Drug discovery in cardiovascular disease identified by text mining and data analysis

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Background: Cardiovascular diseases are currently prevalent in cardiology and vascular surgery in the hospital. The purpose of this study based on text mining and microarray data analysis was designed to find some existing drugs target to gene and expand the potential new drug indications.

Methods: Firstly, we used text mining ("Atherosclerosis") and microarray data analysis (GSE28829) to obtain a common set of genes. Secondly, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analysis performed to these genes, as well as protein-protein interaction (PPI) network. Then, the significant genes clustered in the PPI network were chose to execute gene-drug interaction analysis for potential drug discovery.

Results: We got 1,788 text mining genes (TMGs) and 275 differentially expressed genes (DEGs) through text mining and data analysis, respectively. Ninety-three genes were duplicated between TMGs and DEGs, in which 89 genes were up-regulated genes and four genes were down regulated. Twenty-three genes clustered in the significant gene module. Lastly, the eight out of 23 genes can target 20 existing drugs.

Conclusions: The findings of these eight genes (*VCAM1*, *CSF1R*, *C5AR1*, *CXCR4*, *CD86*, *CCR1*, *ITGB2*, *TLR8*), which were associated with inflammatory response, target to 20 existing drugs may expand drug indications to atherosclerosis-related disease.

Keywords: Text mining; differentially expressed genes (DEGs); genetic diagnosis; drug discovery

Introduction

According to the World Health Organization (WHO) health report, atherosclerotic cardiovascular disease (ASCVD) is one of the most common cardiovascular diseases and one of the leading causes to death around the world (1). In 2012, the death toll of the cardiovascular disease reached 17.5 million, accounting for more than 31% of the global deaths. ASCVD is a chronic disease, which has two primary forms: one is ischaemic heart disease; another is a cerebrovascular disease (2). The occurrence

of ASCVD has multiple intrinsic factors, which include the accumulation of immune cells (such as microphages), production of pro-inflammatory cytokines (*CCL2*, *TNFα*, *IL-1β*, and *IL-6*) and dysfunction of endothelial and vascular smooth muscle cells (3-5). The external risk factors of cardiovascular disease include hypertension, hyperlipidemia, high cholesterol, diabetes, obesity, smoking, and an unhealthy lifestyle. Cardiovascular complications are a major threat to patients with type II diabetes and new updated strategies for prevention are urgently demanded (6). Current available myriad studies have revealed that

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atherosclerosis could occur in any arterial blood vessels, and lead to many related diseases, such as ischemic heart, peripheral artery and chronic kidney diseases, etc. (7-12).

With the development of bioinformatics, text mining and data analysis have been used in many aspects, like as the identification of potential key gene targets, signal pathway, diagnosis of diseases and prediction of the occurrence of diseases. Meanwhile, researchers have discovered a series of mechanisms of atherosclerosis by doing experiments (13). Compared with bioinformatics in the cancer field, there are fewer researches focused on cardiovascular diseases through text mining and data analysis.

In this study, we first used several bioinformatics strategies, such as text mining and microarray data analysis, to obtain the common genes, which their gene expressions have changed between advanced atherosclerosis plaque and early atherosclerosis plaque group, and the correlation between/within genes have existed in these genes. Then we clustered these genes in protein and protein interactions (PPI) and identified significant module genes that have more interactions. Lastly, drug-gene interaction of module genes was carried out in the drug gene interaction database (DGIdb), and aimed at finding some existed drugs to provide new ideas and basis for the prevention and treatment of cardiovascular disease. *Figure 1* shows the framework of this study.

Methods

Text mining, microarray data analysis

Firstly, the open-access website pubmed2ensembl was used to perform text mining (http://pubmed2ensembl. ls.manchester.ac.uk). When a keyword is entered, the pubmed2ensembl website can retrieve and extract all gene symbols found in published PubMed articles related to keyword (14). We input the "Atherosclerosis" keyword into pubmed2ensembl and then extracted all unduplicated genes, these gene sets make up the text mining genes (TMGs).

Secondly, GSE28829 raw expression profiles (.*CEL* format files) and meta clinical information data (.*soft* format file) have been retrieved and downloaded from NCBI-GEO website (15,16), which was executed on the GPL570 platform. GSE28829 datasets contain 16 advanced atherosclerosis plaques (EA) and 13 early atherosclerosis plaques (AA) from carotid artery samples (17,18). We transformed probe identification numbers into official gene symbols via the information built in GPL570 platform, the

non-mRNA probes were removed and the multiple probes to the same gene were deal with to retain the significant value as the gene expression level. Then, we used affy, affyPLM, and limma packages loaded on R/Rstudio to detect the differentially expressed genes (DEGs) between EA and AA samples (19,20). DEGs with the |log2 fold change (FC)| ≥1 and adjust P value <0.05, corrected by the BH method, as the threshold criteria were selected for the follow-up analyses.

The intersection of TMGs and DEGs were overlapping genes, and then for the next further analysis.

Gene ontology (GO) and pathway enrichment analysis

The GO analysis is a ubiquitous and useful method for annotating gene products and its features of functional characteristics. GO is categorized into three groups (biological process, cellular component, molecular function). The Kyoto Encyclopedia of Genes and Genomes (KEGG) (21) is an open access informatic database from Japan for interpreting biological function and characteristics of the organic system, generated by the gene-chip and high-throughput experiments. The GO and KEGG enrichment of Overlapping genes were analyzed via an online tool DAVID, a functional annotation bioinformatics microarray analysis website, used to gene annotation, visualization. P<0.05 was considered as statistically significant as the threshold.

Protein interaction and module analysis

STRING database (version 11.0), covering about 24.6M proteins and more than 3.1 billion interactions originated from 5.09K organisms, was known as the primary source to depict and display the interaction among various proteins. Firstly, we uploaded overlapping genes into the STRING website, and a minimum interaction score >0.4 (low confidence) was set as significant threshold. Then the TSV format file of protein-protein interaction (PPI) was downloaded, and PPI networks were constructed by Cytoscape software. Subsequently, the Molecular Complex Detection (MCODE) and STRING app built in Cytoscape was utilized to classify the significant gene modules (clusters), which have highly interconnected clusters in the PPI network. The parameters in MCODE were set by default. The genes in gene modules were performed drug-gene interaction analysis.
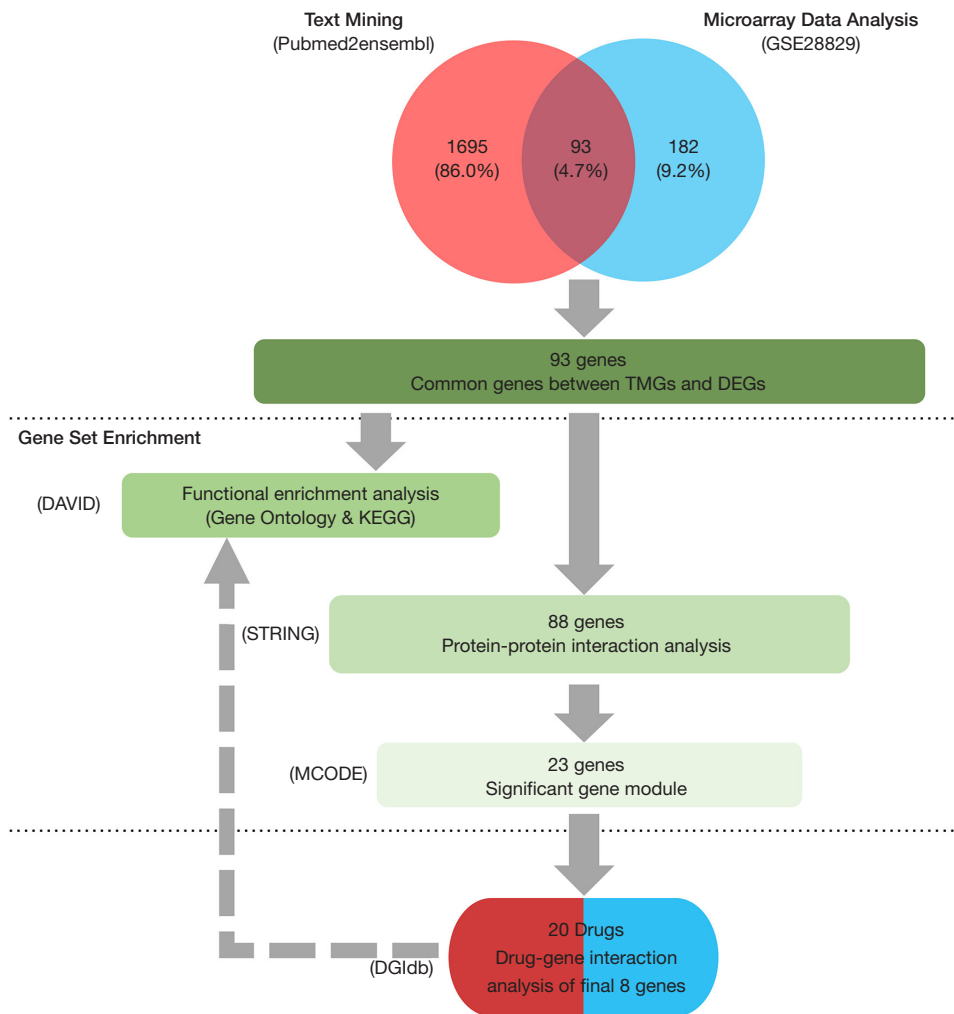
Figure 1 An overview of the workflow. Text mining for TMGs, microarray data analysis for DEGs.

Drug-gene interaction and functional analysis of potential genes

The drug-gene interaction database (DGIdb: http://www.dgidb.org) is an open-source and supports searching, browsing and filtering of information on drug-gene interactions based on over thirty trusted sources. The module genes, as the potential targets, were pasted into the drug-gene database to search for existing drugs or compounds. These potential genes which have matched drugs were obtained and also performed functional enrichment analysis.

Statistics analysis

The moderate *t*-test was applied to identify DEGs,

Fisher's Exact test was used to analyzed GO and KEGG enrichments (22). All statistical analysis was executed in R version 3.6.1 software.

Results

TMGs & DEGs acquisition

Based on the text mining and microarray data analysis strategy that is described in the methods section, 1,788 TMGs were related to atherosclerosis, 275 DEGs were identified in AA compared with EA group, and 93 genes were overlapping between TMGs and DEGs (*Figure 1*). Among these overlapping genes, 89 genes were up-regulated genes and 4 genes were down-regulated genes

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Table 1 The 93 common genes between TMGs and DEGs

| Common genes	Gene names
Up-regulated genes	CHI3L1, PTPRC, RGS1, NCF2, FCGR2A, SLC11A1, IFI16, HLA-DMA, CTSS, VCAM1*, CXCR4*, CP, IL1RN, TNFAIP3, SGK1, CD86*, SOCS3, MARCKS, IL10RA, TIE1, C2, VAMP8, MMP12, C5AR1*, CTSC, PIK3CG, APOE, SCD, UCP2, PLA2G7, CYBA, MAF, PTGS1, LIPA, HP, TNC, PLA2G2A, CCR1*, CD36, CYP1B1, CPM, ABCA1, CX3CR1, ANPEP, EDN1, CD83, LY96, SPP1, CCL19, MMRN1, RAC2, IER3, RALA, CD14, SERPINA1, VSIG4, AIF1, CCL8, CCL5, SLC2A5, CCL18, CCL4, ITGAM, ITGAX, BCL2A1, PLTP, TLR2, CSF1R*, MMP9, CD74, CD209, CXCL2, F13A1, LYN, ITGB2*, TLR8*, CXCL16, PTGER4, C3AR1, CD163, ADAMDEC1, ADAM28, ALOX5AP, FCGR2B, TIAM1, MSR1, CTSB, EPB41L3
Down-regulated genes	RBP4, KCNMA1, MYOCD, CNN1, PPP1R1A

*, the final eight genes.
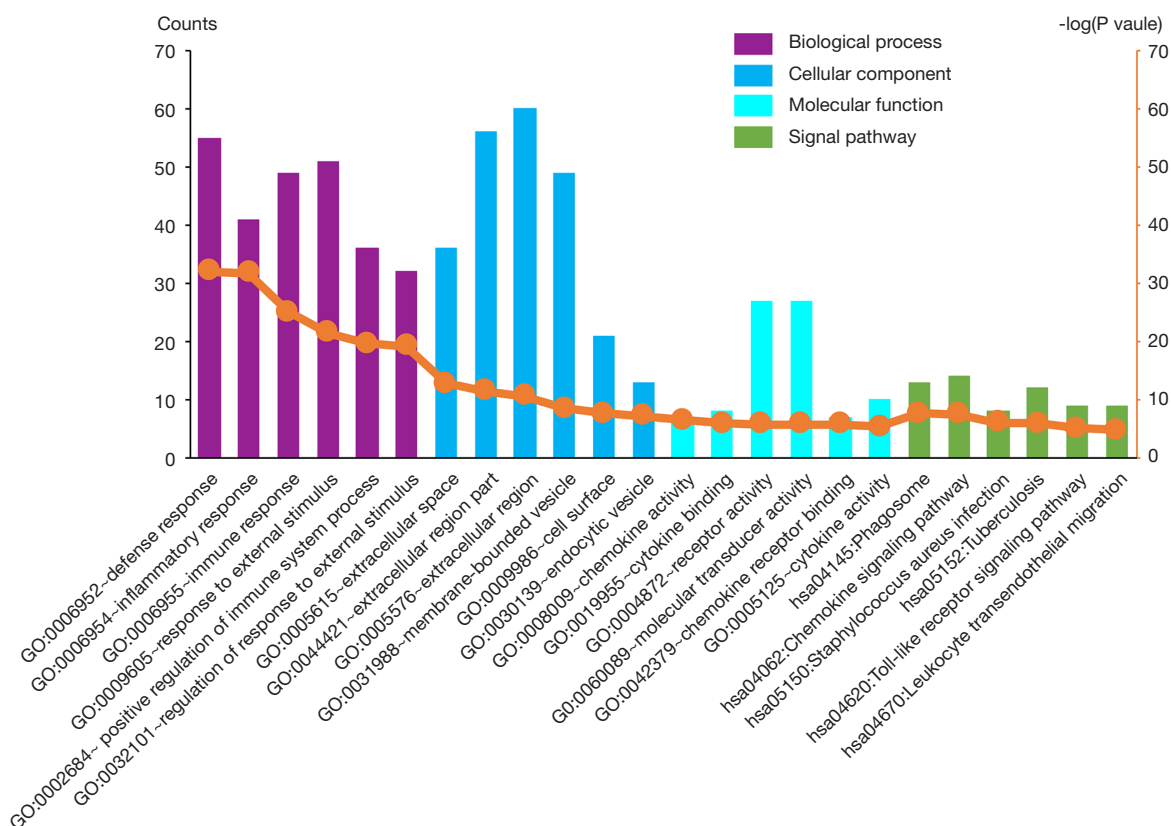
Figure 2 The top six significant GO terms and KEGG pathways of common genes. The bar charts represent the counts of genes classified in the BP, CC, MF and KEGG respectively; the yellow line chart represents the significance of enrichment terms. GO, gene ontology; BP, biological process; CC, cellular component; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

(*Table 1*).

GO and pathway enrichment analysis

In order to demonstrate GO and signal pathway enrichments of common genes, we executed functional

annotation in DAVID website. As shown in *Figure 2*, it showed that the top six significant enrichment terms for BP, CC, MF, and KEGG signal pathways of common genes. In BP category, it was mainly enriched in the defense response, inflammatory response, and response to external stimulus. In CC category, it was significantly enriched
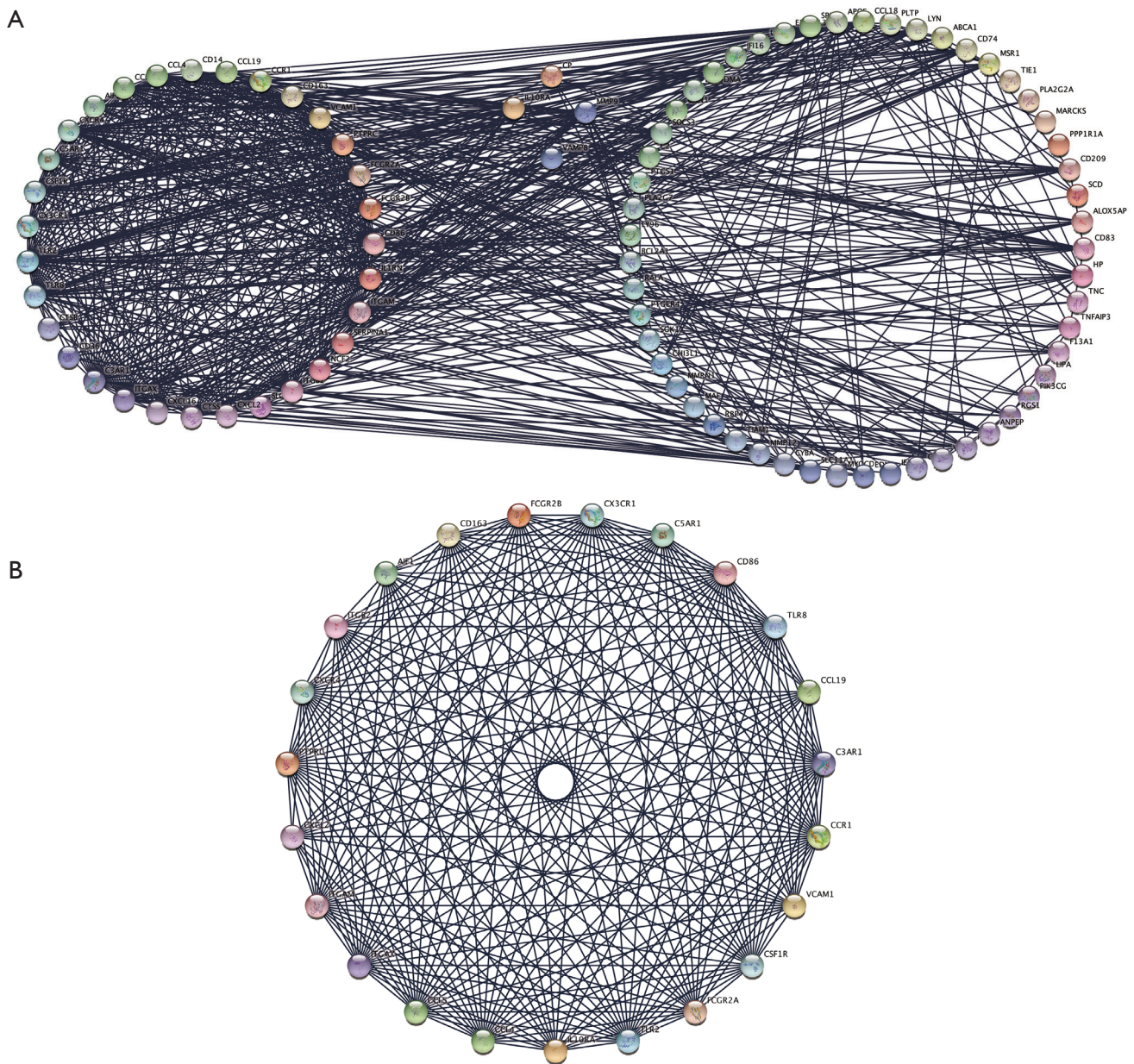
A

B

Figure 3 The PPI networks construction and significant gene module analysis. (A) The entire PPI networks of common genes; (B) the significant gene module, including 23 genes.

in the extracellular space, extracellular region part, and extracellular part. In MF category, it was mainly enriched in the chemokine activity, cytokine binding, and receptor activity. As for signal pathway enrichment, it was mainly involved in the phagosome, chemokine signaling pathway, and staphylococcus aureus infection terms, respectively (*Figure 2*).

Protein interaction and module analysis

All of the common genes were pasted into the STRING website and then analyzed with Cytoscape software. A total of 88 genes/nodes with 705 edges were involved in the construction of the PPI networks, and 5 genes didn't fall into the PPI networks (*Figure 3A*). To get the most significant gene modules, a significant gene module was

selected by using the MCODE application. Module 1 consists 23 genes/nodes with 232 edges/interactions, which all exhibit up-regulated (*Figure 3B*).

Drug-gene interaction and functional analysis of potential genes

The 23 genes clustered in the significant gene module were selected for drug-gene interaction analysis. We found that there were eight genes target to 20 potential existing drugs, which have been divided into 4 drug-gene interaction types and all have their initial drug indications (*Figure 4A, Table 2*). Besides, the functional enrichment terms of those eight gene were mainly involved in 11 GO terms (*Figure 4B, Table 3*), the most significant GO terms were the inflammatory response (BP, adj.pval =3.17E-04), cell surface (CC, adj. pval =1.36E-04), molecular transducer activity (MF, adj.pval =1.13E-02), respectively (*Figure 4B, Table 3*).

Availability of data and materials

The data associated with this article has been deposited in the NCBI-GEO website (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi).

Discussion

Atherosclerotic cardiovascular disease is considered to be a common and chronic inflammatory malady of the arterial intima. In this study, our purposes were to discover new drug indications. As we expected, we finally have found eight genes, namely *VCAM1, CSF1R, C5AR1, CXCR4, CD86, CCR1, ITGB2*, and *TLR8*, target to 20 existing potential drugs which might be used for the treatment of atherosclerosis-related diseases.

In our study, we have identified 20 drugs, which have target to eight genes (*VCAM1, CSF1R, C5AR1, CXCR4, CD86, CCR1, ITGB2*, and *TLR8*). These genes all have correlation with atherosclerosis. Almeda *et al.* team have discovered that the vascular cell adhesion molecule-1 (*VCAM1*) exposed to cytokines have shown potential for drug delivery (23). The inhibition of the progression of atherosclerosis has related to the interaction between miR-155 and B-cell leukemia/lymphoma, when miR-155 suppressed macrophage proliferation by targeting colony-stimulating factor-1 receptor (*CSF1R*) (24). High levels of C5a receptor 1 (*C5AR1*) in human lung tumors were significantly associated with overall survival

and bone metastasis (25). Type 4 chemokine receptor (*CXCR4*) participated in immune cell migration during the progression of atherosclerosis (26) and its expression level has been treated as a promising target for molecular imaging in atherosclerosis (27). *CD86* is expressed on the antigen-presenting cells (APCs) and provides costimulatory signals to T cells. Meletta *et al.* adopt *CD86/CD80* as an imaging probe for atherosclerosis diagnosis (28). Han *et al.* have demonstrated that CC chemokine ligand 7 (CCL7) and its receptors *CCR1, CCR2*, and *CCR3* were high expressions significantly in lung cancer bone metastasis (29). Moreover, CCR1 is also a target of the EGF-AKT-mTOR-STAT3 signaling pathway in breast cancer (30). The differentially methylated regions in integrin subunit beta 2 (*ITGB2*) may mediate genetic risk in inflammatory disease (31).

Toll-like receptor 8 (*TLR8*), a member of TLRs, has correlation with inflammatory disease (32).

A large amount of recent evidence confirms that the occurrence and development of atherosclerotic cardiovascular disease is a chronic inflammatory process. Inflammation is one of the most important intrinsic factors that cause plaque vulnerability. Based on previous scientific research, Janus-activated kinase 2 (JAK2)/signal transducer and activator of transcription 3 (*STAT3*) signaling pathway play an important role in inflammation, chronic pain, and even have an effect on the pathogenesis of neuropathic pain. Proinflammatory cytokines interleukin (*IL-1β*) is a downstream factor involved in JAK2/STAT3 signal transition pathway, which participates in neuron injury and inflammation (33). As the important signal pathway connecting inflammation and tumor, activated signal transduction and transcriptional activation factor 3 (*STAT3*) leads to genetic abnormal expression, gene silencing, genomic instability, etc. in non-small cell lung cancer (NSCLC) cells, and induces therapeutic resistance (34).

Currently, the interaction between the drugs and genes we discovered were divided into four types, namely agonist, inhibitor, antagonist, and binder (*Table 3*). These drugs are classified into anti-inflammatory agent, anti-neoplastic and immunomodulating agents. Although these existing drugs provide a new perspective for us to study cardiovascular diseases, further clinical trials need to be performed for confirmation of its new function and indications.

Conclusions

According to the text mining conception (keyword: atherosclerosis) and microarray data analysis (dataset:
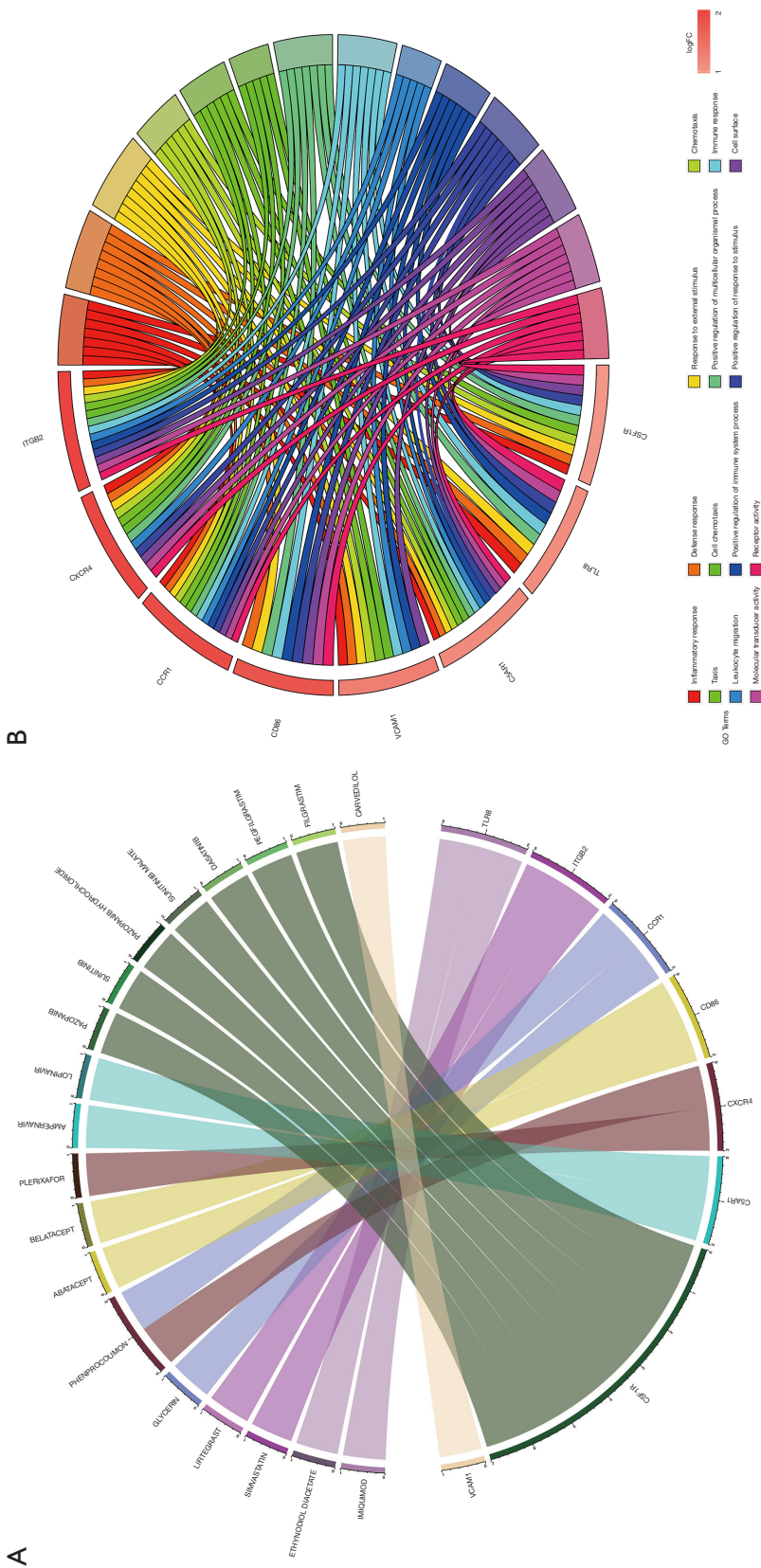
Figure 4 The drugs targeted to genes and its functional enrichment analysis. (A) Chord plot for the connection between 20 drugs and 8 genes; (B) chord plot for functional enrichments of 8 genes.

Table 2 The specified information of drugs and its target genes

Number	Gene	Drug	Interaction	Drug class*
1	TLR8	Imiquimod	Agonist	Anti-wart agent, antineoplastic agent
2	TLR8	Ethynodiol diacetate	Agonist	Contraceptives, oral, synthetic
3	ITGB2	Simvastatin	Other	Antihypertensive agent
4	ITGB2	Lifitegrast	Antagonist	Anti-inflammatory agent
5	CCR1	Glycerin	Agonist	Cryoprotective agents
6	CCR1	Phenprocoumon	Antagonist	Hematologic agents, fibrinolytic agents
7	CD86	Abatacept	Inhibitor, binder, antagonist	Antineoplastic and immunomodulating agents, antirheumatic agents, immunosuppressive agents
8	CD86	Belatacept	Inhibitor	Immunosuppressive agents, antineoplastic and immunomodulating agents
9	CXCR4	Plerixafor	Antagonist, partial agonist	Antineoplastic agent, antineoplastic and immunomodulating agents, anti-retroviral agents, anti-infective agents, anti-HIV agents
10	CXCR4	Phenprocoumon	Antagonist	Hematologic agents, fibrinolytic agents
11	C5AR1	Amprenavir	Antagonist	Not available
12	C5AR1	Lopinavir	Antagonist	Not available
13	CSF1R	Pazopanib	Inhibitor	Antineoplastic agent, moderate risk QTC-prolonging agents, immunosuppressive agents, antineoplastic and immunomodulating agents
14	CSF1R	Sunitinib	Inhibitor	Antineoplastic agent, immunosuppressive agents, hypoglycemia-associated agents, blood glucose lowering agents, antineoplastic and immunomodulating agents, angiogenesis modulating agents
15	CSF1R	Pazopanib hydrochloride	Inhibitor	Not available
16	CSF1R	Sunitinib malate	Inhibitor	Not available
17	CSF1R	Dasatinib	Inhibitor	Not available
18	CSF1R	Pegfilgrastim	Binder	Anti-neutropenic agents, antineoplastic and immunomodulating agents, anti-neutropenic agents
19	CSF1R	Filgrastim	Binder	Anti-neutropenic agents
20	VCAM1	Carvedilol	Inhibitor	Vasodilator agents, neurotransmitter agents, hypotensive agents, cardiovascular agents, bradycardia-causing agents, beta blocking agents, antihypertensive agents, alpha and beta blocking agents, adrenergic agents, antihypertensive agents

*, the drug indications have been approved by FDA.

Table 3 The functional enrichments of the final 8 genes

Category	ID	Term	Genes	adj_pval
BP	GO:0006954	Inflammatory response	*VCAM1, C5AR1, CXCR4, CCR1, ITGB2, TLR8, CSF1R*	3.17E-05
BP	GO:0006952	Defense response	*VCAM1, CD86, C5AR1, CXCR4, CCR1, ITGB2, TLR8, CSF1R*	7.91E-05
BP	GO:0009605	Response to external stimulus	*VCAM1, CD86, C5AR1, CXCR4, CCR1, ITGB2, TLR8, CSF1R*	7.03E-04
BP	GO:0006935	Chemotaxis	*VCAM1, C5AR1, CXCR4, CCR1, ITGB2, CSF1R*	0.00119549
BP	GO:0042330	Taxis	*VCAM1, C5AR1, CXCR4, CCR1, ITGB2, CSF1R*	0.00120627
BP	GO:0060326	Cell chemotaxis	*VCAM1, C5AR1, CXCR4, CCR1, ITGB2*	0.00260446
BP	GO:0051240	Positive regulation of multicellular organismal process	*CD86, C5AR1, CXCR4, CCR1, ITGB2, TLR8, CSF1R*	0.00395928
BP	GO:0006955	Immune response	*VCAM1, CD86, C5AR1, CCR1, ITGB2, TLR8, CSF1R*	0.00638104
BP	GO:0050900	Leukocyte migration	*VCAM1, C5AR1, CXCR4, CCR1, ITGB2*	0.01298062
BP	GO:0002684	Positive regulation of immune system process	*VCAM1, CD86, C5AR1, CCR1, ITGB2, TLR8*	0.0183702
BP	GO:0048584	Positive regulation of response to stimulus	*CD86, C5AR1, CXCR4, CCR1, ITGB2, TLR8, CSF1R*	0.03128537
CC	GO:0009986	Cell surface	*VCAM1, CD86, C5AR1, CXCR4, CCR1, ITGB2, CSF1R*	1.36E-04
MF	GO:0060089	Molecular transducer activity	*CD86, C5AR1, CXCR4, CCR1, ITGB2, TLR8, CSF1R*	0.01136872
MF	GO:0004872	Receptor activity	*CD86, C5AR1, CXCR4, CCR1, ITGB2, TLR8, CSF1R*	0.01136872

GSE28829), we found 20 existing drugs, approved by FDA, target to eight genes, which involved in the inflammatory pathway. These genes might be used for atherosclerosis, as well as its original drug indications.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/apm-20-705). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. For human datasets mentioned in this study, please refer to the original article (PMID: 22388324). We just re-analyzed the open accessed datasets, and no ethical approval was required by the local ethics committees.

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