HCFs) and increased apoptosis. Conversely, knockdown of *MRC2* promoted HCFs cell proliferation number. Additionally, *MRC2* over-expression increased the protein expression level of PPARα, PPARγ, CPT-1, and SIRT3 in Ang II-induced HCFs.

Conclusions: While meeting the need for new biomarkers in the diagnosis and prognosis of AF, this study reveals the inherent limitations of current biomarkers. We identified *MRC2* as a key player as an inhibitory gene in AF, highlighting its role in suppressing AF progression through the PPAR signaling pathway. *MRC2* may not only serve as a diagnostic indicator, but also as a promising therapeutic target for patients with AF, which is expected to be applied in clinical practice and open up new avenues for individualized interventions.

Keywords: Atrial fibrillation (AF); bioinformatics analysis; weighted gene co-expression network analysis (WGCNA); *MRC2*; PPAR signaling pathway

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Introduction

Atrial fibrillation (AF), as a usual arrhythmia phenomenon, refers to severe electrical interference in the atrium, which is more common in the elderly (1). It usually manifests as palpitations, chest tightness, shortness of breath, dizziness, and some patients may also experience syncope, polyuria, angina and so on (2). The pathologic and physiologic mechanisms of AF involve multiple complex electrophysiologic, histologic, and biochemical changes. The interaction between these mechanisms leads to the onset, persistence, and worsening of AF (3). Experimental and clinical data suggest that the pathophysiology of AF is highly complex and involves a large number of important players, including oxidative stress, calcium overload, atrial dilatation, microRNA (miRNA), inflammation, and myofibroblast activation (4). Inflammation contributes to the prothrombotic inflammatory state associated with AF. Inflammatory biomarkers (e.g., C-reactive protein and interleukin-6) contribute to AF-related thrombosis. However, further studies are needed to understand the clinical significance of inflammatory biomarkers in predicting AF-related thromboembolism (5). A study has shown that the disease is closely related to diseases like coronary and congenital heart disease and is regarded as an invisible "killer" of cardiovascular and cerebrovascular diseases (6). AF can also cause cerebral artery embolism, sudden cardiac death, pulmonary embolism, heart failure, peripheral arterial embolism and other complications, which bring certain difficulties to clinical treatment (7). Although some patients can be treated with beta-blockers, calcium channel antagonists, digitalis and other drugs, electrical cardioversion, catheter ablation, and surgical maze surgery to improve the body's homeostasis and restore normal heart rhythm, the prognosis of most patients is still not optimistic (8). Diabetes mellitus (DM) represents an independent risk factor for chronic AF (9). The prevalence of AF among individuals with DM is notably high, reaching alarming levels in recent years. Emerging evidence suggests that DM acts as a chronic inflammatory disease, contributing to the development and progression of AF (10). The presence of a chronic inflammatory state in DM patients with AF further complicates the disease course. A history of DM was identified as one of the components of high-risk AF, and early reporting of biomarkers facilitates its clinical detection (11). And a study proved that ELABELA (peptide hormone essential for cardiac development) is a protective factor in patients with cardiac arrhythmias and can be used as a prognostic marker for AF and its associated complications (12). Therefore, deep knowledge of the AF mechanism is essential to determine its biological markers and improve its treatment.

To better compile the genetic information of disease patients, the National Center for Biotechnology Information (NCBI) creates the Gene Expression Omnibus (GEO) database (13). The database stores a large number of microarray, next-generation sequencing, and other high-throughput sequencing data, which provides convenience for researchers to retrieve gene expression data of any species or man-made (14). Based on this database, high-throughput sequencing has been widely applied to analyze differentially expressed genes (DEGs) and search for hub genes (15). In addition, the methods of bioinformatics analysis focusing on genomics and proteomics are also widely used in the study of human diseases (16). The potential diagnostic key genes unearthed can also provide a theoretical basis for drug sensitivity analysis and targeted drug development for AF (17). Herein, exploring the pathogenesis of AF based on the above methods will provide a more comprehensively diagnostic direction for the treatment of patients.

In this study, to better explore the pathogenesis of AF and discover new potential clinical indicators, we first downloaded the microarray dataset of GSE143924 from the GEO database and conducted DEGs screening and weighted gene co-expression network analysis (WGCNA) for the overlapping genes. Subsequently, these genes were

Highlight box

Key findings

- This study identified *MRC2* as a novel inhibitory gene in atrial fibrillation (AF). *MRC2* downregulation is associated with AF and modulates growth and apoptosis of angiotensin II-induced human cardiac fibroblasts via the PPAR pathway.

What is known and what is new?

- AF involves complex gene networks, but precise mechanisms and implicated genes are not fully understood.
- We discovered a unique role for *MRC2* in AF, implying that its downregulation contributes to the disease. Our findings highlight the importance of *MRC2* as a diagnostic biomarker and therapeutic target for AF.

What is the implication, and what should change now?

- These findings advance AF molecular mechanisms understanding, informing new diagnostic tools and treatments development.
- Further studies should validate the clinical relevance of *MRC2* in AF and understand its disease role.

analyzed by bioinformatics analysis to determine the hub gene. Furthermore, the regulatory mechanisms of the hub gene in AF development were also investigated by cellular experiments. We present this article in accordance with the STREGA and MDAR reporting checklists (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1235/rc).

Methods

Microarray data extraction

We searched the GSE143924 microarray data set from the GEO database in the format of series matrix file for subsequent analysis (18). The GSE143924 data set contained 15 patients in sinus rhythm after surgery (SR) and 15 patients developing postoperative AF after surgery (POAF) samples (19). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification and classification of DEGs

Based on the GSE143924 data set, we compared 30 samples in the data set through GEO2R software in the GEO database (20) and set the selection conditions for DEGs, and the results obtained were displayed in a significantly ranked gene table. Fold change (FC) >1 was the standard for up-regulated DEGs, FC <1 for down-regulated DEGs, and they both met $P<0.05$. Finally, with the help of R software, the cluster distribution of DEGs in 30 samples was drawn into a heat map.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses on DEGs

GO integrates the world's largest source of information about gene functions, and is the basis for large-scale molecular biology and genetics experimental computational analysis in biomedical research (21). It describes the biological functions in three parts: cell component (CC), biological process (BP), and molecular function (MF) (22). The KEGG database has a powerful graphic function, which helps researchers intuitively understand the metabolic pathways and the relationship between the pathways (23). The DAVID database (https://david.ncifcrf.gov/) offers a systematic set of functional annotation tools (24). Herein, we performed an enrichment analysis of DEGs in BP and KEGG based on this database to understand the biological significance behind it.

WGCNA

We performed WGCNA using expression data from GSE143924. In order to conduct this analysis and identify AF-related modules in DEGs, the R package "WGCNA" was employed (25). The adjacency matrix was transformed into a topological overlap matrix using a soft-threshold power with a scale-free R^2 near 0.85 and a slope near 1. For the sake of network design and module identification, we set the soft threshold power to 9. A crucial module was defined as one that has a high association with AF. The key module network of WGCNA was generated by Cytoscape (26). Then, area scale Venn diagrams of key module genes and DEGs were created by Venn Diagram Plotter (https://omics.pnl.gov/software/VennDiagramPlotter.php) to screen the overlapping genes (27).

Gene set enrichment analysis (GSEA) on overlapping genes

GSEA is a calculation method used to determine whether a set of priori-defined genes are statistically displayed (28). It is usually used in conjunction with the Molecular Signatures Database (MSigDB) to study the biological functions of genes (29). In this study, we performed enrichment analysis for ten key genes respectively, and $P<0.05$ was statistically significant.

Expression analysis and receiver operating characteristic (ROC) curve analysis

Peking University created the analytical tool Gene Expression Profiling Interactive Analysis (GEPIA), which includes RNA sequencing (RNA-seq) expression data from 9,736 cancer samples and 8,587 normal tissues (30). Using the "Single Gene Analysis" module of GEPIA, we examined the messenger RNA (mRNA) expressions of overlapping genes in AF for this study. In addition, ROC analysis was used to examine the validity of overlapping genes for AF prognosis prediction. To quantify the ROC, the area under the curve (AUC) was calculated. The accuracy of the prediction increases with AUC values.

Cell culture and transfection

Human cardiac fibroblasts (HCFs) were obtained from the

Chinese Cell Resource Bank and cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), and the environment of the medium was set to 37 ℃, 95% air and 5% CO_2 humid environment. Subsequently, *MRC2* was knocked down [small interfering (si)-*MRC2*-1 and si-*MRC2*-2] and over-expressed (over-*MRC2*), respectively, in HCFs treated with angiotensin II (Ang II)/mock to build the AF model transfected with Lipofectamine 2000 reagent. After a period of time, the next step was performed after transfection.

Quantitative real-time polymerase chain reaction (qRT-PCR) assay

First, total RNA was actually extracted from HCFs cells with Trizol, and then RNA was reverse transcribed by PrimeScript™ RT reagent kit (TaKaRa, Dalian, China) to synthesize cDNA. Subsequently, qRT-PCR was performed on an ABI7500 system (ABI, FosterCity, CA, USA) using SYBR MAsterMix (ABI). Finally, we applied $2^{-\Delta\Delta Ct}$ method to determine the relative expression.

Cell counting kit-8 (CCK-8) assay

HCFs were added to a 96-well plate at a density of 1×10^3 per well plate, and then 10 μL of CCK-8 solution was injected into each well plate. Based on the CCK-8, the cell viability was observed at 24, 48, 72, 96 hours, and the optical density (OD) value of the cells at 450 nm was observed.

Flow cytometry assay

For apoptosis, flow cytometry was used here to detect apoptosis of HCFs treated with Ang II in different groups. Specifically, the transfected cells were first suspended in buffer and washed with phosphate buffered saline (PBS), then stained by Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) assay kit (BD Bioscinge, Carlsbad, CA, USA) and flow cytometry was performed.

Western blotting (WB) assay

First, the protein was acquired from HCFs by radioimmunoprecipitation assay (RIPA) lysis buffer, and the protein concentration was detected and quantified by bicinchoninic acid (BCA) protein. Proteins were then transferred to polyvinylidene fluoride (PVDF) membranes blocked with 5% nonfat milk for 2 hours and detected by primary antibodies at 4 ℃ overnight. Then, PVDF membranes were kept with specific horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 hour at indoor temperature. Finally, the signal was observed by the HyGLO HRP detection kit of Denville (Metuchen, NJ, USA), and the protein level was measured.

Research process

In this study, the GSE143924 microarray dataset was obtained from the GEO database, including 15 samples of patients in SR and 15 samples of patients who developed POAF. First, these 30 samples were compared by GEO2R software to obtain a significantly sorted gene list according to the selection conditions of DEGs, and a heat map of the clustered distribution of DEGs in these 30 samples was plotted using R software. Then, the DEGs were enriched by GO and KEGG using the DAVID database, and then the expression data in GSE143924 were analyzed by WGCNA to identify modules related to AF, and the key module network was obtained by setting appropriate soft thresholds and other parameters using the "WGCNA" package in the R software, and the genes overlapping with DEGs were filtered using the Venn diagram. Genes overlapping with DEGs. The overlapping genes were analyzed by GSEA and combined with the MSigDB to investigate the biological functions of these genes. The mRNA expression of these overlapping genes in AF was investigated using GEPIA, an analytical tool developed by Peking University, and ROC analysis was used to verify the validity of these genes in predicting the prognosis of AF.

At the molecular level, an AF model was constructed using HCFs by cell culture and transfection techniques, and the functions and mechanisms of action of the key genes were investigated by qRT-PCR analysis, CCK-8 experiments, and flow cytometry. The molecular mechanisms and potential therapeutic targets of AF were systematically explored.

Statistical analysis

GraphPadPrismV6 (Prism, San Diego, CA, USA) was used for analysis in all steps of this experiment, and each step was conducted for no less than three times (31). The mean and standard deviation (SD) were used to analyze the data, and statistical significance was inferred when $P<0.05$, indicating significance in a two-sided manner.

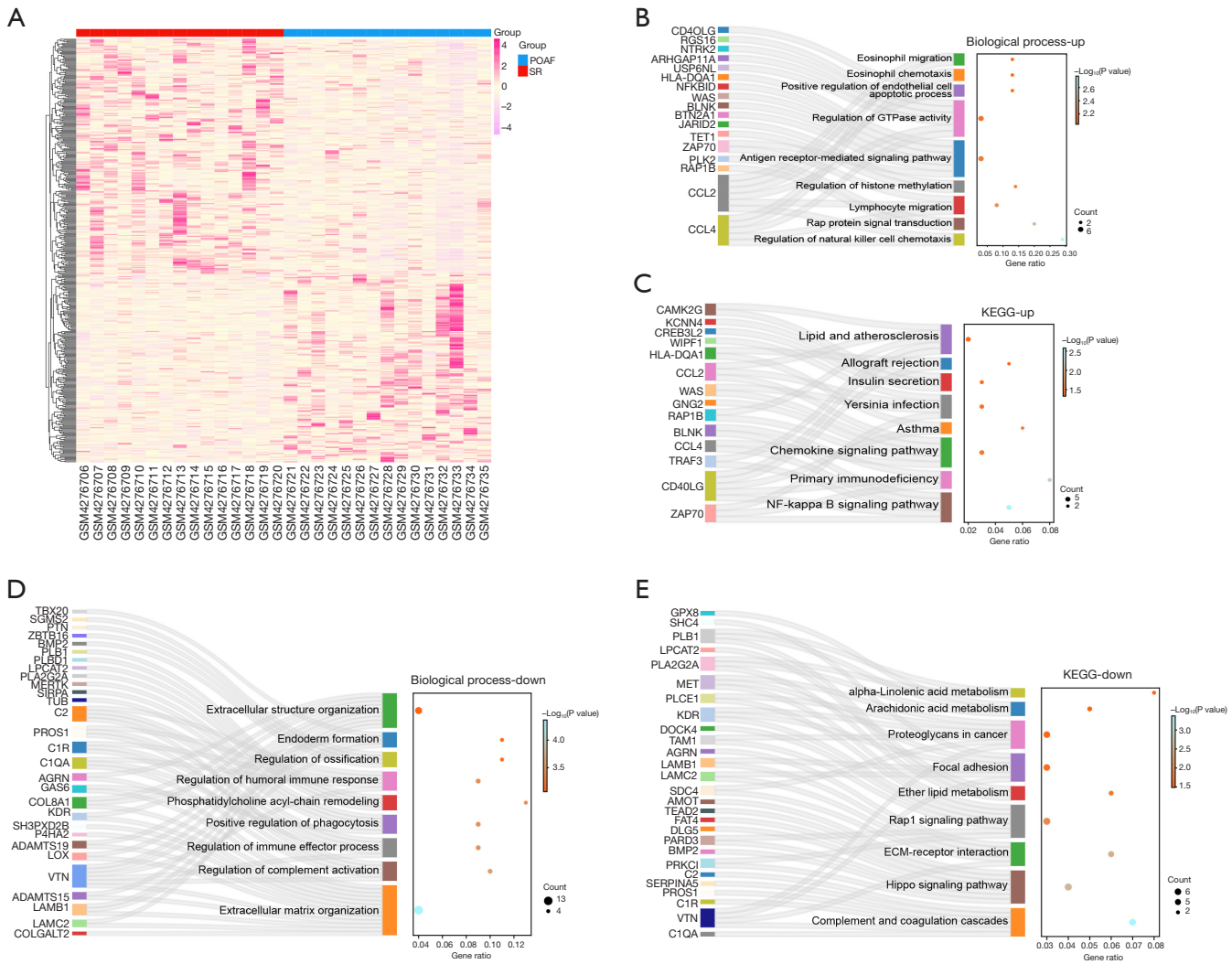

Figure 1 Screening and functional enrichment analysis of GSE143924-DEGs. (A) Heatmap of 15 POAF samples in orange and 15 SR samples in blue. Each column represents sample data and each row a gene. (B,C) Sankey plot and dot plot of the BP and KEGG on up-regulated DEGs. (D,E) Sankey plot and dot plot of the BP and KEGG on down-regulated DEGs. POAF, postoperative atrial fibrillation after surgery; SR, sinus rhythm after surgery; GTPase, guanosine triphosphatase; KEGG, Kyoto Encyclopedia of Genes and Genomes; DEGs, differentially expressed genes; BP, biological process.

Results

GSE143924-DEGs associated with AF and pertinent enrichment analysis

Based on GEO2R, we identified 182 up-regulated and 228 down-regulated DEGs. The heat map in *Figure 1A* showed the cluster distribution of these DEGs in the GSE143924 dataset. The up-regulated DEGs in BP were enriched in eosinophil migration, eosinophil chemotaxis, positive regulation of endothelial cell apoptosis, regulation of guanosine triphosphatase (GTPase) activity, antigen receptor-mediated signaling pathways, and regulation of histone methylation (*Figure 1B*). The enrichment results in KEGG showed the top 8 pathways, namely lipids and atherosclerosis, allograft rejection, insulin secretion, yersinia infection, asthma, chemokine signaling pathway, primary immunity defects and nuclear factor-κB (NF-κB) signaling pathway (*Figure 1C*). Likewise, down-regulated DEGs were enriched in BP, such as extracellular structural organization, endoderm formation, regulation of ossification, regulation

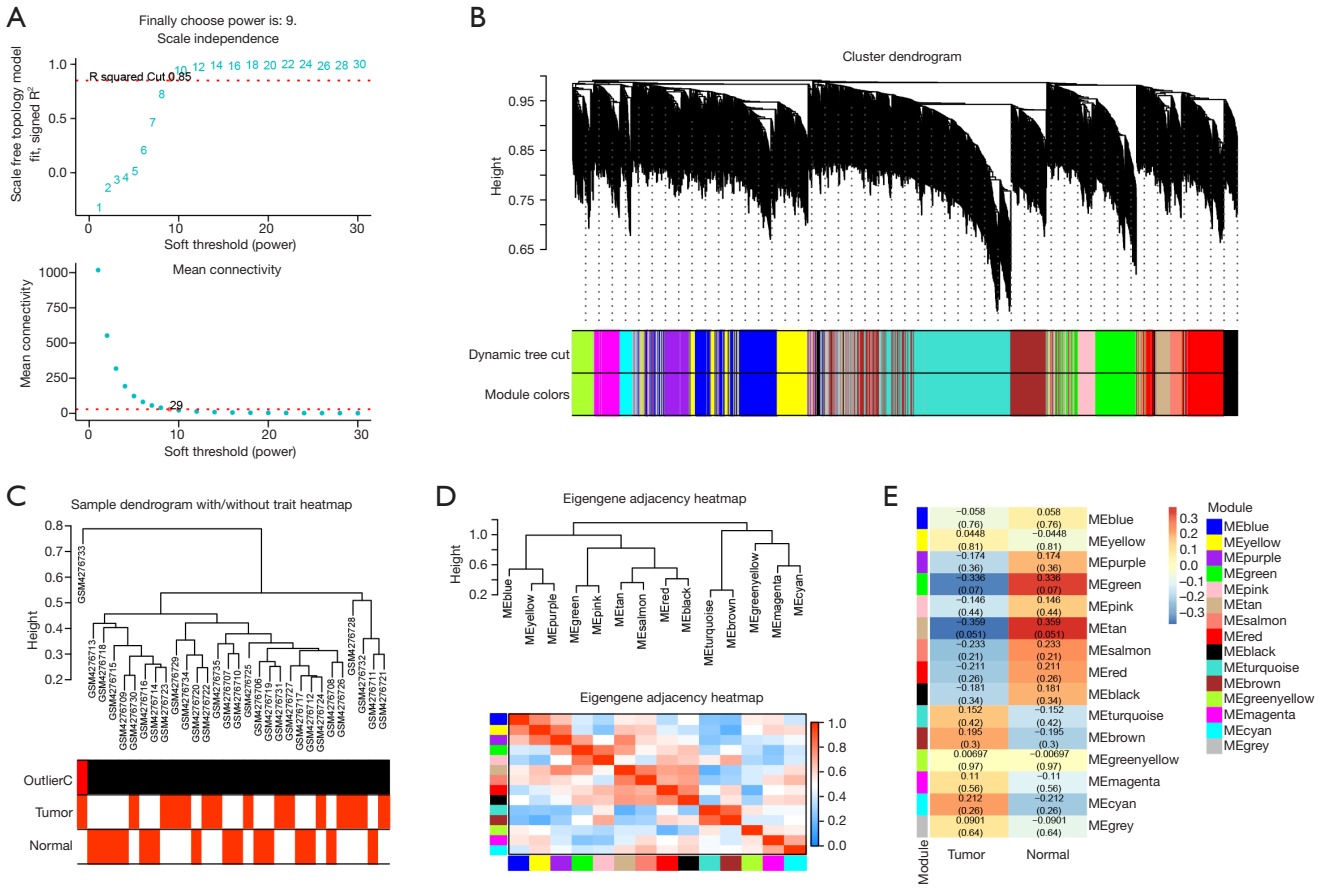

Figure 2 Identification of key module associated with AF in WGCNA. (A) Scale-free topology model (top) and average connectivity (bottom) to determine soft threshold power. (B) Gene clustering tree. (C) Sample clustering to detect outliers. (D) Cluster and eigengene adjacency heatmap for all modules. (E) Heatmap showing the relationship between different modules and clinical samples. AF, atrial fibrillation; WGCNA, weighted gene co-expression network analysis.

of humoral immune responses, phosphatidylcholine acyl chain remodeling (*Figure 1D*). These down-regulated DEGs were also enriched in 9 metabolic pathways in KEGG, namely α-linolenic acid metabolism, arachidonic acid, proteoglycans in cancer, focal adhesions, ether lipid metabolism, extracellular matrix (ECM)-receptor interaction, Hippo signaling pathway, Rap1 signaling and the complement and coagulation cascade (*Figure 1E*).

Tan module was the key module in WGCNA

GSE143924 were then analyzed by WGCNA. When the soft threshold power was 9 (unscaled R^2=0.85, *Figure 2A*), we obtained 15 modules (*Figure 2B-2D*), with non-clustered DEGs in grey modules. From the module-feature correlations, tan module had the highest correlation with AF (correlation coefficient =0.359, P=0.051; *Figure 2E*).

MRC2 was identified as the hub gene associated with AF

Figure 3A was a Venn diagram of tan module genes and GSE143924-DEGs, and 10 overlapping genes were acquired. To investigate potential pathways of these genes, GSEA was first performed (*Figure 3B-3K*). For example, *MRC2* was enriched in primary bile acid biosynthesis [enrichment score (ES) =0.7012, normalized P value (NP) =0.0020], renin-angiotensin system (RAS) (ES =0.6133, NP =0.0145), cytosolic DNA sensing pathway (ES =0.5775, NP =0.0203), and PPAR signaling pathway (ES =0.4419, NP =0.0425; *Figure 3G*). To strengthen the reliability of the overlapping genes identified from the bioinformatics analysis, we first investigated the expressions of these genes

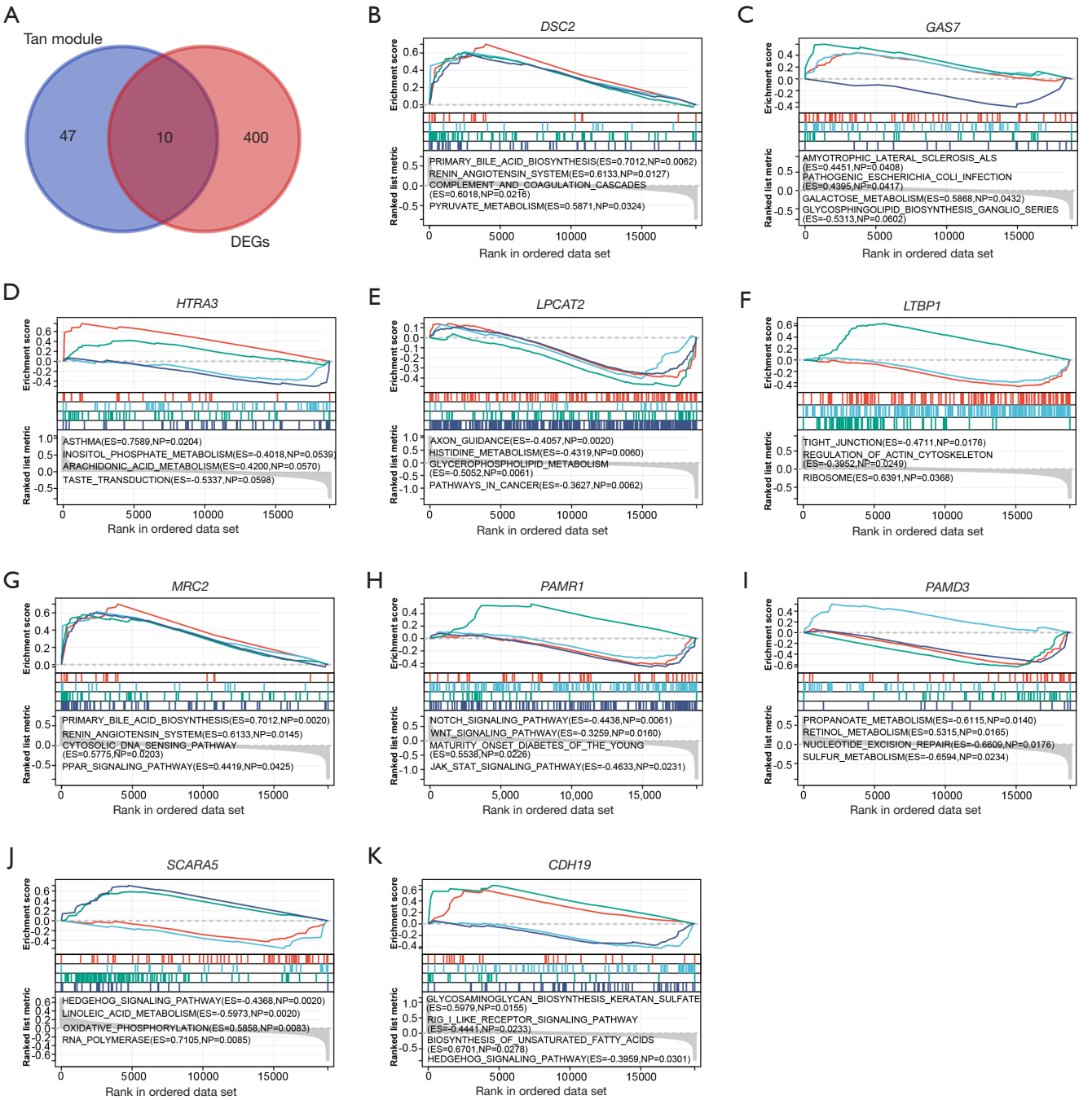

Figure 3 Identification of the overlapping genes from key module and GSE143924-DEGs. (A) Venn diagram of tan modules and DEGs to obtain the overlapping genes. (B-K) GSEA of 10 overlapping genes: (B) *DSC2*, (C) *GAS7*, (D) *HTRA3*, (E) *LPCAT2*, (F) *LTBP1*, (G) *MRC2*, (H) *PAMR1*, (I) *PARD3*, (J) *SCARA5*, (K) *CDH19*. DEGs, differentially expressed genes; ES, enrichment score; NP, normalized P value; GSEA, gene set enrichment analysis.

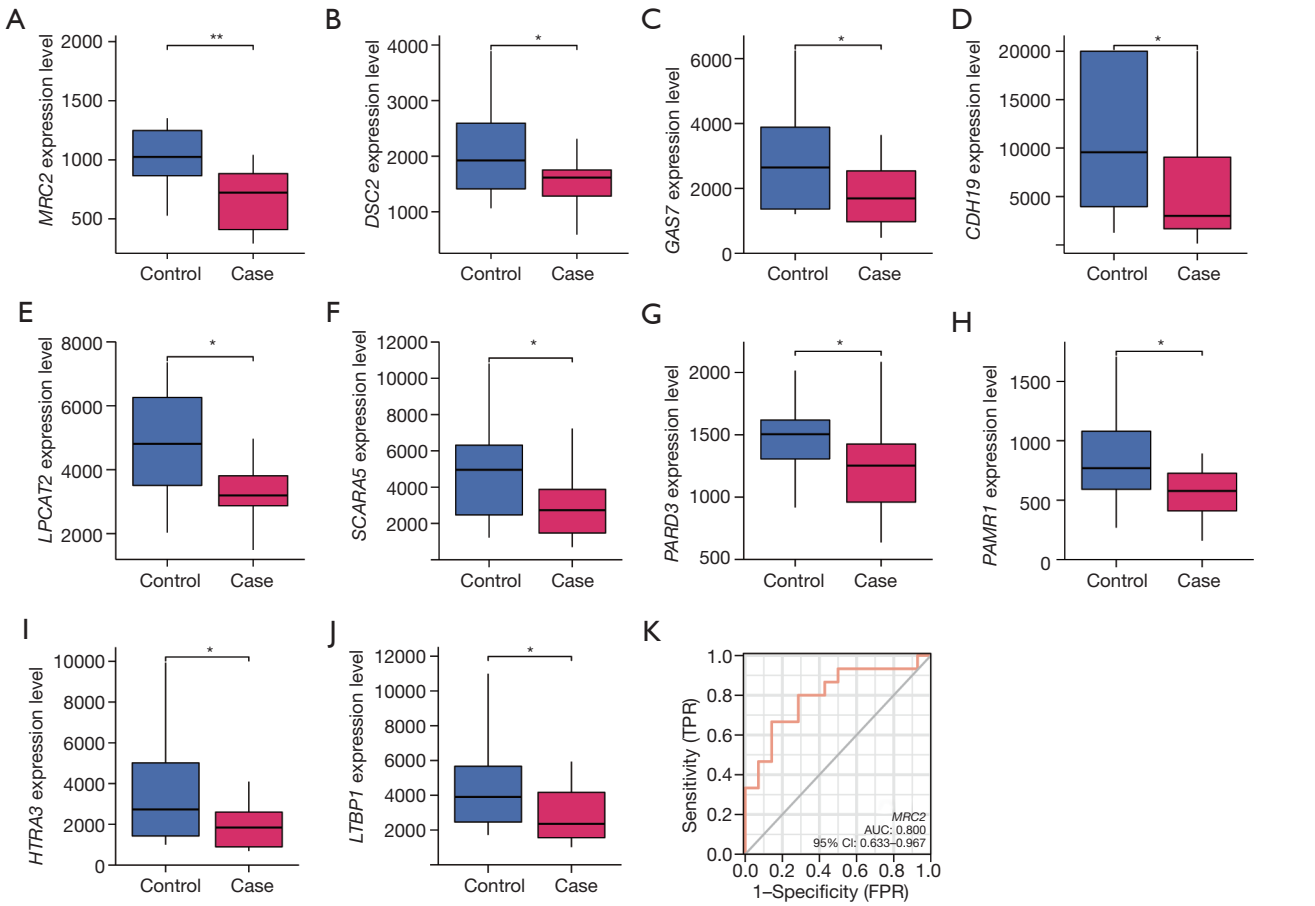

Figure 4 Expression level analysis on 10 overlapping genes. (A) *MRC2*. (B) *DSC2*. (C) *GAS7*. (D) *CDH19*. (E) *LPCAT2*. (F) *SCARA5*. (G) *PARD3*. (H) *PAMR1*. (I) *HTRA3*. (J) *LTBP1*. *, P<0.05; **, P<0.01. (K) ROC curve analysis on *MRC2*. AUC =0.800. TPR, true positive rate; FPR, false positive rate; AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic.

in AF by the online website GEPIA based on The Cancer Genome Atlas (TCGA) data. As shown in *Figure 4A-4J*, the levels of these genes in the case group were closely lower, indicating that all of them were suppressor genes in AF. According to the AUC values of the genes in ROC curve, only *MRC2* had the potential to be diagnostic biomarkers in AF with an AUC value of 0.8 (*Figure 4K*). Based on these findings, we confirmed that *MRC2* was the hub gene.

Over-MRC2 inhibited cell proliferation of Ang II-induced HCFs and promoted cell apoptosis

Based on the results in *Figure 5A,5B*, it appeared that the mRNA and protein expression levels of *MRC2* were lower in Ang II-induced HCFs compared to normal controls. The expression of *MRC2* was subsequently over-expressed and the efficiency was examined by qRT-PCR and WB (*Figure 5C,5D*). In the CCK-8 assay, we compared the cell proliferation between different groups and obtained that the proliferation of AF cells in the *MRC2* overexpression group was significantly inhibited (*Figure 5E*). Following this, we found by flow cytometry that overexpressed *MRC2* promoted the apoptosis of Ang II-induced HCFs (*Figure 5F-5I*).

Knockdown of MRC2 promoted the cell proliferation of Ang II-induced HCFs

Compared with normal control, the level of *MRC2* in HCFs cells after si-*MRC2*-1 and si-*MRC2*-2 transfection was detected by qRT-PCR method, and si-*MRC2*-1 had a more effective efficiency (*Figure 6A*). Afterwards, we constructed

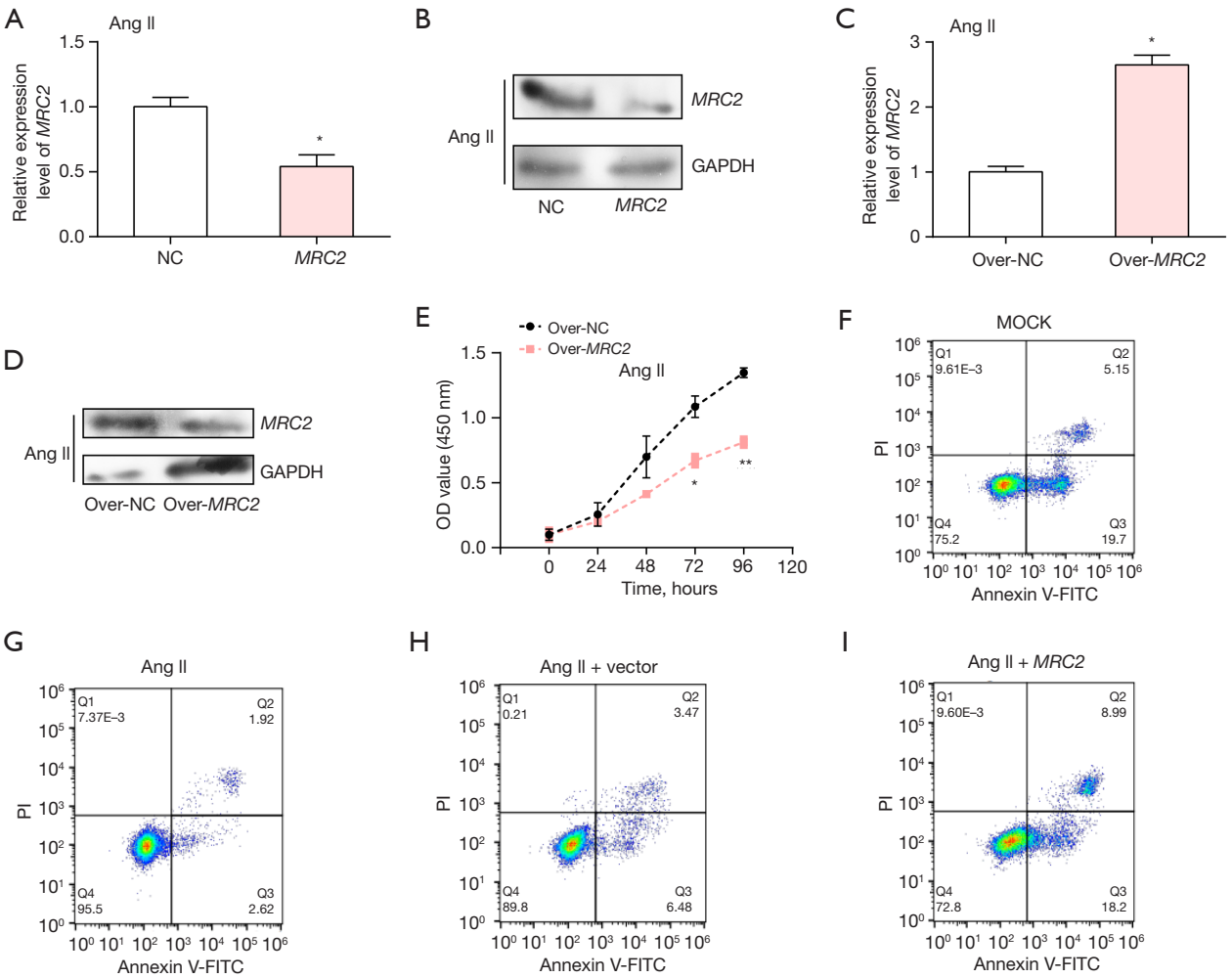

Figure 5 Effect of over-*MRC2* on the cell proliferation and apoptosis of AF. (A,B) The mRNA and protein expression levels of *MRC2* in cells of HCFs induced by Ang II were lower than that of the control group. (C,D) The efficiency of *MRC2* overexpression in cells of HCFs induced by Ang II was examined by qRT-PCR and WB assays. (E) The effect of over-*MRC2* on cell proliferation was detected by CCK-8 in HCFs induced by Ang II. *, P<0.05; **, P<0.01. (F-I) The effect of overexpressed *MRC2* on apoptosis was examined by flow cytometry in HCFs treated with Ang II. Q1 was necrotic cells, Q2 was late apoptotic cells, Q3 was early apoptotic cells, and Q4 was live cells. Ang II, angiotensin II; NC, negative control; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; over-, over-expressed-; OD, optical density; FITC, fluorescein isothiocyanate; PI, propidium iodide; mRNA, messenger RNA; HCFs, human cardiac fibroblasts; qRT-PCR, quantitative real-time polymerase chain reaction; WB, western blotting; CCK-8, cell counting kit-8.

the AF cells by HCFs treated with Ang II. Among the four groups, negative control (NC), Ang II, Ang II + si-NC and Ang II + si-*MRC2*, the expression of *MRC2* was the lowest in Ang II + si-*MRC2* group (*Figure 6B*). In the CCK-8 experiment, the proliferation of HCFs cells was promoted after Ang II treatment, and *MRC2* knockdown promoted Ang II-induced HCFs proliferation (*Figure 6C*).

Protein expression levels of PPARα, PPARγ, CPT-1, SIRT3 in HCFs were increased by over-MRC2 in Ang II-induced HCFs

Based on the GSEA results, PPARα, PPARγ, CPT-1, SIRT3 proteins related to PPAR signaling pathway were selected to verify whether it is related to *MRC2* in AF. In the WB experiments, we detected that the protein levels of these

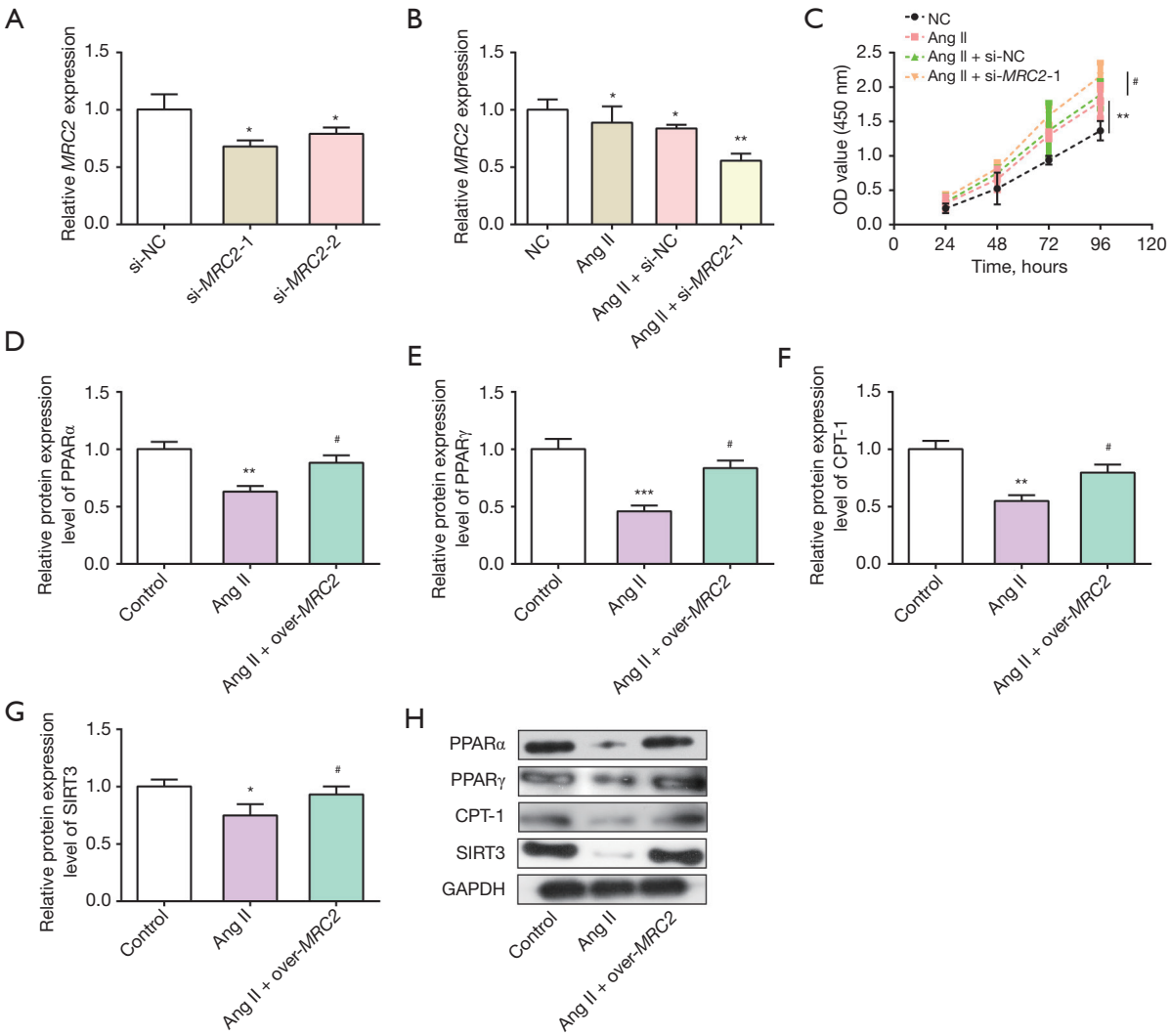

Figure 6 Knockdown of *MRC2* promoted the proliferation of Ang II-induced HCFs. (A) The level of *MRC2* was knocked down in HCFs. (B) *MRC2* knockdown in Ang II-induced HCFs. (C) The effects of Ang II treatment and si-*MRC2*-1 transfection on the proliferation of HCFs were detected by CCK-8. *, P<0.05; **, P<0.01; #, P<0.05, compared between Ang II + si-NC and Ang II + si-*MRC2*-1 groups. (D-G) The protein expression levels of (D) PPARα, (E) PPARγ, (F) CPT-1, and (G) SIRT3 in Ang II-induced HCFs treated with over-*MRC2*. *, P<0.05; **, P<0.01; ***, P<0.001; #, P<0.05, compared with Ang II group. (H) The effect of over-*MRC2* on the protein expression levels of PPARα, PPARγ, CPT-1, and SIRT3 in Ang II-induced HCFs as detected by WB method. Si-, small interfering-; NC, negative control; Ang II, angiotensin II; OD, optical density; over-, over-expressed-; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HCFs, human cardiac fibroblasts; CCK-8, cell counting kit-8; WB, Western blotting.

proteins in HCFs treated with Ang II and over-*MRC2*. The data demonstrated the protein expressions of them in Ang II-induced HCFs were increased by over-*MRC2* (*Figure 6D-6H*). These results indicate PPAR signaling pathway is associated with the mechanism of *MRC2* in AF progression.

Discussion

Although AF itself is not directly life-threatening, its clinical symptoms (such as palpitations, heart failure, etc.) (32) and its complications (such as stroke, etc.) (33) significantly increase disability and mortality possibilities. A study has

shown that AF harms the quality of life by limiting physical activity, dying of social interactions and emotions, reducing happiness, increasing dependence and early retirement (34). Due to the above limitations, improving the efficiency of AF diagnosis and standard treatment is important. Further understanding of the molecular mechanism of AF is a basis to determine the molecular biomarkers of AF and develop promising therapies.

According to the gene expression profile GSE143924, the DEGs were identified between 15 POAF and 15 SR groups. The GO annotation and KEGG indicated the DEGs were closely related to the regulation of GTPase activity, NF-κB signaling pathway, Extracellular structure organization and Arachidonic acid metabolism. Bischoff *et al.* proposed that Rho GTPases operated as a binary switch by cycling between the inactive and active states, and played a vital role in connecting biochemical signals with biophysical cell activity, primarily through the rearrangement of actin and microtubule cytoskeleton (35). Polyakova *et al.* found that atrial fibrosis was an important factor in inducing and maintaining AF. The occurrence of AF is characterized by changes in the structure of atrial muscle cells, including cell hypertrophy, contractile disintegration, glycogen accumulation, myolysis, and changes in the expression of connexin (36). In addition, AF and its duration are related to atrial fibrosis. Atrial fibrosis can cause abnormal atrial conduction, especially in the case of heart failure (37). A recent study has shown that miRNAs are important regulators of several cardiovascular disease processes associated with the heart, and that atrial fibrosis is a hallmark of cardiac remodeling that perpetuates AF (38). Moreover, a study has revealed that in the blood, liver, muscle, and other organ systems, arachidonic acid functions as a structural lipid linked to phospholipids (39).

Next, we performed WGCNA on GSE143924 database to screen the key module, and identify 10 overlapping genes combined with GSE143924-DEGs.The selection of *MRC2* as the hub gene among these 10 overlapping genes was based on the consistency of the results of multiple analyses showing its association with AF. In gene overlap analysis, *MRC2* was found to have 10 overlapping genes with tan module genes and GSE143924-DEG, suggesting its relevance in specific biological contexts. By GSEA analysis, *MRC2* was enriched in several functional pathways such as bile acid biosynthesis, RAS, cell membrane DNA sensing pathway, and PPAR signaling pathway, implying its important role in AF BPs. Gene expression analysis TCGA data and GEPIA tools showed that *MRC2* expression was significantly reduced in AF patients, suggesting that it may have an inhibitory function in AF. It may be related to the pathophysiologic process of AF. In addition, *MRC2* showed potential diagnostic value in biomarker analysis, with its ROC curve analysis determining that *MRC2* has a high AUC value (0.8), results suggesting that it may be a biomarker for AF. Based on these findings, *MRC2* was determined as the target gene in our study. *MRC2* is a constitutive recycling endocytic receptor that belongs to the mannose receptor family. The *MRC2*-encoded protein has a vital function in ECM remodeling by mediating the internalization and lysosomal degradation of collagen ligands. Gai *et al.* found that *MRC2* overexpression predicted poor prognosis in hepatocellular carcinoma (HCC) after hepatectomy, and that *MRC2* might contribute to the upregulation of cell migration and invasion in transforming growth factor-driven HCC (40). Yang *et al.* showed that *MRC2* promoted the epithelial-mesenchymal transition (EMT) process associated with ovarian cancer progression by disrupting epithelial cell-cell interactions (41). Study by López-Guisa *et al.* showed that by slowing the pace of interstitial collagen formation, *MRC2* is crucial in the development of solid organ fibrosis, thereby protecting the parenchyma surrounding the kidney from fibrosis-related damage (42). Herein, we conducted functional experiments to explore the role of *MRC2* in AF cells. The results demonstrated that *MRC2* knockdown promoted the Ang II-induced HCFs cells, which conforms to the finding in expression analysis.

In GSEA, significantly enriched genomes related to *MRC2* were primary bile acid biosynthesis, RAS, cytosolic DNA sensing pathway and PPAR signaling pathway. The study by Mascolo *et al.* suggests that two RAS pathways may counteract the evidence for the beginning of AF by several BPs, including inflammation and the buildup of epicardial adipose tissue (EAT) (43). EAT may cause the start of AF through both direct and indirect pathways, making it a predictor of the condition. In patients with diabetes experiencing AF, a chronic pro-inflammatory state characterized by imbalanced Ang II receptor signaling has been identified. Macrophages, which function as phagocytes and antigen-presenting cells, play a vital role in the secretion of growth factors, immune mediators, effector molecules, as well as the engulfment of pathogens or cellular debris, antigen processing, and presentation (44,45). Diversity and plasticity are hallmarks of macrophages, with two recognized subtypes: classically activated M1 macrophages and alternatively activated M2 macrophages

(46,47). Notably, it has been suggested that the long noncoding RNA Gm9866 in RAW264.7 cells contributes to the enhanced expression of M2 markers, namely *MRC1* and *MRC2*. Moreover, the expression of long noncoding RNA Gm9866 was observed to significantly increase after interleukin-4 treatment, while lipopolysaccharide treatment led to a notable decrease (48). The evidence supports the involvement of novel mechanisms in the upregulation of the long non-coding RNA Dnm3os, disruption of its interaction with nucleolin, and epigenetic modifications of target genes, all of which contribute to the promotion of the inflammatory macrophage phenotype in DM (49). In $CD14^+$ monocytes, *DRAIR* is downregulated by factors like high glucose and palmitic acid but upregulated by anti-inflammatory cytokines and monocyte-to-macrophage differentiation through *KLF4*. *DRAIR* overexpression enhances anti-inflammatory and macrophage differentiation genes while inhibiting proinflammatory genes (50). These findings demonstrate the role of *DRAIR* in modulating the inflammatory phenotype of monocytes/macrophages and its downregulation in promoting chronic inflammation in diabetes.

The study by Zhu *et al.* shows that *ANGPTL4* reverses cardiomyocyte apoptosis, inflammation. At the mechanism level, *ANGPTL4* blocked the expression of PPARα and PPARγ proteins in atrial tissues (51). Namely, *ANGPTL4* weakens Ang II-induced AF and atrial fibrosis by regulating PPARα and PPARγ signaling pathways. Thus, we speculated that *MRC2* might regulate PPAR signaling pathway to suppress AF. In subsequent cellular experiments, we successively confirmed by qRT-PCR, WB, CCK-8 and flow cytometry assays that *MRC2* was down-regulated in HCFs treated with Ang II, overexpression of *MRC2* significantly inhibited the proliferation of Ang II-treated HCFs and promoted cell apoptosis, whereas knockdown of *MRC2* significantly promoted the proliferation of HCFs. Then, we observed the protein levels of four AF-related factors (PPARα, PPARγ, CPT-1, and SIRT3) were higher in Ang II-HCFs cells with over-*MRC2*. Therefore, we proved that PPAR signaling pathway was involved in the regulatory process of *MRC2* in AF.

In the present study, a potential key hub gene, *MRC2*, was identified by mining DEGs. it should be noted that this study has some limitations. First, AF pathogenesis is caused by a combination of multiple genetic and environmental factors. Second, due to the lack of clinical samples, we were unable to perform gene expression validation, and other basic experiments are needed to validate the function and regulatory mechanisms of these genes. Third, without the added validation of *in vivo* experiments, there may be limitations in clinical application.

Conclusions

In this study, we have identified the hub gene *MRC2* for AF and explored the underlying mechanism of AF through a series of bioinformatic analyses. The results of gene expression analysis based on GEPIA show that *MRC2* is a tumor suppressor gene of AF. Over-*MRC2* inhibited the proliferation of Ang II-induced HCFs, promoted cell apoptosis. In contrast, knockdown of *MRC2* expression could promote the proliferation of Ang II-induced HCFs, and the expression levels of 4 PPAR signaling pathway-related proteins in HCFs were correlated with *MRC2* expression. All results show that *MRC2* could suppress AF progression by PPAR signaling pathway, which will pave the way for the progression of effective diagnostic indicators and therapies for AF patients.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-23-1235/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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