Clinical Trial/Experimental Study

Medicine®

OPEN

In silico analysis of the molecular regulatory networks in peripheral arterial occlusive disease

Xuwen Guan, MD[a], Xiaoyan Yang, MD[b], Chunming Wang, PHD[c], Renbing Bi, MD[a,*]

Abstract

Background: Peripheral arterial occlusive disease (PAOD) is a global public health concern that decreases the quality of life of the patients and can lead to disabilities and death. The aim of this study was to identify the genes and pathways associated with PAOD pathogenesis, and the potential therapeutic targets.

Methods: Differentially expressed genes (DEGs) and miRNAs related to PAOD were extracted from the GSE57691 dataset and through text mining. Additionally, bioinformatics analysis was applied to explore gene ontology, pathways and protein–protein interaction of those DEGs. The potential miRNAs targeting the DEGs and the transcription factors (TFs) regulating miRNAs were predicted by multiple different databases.

Results: A total of 59 DEGs were identified, which were significantly enriched in the inflammatory response, immune response, chemokine-mediated signaling pathway and JAK-STAT signaling pathway. Thirteen genes including IL6, CXCL12, IL1B, and STAT3 were hub genes in protein–protein interaction network. In addition, 513 miRNA-target gene pairs were identified, of which CXCL12 and PTPN11 were the potential targets of miRNA-143, and IL1B of miRNA-21. STAT3 was differentially expressed and regulated 27 potential target miRNAs including miRNA-143 and miRNA-21 in TF-miRNA regulatory network.

Conclusion: In summary, inflammation, immune response and STAT3-mediated miRNA-target genes axis play an important role in PAOD development and progression.

Abbreviations: BP = biological process, CC = cellular component, DAVID = database for annotation, visualization and integrated discovery, DEGs = differentially expressed genes, DETFs = differentially expressed TFs, GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes, MF = molecular function, PAOD = Peripheral arterial occlusive disease (PAOD), PPI = protein–protein interaction, TFs = transcription factors (TFs).

Keywords: bioinformatics, miRNAs, regulatory network, peripheral arterial occlusive disease, transcription factor

Editor: Giovanni Tarantino.

This work was supported by the Science Foundation of the People's Hospital of Guangxi Zhuang Autonomous Region (Grant No. QN2017–11).

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

The data used to support the findings of this study are available in the supplementary information. Additionally, the dataset GSE57691 can be obtained from the GEO database (https://www.ncbi.nlm.nih.gov/gds/?term=GSE57691).

[a] Department of Vascular Intervention, [b] Geriatric Department, First People's Hospital of Jingmen City, Jingmen, Hubei Province, [c] Department of Intervention, the People's Hospital of Guangxi Zhuang Autonomous Region, Nanning, Guangxi, China.

[*] Correspondence: Renbing Bi, Department of Vascular Intervention, First People's Hospital of Jingmen City, Jingmen 448000, Hubei Province, China (e-mail: brenbing@163.com)

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Guan X, Yang X, Wang C, Bi R. In silico analysis of the molecular regulatory networks in peripheral arterial occlusive disease. Medicine 2020;99:21(e20404).

Received: 15 January 2020 / Received in final form: 18 April 2020 / Accepted: 24 April 2020

http://dx.doi.org/10.1097/MD.0000000000020404

1. Introduction

Peripheral artery occlusion disease (PAOD) is an atherosclerotic condition involving non-cardiac and non-cerebral arteries. Approximately 202 million cases of PAOD were diagnosed worldwide in 2010,[1] and is estimated to affect almost 41.13 million people in China by 2020 due to a rapidly ageing population.[2] PAOD results in severe arterial stenosis and insufficient blood supply to the distal limb causing intermittent cicada, resting pain, disability and even death. PAOD-related mortality rate increases from $0.07/10^5$ in 40 to 44-year age group to $28.71/10^5$ among those older than 80 years. The highest proportion of patients with disability-adjusted life years in 2010 was $47.88/10^5$ in Western Europe.[3] PAOD is closely associated with coronary events and cerebrovascular disease,[4,5] and other risk factors include smoking, diabetes, hypertension, hypercholesterolaemia, and aging. Although anti-inflammatory, anticoagulant and antiplatelet drugs, surgical intervention and endovascular treatments[6–9] can improve blood supply and reduce PAOD complications, the atherosclerotic lesions and arterial stenosis are unaffected. Gene and cell therapies have been developed in recent years, but the therapeutic outcomes have been less than satisfactory.[10–12] Therefore, it is essential to dissect the molecular basis of PAOD pathogenesis in order to identify novel therapeutic targets.

MicroRNAs (miRNAs) are a class of endogenous single-chain non-coding RNAs consisting of 18 to 22 nucleotides that regulate gene expression at the post-transcriptional level by binding to a seed sequence.[13] miRNAs are associated with a wide range of

physiological and pathological processes, and have been identified as therapeutic targets for various conditions, including cardiovascular diseases.[14] For example, circulating levels of miR-21, miR-218, and miR-211 are potential diagnostic fingerprints of diabetic atherosclerosis.[15] In addition, miR-172 was identified as a target of anti-atherogenic drugs in the ApoE-/- atherosclerotic mouse model.[16] Recently, miRNA-21 and miRNA-143 were identified as biomarkers of peripheral arterial disease and vascular restenosis after endovascular treatment,[17–19] indicating their involvement in the development and progression of PAOD.

Transcription factors (TF) regulate gene expression by binding to the promoter of target genes, and are often dysregulated during pathological conditions. Studies show that some recombinant TFs can promote angiogenic growth factor expression and improve perfusion in limb ischemia and angiogenesis.[20–22] STAT3 is closely related to atherosclerosis, and its inhibition reduced atheromatous plaque formation both in rabbit and murine atherosclerosis models.[23–25] MiRNA-21, the confirmed biomarker of PAOD, is also a target of STAT3.[26] Taken together, a hitherto unknown TF/miRNA axis likely plays an important role in driving PAOD. In this study, we mined gene expression data of PAOD and control specimens from published studies and transcriptomic datasets, and identified crucial signaling pathways, genes and miRNAs involved in PAOD. We also constructed miRNA/target gene and TF/miRNA/target gene networks relevant to PAOD. Our findings provide new insights into the pathogenesis of PAOD and identify potential therapeutic targets.

2. Methods

2.1. Data collection

The GSE57691 dataset based on GPL10558 Illumina HumanHT-12 V4.0 expression beadchip, including gene expression data of aortic specimens from 9 AOD and 10 control donors, was downloaded from the gene expression omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). Differentially expressed genes (DEGs) between the AOD and control aortic specimens were identified using the GEO2R online tool, with $|\text{LogFC}| > 1$ and $P < .05$ as the criteria, and presented in the form of Volcano plots using the ggplot2 package in R software. Genes related to PAOD were mined from published genomic data and MEDLINE literature using pubmed2ensembl (http://pubmed2ensembl.ls.manchester.ac.uk/),[27] with "Peripheral arterial occlusive disease," "Homo sapiens genes (GRCh37) database," and "filter on MEDLINE" as the queries. The genes common to the DEGs in GSE57691 and identified from text mining were analyzed further, and henceforth referred to as PAOD-related genes. The miRNAs associated with PAOD were similarly mined from the PubMed database using search terms "Arterial occlusive disease" [Title/Abstract], "Peripheral arterial occlusive disease" [Title/Abstract], "Lower extremity arterial occlusive disease" [Title/Abstract], "peripheral arterial disease" [Title/Abstract] AND "miRNA" OR "microRNA". Additional approval by an ethics committee was not necessary because the datasets included in the current study were downloaded from public databases, and data acquisition and application were performed according to GEO publication guidelines and data access policies.

2.2. Gene ontology (GO) and pathway enrichment analysis

The database for annotation, visualization and integrated discovery (DAVID) version 6.8 (https://david.ncifcrf.gov/home.jsp) was used for the GO functional annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of the PAOD-related genes identified above. P value $< .05$ was set as the threshold for the enriched GO terms and significant pathways.

2.3. Protein–protein interaction (PPI) network and modular analysis

The PPI network of the relevant genes was constructed using STRING (https://string-db.org/) version 11,[28] and analyzed by the Cytoscape software. The nodes with more than 10 degrees in the PPI were considered hub genes. Modular analysis of PPI was also performed with MCODE app in Cytoscape software, and the subsequent enrichment analysis in DAVID database.

2.4. Prediction of cognate miRNAs of PAOD-related genes and construction of the miRNA-target gene regulatory network

The miRNAs targeting the PAOD-related were predicted using the miRanda (http://www.microrna.org/), miRDB (http://www.mirdb.org/), miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk/), RNA22 (https://cm.jefferson.edu/rna22/Interactive/), and TargetScan (http://www.targetscan.org/vert_72/) databases, and those predicted by at least 4 databases were selected for constructing the miRNA-target gene regulatory network by Cytoscape software.

2.5. Prediction of transcription factors of miRNA and construction of the TF-miRNA-target gene regulatory network

The TFs regulating miRNAs in the miRNA-target gene regulatory network were predicted using literature-curated TF-miRNA regulation data in the TransmiR (http://www.cuilab.cn/transmir) database.[29] The genes common to the predicted TFs and PAOD-related genes were considered differentially expressed TFs (DETFs). The TF-miRNA-target gene, DETF- miRNA-target and PPI hub gene-miRNA-target gene regulatory networks were constructed using Cytoscape software. The TF binding sites on miRNAs and the miRNA binding sites on target genes were predicted by the JASPAR (http://jaspar2018.genereg.net) and microRNA.org (http://www.microrna.org/microrna/) databases respectively.

3. Results

3.1. Identification of PAOD-related genes

A total of 1705 DEGs were identified in the GSE57691 dataset, including 75 up-regulated and 1630 down-regulated genes (Fig. 1 and Supplementary data-1, http://links.lww.com/MD/E277). In addition, 591 genes related to PAOD were identified after text mining (Supplementary data-2, http://links.lww.com/MD/E278). The overlap of both gene sets revealed 59 genes, including 15 up-regulated and 44 down-regulated genes (Fig. 2 and Table 1), that were henceforth analyzed as PAOD-related DEGs. Sixteen miRNAs, including miRNA-320a, miRNA-572, miRNA-21 and miRNA-143, were among the PADO-related DEGs (Supplementary data-3, http://links.lww.com/MD/E279).

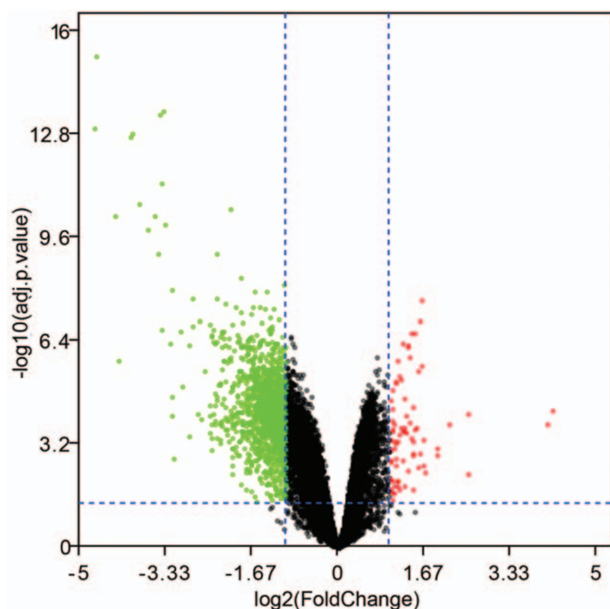

Figure 1. Volcano plot showing DEGs between PAOD and control samples in the GSE57691 dataset. Green nodes indicate down-regulated genes with logFC < -1 and *P* < .05. Red nodes indicate up-regulated genes with logFC > 1 and *P* < .05. FC = fold change.

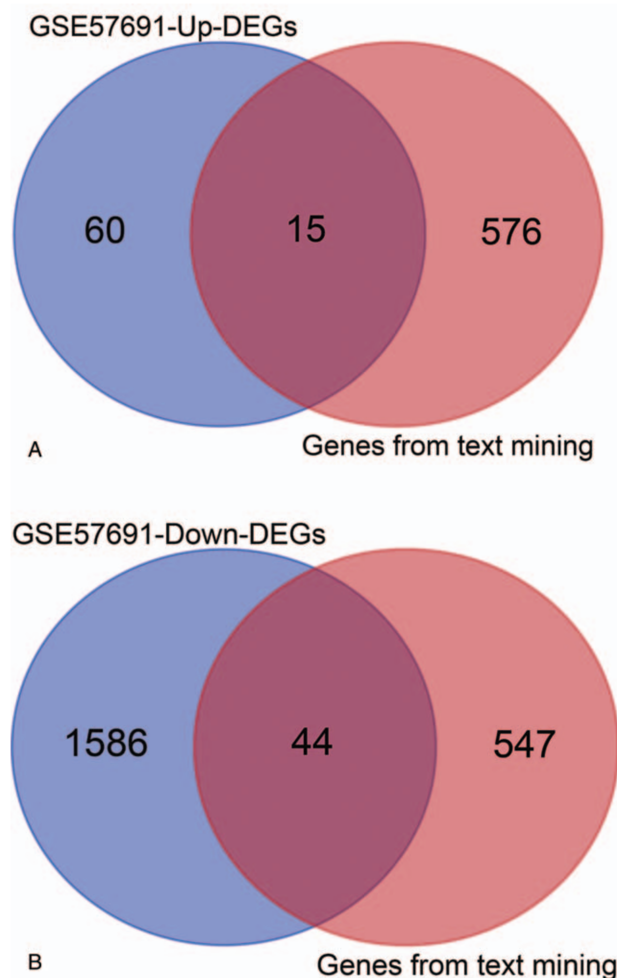

Figure 2. Venn diagram showing DEGs in GSE57691 and from text mining. (A) GSE57691 and text mining respectively identified 60 and 576 up-regulated genes, with 15 overlapping genes, and (B) 1586 and 547 down-regulated genes, with 44 common genes. DEGs = differentially expressed genes.

3.2. GO annotation and KEGG pathway enrichment analyses

To determine the biological relevance of the overlapping PAOD-related DEGs, we next determined the biological process (BP), cellular component (CC) and molecular function (MF) annotations of GO analysis. The significantly enriched BP terms included inflammatory response (GO:0006954, GO:0002675), immune response (GO:0006955), cell adhesion (GO:0007155), regulation of cell proliferation (GO:0008284, GO:0008285), regulation of apoptotic process (GO:0043066), regulation of ERK1 and ERK2 cascade (GO:0070374), regulation of MAPK cascade (GO:0000187, GO:0043410), and cytokine and chemokine-mediated signaling pathway (GO:0019221, GO:0070098) (Fig. 3A and Supplementary data-4, http://links.lww.com/MD/E280). Protein binding (GO:0005515), growth factor activity (GO:0008083), chemokine activity (GO:0008009), cytokine activity (GO:0005125) and cell adhesion molecule binding (GO:0050839) were the significantly enriched MF terms among the DEGs (Fig. 3B and Supplementary data-4, http://links.lww.com/MD/E280). Finally, extracellular space (GO:0005615), extracellular region (GO:0005576), extracellular exosome (GO:0070062), plasma membrane (GO:0005886, GO:0005887), cell surface (GO:0009986) and focal adhesion (GO:0005925) were the significantly enriched CC terms (Fig. 3C and Supplementary data-4, http://links.lww.com/MD/E280). The KEGG pathway analysis showed that DEGs were mainly associated with hsa04510: Focal adhesion, hsa04060: Cytokine-cytokine receptor interaction, hsa04062: Chemokine signaling pathway, hsa04670: Leukocyte transendothelial migration, hsa04151: PI3K-Akt signaling pathway, and hsa04630: Jak-STAT signaling pathway (Fig. 3D and Supplementary data-4, http://links.lww.com/MD/E280).

3.3. PPI network and modular analysis

To determine the potential interactions between the DEGs and identify the hub genes, a PPI network was established using 47 genes (12 up- and 35 down-regulated genes) and 180 edges (Fig. 4). IL6, CXCL12, IL1B, ITGB1, APP, CAV1, STAT3, CTGF, SPARC, CDC42, SOD1, PPARG, and TIMP2 had more than 10 degrees, and were defined as hub genes. In addition, module1 and module2 were clustered from the PPI network. The former had 8 genes and 21 edges that were enriched in regulation of cell proliferation (GO:0008285, GO:0042102), regulation of MAPK cascade (GO:0043410), extracellular region (GO:0005576), positive regulation of JNK cascade (GO:0046330), and hsa04621: NOD-like receptor signaling pathways (Fig. 5A and Supplementary data-5, http://links.lww.com/MD/E281). The latter consisted of 14 genes and 31 edges, and were enriched in chemotaxis (GO:0006935, GO:0070098, GO:0060326, GO:0030593, GO:0008009), immune response (GO:0006955), cell adhesion (GO:0007155, GO:0005925, GO:0050839), inflammatory response (GO:0002523), and hsa04062: Chemokine signaling pathway and hsa05205: Proteoglycans in cancer pathways (Fig. 5B and Supplementary data-5, http://links.lww.com/MD/E281).

Table 1

A total of 59 shared DGEs between GSE57691 dataset and gene sets obtained from text mining were found, including 15 up-regulated and 44 down-regulated genes.

DEGs	Gene symbol	LogFC	Adj.P.Val	Gene symbol	LogFC	Adj.P.Val
Up-regulated	HBA2	4.166594	5.74E-05	SELL	1.30272	0.000277
	IL1B	2.138769	0.000148	PLAUR	1.294895	0.000266
	UCP2	1.901167	0.00142	PADI4	1.274643	2.46E-05
	IL6	1.666395	0.00309	OSM	1.196142	0.000339
	PPBP	1.540993	0.00346	GP9	1.063533	0.00024
	FCGR3B	1.495811	0.000224	ANPEP	1.046855	0.0339
	CD74	1.455451	0.0163	TPSAB1	1.013982	0.00829
	MMP12	1.349807	0.00433			
Down-regulated	PPARG	−1.00974	0.00283	CAV1	−1.3363	0.00998
	TNC	−1.02082	0.00866	MIF	−1.34113	0.000265
	CBS	−1.02935	0.000194	CDC42	−1.37473	8.39E-06
	STAT3	−1.03363	0.00118	VCAN	−1.40439	0.00399
	MTRR	−1.04187	3.62E-05	NME1	−1.41127	0.000145
	TIMP2	−1.06174	0.00164	PCSK5	−1.41626	1.88E-05
	AKTIP	−1.0704	0.000282	PRNP	−1.44203	6.14E-05
	CTGF	−1.07695	0.0171	SPARC	−1.4667	0.00993
	DYM	−1.08247	3.06E-06	DCBLD2	−1.47484	0.000111
	ADD1	−1.08806	0.000168	SCD	−1.50764	0.00672
	ITGB1	−1.13283	0.00107	SOD1	−1.53474	3.21E-05
	PDGFA	−1.14341	4.88E-05	PTS	−1.60866	3.85E-05
	LDLR	−1.16187	0.0171	USF1	−1.60919	1.15E-08
	CX3CL1	−1.17786	0.00196	TFPI	−1.6193	4.44E-06
	ALOX5AP	−1.213	0.0166	ATP5J	−1.64762	3.83E-08
	GYPC	−1.23336	0.000323	APP	−1.72256	2.22E-05
	LRP5	−1.24713	0.00028	PHGDH	−1.75726	0.000439
	BLVRA	−1.24806	4.02E-05	PTPN11	−1.79375	3.97E-05
	GJA1	−1.26923	0.00342	RGS5	−1.87373	0.0119
	ITGA1	−1.2724	0.000249	MCAM	−2.15453	0.000114
	CXCL12	−1.27394	0.00672	SLC25A4	−2.36857	0.000125
	A2M	−1.32275	0.00164	SERPINA3	−3.04854	1.94E-07

DGEs = Differentially expressed genes, LogFC = log fold change, Adj.P.Val = Adjust P value.

3.4. The miRNA-target gene regulatory network

To identify the potential miRNAs involved in PAOD, those targeting the PAOD-related DEGs were predicted using the miRanda, miRDB, miRWalk, RNA22, and TargetScan databases, which revealed 311 miRNAs (Supplementary-data-6, http://links.lww.com/MD/E282) including hsa-miR-143 and hsa-miR-21. A total of 513 miRNA-target gene pairs were identified, including miRNA-143-PTPN11, miR-143-CXCL12 and miRNA-21-IL1B. A PPI network was constructed with 311 miRNAs, 40 target genes and 513 edges (Fig. 6).

3.5. TF-miRNA-target gene regulatory network analysis

The TFs regulating the PAOD-related miRNAs were predicted using TransMir, which revealed 295 TFs, of which STAT3, PPARG, USF1, PDGFA, IL1B, and IL6 were DETFs. The TF-miRNA regulatory network was constructed, and consisted of 434 nodes and 1261 edges including 295 TFs and 139 miRNAs (Fig. 7A and Supplementary data-7, http://links.lww.com/MD/E283). The hub genes identified in the PPI network were also selected to construct the TF-miRNA-target gene regulatory network (Fig. 7B), which showed 251 nodes, 191 TFs, 53 miRNAs, 10 hub genes, and 699 edges (Supplementary data-8, http://links.lww.com/MD/E284). Finally, the DETF-miRNA-target gene network was also constructed, including 39 miRNAs, 22 target genes and 6 DETFs, of which the DETF STAT3 had the highest degree (Fig. 7C and Supplementary data-9, http://links.lww.com/MD/E285). Hsa-miR-143 and hsa-miR-21 were predicted as the targets of STAT3 (Fig. 8A and B), PTPN11 as a target DEG of hsa-miR-143, and STAT3 and IL1-B as the target DEGs of hsa-miR-21 (Fig. 8C).

4. Discussion

Peripheral arterial occlusive disease (PAOD) has become more frequent due to an ageing population, and often leads to distal limb ischemia that results in reduced quality of life and death. Furthermore, PAOD is an occult condition that goes undetected in the early stages. There is no effective cure at present, and traditional open surgery and endovascular treatment has no retarding effect on the lesion. Therefore, the underlying pathological mechanism of PAOD, and the potential pathways and core genes, have gained considerable attention in recent years with the aim of identifying potential therapeutic targets. In our study, we discovered several putative PAOD-associated genes and pathways, and built miRNA-target gene and TF-miRNA-target gene regulatory networks. The inflammatory response, immune response, cell adhesion change, JAK-STAT signaling pathway and chemokine-mediated signaling pathway were significantly associated with PAOD progression. Furthermore, STAT3, IL6, IL1B and CXCL12, and the STAT3-miRNA-143/PTPN11, miR-143/CXCL12 and STAT3-miRNA-21/IL1B regulatory pairs were likely involved in PAOD development and progression.

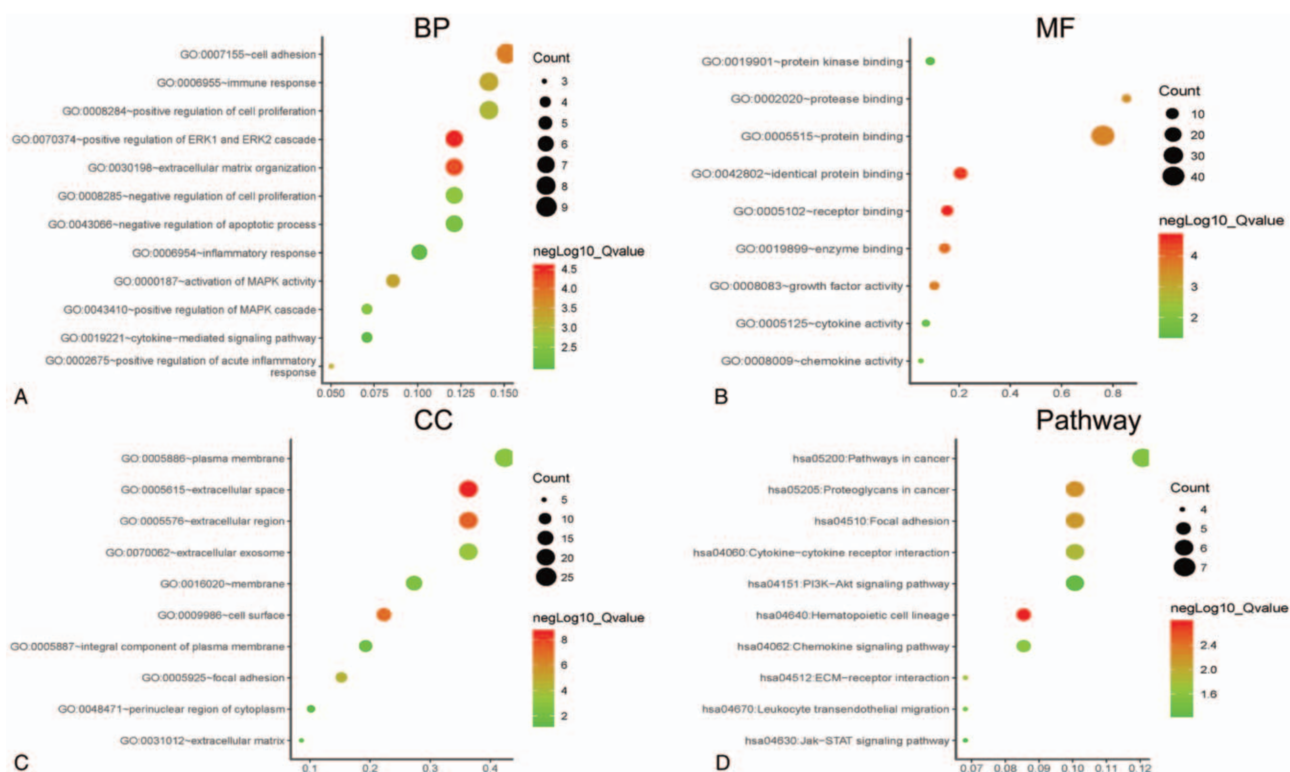

Figure 3. GO and KEGG pathway enrichment of PAOD-related DEGs. The significantly enriched (A) BP, (B) MF and (C) CC terms, and (D) pathways. BP = biological process, CC = cellular component, DEGs = differentially expressed genes, MF = molecular function.

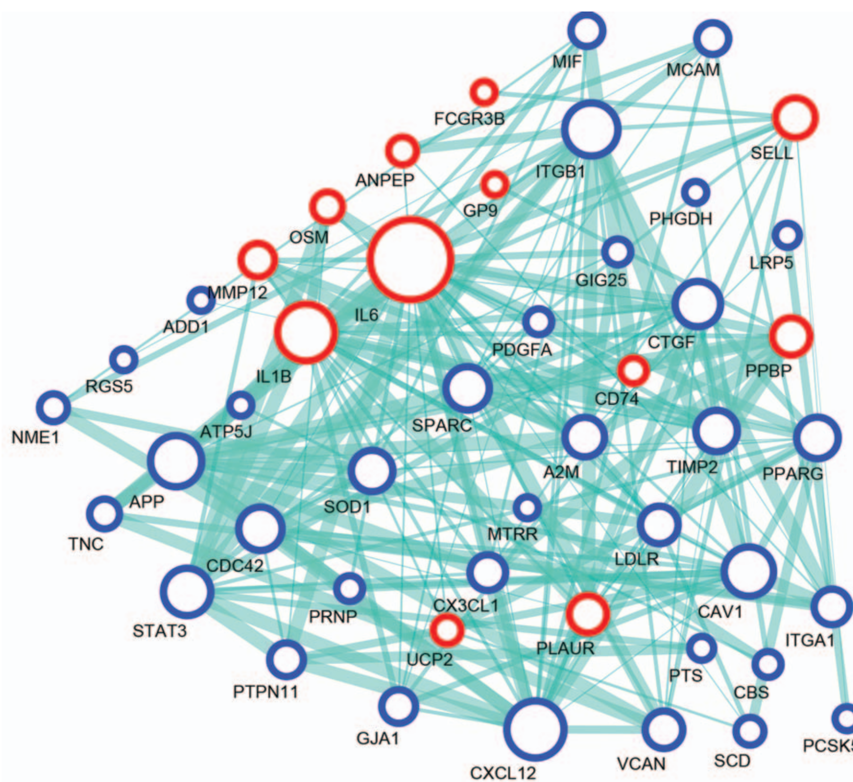

Figure 4. PPI network of PAOD-related DEGs showing 47 nodes and 180 edges. Red and blue nodes represent the up- and down-regulated genes respectively. Greater node size corresponds to higher degree. Greater edge size and darker color correspond to higher combined score. DEGs = differentially expressed genes, PPI = protein–protein interaction.

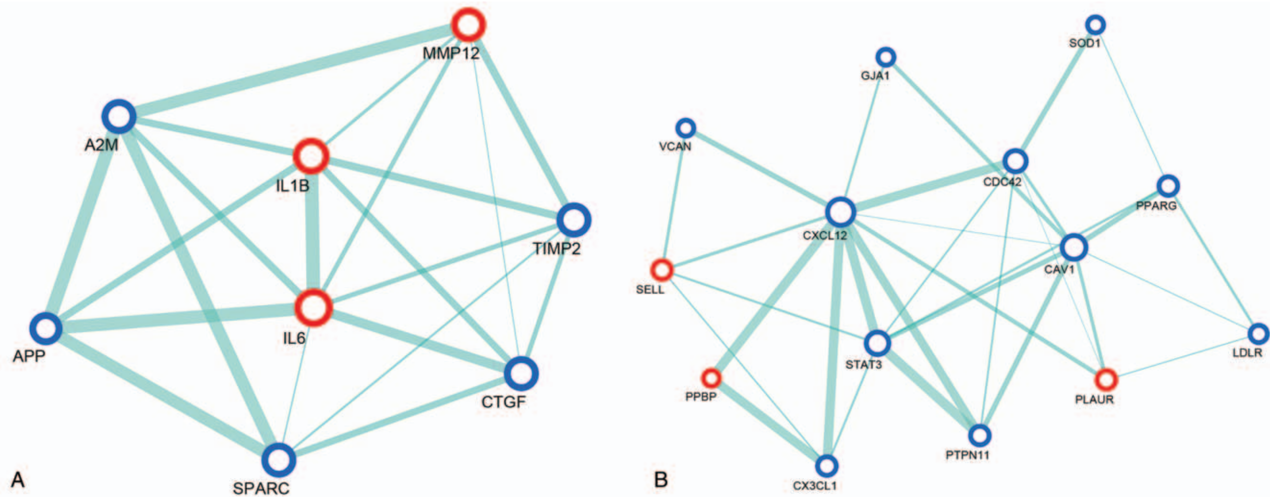

Figure 5. Module analysis of PPI network. (A) module1 and (B) module2.PPI = protein–protein network.

The major risk factors of PAOD include arteriosclerosis occlusion, thromboangiitis obliterans and multiple arteritis.[30,31] The immune-inflammatory responses and cell adhesion play important roles in all stages of arteriosclerosis,[32–34] and targeting either can prevent and attenuate disease development and progression.[35–37] In this study, inflammation, cell adhesion, and immune-related BP terms and pathways were significantly enriched in the PDAO-related DEGs, indicating a similar pathological mechanism as arteriosclerosis and the possibility of an anti-inflammatory therapeutic strategy. Consistent with our findings, Tan et al reported that the DEGs between advanced and early carotid atherosclerotic plaques were also associated with the immune system, chemokine signaling pathway and focal adhesion.[38] Zhang et al found that the chemokine signaling pathway, focal adhesion and JAK-STAT signaling pathway were enriched in the DEGs between coronary artery disease (CAD) and

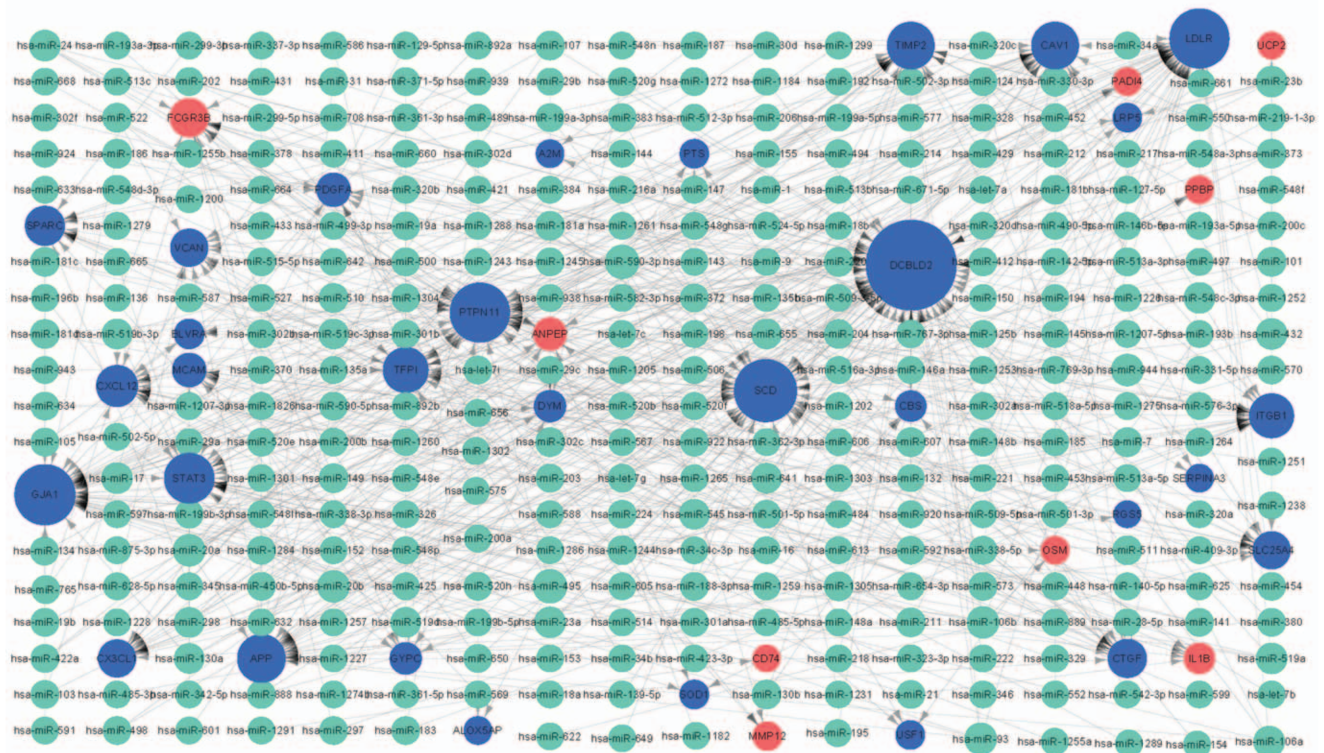

Figure 6. miRNA-target gene regulatory network. Green nodes represent miRNAs. Red and blue nodes represent up- and down-regulated target genes respectively. Greater node size corresponds to higher degree. Arrows indicate miRNA-target gene relationship.

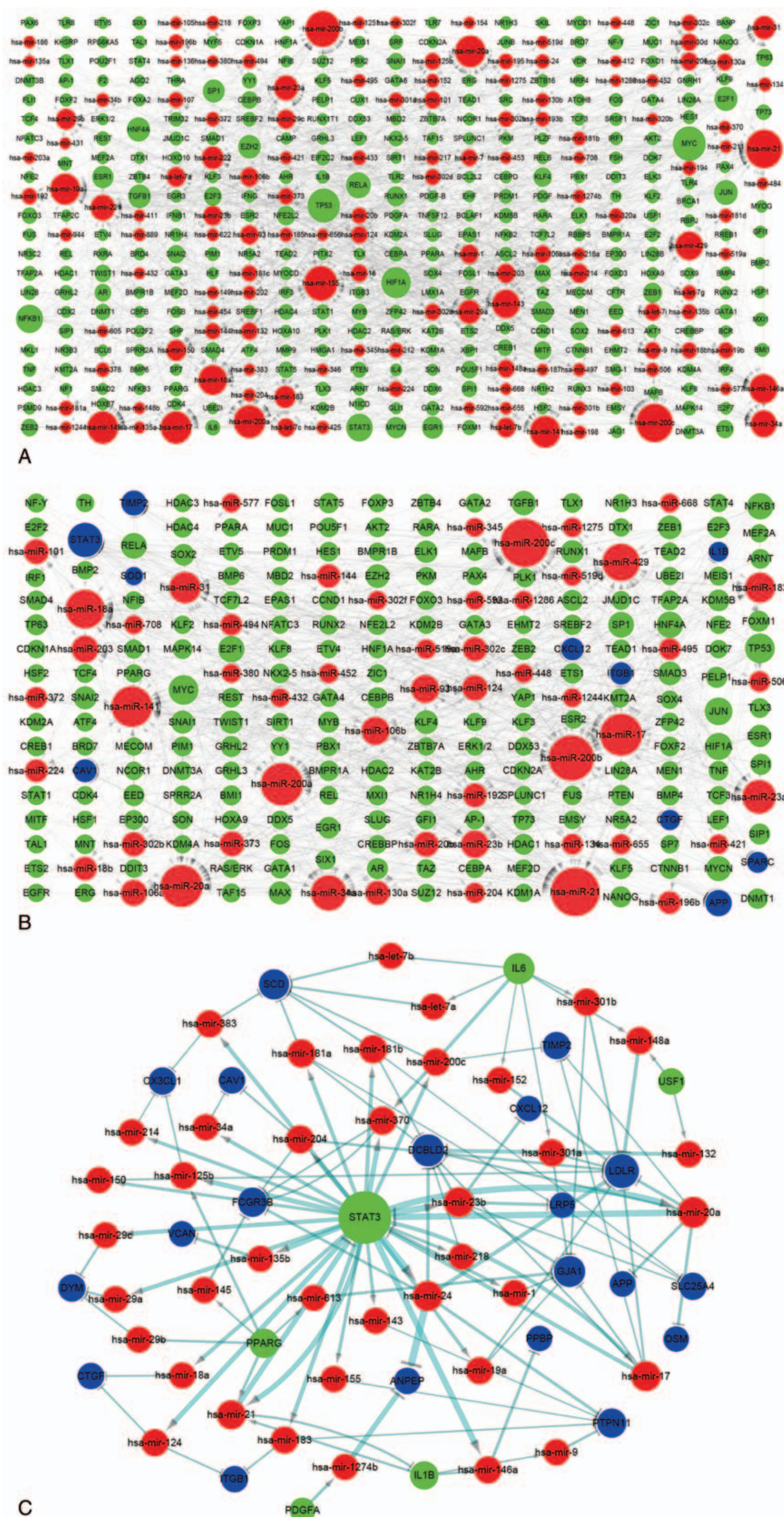

Figure 7. Molecular regulatory networks in PAOD. (A) TF-target miRNA network. (B) TF-target miRNA-target hub gene network. Blue nodes represent hub genes in PPI network. (C) DETF-target miRNA-target gene network. Red, green and blue nodes represent miRNAs, TFs and target genes respectively. Greater node size corresponds to higher degree. Arrows indicate TF-target miRNA relationship. "T" shape indicates miRNA-target gene relationship. DETF = differential expression transcription factor, PPI = protein-protein network, TF = transcription factor.

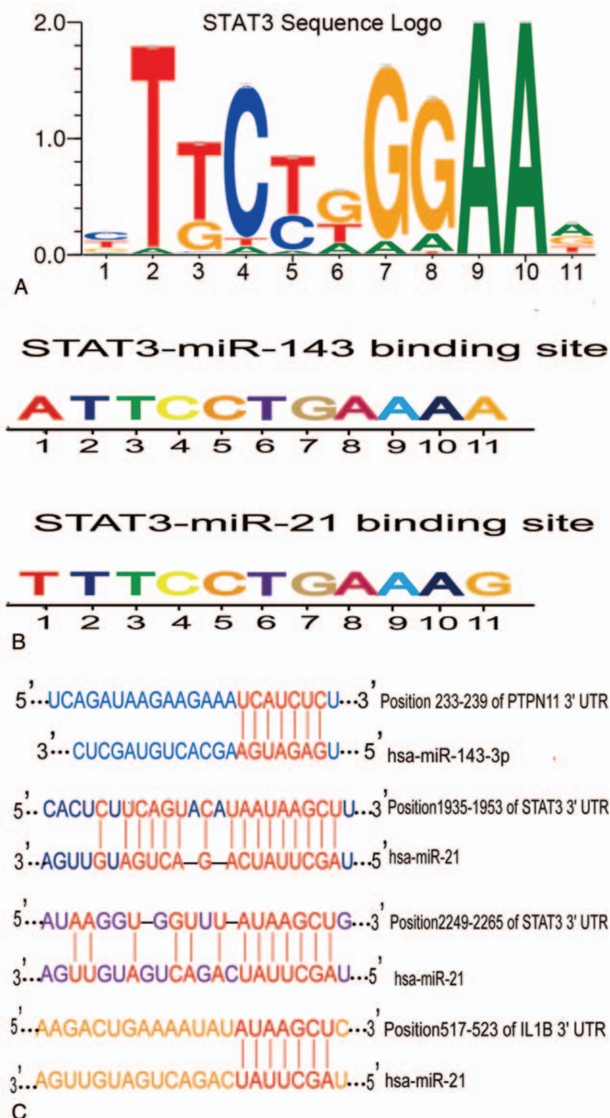

Figure 8. STAT3-target miRNA-target gene axis. (A) Binding site consensus sequence of STAT3. (B) Binding site between STAT3 and miR-143 and between STAT3 and miR-21. (C) Binding site between miR-143 and PTPN11 and between miR-21 and STAT3, IL1B.

healthy tissue samples.[39] In an animal model of atherosclerosis also, these pathways were significantly enriched,[40,41] further underscoring their therapeutic potential.

Important hub genes in the PPI network of the PAOD-related DEGs included those encoding for the pro-inflammatory cytokines IL-6 and IL-1B, the chemokine CXCL12, and the transcription factor STAT3. Polymorphisms in the IL6 promoter region are correlated to the plasma levels of atherogenic markers like fibrinogen, high sensitivity C-reactive protein, apolipoprotein A1 and high density lipoprotein (HDL) cholesterol, all of which are risk factors of CAD and atherosclerosis.[42,43] IL-6 gene polymorphisms are independent risk factors in PAOD as well, with the GG genotype of the 174 locus present at a higher frequency in PAOD patients compared to the controls, and associated with greater disease severity.[44] Similarly, the 511C/T polymorphism in IL-1B gene is associated with a greater risk of CAD among the Chinese.[45]

CXCL12 regulates the function of several immune and inflammatory cells by interacting with its cognate receptor, and its levels are increased in the sera and atherosclerotic lesions of patients with coronary artery occlusion.[46,47] Döring et al reported an athero-protective role of CXCL12, and found that CXCL12/CXCR4 signaling recruited the endothelial progenitor cells and plaque-stabilizing vascular smooth muscle progenitor cells to the lesions. However, it also activates inflammatory and immune cells, which are known to promote atherosclerosis.[48] In this study, CXCL12 was up-regulated in PAOD, and its exact role likely depended on the lesion area and the severity of atherosclerosis. In addition, CXCL12 was predicted as a target of miR143, which is correlated to postoperative stent restenosis in lower extremity AOD.[19] Therefore, the role and potential mechanism of miR-143/CXCL12 axis in PAOD needs further study.

STAT3 is a pleiotropic TF that can be activated by various cytokines. It is downregulated in the atherosclerotic lesions of the ApoE-/- mice, and its forced overexpression decrease inflammation, lipid accumulation and vascular smooth muscle cell proliferation, indicating an athero-protective function.[49] However, phosphorylated STAT3 and JAK2 aggravated IL-1B-induced adventitial inflammation in a rat model of atherosclerosis, resulting in intimal proliferation.[50] Pravastatin treatment decreased the levels of phosphorylated STAT3 in the ApoE-/- knockout mice, suggesting that STAT3 activation promotes atherosclerotic progression.[24] Taken together, STAT3 has a complex role in atherosclerosis development and progression. In our study, STAT3 expression was down-regulated and the JAK-STAT signaling pathway was also enriched, indicating a protective role in PAOD. Another study showed that STAT3 activation and the vascular endothelial growth factor receptor 1 (VEGFR1)-STAT3 signaling pathway were inhibited both in human and murine experimental peripheral arterial disease. In contrast, activation of STAT3 and the endothelial growth factor receptor 1 (EGFR1)-STAT3 pathway increased perfusion in these mice, indicating that STAT3 and its signaling pathways are potential therapeutic targets in PAOD.[51] STAT3 was the target of 15 putative miRNAs, including miRNA-21 which is increased during vascular restenosis in lower extremity AOD, and is a diagnostic marker of the same.[18] Interestingly, in the DETF-miRNA-target gene network, miRNA-21 and miRNA-143 were also predicted as target miRNAs of STAT3 and IL-1B, while PTPN11 was the target gene of both miRNAs. Chen et al reported that STAT3 upregulated miRNA-21 by binding to its promoter region, and promoted AngII-induced angiogenesis in human microvascular endothelial cells.[26] In addition, serum levels of miRNA-143 are down-regulated in patients with atherosclerosis, CAD and PAOD, and predictive of in-stent restenosis for PAOD patients.[19,52,53] Furthermore, Bai et al reported that STAT3 up-regulation induced miRNA-143 while its silencing had the opposite effect.[54] PTPN11 is involved in low-density lipoprotein cholesterol metabolism and CAD progression,[55–57] although its role in PAOD or atherosclerosis is unknown. Taken together, the STAT3/miRNA-21/IL1B and STAT3/miRNA-143/PTPN11 axes play important roles in PAOD progression.

There are several limitations in our study that should be emphasized. First, our conclusions were based on in silico data, and need to be further verified in experimental studies. In addition, the DEGs were obtained from text mining and gene expression profiles, which are derived from small samples, resulting in possible bias.

5. Conclusions

Our findings indicate that inflammation and immune responses, and chemokine-mediated signaling are pivotal in PAOD development and progression. The miR-143/CXCL12 axis and the STAT3/miR-21/miR-143/target gene axis also appear to be pathologically relevant, and should be studied further to determine their therapeutic utility.

5.1. Ethics approval and consent to participate

Approval by an ethics committee was not necessary since the datasets included in the current study were downloaded from public databases.

Acknowledgments

The authors thanked the contributors of GSE57691 dataset for sharing the gene expression data of aortic specimens from AOD and control donors on open access.

Author contributions

Renbing Bi conceived and instructed the work. Renbing Bi, Xuwen Guan, Xiaoyan Yang checked the associated database and analyze raw data. Xuwen Guan wrote and revised the manuscript. Chunming Wang provided fund support. All of the authors read and approved the final manuscript.

References

[1] Fowkes FGR, Rudan D, Rudan I, et al. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. Lancet 2013;382:1329–40.
[2] Song P, Rudan D, Wang M, et al. National and subnational estimation of the prevalence of peripheral artery disease (PAD) in China: a systematic review and meta-analysis. J Glob Health 2019;9:010601.
[3] Sampson UK, Fowkes FG, McDermott MM, et al. Global and regional burden of death and disability from peripheral artery disease: 21 world regions, 1990 to 2010. Glob Heart 2014;9:145–58. e121.
[4] Merino J, Planas A, De Moner A, et al. The association of peripheral arterial occlusive disease with major coronary events in a mediterranean population with low coronary heart disease incidence. Eur J Vasc Endovasc Surg 2008;36:71–6.
[5] Mostaza JM, Manzano L, Suarez C, et al. Prevalence of asymptomatic peripheral artery disease detected by the ankle-brachial index in patients with cardiovascular disease. MERITO II study. Med Clin 2008;131: 561–5.
[6] Antonopoulos AS, Papanikolaou E, Vogiatzi G, et al. Anti-inflammatory agents in peripheral arterial disease. Curr Opin Pharmacol 2018;39:1–8.
[7] Vos CG, Vahl AC. Anticoagulation and antiplatelet therapy in patients with peripheral arterial disease of the femoropopliteal arteries. J Cardiovasc Surg 2018;59:164–71.
[8] Vartanian SM, Conte MS. Surgical intervention for peripheral arterial disease. Circul Res 2015;116:1614–28.
[9] Kudagi VS, White CJ. Endovascular stents: a review of their use in peripheral arterial disease. Am J Cardiovasc Drugs 2013;13:199–212.
[10] Frangogiannis NG. Cell therapy for peripheral artery disease. Curr Opin Pharmacol 2018;39:27–34.
[11] Hammer A, Steiner S. Gene therapy for therapeutic angiogenesis in peripheral arterial disease - a systematic review and meta-analysis of randomized, controlled trials. VASA V 42 2013;331–9.
[12] Mughal NA, Russell DA, Ponnambalam S, et al. Gene therapy in the treatment of peripheral arterial disease. Br J Surg 2012;99:6–15.
[13] Carthew RW, Sontheimer EJ. Origins and Mechanisms of miRNAs and siRNAs. Cell 2009;136:642–55.
[14] Nouraee N, Mowla SJ. miRNA therapeutics in cardiovascular diseases: promises and problems. Front Genet 2015;6:232.
[15] Zhang JY, Gong YL, Li CJ, et al. Circulating MiRNA biomarkers serve as a fingerprint for diabetic atherosclerosis. Am J Transl Res 2016; 8:2650–8.
[16] Kheirolomoom A, Kim CW, Seo JW, et al. Multifunctional nanoparticles facilitate molecular targeting and miRNA delivery to inhibit Atherosclerosis in ApoE(-/-) Mice. ACS Nano 2015;9:8885–97.
[17] Stather PW, Sylvius N, Wild JB, et al. Differential microRNA expression profiles in peripheral arterial disease. Circulation. Cardiovasc Genet 2013;6:490–7.
[18] Zhang B, Yao Y, Sun QF, et al. Circulating mircoRNA-21 as a predictor for vascular restenosis after interventional therapy in patients with lower extremity arterial occlusive disease. Biosci Rep 2017;37:
[19] Yu ZH, Wang HT, Tu C. Diagnostic value of microRNA-143 in predicting in-stent restenosis for patients with lower extremity arterial occlusive disease. Eur J Med Res 2017;22:2.
[20] Hashiya N, Jo N, Aoki M, et al. In vivo evidence of angiogenesis induced by transcription factor Ets-1: Ets-1 is located upstream of angiogenesis cascade. Circulation 2004;109:3035–41.
[21] Dai Q, Huang J, Klitzman B, et al. Engineered zinc finger-activating vascular endothelial growth factor transcription factor plasmid DNA induces therapeutic angiogenesis in rabbits with hindlimb ischemia. Circulation 2004;110:2467–75.
[22] Li Y, Hazarika S, Xie D, et al. In mice with type 2 diabetes, a vascular endothelial growth factor (VEGF)-activating transcription factor modulates VEGF signaling and induces therapeutic angiogenesis after hindlimb ischemia. Diabetes 2007;56:656–65.
[23] Dutzmann J, Daniel JM, Bauersachs J. Hilfiker-Kleiner D and Sedding DG: Emerging translational approaches to target STAT3 signalling and its impact on vascular disease. Cardiovasc Res 2015; 106:365–74.
[24] Zhou X, Li D, Yan W, et al. Pravastatin Prevents Aortic Atherosclerosis via Modulation of Signal Transduction and Activation of Transcription 3 (STAT3) to Attenuate Interleukin-6 (IL-6) Action in ApoE Knockout Mice. Int J Mol Sci 2008;9:2253–64.
[25] Chithra PK, Jayalekshmy A, Helen A. Petroleum ether extract of Njavara rice (Oryza sativa) bran upregulates the JAK2-STAT3-mediated anti-inflammatory profile in macrophages and aortic endothelial cells promoting regression of atherosclerosis. Biochem Cell Biol 2017;95: 652–62.
[26] Chen LY, Wang X, Qu XL, et al. Activation of the STAT3/microRNA-21 pathway participates in angiotensin II-induced angiogenesis. J Cell Physiol 2019;234:19640–54.
[27] Baran J, Gerner M, Haeussler M, et al. pubmed2ensembl: a resource for mining the biological literature on genes. PLoS One 2011;6:e24716.
[28] Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucl Acids Res 2019; 47:D607–13.
[29] Tong Z, Cui Q, Wang J, et al. TransmiR v2. 0: an updated transcription factor-microRNA regulation database. Nucl Acids Res 2019;47: D253–8.
[30] Le Hello C, Blacher J, Conard J, et al. Thrombophilias and peripheral arterial occlusive disease. J Mala Vasc 2008;33:126–36.
[31] Baumgartner I. Systemic antiatherosclerotic treatment for the peripheral arterial occlusive disease patient. Expert Opin Pharmacother 2005;6: 2181–92.
[32] Chistiakov DA, Orekhov AN, Bobryshev YV. Immune-inflammatory responses in atherosclerosis: Role of an adaptive immunity mainly driven by T and B cells. Immunobiology 2016;221:1014–33.
[33] Nus M, Mallat Z. Immune-mediated mechanisms of atherosclerosis and implications for the clinic. Exp Rev Clin Immunol 2016;12:1217–37.
[34] Chi Z, Melendez AJ. Role of cell adhesion molecules and immune-cell migration in the initiation, onset and development of atherosclerosis. Cell Adhes Migr 2007;1:171–5.
[35] Hedin U, Matic LP. Recent advances in therapeutic targeting of inflammation in atherosclerosis. J Vasc Surg 2019;69:944–51.
[36] Zhao TX, Mallat Z. Targeting the immune system in atherosclerosis: JACC state-of-the-art review. J Am Coll Cardiol 2019;73:1691–706.
[37] Sato K, Yamashita T, Shirai R, et al. Adropin contributes to anti-atherosclerosis by suppressing monocyte-endothelial cell adhesion and smooth muscle cell proliferation. Int J Mol Sci 2018;19.
[38] Tan X, Zhang X, Pan L, et al. Identification of key pathways and genes in advanced coronary atherosclerosis using bioinformatics analysis. BioMed Res Int 2017;2017:4323496.

[39] Zhang X, Cheng X, Liu H, et al. Identification of key genes and crucial modules associated with coronary artery disease by bioinformatics analysis. Int J Mol MedV 34 2014;863–9.
[40] Moreno-Viedma V, Amor M, Sarabi A, et al. Common dysregulated pathways in obese adipose tissue and atherosclerosis. Cardiovasc Diabetol 2016;15:120.
[41] Ramsey SA, Vengrenyuk Y, Menon P, et al. Epigenome-guided analysis of the transcriptome of plaque macrophages during atherosclerosis regression reveals activation of the Wnt signaling pathway. PLoS Genet 2014;10:e1004828.
[42] Hulkkonen J, Lehtimaki T, Mononen N, et al. Polymorphism in the IL6 promoter region is associated with the risk factors and markers of subclinical atherosclerosis in men: the cardiovascular risk in young finns study. Atherosclerosis 2009;203:454–8.
[43] Maitra A, Shanker J, Dash D, et al. Polymorphisms in the IL6 gene in Asian Indian families with premature coronary artery disease–the Indian Atherosclerosis Research Study. Thromb Haemost 2008;99:944–50.
[44] Flex A, Gaetani E, Angelini F, et al. Pro-inflammatory genetic profiles in subjects with peripheral arterial occlusive disease and critical limb ischemia. J Intern Med 2007;262:124–30.
[45] Zhao N, Wang X, Zhang R. Associations of interleukin gene IL1B-511C/T and IL1RN+8006T/C polymorphisms with coronary artery disease in Chinese population: meta-analysis. Chin J Cell Mol Immunol 2017; 33:1409–14.
[46] Merckelbach S, van der Vorst EPC, Kallmayer M, et al. Expression and cellular localization of CXCR4 and CXCL12 in human carotid atherosclerotic plaques. Thromb Haemos 2018;118:195–206.
[47] 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. Int J Cardiol 2017;233:23–8.
[48] Doring Y, Pawig L, Weber C, et al. The CXCL12/CXCR4 chemokine ligand/receptor axis in cardiovascular disease. Front Physiol 2014; 5:212.
[49] Wang R, Zhang Y, Xu L, et al. Protein Inhibitor of Activated STAT3 Suppresses Oxidized LDL-induced Cell Responses during Atherosclerosis in Apolipoprotein E-deficient Mice. Sci Rep 2016; 6:36790.
[50] Wang X, Chen L, Liu J, et al. In vivo treatment of rat arterial adventitia with interleukin1beta induces intimal proliferation via the JAK2/STAT3 signaling pathway. Mol Med Rep 2016;13:3451–8.
[51] Ganta VC, Choi M, Kutateladze A, et al. VEGF165b modulates endothelial VEGFR1-STAT3 signaling pathway and angiogenesis in human and experimental peripheral arterial disease. Circul Res 2017;120:282–95.
[52] Liu K, Xuekelati S, Zhang Y, et al. Expression levels of atherosclerosis-associated miR-143 and miR-145 in the plasma of patients with hyperhomocysteinaemia. BMC Cardiovasc Disor 2017;17:163.
[53] He M, Gong Y, Shi J, et al. Plasma microRNAs as potential noninvasive biomarkers for in-stent restenosis. PLoS One 2014;9:e112043.
[54] Bai Y, Zhang Y, Hua J, et al. Silencing microRNA-143 protects the integrity of the blood-brain barrier: implications for methamphetamine abuse. Sci Rep 2016;6:35642.
[55] Han X, Zhang L, Zhang Z, et al. Association between phosphatase related gene variants and coronary artery disease: case-control study and meta-analysis. Int J Mol Sci 2014;15:14058–76.
[56] Zhang H, Mo XB, Xu T, et al. Detecting novel genes for low-density lipoprotein cholesterol in European population using bioinformatics analysis. Pers Med 2016;13:225–31.
[57] Zhang H, Mo XB, Xu T, et al. Novel genes affecting blood pressure detected via gene-based association analysis. G3 (Bethesda Md) 2015;5:1035–42.