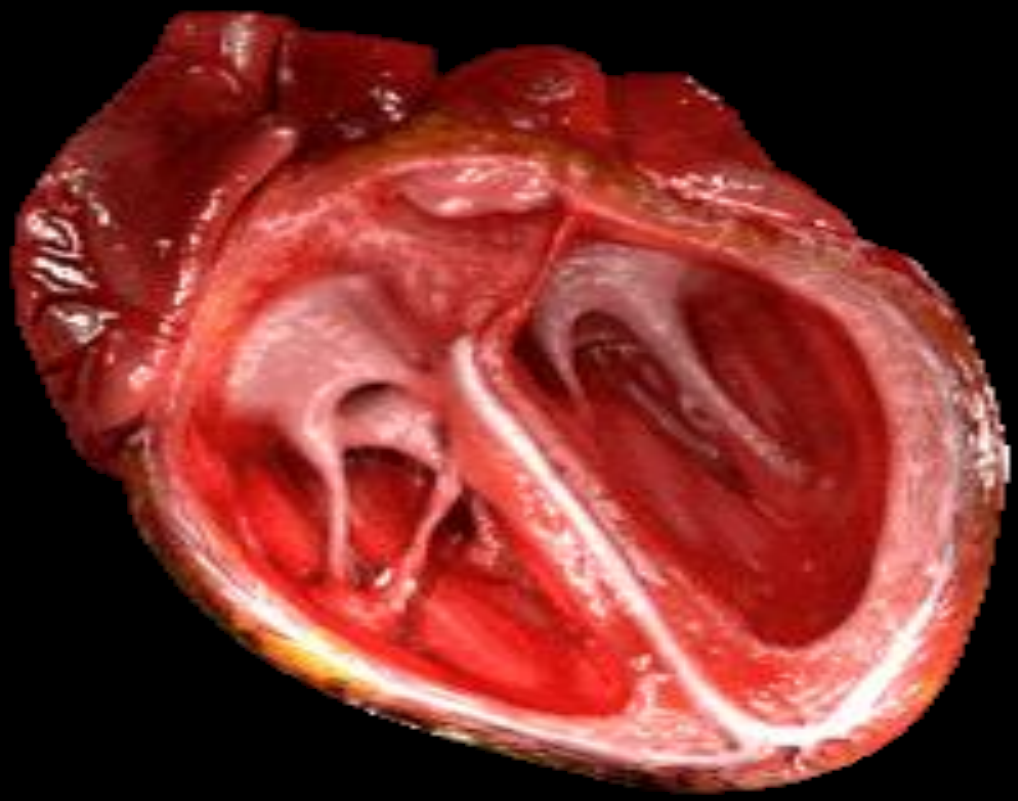

Exosome in cardiovascular diseases:

Circulating exosome-derived miR-122-5p is a novel biomarker for prediction of postoperative atrial fibrillation

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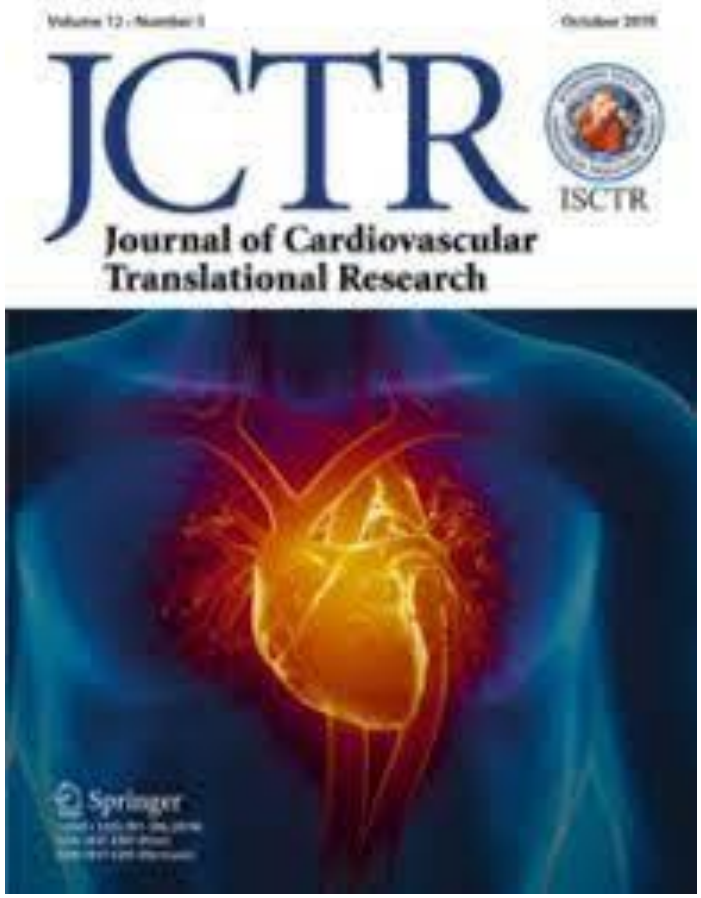
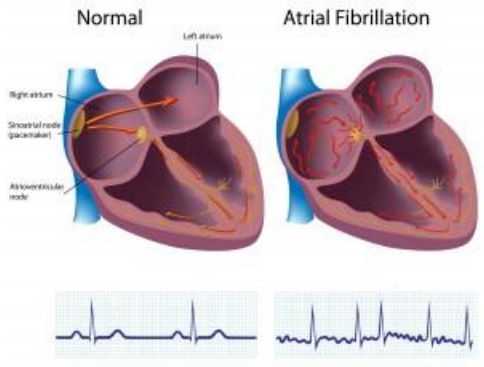


Introduction

- New-onset postoperative atrial fibrillation (POAF) is a frequent complication following approximately 18–57% of cardiac Surgery



- POAF is associated with:
- Increased perioperative mortality and morbidity
- Expenses, prolonged hospital stays
- Decreased survival

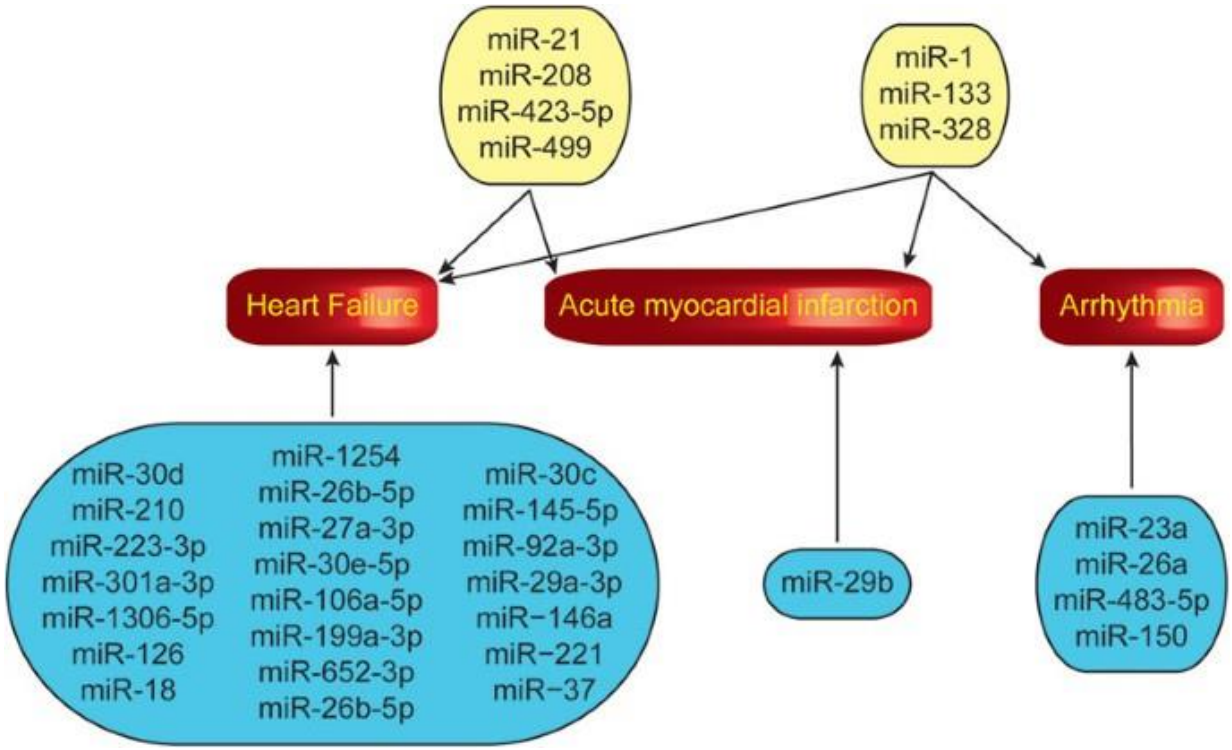
- Diagnostic methods, twelve lead electrocardiography and ambulatory electrocardiographic recording devices are not very efficient

- The development effective diagnostic tools is of great value for the early diagnosis and prevention of POAF

Clinical biomarkers

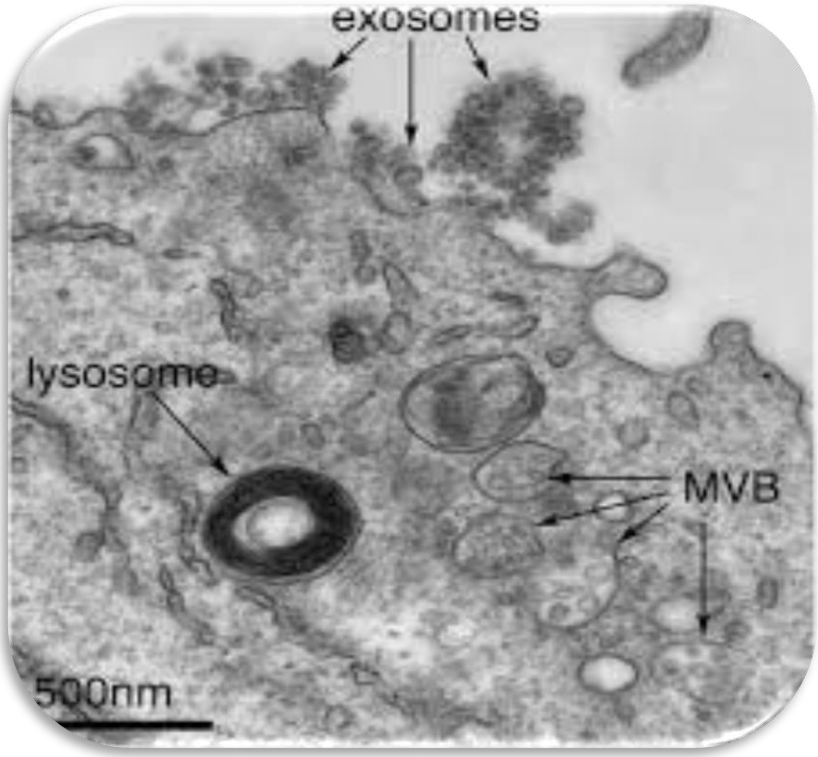
microRNAs (miRNAs)

- Circulating microRNAs (miRNAs) serve as potential diagnostic biomarkers for cardiovascular disease

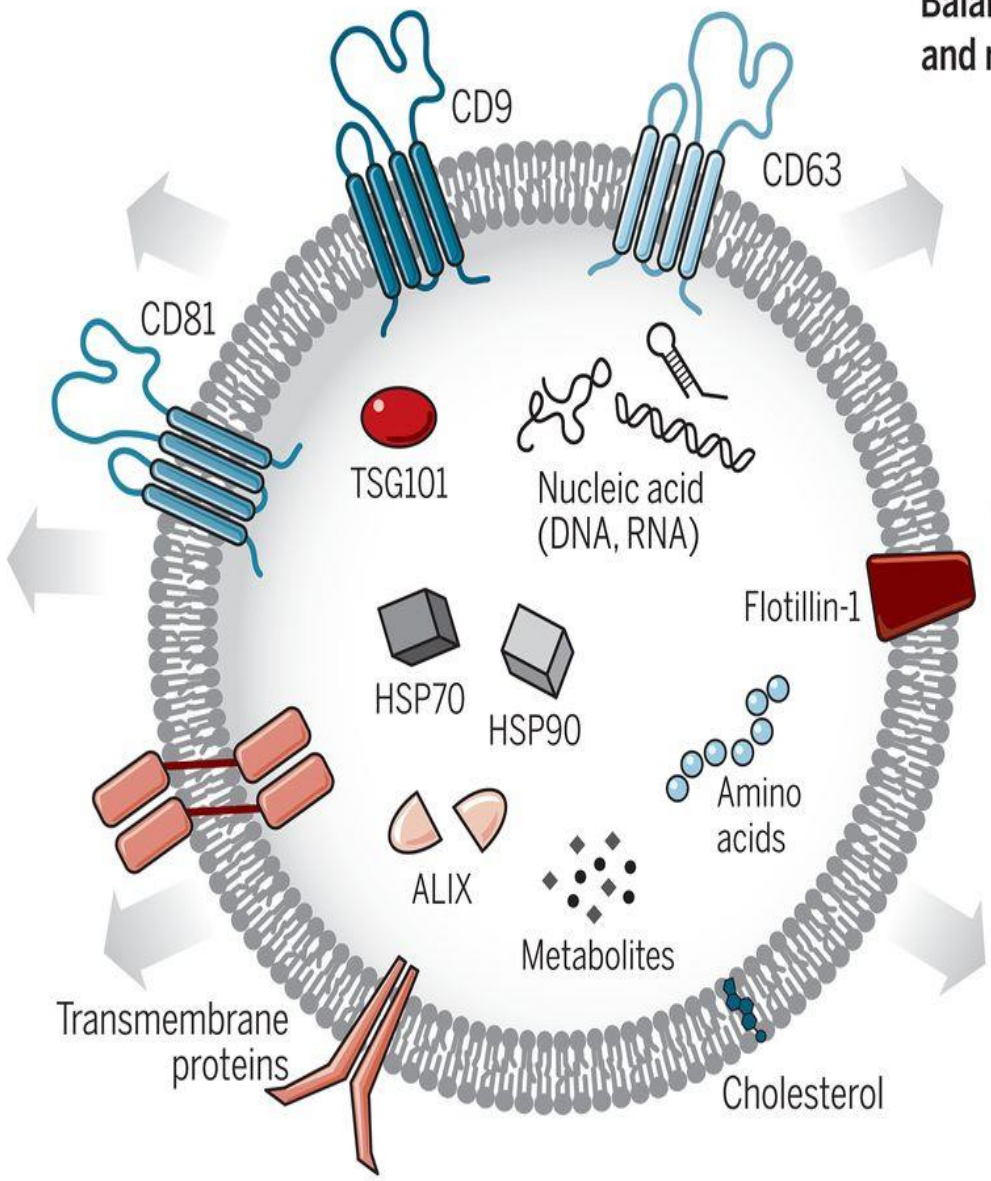


Exosomes

- Ranging in size from 30 -150 nm
- Rich in bioactive molecules (cargos):
- DNA, mRNAs, microRNAs (miRNAs), and proteins
- Secreted by many cell types and into all biological fluids such: plasma, serum, saliva, breast milk, urine and cell culture media
- Cell–cell and cell–environment communications



Hallmarks of exosomes



Regulation of gene transcription and translation

Survival and proliferation

Reproduction and development

Angiogenesis and wound healing

Waste management

Host-microbiome interaction and viral immunity

Balance of immune response and regulation of central and peripheral immunity

Receptor-ligand signaling

Apoptosis

Cellular differentiation and neoplasia

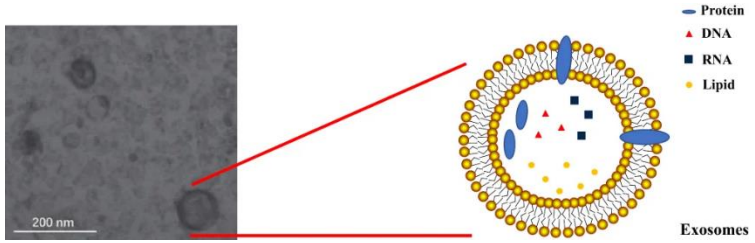
Cellular migration and metastatic disease

Metabolic reprogramming and regulation

Advantage of Exosomes over Free Circulation Markers

- Cargo ⟹ parent cell fingerprint

- Most circulating miRNAs are enclosed in EXOs
- EXOs isolation amplifications the isolation of miRNA from biological fluids
- EXOs protect their cargo from damage
  - Double-layered membrane
  - Half-life



Aims

- In this study, we aimed to identify differences in exosomal miRNAs in POAF patients
- Then, the relative levels of differential expression of exosomal miRNAs in POAF and non-POAF patients were verified by the real-time PCR method
- These findings can help identify the underlying mechanisms of POAF and help develop more promising therapeutic targets

Material and Methods
Patient recruitment and specimen collection

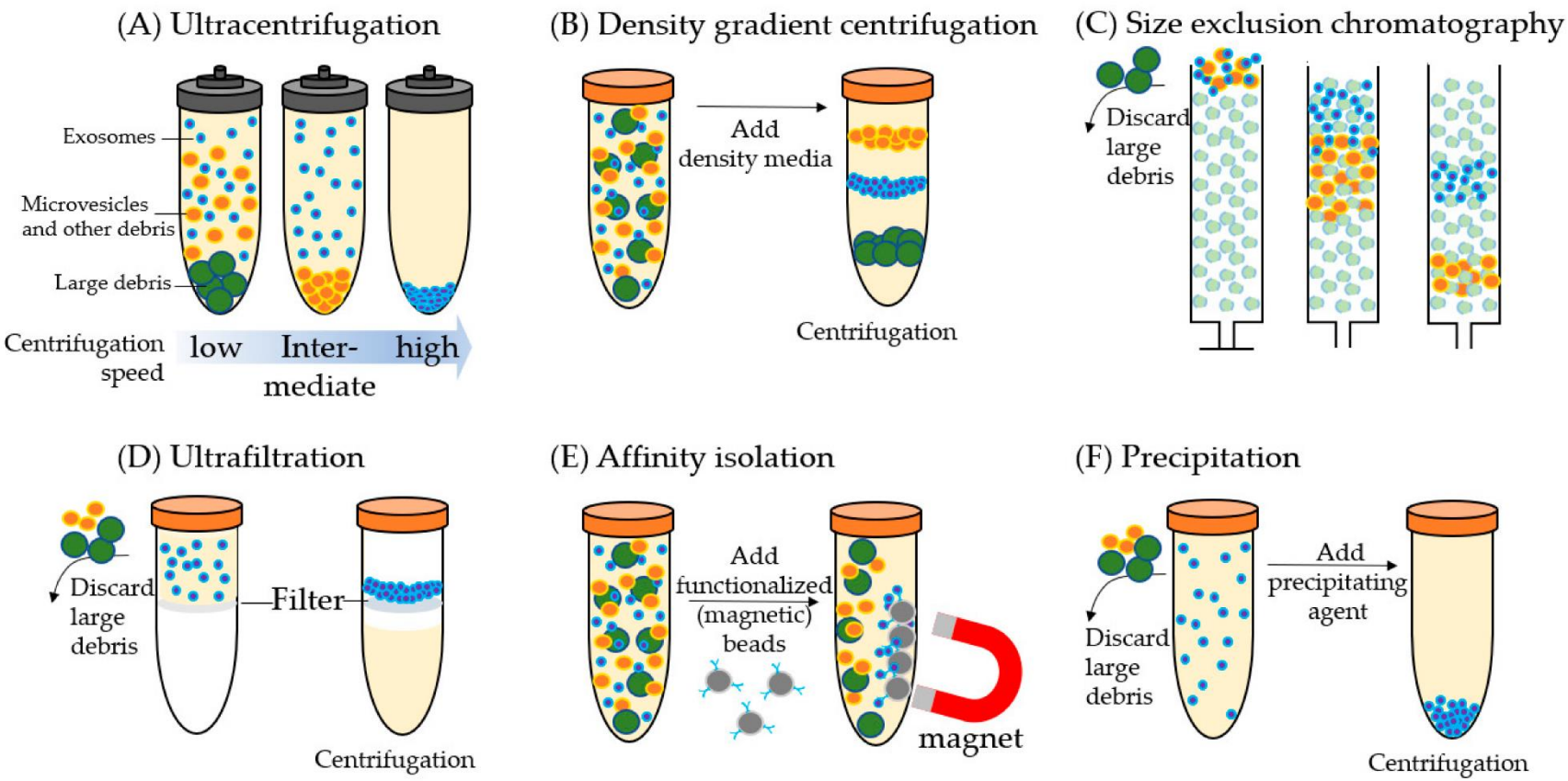
- Participants were:
- Elective coronary artery bypass grafting (CABG) surgery in Hospital
- Aged 18–90 years,
- Without heart failure (HF) or atrial fibrillation (AF)

- POAF was defined as a new-onset irregular rhythm with no apparent P waves lasting at least 30 s, detected in patients by telemetry based continuous electrocardiographic (ECG) monitoring

- Whole blood samples were obtained from all participants 24 h before surgery and in the morning before breakfast

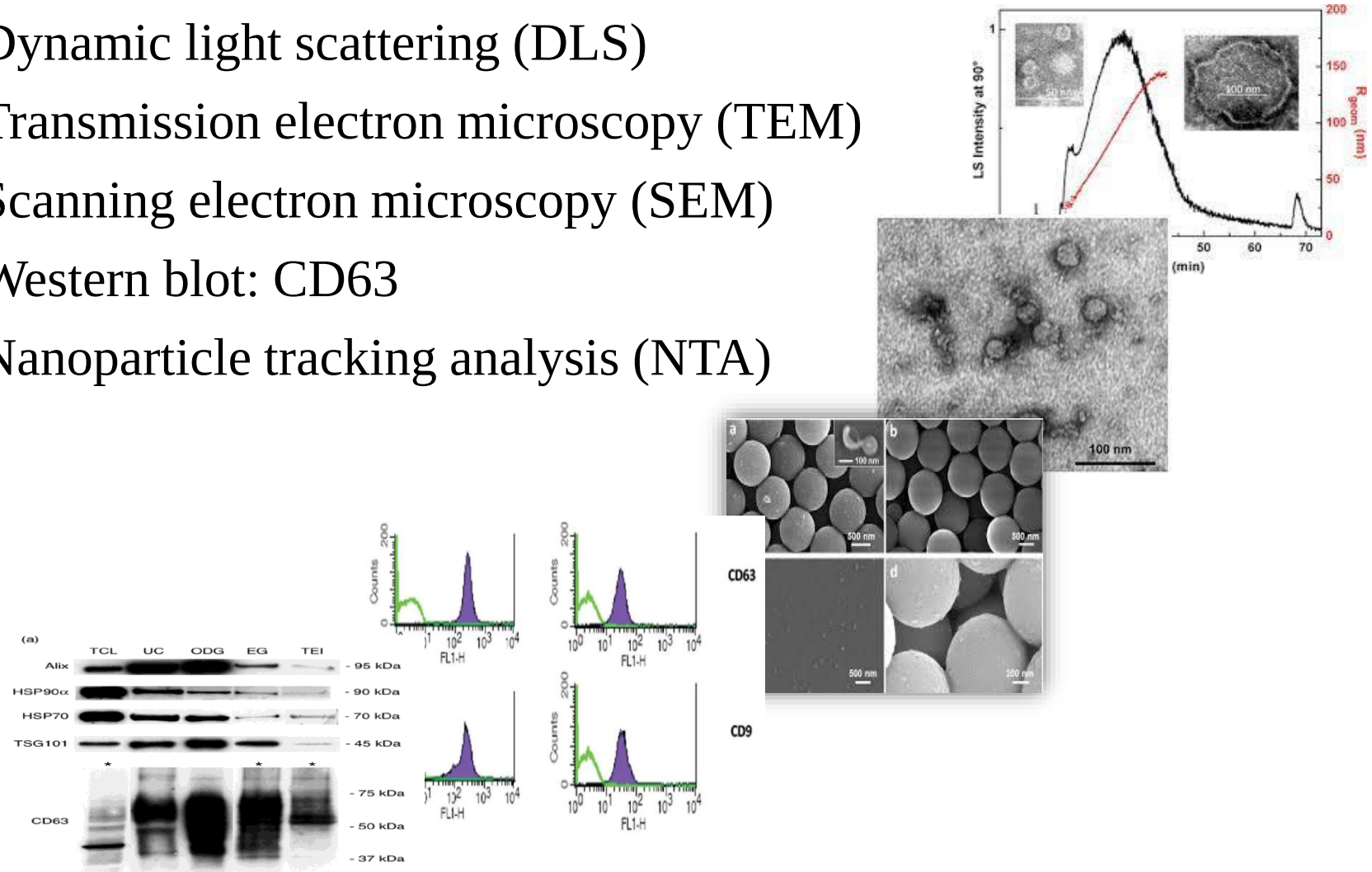
- stored at − 80 °C before exosome extraction

Exosome Isolation



Exosome Characterization

- Dynamic light scattering (DLS)
- Transmission electron microscopy (TEM)
- Scanning electron microscopy (SEM)
- Western blot: CD63
- Nanoparticle tracking analysis (NTA)



Exosomal RNA extraction and miRNA sequencing

- Total RNA was extracted from isolated exosomes using the miRNeasy Mini Kit
- RNA concentration was measured using the RNA Nano 6000 Assay Kit
- cDNA synthesis
- PCR amplification
- The construction of RNA libraries with the QIAseq miRNA library kit
- Then, RNA sequencing was performed on the Illumina-HiSeq2500 sequencing platform (Illumina, CA, USA)

Prediction and gene function enrichment analysis of miRNA target genes

- Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment:

- Identify biological processes (BP)
- Molecular function (MF)
- Cellular components (CC)
- KEGG pathways of the predicted target genes involved

Real-time PCR validation

- Reverse transcription of total exosomal RNA to cDNA using the PrimeScript™ RT reagent kit

Results
Clinical characteristics of patients

- Among these parameters, the left ventricular end-diastolic dimension (LVEDD) and the left ventricular end-systolic dimension (LVSDD) were significantly different: which indicated that changes in cardiac structure and heart function may be related to POAF progression

- In-hospital days were significantly different between the POAF and non-POAF groups ($p < 0.05$), and POAF patients stayed longer than non-POAF patients

- Furthermore, among the important risk factors for POAF, such as hypokalemia, hypoxia, hypoglycemia, hypovolemia, pain and anemia, hypovolemia was significantly different between the POAF and non-POAF groups ($p < 0.05$), which indicated that hypovolemia can increase the risk of POAF

Characterization of plasma exosomes

- TEM revealed that the exosome-shaped particle structures, oval or bowlshaped capsules
- NTA analysis revealed that the mean size of the exosomes was about 30–150 nm
- The exosomal surface protein markers CD9, CD63, and Tsg101, were identified by Western blotting

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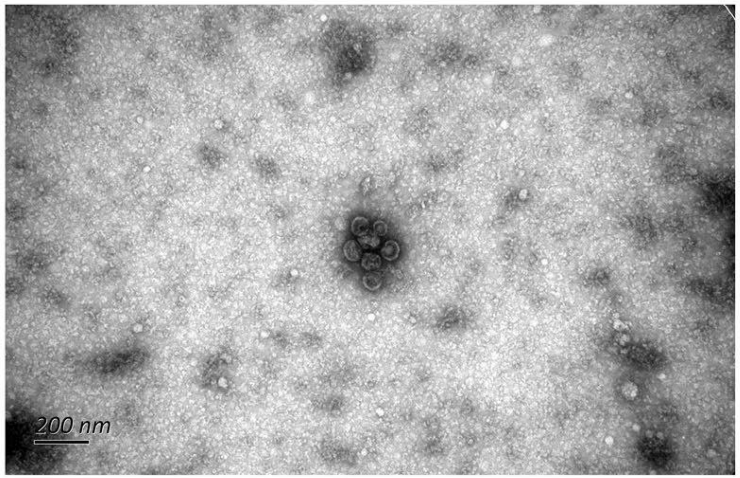

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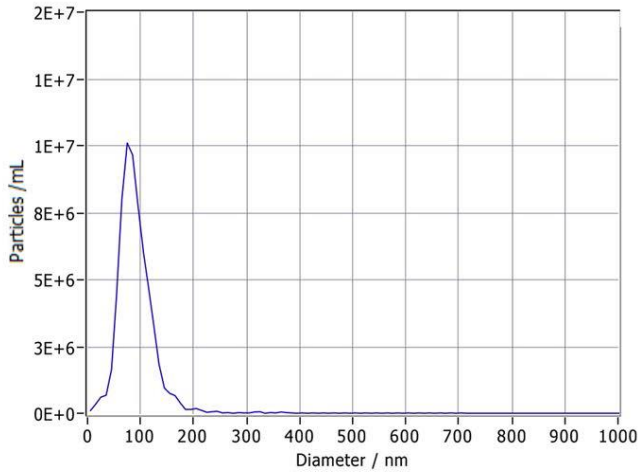

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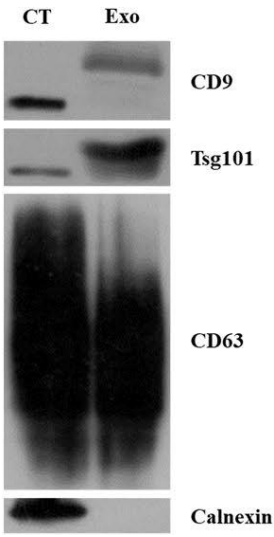
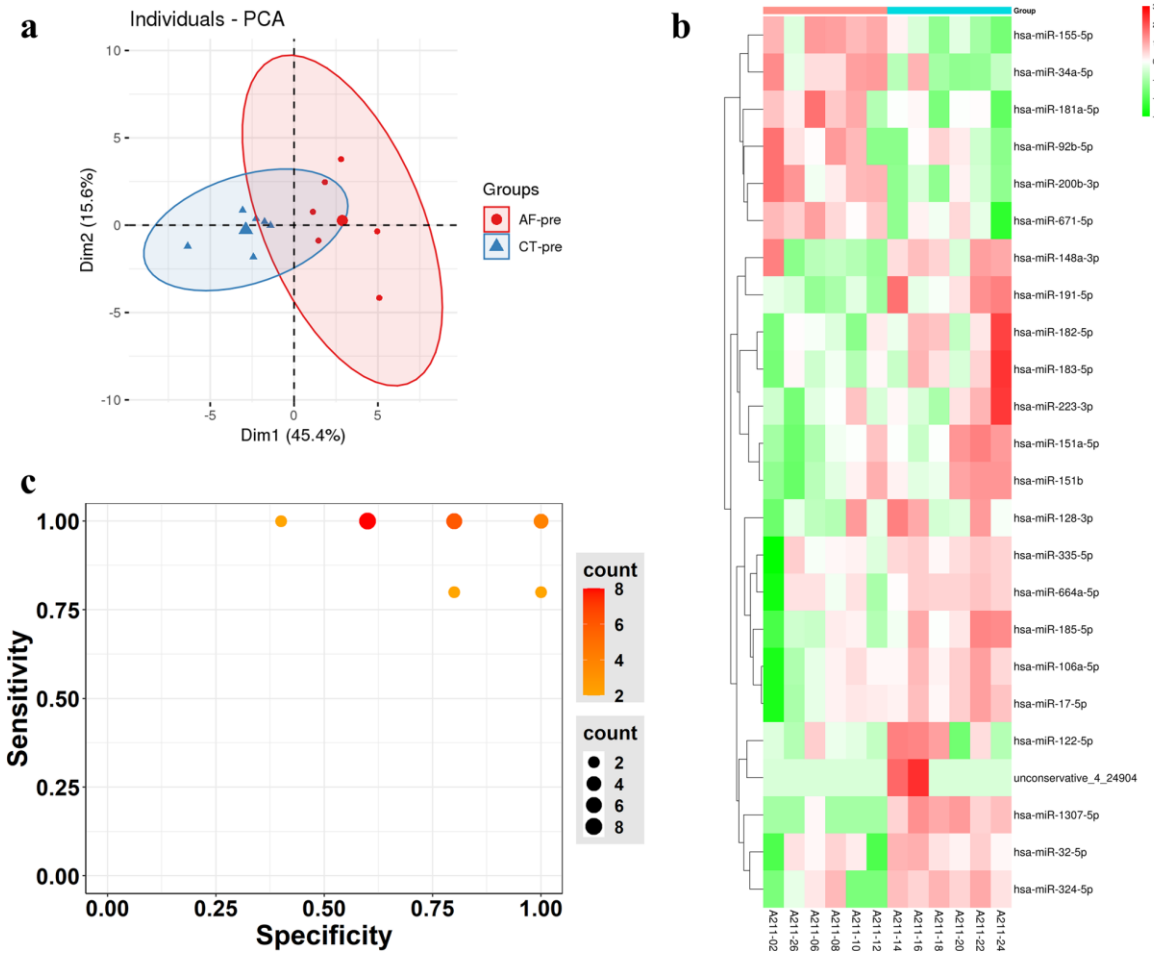


Identification of exosomal differentially expressed miRNAs

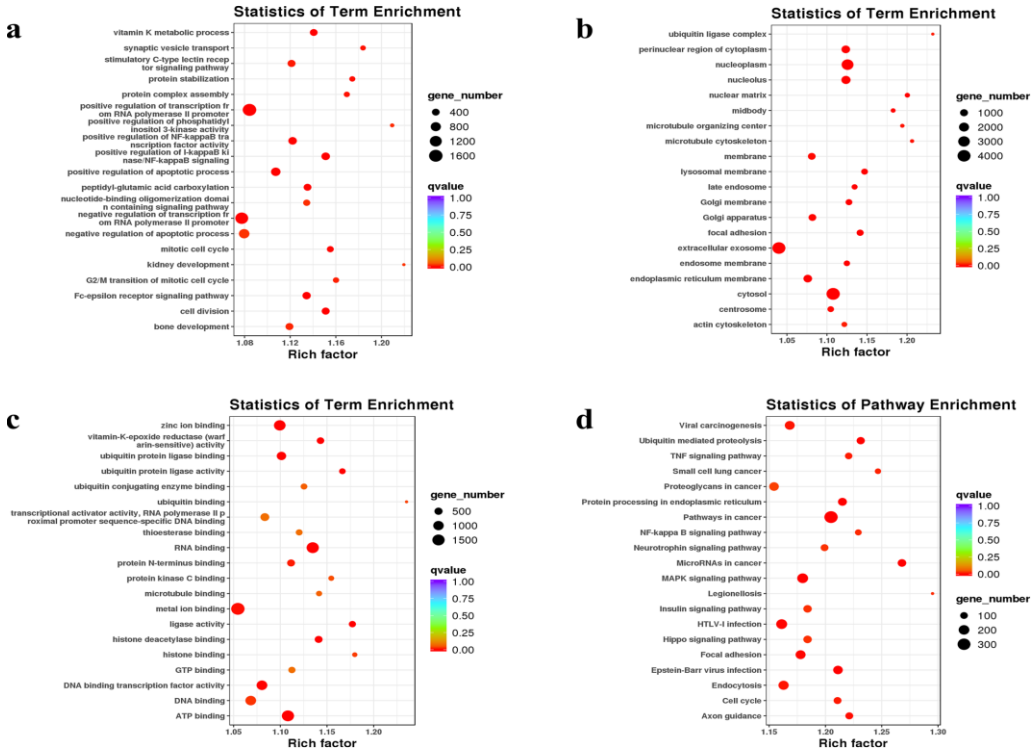
- The miRNA sequencing was performed to identify exosomal differentially expressed miRNAs (DEMs) between the POAF and non-POAF
- Finally, a total of 23 exosomal miRNAs were found to be differentially expressed between the POAF and non-POAF groups
- 17 miRNAs were upregulated and 6 miRNAs were down-regulated



- Identification of exosomal differentially expressed miRNAs (DEMs). (**a**) PCA analysis of the POAF group (AF-pre) and non- POAF group (CT-pre) of patients. (**b**) The specificity and sensitivity of each DEM in identifying exosomes from POAF patients. (**c**) Heatmap of DEMs in the POAF group (AF-pre) and non-POAF group (CT-pre) of the patients. The intensity plot shows the relatively higher expression (red) and the lower expression (green)

Gene ontology (GO) enrichment and KEGG annotation analysis

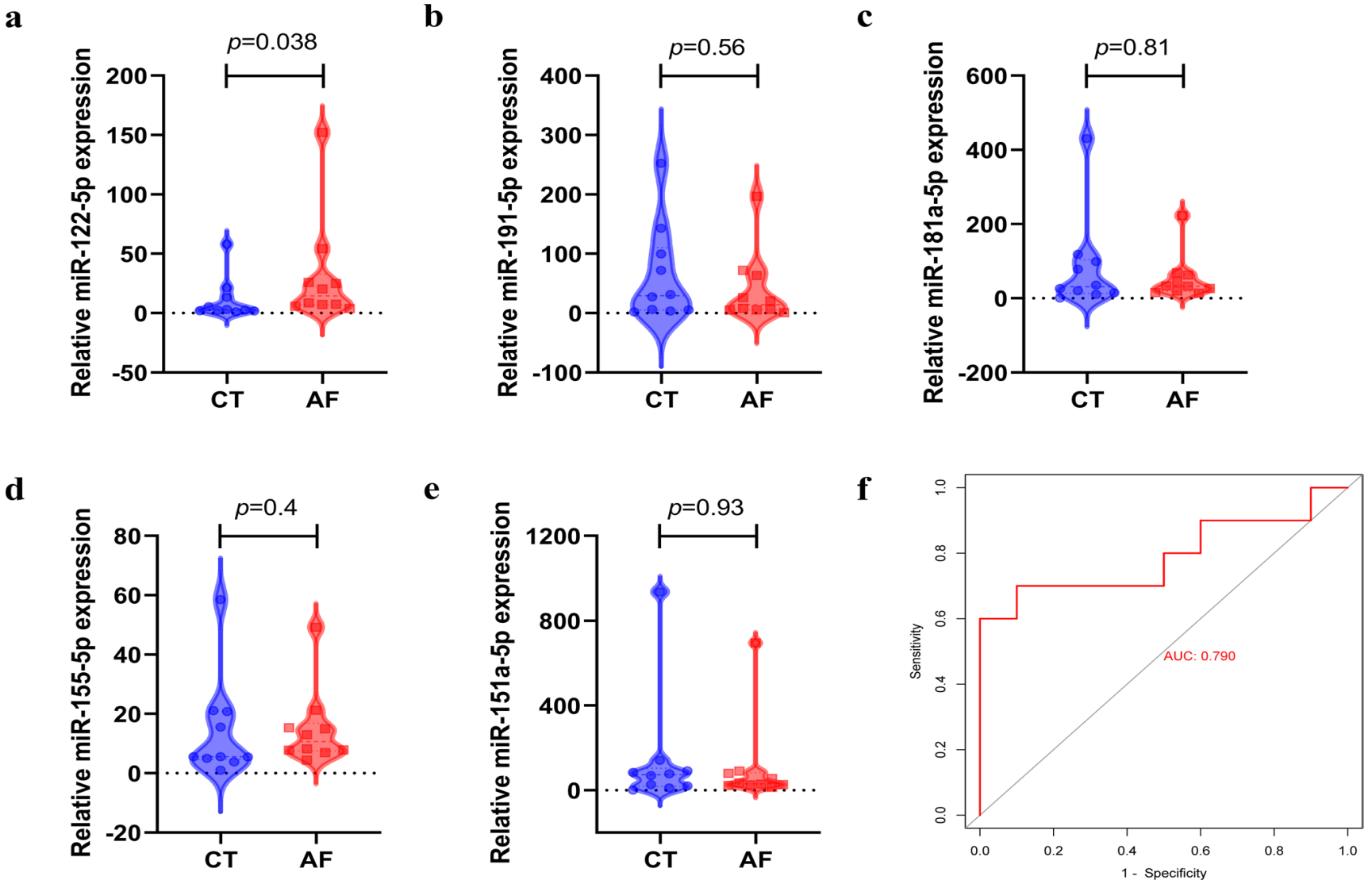
- To understand the comprehensive function of exosoma miRNAs, DEMs target genes were predicted using the multiMiR package, followed by GO and KEGG analysis through the GO and KEGG databases



qRT-PCR verification of exosomal differentially expressed miRNAs

- the DEMs, miR-122-5p, miR-191-5p, miR-181a-5p, miR-155-5p, and miR-151a-5p were selected for qRT-PCR validation
- miR-122-5p was up-regulated in POAF patients (AF group) compared to non-POAF patients no significant changes in miR-191-5p, miR-181a-5p, miR-155-5p and miR-151a-5p
- To evaluate the potential diagnostic value of miR-122-5p, a ROC curve was generated for miR-122-5p levels in plasma samples from POAF patients
- The area under the ROC curve (AUC) was 0.79 ($p = 0.028$), and the 95% confidence interval is 0.58 to 1.00

qRT-PCR verification of exosomal differentially expressed miRNAs

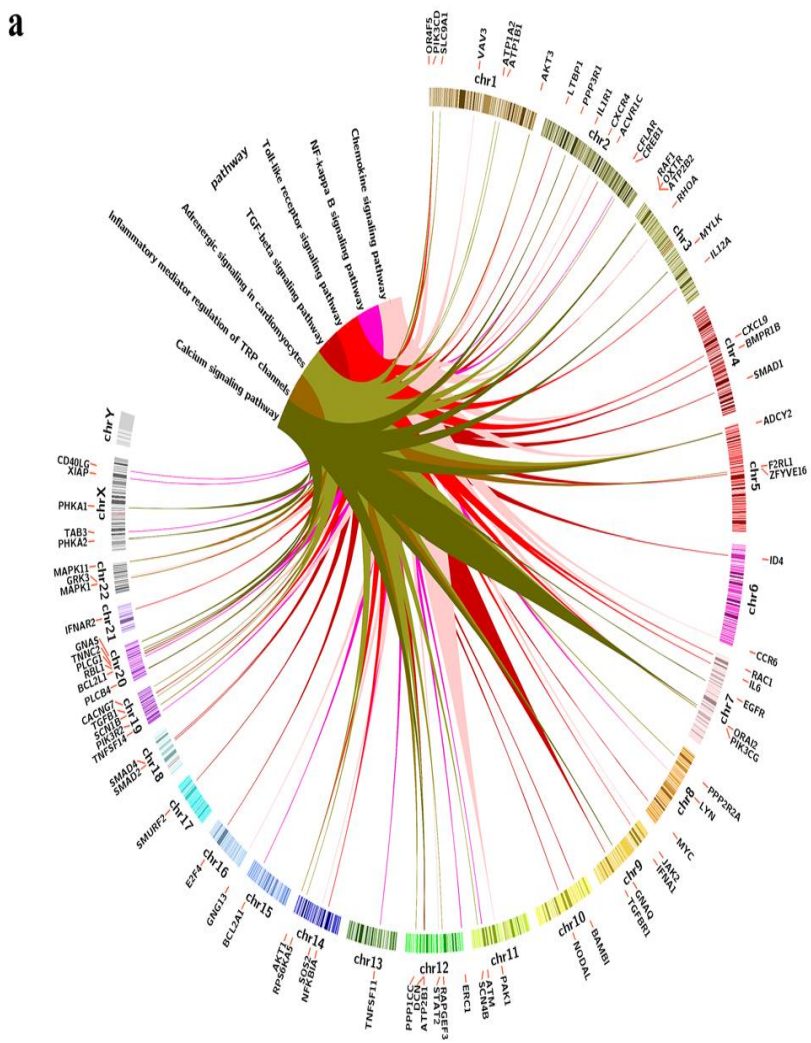


Functional analysis and determination of the miR-122-5p target genes

- To gain further insight into the functions of miR-122-5p and its target genes, GO enrichment and KEGG annotation analysis were performed focused on heart functions:

- NF-kappa B signaling pathway (NFKB1A, IL1R1, ERC1 and TNFSF11)
- Toll-like receptor signaling pathway (MAPK1, IL6, AKT1, CXCL9)
- The TGF-beta signaling pathway (MAPK1, TGFB1, TGFBR1 and MYC)
- The regulation of cardiac muscle contraction by regulation of the release of sequestered calcium ion (TNNC2, CALM2, CLIC1 and CLIC4)
- The Negative regulation of toll-like receptor signaling pathway (PDK4, IRAK3, PDPK1)
- The Regulation of oxidative stress-induced intrinsic apoptotic signaling pathway (AKT1, MAPK1, NFE2L1 and NFE2L3)
- Related to cardiac functions to promote apoptosis, fibrosis, and hypertrophy, and play a promoting role in cardiac fibrosis
- miR-122-5p directly regulates the PDK4 gene, which is involved in the regulation of toll-like receptor signaling pathway

Functional analysis and determination of the miR-122-5p target genes



Discussion

- Previous studies have shown that miR-122 can predict the risk of AF
- (Upregulation of miR-122 is associated with cardiomyocyte apoptosis in atrial fibrillation, Myocardial Interstitial Fibrosis in Heart Failure: Biological and Translational Perspectives)

- On the one hand, miR-122 levels increased significantly in the AF mice model (Long non-coding RNA UCA1 relieves cardiomyocytes H9c2 injury aroused by oxygen-glucose deprivation via declining miR-122)

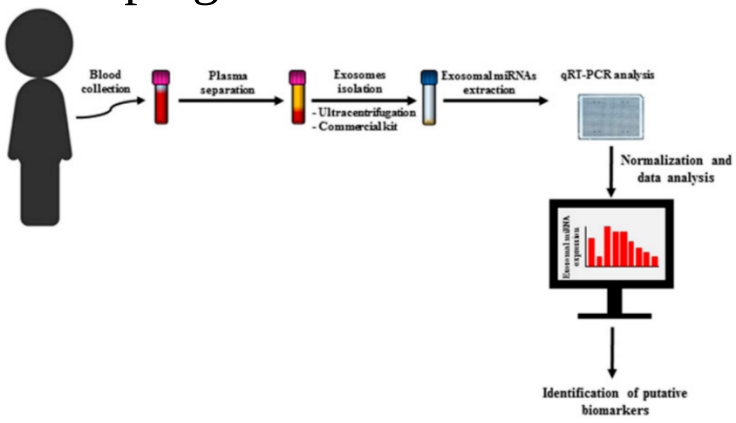
- The high expression of miR-122 involved in the proliferation and apoptosis of CMs by regulating the expression of anti-apoptotic proteins, such as Bcl-2 and caspase-3, in atrial fibrillation (Upregulation of miR-122 is associated with cardiomyocyte apoptosis in atrial fibrillation)

Discussion

- The peripheral monocyte Toll-like receptor (TLR) expression was associated with AF presence, indicating that TLR-mediated inflammation plays an important role in the pathogenesis of AF (Monocyte Toll-Like Receptor Expression in Patients With Atrial Fibrillation)

Conclusion

- The miR-122-5p may be related to many signaling pathways that can affect atrial function and structure, oxidative stress, and fibrosis involved in the progression of POAF
- Exosomal miRNAs have great potential as novel biomarkers to assess the severity or prognostic of POAF, which may contribute to risk stratification, individualized therapeutic strategy, drug intervention, and evaluation of POAF
- Although exosomes show attractive possibilities in the diagnosis and treatment of cardiovascular diseases, these new methods are still undeveloped areas that we are committed to developing



Thank You For Your Attention