Abnormal expression of TGFBR2, EGF, LRP10, and IQGAP1 is involved in the pathogenesis of coronary artery disease

Yanwei Du1,2,†, Yanan Hu2,†, Naiyan Wen3, Shuang Fu2, Guorong Zhang2, Li Li2, Tiantian Liu2, Xuejiao Lv1,*, Wenfeng Zhang2,†

1 Department of Respiratory and Critical Care Medicine, the 2nd Hospital of Jilin University, 130041 Changchun, Jilin, China
2 Department of Prescriptions, Pathology and Pathophysiology, Changchun University of Chinese Medicine, 130117 Changchun, Jilin, China
3 Department of Nursing, Changchun University of Chinese Medicine, 130117 Changchun, Jilin, China

*Correspondence: wenfzh@163.com (Wenfeng Zhang); lvxuejiao0311@163.com (Xuejiao Lv)
† These authors contributed equally.
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Coronary artery disease (CAD) is the most common cardiovascular disease worldwide. In this study, we investigated the pathogenesis of CAD. We downloaded the GSE98583 dataset, including 12 CAD samples and 6 normal samples, from the Gene Expression Omnibus (GEO) database and screened differentially expressed genes (DEGs) in CAD versus normal samples. Next, we performed functional enrichment analysis, protein-protein interaction (PPI) network, and functional module analyses to explore potential functions and regulatory functions of identified DEGs. Next, transcription factors (TFs) and microRNAs (miRNAs) targeting DEGs were predicted. In total, 456 DEGs were identified in CAD and normal samples, including 175 upregulated and 281 downregulated genes. These genes were enriched in the intestinal immune network for immunoglobulin A production and the mitogen-activated protein kinase signaling pathway (e.g., TGFBR2 and EGF). The PPI network contained 212 genes, and HIST1H2B, HIST1H2AC, EGF, and EP300 were hub genes with degrees higher than 10. Four significant modules were identified from the PPI network, with genes in the modules mainly enriched in the inflammatory response, protein ubiquitination involved in ubiquitin-dependent protein catabolic processes, protein transport, and mitochondrial translational elongation, respectively. Two TFs (E2F1 and FOXK1) and five miRNAs (mir-122A, mir-316-5p, mir-507, mir-342, and mir-520F) were predicted to target 112 DEGs. mir-122A reportedly targets both LRPS and IQGAP1 in the TF-miRNA target regulatory network. The abnormal expression of TGFBR2, EGF, LRP10, and IQGAP1 may be implicated in CAD pathogenesis. Our study provides targets and potential regulators for investigating CAD pathogenesis.

Keywords
Coronary artery disease, Transcription factor, microRNAs, MAPK signaling pathway, Pathogenesis

1. Introduction
Coronary artery disease (CAD), also known as ischemic heart disease, is a group of diseases that includes sudden cardiac death, myocardial infarction, and stable and unstable angina [1]. CAD is the most common cardiovascular disease and is usually characterized by chest discomfort or chest pain [2]. For CAD, primary risk factors include a lack of exercise, smoking, excessive alcohol consumption, high blood pressure, obesity, depression, and poor diet [3, 4]. Additionally, genetics is considered a risk factor for developing CAD [5]. In clinical practice, CAD can be diagnosed by employing coronary angiography, electrocardiogram, coronary computed tomographic angiography, and cardiac stress testing [6]. In 2015, 110 million CAD cases were reported, leading to 8.9 million deaths, thus making it the leading cause of disease-related deaths globally [7]. Therefore, elucidating mechanisms that underlie CAD is of considerable importance and significance.

Kalirin (KALRN) reportedly inhibits the activities of guanine-exchange factor and inducible nitric oxide synthase, which play important roles in the CAD mechanism via the Rho GTPase signaling pathway [8]. Decreased adiponectin and increased interleukin-6 (IL-6) levels promote CAD progression in epicardial adipose tissues [9, 10]. Moreover, levels of neuregulin-4 (Nrg4) are found to be inversely related to the development and severity of CAD [11]. Transforming growth factor-β1 (TGF-β1) is involved in the pathogenesis of restenosis, including thrombogenesis and inflammation. In patients with CAD, polymorphisms and TGF-β1 levels are independent risk factors for developing in-stent restenosis after coronary bare-metal stent implantation [12]. MiR-214 is known to inhibit the expression of vascular endothelial growth factor (VEGF), as well as activities of endothelial progenitor cells; therefore, circulating miR-214 could be employed as a novel biomarker and a diagnostic factor for CAD [13]. MiR-34a mediates sirtuin 1 (SIRT1) in endothelial progenitor cells, and atorvastatin reportedly improves endothelial function by promoting SIRT1 expression by suppressing miR-34a [14, 15]. Serum levels of miR-126, miR-197, and miR-223 are reportedly increased in patients with CAD, and both miRNA-197 and miRNA-223 can predict cardiovascular death [16]. According to a report by Bai et al. [17], the MEG3-miR-26a-Smad1 regulatory axis can be implicated in regulating the proliferation/apoptosis balance of vascular smooth...
muscle cells during atherosclerosis. Although these studies have focused on CAD pathogenesis, key genes and miRNAs associated with CAD remain unclear.

Microarray studies of human diseases, including CAD, are limited owing to a lack of human disease tissues or appropriate disease models. Peripheral blood plays a crucial role in mediating immune responses, metabolism, and intercellular communication, as well as affords convenient sample collection; accordingly, it is an ideal tissue for biomarker detection [18, 19]. Moreover, gene expression in peripheral blood could reflect CAD severity [20, 21]. Additionally, Taurino et al. [22] revealed that analyzing gene expression in whole blood is useful for detecting genes that determine cardiovascular phenotypes, including those implicated in the pathogenesis and progression of CAD. In the present study, we utilized the microarray dataset GSE98583, contributed by Kumar and Kashyap et al. [23]. In the study by Kumar and Kashyap et al. [23], differentially expressed genes (DEGs) of different disease severities were identified, followed by functional enrichment and other analyses to explore candidate genes and pathways contributing to CAD severity. In the present study, we primarily identified DEGs in CAD and control samples, followed by functional enrichment and prediction of transcription factors (TFs) and microRNAs (miRNAs) regulating these DEGs, to elucidate potential genes and their corresponding regulators involved in CAD pathogenesis. This study will provide deeper insights into the pathogenesis of CAD and provide a theoretical basis for developing targeted therapy.

2. Methods

2.1 Ethical approval

In the present study, all datasets were downloaded from public databases, which allowed researchers to download and analyze public datasets for scientific purposes. Therefore, ethical approval was not required.

2.2 Data source

We downloaded and used the microarray dataset GSE98583 from the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) database, which is based on the GPL571 [HG-U133A_2] Affymetrix Human Genome U133A 2.0 Array. The GSE98583 dataset included 12 whole blood samples from non-diabetic male patients with CAD based on their coronary angiogram results. Of the 12 patients, 6 presented single-vessel disease (stenosis >95% in the left anterior descending artery, Gensini score 20–30), and 6 had triple vessel disease (stenosis >95% in all three major epicardial vessels, Gensini score 50–60). Additionally, six whole blood samples from control subjects with atypical angina and normal coronary angiograms were included.

2.3 Data preprocessing and differential expression analysis

The original CEL files were downloaded and preprocessed using the R package Oligo (version 1.34.0, http://biocoductor.org/help/search/index.html?q=oligo/), Johns Hopkins University, Baltimore, MD, USA.) [24]. Data preprocessing involved data format conversion, filling missing data, background correction, and data standardization. Next, the probes were annotated and combined with platform annotation files. Probes that could not be matched to gene symbols were filtered out. For multiple probes mapped to one gene symbol, the average value of the probes was obtained as the expression value of the corresponding gene symbol. Using the R package Limma (version 3.10.3, http://www.biocoductor.org/packages/release/limma/html/limma.html, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia) [25], we analyzed DEGs between CAD and control samples. Genes with a P-value of <0.05 were defined as DEGs.

2.4 Enrichment analysis

Based on the Database for Annotation, Visualization and Integrated Discovery (DAVID, version 6.8, https://david.ncifcrf.gov/, Laboratory of Human Retrovirology and Immunoinformatics, USA) tool [26], Gene Ontology (GO) terms [27] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [28] enrichment analyses were performed for identified DEGs. The number of genes involved in each term was set at ≥2, and a P-value < 0.05 was established as the significant threshold.

2.5 PPI network analysis

Combined with the STRING (version 10.0, http://string-db.org/) database [29], PPI pairs were used to predict proteins encoded by identified DEGs. The PPI score was set at 0.7. Next, a PPI network was constructed for DEGs using the Cytoscape software (version 3.2.0, http://www.cytoscape.org, National Institute of General Medical Sciences, USA) [30]. Using the CytoNCA plug-in (version 2.1.6, http://apps.cytoscrape.org/apps/cytocentracal South University, Changsha, China) [31] in Cytoscape with parameter set as: without weight, we performed network topology analysis to identify the hub network nodes. Furthermore, significant modules with a score ≥ 5 were selected from the PPI network using the MCODE plug-in (version 1.4.2, http://apps.cytocscape.org/apps/MCODE, University of Toronto, Canada) [32] in Cytoscape.

2.6 TF-miRNA target regulatory network analysis

Using the iRegulon plug-in (version 1.3, http://apps.cytoscrape.org/apps/iRegulon, Laboratory of Computational Biology, KU Leuven, Belgium) [33] in Cytoscape, we performed a TF-target prediction for the PPI network nodes. The parameters “minimum identity between orthologous genes” and “maximum false discovery rate on motif similarity” were set at 0.05 and 0.001, respectively. Results with a normalized enrichment score (NES) of >4 were selected. Using the WebGestalt GAST (version: update 2013, http://www.webgestalt.org/option.php, Baylor College of Medicine, Houston, TX, USA) tool [34], we predicted target miRNAs for PPI network nodes by employing the overrepresentation enrichment analysis (ORA) method. The least number of enriched genes was
Fig. 1. The box diagram presents the distribution of expression values after data normalization. Red and white represent disease samples and control samples, respectively.

set at two, and the top five results are presented. Finally, the results of TF-target prediction and miRNA-target prediction were merged to build the TF-miRNA target regulatory network using Cytoscape [30].

2.7 Validation using the Comparative Toxicogenomics Database

The etiology of several chronic diseases is based on interactions between environmental chemicals and genes regulating physiological processes [35]. The Comparative Toxicogenomics Database (CTD, http://ctdbase.org/, NC State University, Raleigh, NC, USA) is a publicly available database for identifying chemical-gene-disease networks [36]. We conducted a CTD search to identify genes and pathways associated with CAD. Next, we performed a Venn analysis to identify overlapping genes and pathways between the CTD database and the microarray dataset GSE98583.

3. Results

3.1 Differential expression analysis

The distribution of expression values after data normalization is shown in Fig. 1. The medians were at the same level, indicating that data preprocessing results were good. According to the screening threshold, a total of 456 DEGs (175 upregulated and 281 downregulated genes) were identified. The clustering heatmap indicated that DEGs could help distinguish samples with different disease statuses (Fig. 2).

3.2 Enrichment analysis

Multiple GO functional terms were enriched for upregulated and downregulated genes. For upregulated genes, positive regulation of proteins targeting mitochondria, the glutathione derivative biosynthetic process, and mitochondrial translational termination were the primary functional terms that were enriched (Fig. 3). In contrast, cellular response to mechanical stimulus, centrosome localization, and thymus development were potential functions of downregulated genes (top 20 listed, Fig. 4). Meanwhile, the upregulated genes were implicated in 3 pathways (such as the intestinal immune network for immunoglobulin A (IgA) production, $P = 2.10 \times 10^{-2}$), whereas the downregulated genes were implicated in 15 pathways (such as endocytosis, $P = 4.61 \times 10^{-4}$; mitogen-activated protein kinase (MAPK) signaling pathway, $P = 1.22 \times 10^{-2}$) (Table 1). In particular, the downregulated transforming growth factor-beta receptor 2 (TGFBR2) and epidermal growth factor (EGF) were enriched in the MAPK signaling pathway.

3.3 PPI network analysis

The PPI network is shown in Fig. 5, presenting 212 nodes and 332 edges. In the PPI network, histone cluster 1, H2bj (HIST1H2BJ, down, degree = 18), histone cluster 1, H2ac (HIST1H2AC, down, degree = 17), EGF (down, degree = 16), and E1A binding protein p300 (EP300, down, degree = 15) were nodes with degrees higher than 10 and were thus con-
sidered hub nodes. Moreover, four network modules were screened, including module A (score = 7; with seven nodes and 21 edges), module B (score = 6; with six nodes and 15 edges), module C (score = 6; with 6 nodes and 15 edges), and module D (score = 5.6; with 6 nodes and 14 edges) (Fig. 6).

The results of the GO functional enrichment analysis of module nodes are listed in Table 2. The nodes in modules A, B, C, and D were mainly enriched in the inflammatory response ($P = 3.70 \times 10^{-6}$), protein ubiquitination involved in ubiquitin-dependent protein catabolic process ($P = 2.95 \times 10^{-6}$), protein transport ($P = 5.27 \times 10^{-3}$), and mitochondrial translational elongation ($P = 3.05 \times 10^{-9}$), respectively.

Fig. 2. The clustering heatmap of the differentially expressed genes (DEGs). Y-axis represents all DEGs, and X-axis represents all samples; the green and purple blocks in upper represent control group and CAD group, respectively.
**Fig. 3.** The Gene Ontology (GO) terms enriched for upregulated genes. The size of a circle indicates the number of genes involved in the respective term. The color change from green to red suggests that the $-\log_{10}(P$-value$)$ changes from small to large.

**Table 1. Pathways enriched separately for the up- and down-regulated genes.**

| Category | Pathway ID | Pathway name | Count | $P$ value | Genes |
|----------|------------|--------------|-------|-----------|-------|
| UP       | hsa04672   | Intestinal immune network for IgA production | 4     | $2.10 \times 10^{-2}$ | CD86, IL15, HLA-DMB, HLA-DMA |
|          | hsa05130   | Staphylococcus aureus infection | 4     | $3.02 \times 10^{-2}$ | CIAR1, C3, HLA-DMB, HLA-DMA |
|          | hsa00561   | Glycerolipid metabolism | 4     | $3.62 \times 10^{-2}$ | AKR1A1, DGKH, AGK, ALDH3A2 |
| DOWN     | hsa04144   | Endocytosis | 14    | $4.61 \times 10^{-4}$ | CHMP2A, RAB5B, TGFBR2, CXCR1, CXCR2, VPS37C, EPS15LI, KIT, KIF21, HSPA6, GIT2, RAB11A, EGF, IQSEC1 |
|          | hsa05220   | Chronic myeloid leukemia | 7     | $1.42 \times 10^{-3}$ | E2F3, GAB2, SOS1, STAT5B, TGFBR2, SOS2, RAF1 |
|          | hsa04068   | FOXO signaling pathway | 9     | $2.09 \times 10^{-3}$ | EP300, STIP4, SOS1, PRKAB2, TGFBR2, SOS2, RAF1, EGF, BCL2L1 |
|          | hsa05219   | Bladder cancer | 5     | $5.18 \times 10^{-3}$ | RPS6KA5, E2F3, DAPK2, RAF1, EGF |
|          | hsa04010   | MAPK signaling pathway | 11    | $1.22 \times 10^{-2}$ | RPS6KA5, SOS1, TGFBR2, SOS2, MAP2K4, HSPA6, RAF1, CACNB4, RAPGEF2, EGF, DUSP9 |
|          | hsa05223   | Non-small cell lung cancer | 5     | $1.54 \times 10^{-2}$ | E2F3, SOS1, SOS2, RAF1, EGF |
|          | hsa05221   | Acute myeloid leukemia | 5     | $1.54 \times 10^{-2}$ | SOS1, STAT3B, SOS2, RAF1, KIT |
|          | hsa04012   | ErbB signaling pathway | 6     | $1.68 \times 10^{-2}$ | SOS1, STAT3B, SOS2, MAP2K4, RAF1, EGF |
|          | hsa05215   | Prostate cancer | 6     | $1.75 \times 10^{-2}$ | E2F3, EP300, SOS1, SOS2, RAF1, EGF |
|          | hsa05212   | Pancreatic cancer | 5     | $2.53 \times 10^{-2}$ | E2F3, RLBP1, TGFBR2, RAF1, EGF |
|          | hsa05214   | Glioma | 5     | $2.53 \times 10^{-2}$ | E2F3, SOS1, SOS2, RAF1, EGF |
|          | hsa06664   | Fc epsilon RI signaling pathway | 5     | $2.93 \times 10^{-2}$ | GAB2, SOS1, SOS2, MAP2K4, RAF1 |
|          | hsa05200   | Pathways in cancer | 13    | $3.74 \times 10^{-2}$ | E2F3, RLBP1, TGFBR2, STAT5B, RAF1, FADD, KIT, DAPK2, EP300, SOS1, SOS2, TPR, EGF |
|          | hsa05161   | Hepatitis B | 7     | $3.87 \times 10^{-2}$ | E2F3, EP300, DDXX, STAT5B, MAP2K4, RAF1, FADD |
|          | hsa00531   | Glycosaminoglycan degradation | 3     | $4.16 \times 10^{-2}$ | HYAL2, IDS, GALNS |
3.4 TF-miRNA target regulatory network analysis

Following the prediction of 2 TFs (E2F1 and FOXK1) and 5 miRNAs (miR-122A, miR-516-5P, miR-507, miR-342, and miR-520F), 172 regulatory pairs were obtained (involving 29 up-regulated and 83 downregulated genes). Subsequently, we built a TF-miRNA target regulatory network (Fig. 7). In the regulatory network, miR-122A can target both low-density lipoprotein (LDL) receptor-related protein 10 (LRP10) and IQ motif-containing GTPase-activating protein 1 (IQGAP1).

3.5 Validation with CTD database

In the CTD database, a total of 25,384 genes and 149 pathways were found to be associated with CAD. The Venn analysis identified 429 overlapping genes between CAD-associated genes and 456 DEGs, including TGFBR2, EGF, LRP10, and IQGAP1 (Fig. 8A and Supplemental Table 1). Similarly, we screened 10 overlapping pathways between CAD-associated pathways and 18 significant KEGG pathways, including the MAPK signaling pathway (Fig. 8B and Table 3). These results suggest that identified genes and pathways are important in CAD and could be implicated in CAD pathogenesis.

4. Discussion

In the present study, we identified 456 DEGs (including 175 upregulated and 281 downregulated genes) between CAD and control samples. In the PPI network, EGF (down, degree = 16) was a hub node. Additionally, we screened four significant network modules (modules A, B, C, and D) and observed that each node was individually implicated in the inflammatory response, protein ubiquitination involved in ubiquitin-dependent proteasome catabolic process, protein transport, and mitochondrial translational elongation. Furthermore, we built a TF-miRNA target regulatory network.

In patients with CAD, TGFBR2 polymorphism is correlated with the risk of sudden cardiac arrest induced by ventricular arrhythmias, suggesting that genetic variations in the TGF signaling pathway could influence susceptibility to sudden cardiac arrest [37]. TGFBR1 is reportedly overexpressed in patients with left ventricular dysfunction and is thus considered a potential prognostic factor after acute myocardial infarction [38]. The mRNA expression levels of EGFR in athrombotic lesions could be a promising prognostic biomarker for predicting the stimulatory growth factor-induced increase in smooth muscle cell proliferation [39].
Circulating miR-23a could serve as a diagnostic biomarker to indicate the presence and severity of coronary lesions in patients with CAD. Moreover, miR-23a regulates vasculogenesis in CAD by inhibiting EGFR expression [40]. In the present study, both TGFBR2 and EGF were involved in the MAPK signaling pathway, thus indicating their potential roles in CAD development.

Plasma levels of miR-122 and miR-370, which are upregulated in patients with hyperlipidemia, are positively associated with CAD severity; therefore, they may be correlated with the development and progression of CAD in patients with hyperlipidemia [41]. The expression of circulating miR-122-5p is reportedly elevated in patients with acute myocardial infarction, suggesting its application as a promising biomarker [42, 43]. The plasma levels of miR-122, miR-140-3p, miR-720, miR-2861, and miR-3149 are higher in acute coronary syndrome samples than in control samples; thus, they can be employed as potential markers in patients with acute coronary syndrome [44]. These results indicate that miR-122 plays a critical role in CAD pathogenesis.

LRP is known to possess biological functions in multiple vascular biology-associated processes. Moreover, LRP poly-
morphisms are risk factors, especially in Caucasians with premature CAD [45]. In cardiac fibroblasts, LRP1 contributes to the expression of matrix metalloproteinase 9 (MMP9), which has been associated with ventricular remodeling following myocardial infarction [46]. IQGAPI reportedly affects neovascularization after ischemia by mediating endothelial cell-regulated angiogenesis, macrophage infiltration, and reactive oxygen species production; therefore, IQGAPI is a valuable therapeutic target for ischemic cardiovascular diseases [47, 48]. As a scaffold for the extracellular signal-regulated kinase (ERK)1/2 cascade, IQGAPI mediates the integration of hypertrophy and survival signals in the heart, facilitating left ventricular remodeling following pressure overload [49]. Therefore, both LRP10 and IQGAPI were targeted by miR-122A in the regulatory network, implying a probable correlation between miR-122A and CAD via the regulation of LRP10 and IQGAPI.

5. Conclusions

In total, 456 DEGs were screened in CAD samples. Herein, we revealed the probable involvement of TGFBR2, EGF, LRP10, IQGAPI, and miR-122 in CAD pathogenesis. The functions of these genes and miRNAs in CAD pathogenesis need to be comprehensively validated in future experimental research.

Author contributions

Conception and design of the research: WZ and XL; acquisition of data: NW, TL and GZ; analysis and interpretation of data: SF and LL; Statistical analysis: XL and YH; drafting the manuscript: YD; revision of manuscript for important intellectual content: WZ. All authors read and approved the final manuscript.
Table 2. The GO functional terms enriched for the nodes in modules A, B, C, and D.

| Module | Biological process | GO ID | Term | Count | P value | Genes |
|--------|-------------------|-------|------|-------|---------|-------|
| Module A | GO:0006954 inflammatory response | GO:0070125 | mitochondrial translation elongation | 5 | 3.05E-06 | CSAR1, CSAR1, C3, CCR1, CXCR2 |
| GO:0045766 positive regulation of angiogenesis | 4 | 6.17E-06 | CSAR1, CSAR1, C3, CXCR2 |
| GO:0006935 | 4 | 7.37E-06 | CSAR1, CSAR1, CXCR1, CXCR2 |
| GO:0007204 | 4 | 9.77E-06 | CSAR1, CSAR1, SIPR4, CXCR2 |
| GO:0090023 positive regulation of neutrophil chemotaxis | 3 | 2.45E-06 | CSAR1, CSAR1, CXCR2 |
| GO:0010575 | 3 | 3.72E-05 | CSAR1, CSAR1, C3 |
| GO:0030449 regulation of complement activation | 3 | 4.61E-05 | CSAR1, CSAR1, C3 |
| GO:0007200 phospholipase C-activating G-protein coupled receptor signaling pathway | 3 | 2.26E-04 | CSAR1, CSAR1, CXCR2 |
| Module B | GO:0038112 interleukin-8 mediated signaling pathway | GO:0006412 | translation | 4 | 3.31E-04 | |
| GO:0070126 mitochondrial translational termination | 5 | 3.19E-05 | |
| GO:0007173 epidermal growth factor receptor signaling pathway | 2 | 1.66E-04 | |
| GO:0043161 proteasome-mediated ubiquitin-dependent protein catabolic process | 2 | 3.92E-03 | |
| GO:0000209 protein polyubiquitination | 2 | 6.06E-03 | CXCR1, CXCR2 |
| Module C | GO:00301623 receptor internalization | GO:0000655 | immune response | 2 | 8.80E-03 | |
| GO:000324 dendritic cell chemotaxis | 2 | 9.26E-03 | |
| GO:0006968 cellular defense response | 2 | 9.53E-02 | CXCR1, CXCR2 |
| GO:00063026 cell chemotaxis | 2 | 2.20E-02 | |
| GO:0010575 | 2 | 2.30E-02 | CSAR1, CSAR1 |
| GO:00035939 neutrophil chemotaxis | 2 | 2.34E-02 | |
| GO:00070098 chemokine-mediated signaling pathway | 2 | 2.51E-02 | |
| GO:00050900 leukocyte migration | 2 | 4.28E-02 | |
| Module D | GO:00042787 protein ubiquitination involved in ubiquitin-dependent protein catabolic process | GO:0002009 | protein polyubiquitination | 4 | 5.14E-06 | SIAH1, RNF19B, RNF111 |
| GO:0043161 proteasome-mediated ubiquitin-dependent protein catabolic process | 2 | 4.75E-02 | SIAH1, RNF111 |
| Module E | GO:0015031 protein transport | GO:0015031 | protein transport | 3 | 5.27E-03 | |
| GO:007173 epidermal growth factor receptor signaling pathway | 2 | 1.66E-02 | |
| Module F | GO:00070212 mitochondrial translational elongation | GO:00070122 mitochondrial translational termination | 5 | 3.05E-09 | |
| GO:0006412 translation | 4 | 3.31E-05 | | 

Table 3. The 10 overlapped pathways in VENN analysis for pathways.

| Disease name | Disease ID | Category | Term | Count | P value | Genes |
|--------------|------------|----------|------|-------|---------|-------|
| CAD | MESH:0003324 KEGG_PATHWAY | has04672: Intestinal immune network for IgA production | 4 | 2.10E-10 | CD86, IL15, HLA-DMB, HLA-DMA |
| CAD | MESH:0003324 KEGG_PATHWAY | has05611: Glycolipid metabolism | 4 | 3.62E-10 | AKR1A1, DGKH, AGK, ALDH5A2 |
| CAD | MESH:0003325 KEGG_PATHWAY | has04144: Endocytosis | 14 | 4.61E-10 | CHMP2A, RAB5B, TGFBR2, CXCR1, CXCR2, VPS7C, EPC1SL1, KIT, IGF2B1, HSPA6, GIT2, RAB11A, EGF, IQSEC1 |
| CAD | MESH:0003326 KEGG_PATHWAY | has04068: FoxO signaling pathway | 9 | 2.09E-09 | | |
| CAD | MESH:0003327 KEGG_PATHWAY | has05219: Bladder cancer | 5 | 5.18E-10 | RPS6KA5, EIF3, RAF1, MAP2K2, EGFR |
| CAD | MESH:0003328 KEGG_PATHWAY | has04010: MAPK signaling pathway | 11 | 1.22E-10 | RPS6KA5, SOS1, TGFBR2, SOS2, MAP2K4, HSPA6, RAF1, CACNB4, RAPGEF2, EGF, DUSP6 |
| CAD | MESH:0003329 KEGG_PATHWAY | has05215: Prostate cancer | 6 | 1.75E-10 | EIF3, EP300, SOS1, SOS2, RAF1, EGFR |
| CAD | MESH:0003330 KEGG_PATHWAY | has05212: Pancreatic cancer | 6 | 2.53E-10 | EIF3, RAF1B, TGFBR2, RAF1, EGFR |
| CAD | MESH:0003331 KEGG_PATHWAY | has05200: Pathways in cancer | 13 | 3.74E-10 | EIF3, RAF1B, TGFBR2, STAT5B, RAF1, FADD, KIT, MAP2K2, EP300, SOS1, SOS2, TPR, EGF |
| CAD | MESH:0003332 KEGG_PATHWAY | has05161: Hepatitis B | 7 | 3.87E-10 | EIF3, EP300, DDX3X, STAT5B, MAP2K4, RAF1, FADD |

CAD, coronary artery disease.
Fig. 7. The transcription factor (TF)-microRNA (miRNA) target regulatory network. Yellow circles and green prisms indicate upregulated and downregulated genes, respectively. Blue hexagons and red triangles indicate TFs and miRNAs, respectively. Arrows indicate regulatory directions.

Fig. 8. The results of Venn analysis using the CTD database. (A) Venn diagram showing 429 overlapping genes between CAD-associated genes and differentially expressed genes. (B) Venn diagram showing 10 overlapping pathways between CAD-associated pathways and 18 significant KEGG pathways. CTD, Comparative Toxicogenomics Database; CAD, Coronary artery disease; KEGG, Kyoto Encyclopedia of Genes and Genomes.
Ethics approval and consent to participate
Not applicable.

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Conflict of interest
The authors declare no conflict of interest.

Supplementary material
Supplementary material associated with this article can be found, in the online version, at https://rcm.imrpress.com/E N/10.31083/j.rcm2203103.

Data availability
The data used to support the findings of this study are available from the corresponding author upon request.

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