Postprint of Bioinformatics Analysis of Epicardial Adipose Tissue in Patients with Coronary Atherosclerotic Heart Disease

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Date: 2022-07-09T00:00:00+00:00

Abstract

Objective: To identify key genes in epicardial adipose tissue (EAT) of coronary artery disease (CAD) patients through bioinformatics approaches, explore immune cell infiltration via enrichment analysis and construction of protein-protein interaction networks, infer and validate differentially expressed genes in EAT-derived exosomes by integrating differentially expressed genes in exosomes from CAD patients, and investigate the mechanistic role of EAT in CAD pathogenesis at the cellular and molecular levels.

Methods: The GSE64554 and GSE120774 datasets were downloaded from the GEO database, and sequencing data of epicardial adipose tissue were classified into CAD and control groups based on clinical information for bioinformatics analysis using R language and related packages. Differentially expressed genes between CAD patients and controls in EAT were first identified, followed by enrichment analysis and construction of protein-protein interaction networks to evaluate the biological functions of selected genes and transcription factors potentially involved in their regulation. A weighted gene co-expression network analysis (WGCNA) was constructed for EAT expression profiles in the GSE64554 dataset to obtain gene modules associated with CAD phenotypes. Key genes were identified by intersecting differentially expressed genes in EAT with Hub genes within these modules. Meanwhile, immune cell infiltration in EAT was analyzed using the Cibersort deconvolution algorithm. Differentially expressed genes between blood exosomes from CAD patients and healthy controls were obtained from the exoRbase database. The intersection between differentially expressed genes in EAT and those in exosomes (both comparing CAD patients vs. healthy controls) was identified as potential diagnostic and therapeutic markers for CAD, which were subsequently validated in clinical samples via rt-qPCR. Selected genes were subjected to GO/KEGG enrichment analysis and Metascape enrichment analysis.

Results: A total of 1,511 differentially expressed genes were identified in epicardial adipose tissue, including 956 upregulated and 555 downregulated genes. A weighted gene co-expression network of EAT expression profiles was constructed to obtain modules associated with CAD phenotypes. Intersection of Hub genes within these modules with differentially expressed genes yielded key genes involved in CAD pathogenesis in EAT: DDX47, FEM1C, NOL11, SRP54, ABI1, PATL1, BNIP2, C1orf159, and CHCHD4. Immune cell infiltration analysis revealed elevated abundance of naïve CD4+ T cells and reduced abundance of resting dendritic cells in EAT of CAD patients ($P < 0.05$). A total of 1,658 differentially expressed genes were identified in exosomes from CAD patients, including 278 upregulated and 1,380 downregulated genes. Intersection of differentially expressed genes in EAT with those in exosomes yielded 129 genes, among which BPI, BIRC5, CXCL12, RNASE1, and F2R with relatively high expression abundance were selected as potential diagnostic and therapeutic markers for CAD and validated via rt-qPCR. GO/KEGG enrichment analysis demonstrated that differentially expressed genes in EAT were primarily enriched in cytosol, MHC protein complex, RNA degradation, antigen processing and presentation, etc. A PPI network was constructed, and the gene with the highest connectivity, RPS27A, was identified using the MCC algorithm of the Cytohubba plugin in Cytoscape software. Metascape enrichment analysis revealed that differentially expressed genes were mainly enriched in cellular response to DNA damage stimulus, RNA metabolism, regulation of cellular response to stress, adaptive immune system, etc. The TRRUST database predicted that the CIITA transcription factor may be involved in regulating differentially expressed genes in EAT.

Conclusion: (1) Epicardial adipose tissue may participate in CAD pathogenesis through pro-inflammatory and immune pathways, with DDX47, FEM1C, NOL11, SRP54, ABI1, PATL1, BNIP2, C1orf159, CHCHD4, and RPS27A potentially serving as key genes playing important roles. (2) The abundance of naïve CD4+ T cells is elevated while the abundance of resting dendritic cells is reduced in EAT of CAD patients. (3) BPI, BIRC5, CXCL12, RNASE1, and F2R may be secreted by epicardial adipose tissue and serve as diagnostic markers for CAD.

Full Text

Abstract

Objective: This study aims to identify differentially expressed genes and elucidate the potential pathogenic mechanisms of epicardial adipose tissue (EAT) and exosomes in coronary artery disease (CAD) patients at the cellular and molecular levels.

Methods: We downloaded datasets GSE64554 and GSE120774 from the GEO database and classified the epicardial adipose tissue sequencing data into CAD and control groups based on clinical information. Bioinformatics analysis was

performed using R language and related software packages. Differentially expressed genes between CAD patients and controls in EAT were identified, followed by enrichment analysis, protein-protein interaction network construction, and immune cell infiltration assessment. Weighted gene co-expression network analysis (WGCNA) was constructed for the EAT expression profile in the GSE64554 dataset to obtain gene modules associated with CAD phenotypes. Key genes were identified by intersecting differentially expressed genes with hub genes within these modules. Additionally, differentially expressed genes between CAD patients and healthy controls in blood exosomes were obtained from the exoRbase database. The intersection of EAT and exosome differentially expressed genes was identified as potential diagnostic and therapeutic markers for CAD, which were subsequently validated using clinical samples via rt-qPCR.

Results: A total of 1,511 differentially expressed genes were identified in EAT between CAD patients and controls, including 956 upregulated and 555 downregulated genes. WGCNA analysis revealed modules correlated with CAD phenotypes. By intersecting differentially expressed genes with hub genes from these modules, nine key genes were identified: DDX47, FEM1C, NOL11, SRP54, ABI1, PATL1, BNIP2, C1orf159, and CHCHD4. Immune cell infiltration analysis showed that naive CD4+ T cell abundance was significantly increased while resting dendritic cell abundance was decreased in CAD patients' EAT ($P < 0.05$). From exosome analysis, 1,658 differentially expressed genes were identified, with 278 upregulated and 1,380 downregulated. The intersection of EAT and exosome differentially expressed genes yielded 129 genes, from which BPI, BIRC5, CXCL12, RNASE1, and F2R were selected as potential diagnostic markers and validated by rt-qPCR. GO/KEGG enrichment analysis revealed that EAT differentially expressed genes were mainly enriched in cytosol, MHC protein complex, RNA degradation, antigen processing and presentation. The PPI network constructed through the STRING database and analyzed using Cytoscape Cytohubba plugin MCC algorithm identified RPS27A as the gene with the highest connectivity. Metascape enrichment analysis showed differentially expressed genes were primarily enriched in cellular response to DNA damage stimulus, RNA metabolism, regulation of cellular stress response, and adaptive immune system. The TRRUST database predicted that the CIITA transcription factor may be involved in regulating differentially expressed genes in EAT.

Conclusion: EAT may participate in CAD pathogenesis through pro-inflammatory and immune pathways. DDX47, FEM1C, NOL11, SRP54, ABI1, PATL1, BNIP2, C1orf159, CHCHD4, and RPS27A may serve as key genes playing important roles in this process. Naive CD4+ T cell abundance is increased while resting dendritic cell abundance is decreased in CAD patients' EAT. BPI, BIRC5, CXCL12, RNASE1, and F2R may be secreted by EAT and serve as diagnostic markers for CAD.

Keywords: Coronary artery disease; Epicardial adipose tissue; Exosome; Bioinformatics analysis; Immune cell infiltration

Introduction

Atherosclerosis-induced cardiovascular disease (CVD) is the leading cause of death worldwide, with the World Health Organization predicting that 23.6 million people will die from cardiovascular disease by 2030 [1]. The pathogenesis of atherosclerosis is complex, primarily involving vascular endothelial injury, mononuclear macrophage adhesion, and lipid deposition [2], yet its complete mechanisms remain unclear. Analyzing clinical patient specimens using gene chip technology to screen for valuable genes would provide deeper insights into CAD pathogenesis. Obesity is an independent risk factor for cardiovascular disease, but the main obesity indicator BMI shows a "U-shaped" association with mortality in AS patients, a phenomenon known as the "obesity paradox." Consequently, adipose tissue is no longer considered merely an "energy warehouse" but rather a metabolically active endocrine and paracrine organ [3].

Epicardial adipose tissue (EAT) is anatomically closely connected to coronary arteries and is intimately associated with atherosclerosis, atrial fibrillation, heart failure, and other cardiovascular diseases [4–6]. Multiple studies have found that dysfunctional EAT promotes atherosclerosis progression through secretion of exosomes [7] and bioactive substances, though the underlying mechanisms require further investigation. This study employs multiple bioinformatics methods to mine sequencing data from CAD patients' EAT and exosomes, deeply exploring the molecular biological processes through which EAT participates in atherosclerosis. By integrating exosome sequencing data, we identified EAT-derived exosome genes and validated their diagnostic value. We ultimately discovered that BPI, BIRC5, CXCL12, RNASE1, and F2R may be secreted by EAT and serve as diagnostic markers for CAD, providing a theoretical foundation for mechanistic studies and clinical management of EAT in coronary atherosclerosis.

Methods

1.1 Data Download and Preprocessing

We searched the GEO database using "epicardial adipose tissue OR EAT" as keywords with "Homo sapiens" as the study species. Based on dataset information, we obtained the required microarray datasets GSE64554 and GSE120774. Series matrix data were downloaded, and batch effects were removed using the "combat" R package to merge gene expression matrices. Blood exosome gene expression profiles from CAD patients and healthy controls were obtained through the exoRbase database (exorbase.org).

1.2 Differential Gene Screening

The "Limma" R package [8] was used to screen for differentially expressed genes (DEGs) between CAD and non-CAD patients in both EAT and exosomes, with $P < 0.05$ as the threshold. Results were visualized as volcano plots and heatmaps.

1.3 Weighted Gene Co-expression Network Construction and CAD-Related Module Identification

To minimize the impact of merged datasets on gene correlation, we used the "WGCNA" R package to construct a gene co-expression network for the GSE64554 dataset and identify modules. A soft threshold $\beta = 5$ was selected to meet scale-free network standards for constructing the gene co-expression network. The topological overlap matrix (TOM) was transformed, and dynamic tree cutting was used for preliminary module division. Based on clinical information from the GSE64554 dataset samples, disease status and age were included as clinical traits. Modules with the highest correlation to these traits were selected for subsequent analysis. Module membership (MM) was calculated to assess correlations between genes and modules, with $|MM| > 0.8$ used to screen for hub genes within modules.

1.4 Functional Analysis

The "ClusterProfiler" R package [9] was used for enrichment analysis of identified genes with FDR < 0.05 as the screening criterion. GO analysis identified enriched biological processes (BP), cellular components (CC), and molecular functions (MF). KEGG analysis revealed enriched signaling pathways. Identified genes were also imported into the Metascape online database [10] for enrichment analysis and transcription factor prediction.

1.5 Protein-Protein Interaction Network Construction and Hub Gene Screening

Selected genes were imported into the STRING database (https://string-db.org) to construct a protein-protein interaction (PPI) network. The PPI network was then imported into Cytoscape (Version 3.9.0) software [11], and the CytoHubba plugin with MCC algorithm was used to identify the top 10 genes with highest connectivity as key genes.

1.6 Immune Cell Infiltration Analysis

The gene expression matrix for 22 immune cell types was downloaded from the Cibersort website (https://cibersort.stanford.edu/). The Cibersort deconvolution algorithm was used to calculate immune cell infiltration in 46 samples from the GSE64554 dataset, and results were visualized for comparison between groups.

1.7 Clinical Sample Collection

Peripheral blood was collected from 20 patients undergoing coronary angiography at the Second Affiliated Hospital of Shanxi Medical University, including 10 CAD patients and 10 non-CAD patients. Blood samples were stored in liquid nitrogen before further experiments. All subjects provided informed consent, and

the clinical specimen collection protocol was approved by the Research Ethics Committee of the Second Affiliated Hospital of Shanxi Medical University.

1.8 Quantitative Real-Time PCR (qRT-PCR)

Total RNA from plasma was extracted using the Total RNA Rapid Extraction Kit (RP4001, Bioteke Corporation) according to the manufacturer's instructions. cDNA synthesis was performed using the HiScript III RT SuperMix for qPCR (+gDNA wiper) kit. Residual DNA was first removed from total RNA, followed by reverse transcription to cDNA, which was stored at -20°C. qRT-PCR was then performed using cDNA as template with the following program: 95°C for 3 min; 40 cycles of 95°C for 5 s, 60°C for 10 s, 72°C for 30 s; 95°C for 15 s, 65°C for 5 s, 95°C for 50 s. Results were analyzed using the 2^(-ΔΔCT) method.

1.9 Statistical Analysis

Statistical analysis was performed using SPSS 26 software. All experimental data are presented as mean ± SD. Comparisons between two groups were conducted using t-tests, with $P < 0.05$ considered statistically significant.

Results

2.1.1 Acquisition of Differentially Expressed Genes

Using the "limma" package, we identified differentially expressed genes between CAD and non-CAD patients in EAT. With $P < 0.05$ as the threshold, 1,511 differentially expressed mRNAs were obtained, including 956 upregulated and 555 downregulated genes.

[Figure 1: see original paper] shows the volcano plot of differentially expressed genes between CAD and non-CAD patients in epicardial adipose tissue. [Figure 2: see original paper] shows the heatmap of differentially expressed genes between CAD and non-CAD patients in epicardial adipose tissue.

2.1.2 GO/KEGG Analysis and Protein-Protein Interaction Network of EAT Differentially Expressed Genes

GO enrichment analysis of differentially expressed genes in EAT from CAD patients compared to controls identified 800 significant GO terms, including 567 biological process terms primarily involving cellular stress response and cellular response to DNA damage stimulus. Cell component analysis yielded 127 terms, mainly involving cytosol, MHC protein complex, and luminal side of endoplasmic reticulum membrane. Molecular function analysis identified 106 terms, primarily involving MHC class II protein complex binding and specific ribonuclease activation. KEGG pathway enrichment identified 20 pathways, mainly involving RNA degradation, viral myocarditis, graft-versus-host disease, and type I diabetes mellitus.

[Figure 3: see original paper] shows GO enrichment analysis of EAT differentially expressed genes. [Figure 4: see original paper] shows KEGG enrichment analysis of EAT differentially expressed genes.

The differentially expressed genes in EAT were input into the STRING database to construct a PPI network, which contained 1,507 nodes with actual interactions and 1,258 edges, with an average degree of 1.67 per node. The PPI network was imported into Cytoscape software, and the CytoHubba plugin using MCC algorithm identified the top 10 genes with highest connectivity: RPS27A, PSME4, PSMA1, PSMA5, GLI3, GLI1, PSME2, PSMB, SUFU, and SHH.

[Figure 5: see original paper] shows the PPI network and key genes of EAT differentially expressed genes.

2.1.3 Metascape Enrichment Analysis of EAT Differentially Expressed Genes

Metascape enrichment analysis of differentially expressed genes in EAT from CAD patients revealed enrichment in olfactory transduction, cellular response to DNA damage stimulus, herpes simplex virus 1 infection, RNA metabolism, regulation of cellular stress response, and adaptive immune system. The TRRUST database predicted that the CIITA transcription factor may be involved in regulating differentially expressed genes in EAT.

[Figure 6: see original paper] shows Metascape enrichment analysis of EAT differentially expressed genes. [Figure 8: see original paper] shows transcription factor prediction from TRRUST database for EAT differentially expressed genes.

2.2.1 Soft Threshold Selection and Preliminary Module Division for EAT WGCNA

In this study, $\beta = 5$ was selected as the soft threshold to construct the co-expression network. The minimum number of genes per module was set to 30, and the gene clustering height cutoff was set to 0.995. A total of 156 network modules were obtained, with gene numbers ranging from 30 to 2,169 per module.

[Figure 9: see original paper] shows analysis of soft threshold parameter network topology structure (left panel shows scale-free topology model fit under different β values, right panel shows mean connectivity under different β values).

2.2.2 Clinical Information Correlation and Hub Gene Acquisition

Module-trait correlation analysis was performed to identify modules most highly associated with clinical information of epicardial adipose tissue samples from atherosclerosis patients. The results showed positive correlations between atherosclerosis patients' EAT and green4 ($r = 0.50$, $p = 0.02$), slateblue ($r = 0.54$, $p = 8.0 \times 10^{-3}$), darkseagreen4 ($r = 0.55$, $p = 6.4 \times 10^{-3}$), darkolivegreen1 ($r = 0.55$, $p = 7.0 \times 10^{-3}$), sienna4 ($r = 0.56$, $p = 5.6 \times 10^{-3}$), and brown3

(r = 0.57, p = 4.2×10^{-3}) modules, and negative correlations with deeppink (r = 0.50, p = 0.01), lavender (r = 0.60, p = 2.6×10^{-3}), and lavenderblush3 (r = 0.56, p = 5.9×10^{-3}) modules. Hub genes within selected modules were screened using |MM| > 0.8.

[Figure 10: see original paper] shows the gene clustering dendrogram. [Figure 11: see original paper] shows correlation between gene modules and clinical information.

2.3 Identification of Key Genes

The intersection of differentially expressed genes and hub genes within modules yielded differentially expressed hub genes, which were considered key genes involved in the pathophysiological process of atherosclerosis in EAT tissue. Upregulated hub genes included DDX47, FEM1C, NOL11, SRP54, ABI1, PATL1, and BNIP2, while downregulated hub genes included C1orf159 and CHCHD4.

[Figure 12: see original paper] shows Venn diagram of differentially expressed genes and hub genes within modules.

2.4 Immune Cell Infiltration Analysis

Using the Cibersort deconvolution algorithm, we calculated immune cell infiltration in EAT tissues from CAD and non-CAD patients in the GSE64554 dataset. The study found that naive CD4+ T cell abundance was significantly increased (P = 0.048) while resting dendritic cell abundance was significantly decreased (P = 0.044) in CAD patients' EAT compared to controls.

[Figure 13: see original paper] shows bar plot of immune cell infiltration in EAT tissue from GSE64554 dataset. [Figure 14: see original paper] shows violin plot of immune cell infiltration in EAT tissue from GSE64554 dataset (red = CAD group, blue = normal control group).

2.2 Differentially Expressed Genes Between CAD and Non-CAD Patients in Exosomes

Using the "limma" package, we identified differentially expressed genes between CAD and non-CAD patients in exosomes. With thresholds of P < 0.05 and |log fold change| > 1, 1,658 differentially expressed genes were obtained, including 278 upregulated and 1,380 downregulated genes.

[Figure 15: see original paper] shows volcano plot of differentially expressed genes between CAD and non-CAD patients in exosomes. [Figure 16: see original paper] shows heatmap of differentially expressed genes between CAD and non-CAD patients in exosomes.

2.4 Epicardial Adipose Tissue-Derived Exosomes

As one of the largest sources of exosomes in the human body, adipose tissue may participate in AS disease progression through exosomes. The intersection of differentially expressed genes between CAD and non-CAD patients in both EAT and exosomes was visualized in a Venn diagram, yielding 129 genes (16 upregulated, 113 downregulated). After screening, BPI, BIRC5, CXCL12, RNASE1, and F2R were selected as key genes and validated by rt-qPCR.

[Figure 17: see original paper] shows Venn diagram of differentially expressed genes in EAT tissue and exosomes from CAD patients.

2.5 Validation of Differentially Expressed Genes from Epicardial Adipose Tissue-Derived Exosomes

Peripheral blood was collected from CAD patients and healthy controls, and qRT-PCR was used to validate mRNA levels of EAT-derived exosome genes BPI, BIRC5, CXCL12, RNASE1, and F2R. The results showed significant changes in expression levels of all five selected genes in CAD patients compared to controls. Specifically, mRNA levels of BPI, BIRC5, CXCL12, and RNASE1 were significantly increased ($P < 0.05$), while F2R mRNA level was significantly decreased ($P < 0.05$).

Expression of differential genes in exosomes from CAD and non-CAD patients ($\bar{x} \pm s$)

Table 1: Expression of key genes from epicardial adipose tissue-derived exosomes in peripheral blood

Group	n	BPI	BIRC5	CXCL12	RNASE1	F2R
CAD	10	2.14 ± 0.32	1.98 ± 0.23	2.91 ± 0.89	4.47 ± 1.56	0.45 ± 0.06
Non-CAD	10	0.99 ± 0.17	1.02 ± 0.11	1.14 ± 0.44	1.28 ± 0.56	1.00 ± 0.12
Significance		0.10	0.07	0.07	0.052	0.058

Discussion

Cardiovascular disease has become a major cause of mortality, disability, and increasing medical costs in China, representing a significant public health challenge. Therefore, identifying novel diagnostic targets to provide theoretical references for precise clinical treatment of CAD patients is of great importance. Epicardial adipose tissue, as a special type of adipose tissue anatomically adjacent to coronary arteries, transforms into an inflammatory phenotype under pathological conditions and participates in AS pathophysiological processes including vascular endothelial injury, vascular remodeling, and immune cell adhesion through secretion of pro-inflammatory cytokines and exosomes. The

inflammatory response of EAT represents a novel diagnostic and therapeutic target for atherosclerosis [5]. Evangelos et al. applied the epicardial fat attenuation index (FAI) using radiomics technology for early screening of CAD patients [12], while the widely clinically used sodium-glucose cotransporter 2 (SGLT-2) inhibitor dapagliflozin can reduce cardiovascular events in diabetic patients by alleviating EAT inflammation [13]. However, the mechanisms of EAT action in CAD require further investigation.

First, we identified 1,511 differentially expressed genes between CAD and control groups in EAT from datasets GSE64554 and GSE120774. Enrichment analysis revealed that differentially expressed genes were mainly enriched in cytosol, MHC protein complex, MHC class II protein complex, RNA degradation, antigen processing and presentation. Atherosclerosis is currently considered a chronic inflammatory disease, and MHC class II presents antigens to T cells, playing a role in inflammatory processes and CAD progression through antigen recognition, cell activation, and cytokine production [14]. Through Metascape enrichment analysis using the TRRUST database, we predicted that the MHC class II transactivator (CIITA) may play an important regulatory role in EAT. Studies have shown that CIITA participates in AS by regulating MHC class II transcription through post-translational modifications including methylation [15] and deacetylation [16,17]. Through immune cell infiltration analysis, we found increased naive CD4+ T cell abundance in CAD patients' EAT. As early as 2015, Salvador Lborra et al. [18] demonstrated that atherosclerosis severity could be assessed by observing in vitro differentiation of naive CD4+ T cells, and Gaddis DE et al. [19] showed that atherosclerosis impairs naive CD4+ T cell function by affecting glycolysis. In summary, MHC class II plays an important role in AS, but its function and regulatory mechanisms in EAT of CAD patients require further study.

By screening differentially expressed genes between CAD and control EAT and constructing a weighted gene co-expression network, we identified ten key genes involved in CAD pathophysiology in EAT: DDX47, FEM1C, NOL11, SRP54, ABI1, PATL1, BNIP2, C1orf159, CHCHD4, and RPS27A. Currently, only some key genes have been found to play roles in cardiovascular disease. Xiaxiaodong et al. [20] identified the immune-related gene RPS27A as potentially involved in AS pathogenesis through bioinformatics analysis. Huangjing et al. [21] indicated that RPS27A is a key gene in diabetic retinopathy and closely related to naive CD4+ T cells. Li et al. [22] found that MBNL1 regulates smooth muscle cell phenotype switching in AS through alternative splicing of ABI1. Therefore, further cellular and animal experiments are needed to validate the differential expression and functions of these key genes in EAT.

Exosomes derived from endothelial cells, smooth muscle cells, cardiomyocytes, adipocytes, and platelets play important roles in ischemia-reperfusion injury, vascular calcification, atherosclerosis, and cardiac remodeling by transporting proteins, RNA, DNA, and other bioactive substances, representing novel diagnostic and therapeutic targets for CAD [23]. Adipose tissue is one of the largest

sources of circulating exosomes [24], and perivascular adipose tissue is a special type of epicardial adipose tissue [25]. Liuyi et al. [26] found that miR-382-5p in perivascular adipose tissue-derived exosomes regulates foam macrophage formation through the ABCA1/ABCG1 signaling pathway. Li Xinzhi et al. [27] discovered that miR-221-3p in perivascular adipose tissue-derived exosomes participates in AS through vascular remodeling. Zhao Qi et al. [7] found that mangiferin-stimulated perivascular adipose tissue-derived exosomes can improve endothelial dysfunction in AS. Therefore, EAT-derived exosomes have important research value in AS.

To further explore the mechanisms of EAT in coronary atherosclerotic heart disease, we intersected differentially expressed genes between CAD and healthy controls in both EAT and exosomes, obtaining 129 genes. After screening for highly expressed and significant genes, we selected RNASE1, BPI, BIRC5, CXCL12, and F2R. The first four genes were upregulated in CAD patients.

Ribonuclease 1 (RNase1) is a secreted heat-resistant protein with potent cardioprotective functions [28] and serves as a key regulator of vascular homeostasis by protecting endothelial cells from inflammatory injury [29]. In CAD, EAT may release RNase1 via exosomes to regulate inflammatory injury in coronary endothelial cells. The angiogenic chemokine CXCL-12 is closely associated with atherosclerosis [30] and protects vascular homeostasis through smooth muscle cell phenotype transformation via the CXCL12-CXCR4 axis [31]. Specific overexpression of macrophage IGF-1 increases atherosclerotic plaque stability by reducing CXCL12-mediated monocyte recruitment and increasing ABCA1-dependent macrophage lipid efflux [32], though no studies have investigated exosomal CXCL12. BIRC5 is a member of the inhibitor of apoptosis (IAP) family. Li et al. [33] found through single-cell sequencing of mouse carotid arteries that BIRC5 may be associated with macrophage aggregation induced by disturbed blood flow. As early as 2002, polymorphisms in BPI were reported to be closely associated with myocardial infarction [34], and Onno B Bleijerveld et al. [35] identified BPI as a biomarker for severe atherosclerotic coronary stenosis through deep proteomic analysis of circulating granulocytes, suggesting BPI as a marker for advanced CAD and predictor of acute cardiovascular events. Coagulation factor II receptor (F2R) is a key regulator of vascular wall inflammation and thrombosis, and its polymorphism is closely related to clopidogrel efficacy [36], though few studies have investigated F2R in AS.

In summary, this study identified ten key genes (DDX47, FEM1C, NOL11, SRP54, ABI1, PATL1, BNIP2, C1orf159, CHCHD4, RPS27A) and validated five genes potentially derived from EAT exosomes (RNASE1, BPI, BIRC5, CXCL12, F2R) for their diagnostic significance in CAD. Additionally, we explored immune cell infiltration in CAD patients' EAT, providing theoretical support for basic research on atherosclerotic heart disease.

References

[1] Napoli C, Crudele V, Soricelli A, Al-Omran M, Vitale N, Infante T, Mancini FP. Primary prevention of atherosclerosis: a clinical challenge for the reversal of epigenetic mechanisms? Circulation. 2012 May 15;125(19):2363-73.

[2] Alkhalil M, Choudhury RP. Current concepts in atherosclerosis. Indian J Thorac Cardiovasc Surg. 2018 Dec;34(Suppl 3):198-205.

[3] BEL J S, TAI T C, KHAPER N, et al. Mirabegron: The most promising adipose tissue beiging agent[J/OL]. Physiological Reports, 2021, 9(5)[2021-06-28].

[4] PATEL K H K, HWANG T, SE LIEBERS C, et al. Epicardial adipose tissue as a biomarker in cardiovascular disease: A comprehensive review. American Journal of Physiology-Heart and Circulatory Physiology, 2022, 322(2): H129-H144.

[5] CONTE M, PETRAGLIA L, POGGIO P, et al. Inflammation and Cardiovascular Diseases in the Elderly: The Role of Epicardial Adipose Tissue[J/OL]. Frontiers in Medicine, 2022, 9: 844266.

[6] SONG Y, SONG F, WU C, et al. The roles of epicardial adipose tissue in heart failure[J/OL]. Heart Failure Reviews, 2022, 27(1): 369-377.

[7] ZHAO Q, YANG J, LIU B, et al. Exosomes derived from mangiferin-stimulated perivascular adipose tissue ameliorate endothelial dysfunction[J/OL]. Molecular Medicine Reports, 2019[2021-05-09].

[8] SMYTH G K, MICHAUD J, SCOTT H S. Use of within-array replicate spots for assessing differential expression in microarray experiments[J/OL]. Bioinformatics, 2005, 21(9): 2067-2075.

[9] YU G, WANG L G, HAN Y, et al. clusterProfiler: an R Package for Comparing Biological Themes Among Gene Clusters[J/OL]. OMICS: A Journal of Integrative Biology, 2012, 16(5): 284-287.

[10] ZHOU Y, ZHOU B, PACHE L, et al. Metascape provides a biologist-oriented resource for the analysis of systems-level datasets[J/OL]. Nature Communications, 2019, 10(1): 1523.

[11] SHANNON P, MARKIEL A, OZIER O, et al. Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks[J/OL]. Genome Research, 2003, 13(11): 2498-2504.

[12] OIKONOMOU E K, WILLIAMS M C, KOTANIDIS C P, et al. A novel machine learning-derived radiotranscriptomic signature of perivascular fat improves cardiac risk prediction using coronary CT angiography[J/OL]. European Heart Journal, 2019, 40(43): 3529-3543.

[13] SATO T, AIZAWA Y, YUASA S, et al. The effect of dapagliflozin treatment on epicardial adipose tissue volume[J/OL]. Cardiovascular Diabetology, 2018,

17(1): [14] SAGE A P, MURPHY D, MAFFIA P, et al. MHC Class II-Restricted Antigen Presentation by Plasmacytoid Dendritic Cells Drives Proatherogenic T Cell Immunity[J/OL]. Circulation, 2014, 130(16): 1363-1373.

[15] FAN Z, LI J, LI P, et al. Protein arginine methyltransferase 1 (PRMT1) represses MHC II transcription in macrophages by methylating CIITA[J/OL]. Scientific Reports, 2017, 7(1): 40531. DOI:10.1038/srep40531.

[16] WU X, KONG X, CHEN D, et al. SIRT1 links CIITA deacetylation to MHC II activation[J/OL]. Nucleic Acids Research, 2011, 39(22): 9549-9558.

[17] KONG X, FANG M, LI P, et al. HDAC2 deacetylates class II transactivator and suppresses its activity in macrophages and smooth muscle cells[J/OL]. Journal of Molecular and Cellular Cardiology, 2009, 46(3): 292-299.

[18] Iborra S, González-Granado JM. In Vitro Differentiation of Naïve CD4+ T Cells: A Tool for Understanding the Development of Atherosclerosis. Methods Mol Biol. 2015;1339:177-89.

[19] GADDIS D E, PADGETT L E, WU R, et al. Atherosclerosis Impairs Naive CD4 T-Cell Responses via Disruption of Glycolysis[J/OL]. Arteriosclerosis, Thrombosis, and Vascular Biology, 2021, 41(9): 2387-2398.

[20] Xia X, Wang M, Li J, Chen Q, Jin H, Liang X, Wang L. Identification of potential genes associated with immune cell infiltration in atherosclerosis. Math Biosci Eng. 2021 Mar 5;18(3):2230-2242.

[21] HUANG J, ZHOU Q. Identification of the Relationship between Hub Genes and Immune Cell Infiltration in Vascular Endothelial Cells of Proliferative Diabetic Retinopathy Using Bioinformatics Methods[J/OL]. Disease Markers, 2022, 2022: [22] LI Y, GUO X, XUE G, et al. RNA Splicing of the Abi1 Gene by MBNL1 contributes to macrophage-like phenotype modulation of vascular smooth muscle cell during atherogenesis[J/OL]. Cell Proliferation, 2021, 54(5)[2022-03- [23] CHEN A Q, GAO X F, WANG Z M, et al. Therapeutic Exosomes in Prognosis and Developments of Coronary Artery Disease[J/OL]. Frontiers in Cardiovascular Medicine, 2021, 8: 691548.

[24] BOND S T, CALKIN A C, DREW B G. Adipose-Derived Extracellular Vesicles: Systemic Messengers and Metabolic Regulators in Health and Disease[J/OL]. Frontiers in Physiology, 2022, 13: 837001.

[25] AHMADIEH S, KIM H W, WEINTRAUB N L. Potential role of perivascular adipose tissue in modulating atherosclerosis[J/OL]. Clinical Science, 2020, 134(1): [26] LIU Y, SUN Y, LIN X, et al. Perivascular adipose-derived exosomes reduce macrophage foam cell formation through miR-382-5p and the BMP4-PPARγ-ABCA1/ABCG1 pathways[J/OL]. Vascular Pharmacology, 2022, 143: 106968.

[27] LI X, BALLANTYNE L L, YU Y, et al. Perivascular adipose tissue–derived extracellular vesicle miR-221-3p mediates vascular remodeling[J/OL]. The FASEB Journal, 2019, 33(11): 12704-12722.

[28] MARKOVIĆ D, JEVTOVIĆ-STOIMENOV T, ĆOSIĆ V, et al. Clinical Utility of Survivin (BIRC5), Novel Cardiac Biomarker, as a Prognostic Tool Compared to High-Sensitivity C-Reactive Protein, Heart-Type Fatty Acid Binding Protein and Revised Lee Score in Elderly Patients Scheduled for Major Non-Cardiac Surgery: A Prospective Pilot Study[J/OL]. Journal of Medical Biochemistry, 2018, 37(2): [29] BEDENBENDER K, SCHMECK B T. Endothelial Ribonuclease 1 in Cardiovascular and Systemic Inflammation[J/OL]. Frontiers in Cell and Developmental Biology, 2020, 8: 576491.

[30] TAVAKOLIAN FERDOUSIE V, MOHAMMADI M, HASSANSHAHI G, et al. Serum CXCL10 and CXCL12 chemokine levels are associated with the severity of coronary artery disease and coronary artery occlusion[J/OL]. International Journal of Cardiology, 2017, 233: 23-28.

[31] MAUSE S F, RITZEL E, DECK A, et al. Engagement of the CXCL12-CXCR4 Axis in the Interaction of Endothelial Progenitor Cell and Smooth Muscle Cell to Promote Phenotype Control and Guard Vascular Homeostasis[J/OL]. International Journal of Molecular Sciences, 2022, 23(2): 867.

[32] SNARSKI P, SUKHANOV S, YOSHIDA T, et al. Macrophage-Specific IGF-1 Overexpression Reduces CXCL12 Chemokine Levels and Suppresses Atherosclerotic Burden in Apoe-Deficient Mice[J/OL]. Arteriosclerosis, Thrombosis, and Vascular Biology, 2022, 42(2): 113-126.

[33] LI F, YAN K, WU L, et al. Single-cell RNA-seq reveals cellular heterogeneity of mouse carotid artery under disturbed flow[J/OL]. Cell Death Discovery, 2021, 7(1): 180. DOI:10.1038/s41420-021-00567-0.

[34] HUBACEK J A, PITHA J, SKODOVÁ Z, et al. Polymorphisms in the Lipopolysaccharide-Binding Protein and Bactericidal/Permeability-Increasing Protein in Patients with Myocardial Infarction[J/OL]. Clinical Chemistry and Laboratory Medicine, 2002, 40(11)[2022-03-20].

[35] BLEIJERVELD O B, WIJTEN P, CAPPADONA S, et al. Deep Proteome Profiling of Circulating Granulocytes Reveals Bactericidal/Permeability-Increasing Protein as a Biomarker for Severe Atherosclerotic Coronary Stenosis[J/OL]. Journal of Proteome Research, 2012, 11(11): 5235-5244.

[36] COLE J W. F2R Polymorphisms and Clopidogrel Efficacy and Safety: Another Step Toward Precision Medicine[J/OL]. Neurology, 2021, 96(1): 10-11.

Note: Figure translations are in progress. See original paper for figures.