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Identification and Validation of Vascular-Associated Biomarkers for the Prognosis and Potential Pathogenesis of Hypertension Using Comprehensive Bioinformatics Methods

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Abstract

Background: Hypertension, also known as increased blood pressure, is a phenomenon in which blood flows in blood vessels and causes persistently higher-than-normal pressure on the vessel wall. The identification of novel prognostic and pathogenesis biomarkers plays a key role in the management of hypertension. **Methods:** The GSE7483 and GSE75815 datasets from the gene expression omnibus (GEO) database were used to identify the genes associated with hypertension that were differentially expressed genes (DEGs). The functional role of the DEGs was elucidated by gene body (GO) enrichment analysis. In addition, we performed an immune infiltration assay and GSEA on the DEGs of hypertensive patients and verified the expression of novel DEGs in the blood of hypertensive patients by RT-qPCR. **Results:** A total of 267 DEGs were identified from the GEO database. GO analysis revealed that these genes were associated mainly with biological processes such as fibroblast proliferation, cell structural organization, extracellular matrix organization, vasculature development regulation, and angiogenesis. We identified five possible biomarkers, Ecm1, Sparc, Sphk1, Thbsl, and Mecp2, which correlate with vascular development and angiogenesis characteristic of

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hypertension by bioinformatics, and explored the clinical expression levels of these genes by RT-qPCR, and found that Sparc, Sphk1, and Thbs1 showed significant up-regulation, in agreement with the results of the bioinformatics analysis. **Conclusion:** Our study suggested that Sparc, Sphk1 and Thbs1 may be potential novel biomarkers for the diagnosis, treatment and prognosis of hypertension and that they are involved in the regulation of vascular development and angiogenesis in hypertension.

Keywords

Hypertension, Biomarkers, Differentially Expressed Genes, Vascular Development and Angiogenesis, Bioinformatics Analysis

1. Introduction

Hypertension is a major risk factor for cardiovascular diseases and end-stage kidney damage and currently affects approximately 40% of the global adult population [1]. Hypertension is a risk factor for the development of cardiovascular diseases such as coronary artery disease (CAD), left ventricular hypertrophy, valvular heart disease, atrial fibrillation, stroke and arrhythmias, without timely diagnosis and appropriate treatment, it may lead to disease or death [2] [3]. It is estimated that 1.4 billion people worldwide suffer from high blood pressure, but only 14% of patients have their condition under control [4], and the prevention and control situation is very grim. Antihypertensive drugs are currently the main means of treating hypertension, but there are significant individual differences in the efficacy of these drugs, and there are many adverse reactions [5]. Existing studies have shown that differences in individualized medication used caused by genetic polymorphisms are the main reason for the low efficacy and greater side effects of current antihypertensive drugs [6].

Bioinformatics is a multidisciplinary field that applies information technology and computer science principles to the study of the molecular aspects of biology and immunology [7]. Over the past decade, single-cell RNA sequencing (SCS) has emerged as a powerful tool for characterizing different cellular functions and states [8] [9]. In recent years, with the cross-fusion of computer science and medical fields, an increasing number of algorithms have been used in the diagnosis, prediction and prognosis of clinical diseases [10]. Weighted gene coexpression network analysis (WGCNA) has been widely used to identify central genes in clinically important modules by constructing gene coexpression networks [11]. Microarray data and high-throughput sequencing technology are widely used in gene sequencing for screening differentially expressed genes and have shown enormous potential in medical research because of their high diagnostic performance for various diseases [12] [13]. However, to date, a multibiomarker-based diagnostic model has not been developed for the diagnosis of hypertension.

In this study, we used bioinformatics methods to identify and validate genes associated with hypertension. This study explored the biological functions of genes associated with hypertension through the use of public databases and constructed a prediction model and novel biomarker interaction network. The findings can aid in the prediction and treatment of hypertension risk. The data of hypertension patients were downloaded from the GEO dataset and analyzed using R software to identify DEGs. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genomes (KEGG) pathway analysis methods were used to analyze the overlapping DEGs in the dataset. Using a variety of bioinformatics methods, novel biomarkers associated with the prognosis and potential pathogenesis of hypertension were screened.

2. Materials and Methodology

2.1. Data Sources

The hypertension-related datasets GSE7483 and GSE75815 were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The GSE7483 dataset utilizes the GPL890 platform and comprises both treated and untreated Wistar Kyoto (WKY) rats, as well as treated and untreated spontaneously hypertensive rats (SHR). From this dataset, we specifically isolated 12 hypertensive samples (untreated SHR rats) and 12 control samples (untreated WKY rats). Regarding the GSE75815 dataset, analysis was performed utilizing the GPL6887 platform. This dataset investigates an animal model of AngII-induced hypertension utilizing Illumina WG-6 v2.0 microarrays. Specifically, the thoracic aorta, abdominal aorta, and mesenteric arteries of C57BL/6J mice infused with AngII (490 ng/kg/min) for 2 consecutive weeks were analyzed. From this dataset, our focus was on the transcriptome of hypertensive mice and control mice. We selected 3 hypertensive samples (thoracic aorta of C57BL/6J wild-type hypertensive mice infused with AngII (490 ng/kg/min) for 2 weeks) and 3 control samples (thoracic aorta of C57BL/6J wild-type hypertensive mice infused with an AngII carrier for 2 weeks).

2.2. Differential Gene Analysis

R software was used for statistical analysis and visualization of the data. The data were downloaded from the GEO database through the GEO query package. Probes with one probe corresponding to multiple molecules were removed. When probes corresponding to the same molecule were encountered, only probes with the maximum signal value were retained. The DESeq2 package was used to normalise the counts. The limma package was used for difference analysis between two groups, and data visualization was performed. The threshold for identifying DEGs was set to $P < 0.05$. A volcano map and heatmap were generated to display the differential gene expression results. Overlap analysis of the different gene sets in this paper was performed using Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/index.html).

2.3. Gene Ontology (GO) Analysis

GO enrichment analysis was performed using R software. GO annotation analysis (molecular function, cellular component, and biological process) of the DEGs was performed using the cluster Profiler package, loading species genomes using the Annotation Hub package, and the database for GO analysis was called from https://www.geneontology.org/. $P < 0.05$ was considered to indicate statistical significance.

2.4. Immune Infiltration Assay

Immune infiltration analysis was performed using R software, and the gene expression data of each sample were compared with those of immune cells through the mMCP-counter package to assess the infiltration level of immune cells.

2.5. Gene Set Enrichment Analysis (GSEA)

R software was used for GSEA conversion of rat genes to mouse genes using the biomaRT software package and the cluster profiler package was used to analyze the risk signature-related biological pathways and functions of the DEGs. The GMT files used were obtained from https://www.gsea-msigdb.org/gsea/downloads.jsp, and the "gene set database" was selected as follows: "M2: curated gene sets (CP: Canonical pathways)". Based on the gene expression profiles and phenotype grouping, according to the description of clusterprofiler (https://bioconductor.org/packages/release/bioc/html/clusterProfiler.html), the minimum gene set was set to 5, the maximum gene set was 5000, and one thousand repetitions were used for sampling. A $P < 0.05$ and an $FDR < 0.25$ were considered significant, and visual analysis were performed with the enrichplot package.

2.6. Clinical Sample Collection

A total of 102 blood samples were collected from healthy controls and hypertensive patients at Yangbi Yi Autonomous County People's Hospital. The study was conducted with the informed consent of the patients and with the approval of the Ethics Committee of Yangbi Yi Autonomous County People's Hospital.

2.7. RNA Extraction and RT-qPCR

Total RNA was extracted using a magnetic bead method and a total RNA extraction kit (G3611-50T, Servicebio, China) and synthesized using a SweScript RT I first-strand cDNA synthesis kit (G3331-50, Servicebio, China) according to the manufacturer's instructions. cDNA was synthesized, and RT-qPCR was performed using SYBR Green qPCR Master Mix (G3320-01, Servicebio, China). The RT-qPCR amplification reaction program was set as follows: amplification at 95˚C for 10 min, amplification at 95˚C for 15 s, amplification at 60˚C for 30 s, and 40 cycles. Using GAPDH as an internal reference gene, the relative expres-

sion levels were calculated by the $2^{-\Delta\Delta ct}$ method. The sequence of primers is shown in Table 1.

3. Results

3.1. DEGs That Were Significantly Differentially Expressed

A total of 2357 DEGs that were differentially expressed according to the GSE7483 dataset were obtained. A total of 1181 genes were upregulated, and 1176 genes were downregulated (Figure 1(a)). A total of 1416 DEGs were generated from the GSE75815 database, of which 735 were upregulated and 681 were downregulated (Figure 1(b)), additionally, heatmaps were drawn (Figure 1(c) and Figure 1(d)). Additionally, 267 overlapping DEGs were identified in the 2 datasets using Venny 2.1. (Figure 1(e)).

3.2. Enrichment Analysis

The results of GO enrichment analysis showed that the biological processes involved mainly included chemotactic positive regulation, actin filament organization, actin filament assembly, fibroblast proliferation, extracellular structure organization, extracellular matrix organization, positive regulation of response to external stimuli, regulation of vascular system development, regulation of angiogenesis, and chemotactic regulation. The main molecular functions include cell adhesion molecule binding, collagen binding, carbohydrate binding, sulfur binding, heparin binding, growth factor binding, extracellular matrix binding, extracellular matrix structural components, integrin binding, glycosaminoglycan

Table 1. Primer sequences.

Genes	Sequence (F: Forward primer, R: Reversed primer)
Ecm1	F: 5'-AGAGCACTTTCAUGUGTTGG-3' R: 5'-GAGTCAGGGTGATCCATGG-3'
Mecp2	F: 5'-TGCTGGGAAGTATGATGTG-3' R: 5'-CACTTTAGAGCGAAAGGCT-3'
Sparc	F: 5'-CCTGGTCACCCTGTATGAG-3' R: 5'-TCATGGATCTTCTTCACCCG-3'
Sphk1	F: 5'-TATGCTGGCTATGAGCAGG-3' R: 5'-AGAGACAGCAGGTTCATGG-3'
Thbs1	F: 5'-CGATAGCCTCAACAACCGA-3' R: 5'-GCCATCCTGTTTAAUTCTC-3'
GAPDH	F: 5'-TCAAGATCATCAGCAATGCC-3' R: 5'-CGATACCAUAGTTGTCATGGA-3'

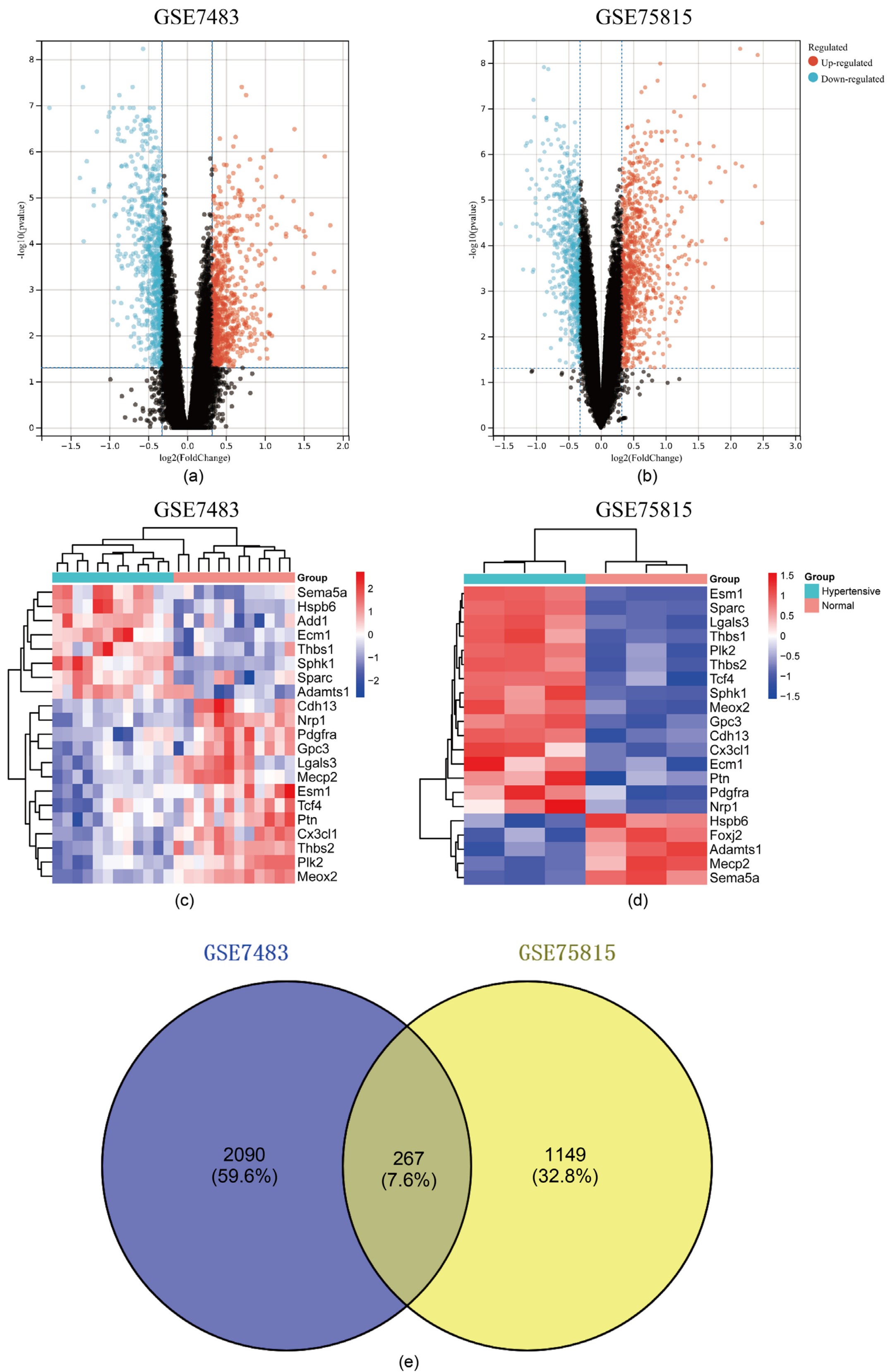

Figure 1. Significantly differentially expressed DEGs. (a), (b): Volcano maps; (c), (d): Heatmaps; (e): Dataset intersection DEGs.

binding, etc. The cellular components mainly included collagen-containing extracellular matrix, cell-matrix junctions, β-catenin-TCF complexes, membrane domains of cell adhesion protein complexes, membrane microdomains of integrin complexes, platelet α-granules, basement membrane, etc. (Figures 2(a)-(f)). Through GO enrichment analysis, we found that the DEGs were significantly associated mainly with angiogenesis, vascular development, and intercellular junctions, and the key genes are listed in Table 2.

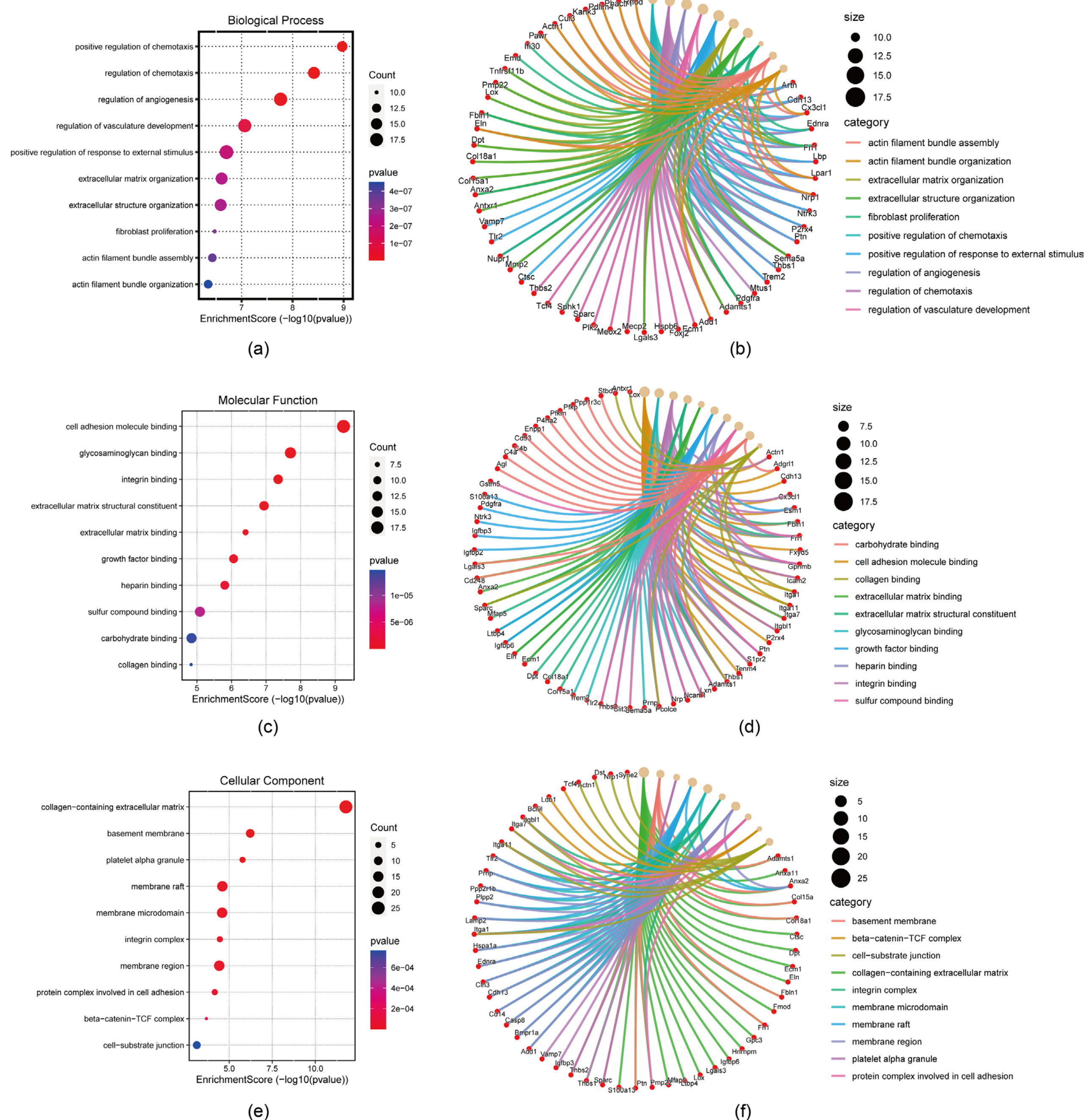

Figure 2. Enrichment analysis. (a), (b): Biological processes (BPs); (c), (d): Molecular functions (MFs); (e), (f): Cellular components (CCs).

Table 2. Key genes at the intersection of DEGs.

Name	logFC	
	GSE7483	GSE75815
Ecm1	0.435	0.478
Mecp2	−0.249	−0.445
Sparc	0.253	0.916
Sphk1	0.561	1.22
Thbs1	0.205	0.595

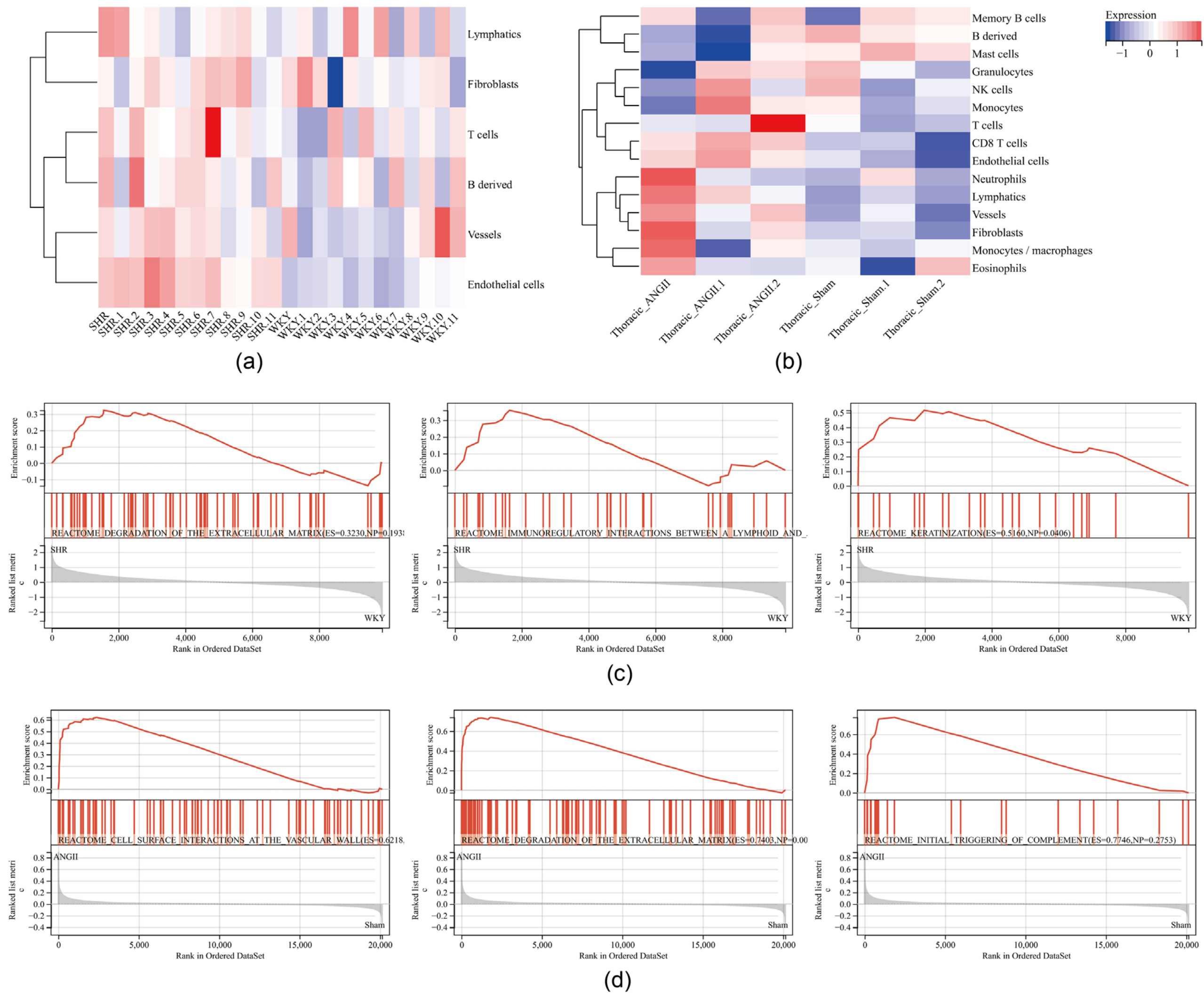

Figure 3. Immune infiltration assay. (a): Immune infiltration assay of GSE7483; (b): Immune infiltration assay of GSE75815; (c): GSEA analysis of GSE7483; (d): GSEA analysis of GSE75815.

3.3. Immune Infiltration Assay

To determine whether hypertensive patients have different immune infiltrates, we compared immune infiltration among hypertensive patients. As shown in Figure 3(a) and Figure 3(b), the immune cells (lymphatics, fibroblasts, T cells, B cells, vessels, endothelial cells, memory B cells, mast cells, granulocytes, NK

cells, monocytes, cd8 T cells, neutrophils, macrophages and eosinophils) of the hypertension group and the normal group were significantly different, indicating that hypertension is significantly associated with immune infiltration. The GSEA technique was used to investigate the changes in patients with hypertension and the possible underlying mechanisms. The results showed that multiple signaling pathways were significantly enriched, but the enrichment modes were different. In the hypertension group, genes associated with keratinocyte, immunoregulatory interactions between a lymphoid cell and a nonlymphoid cell, degradation of the extracellular matrix, initial triggering of complement, degradation of the extracellular matrix, and cell surface interactions at the vascular wall were highly aggregated (**Figure 3(c)**). We selected five differential DEGs (Ecm1, Sparc, Sphk1, Thbsl and Mecp2) that are highly correlated with vascular development and angiogenesis for subsequent experimental validation.

3.4. RT-qPCR Validation of the Five DEGs in the Blood of Patients with Clinical Hypertension

To further validate the expression of the five novel key DEGs enriched above in the blood of hypertensive patients, we detected their expression in 51 hypertensive blood samples and 51 non-hypertensive blood samples. The RT-qPCR results for Sparc ($P < 0.05$), Sphk1 ($P < 0.05$), and Thbs1 ($P < 0.05$) in the blood of hypertensive patients were consistent with the results of the database analysis, revealing a significant upregulation trend. In addition, RT-qPCR results showed that there was no significant difference in the expression of Ecm1 and Mecp2 in the blood of hypertensive patients and non-hypertensive blood (**Figure 4**).

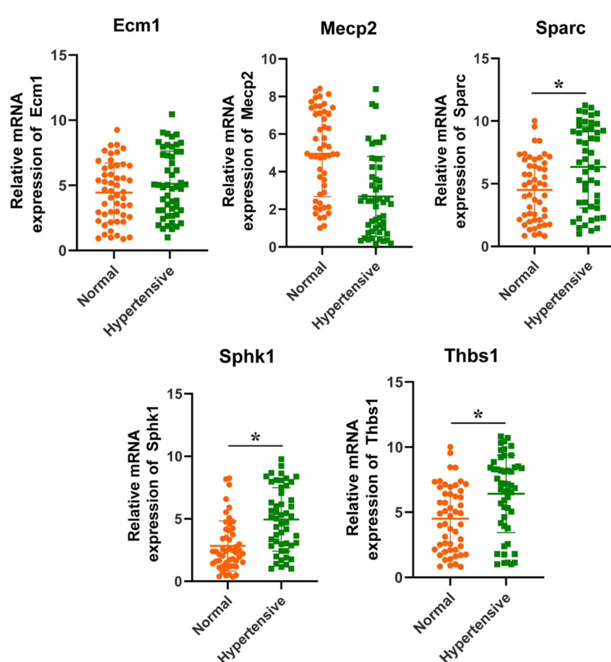

Figure 4. RT-qPCR validation of five DEGs in the blood of patients with clinical hypertension. A: The expression of the Ecm1, Mecp2, Sparc, Sphk1, and Thbs1 genes was detected by RT-qPCR, $*P < 0.05$.

4. Discussion

In the field of vascular biology, animal models play a critical role in the discovery of the basic mechanisms of vascular health and the development of treatments for various diseases [14]. The development and formation of blood vessels is a complex process that involves the regulation of various cell types and molecular signaling pathways and includes multiple steps, such as the proliferation and migration of vascular endothelial cells, tube formation, and vascular differentiation and maturation [15] [16] [17]. Studies have shown that vascular endothelial growth factor (VEGF) and its receptor (VEGFR) play key roles in tumor angiogenesis [18]. In addition, gestational hypertension is associated with changes in vascular function during pregnancy, and these changes may affect the future cardiovascular health of the mother and the child [19]. Clinical noninvasive methods for studying human endothelial function and vascular tension have proven that patients with hypertension exhibit impaired endothelium-dependent vasodilation, enhanced vasoreactivity, and increased contractility [20]. In addition, some studies have shown that promoting the development and formation of blood vessels can ameliorate the development and progression of hypertension [21]. Therefore, regulating angiogenesis may provide a new therapeutic strategy for the treatment of hypertension. In this study, we investigated the expression levels of the Ecm1, Mecp2, Sparc, Sphk1, and Thbs1 genes, which are associated with angiogenesis and angiogenesis in hypertensive patients, and proposed that Sparc, Sphk1 and Thbs1 may be significant novel targets.

SphK1 is a key rate-limiting enzyme and intracellular signal transduction enzyme involved in sphingolipid metabolism, and it is involved in the regulation of various physiological processes, such as Ca^{2+} homeostasis, cell survival, migration, and inflammation [22] [23]. Under inflammatory conditions, abnormally expressed SphK1 is involved in the regulation of the inflammatory response and the management of various immune cell functions. Abnormal inflammation can activate SphK1, destroying its activity and balance [24] [25]. Moreover, SphK1 triggers proinflammatory signals, regulates the chemotactic response of immune cells and activates vascular endothelial cells (VECs) [26]. Our studies showed that the expression of SphK1 in the blood of patients with hypertension was significantly upregulated. Further bioinformatics, KEGG, enrichment, and MF analyses confirmed that SphK1 was involved in the regulation of angiogenesis and angiogenesis inhibition in patients with hypertension.

Thrombospondin-1 (Thbs1) is associated with various cardiovascular diseases. The association between Thbs1 and endothelial dysfunction suggests that Thbs1 plays an important role in hypertension [27]. In addition, Thbs1 is an important endogenous inhibitor of angiogenesis that contributes to microcirculation and capillary density by regulating VEGF signaling [28]. Our results showed that Thbs1 levels increase under hypertensive conditions, which prompted us to further investigate the molecular importance of Thbs1 in the development of hypertension. Using bioinformatics methods, we used the GSE7483 and GSE75815

datasets to determine the Thbs1 level in patients with hypertension. Regulatory mechanisms of angiogenesis and angiogenesis. These results confirmed that Thbs1 obstructed the development and production of blood vessels in cells, thereby aggravating the increase in human blood pressure. Moreover, GO enrichment analysis and KEGG pathway analysis revealed that Ecm1, Mecp2, Sparc, Sphk1, and Thbs1 were involved in the processes of cellular immune infiltration and angiogenesis.

Hypertension is a common chronic disease that affects a large population worldwide. A growing body of evidence shows a strong link between the immune system and high blood pressure [29] [30]. In the pathological process of hypertension, immune cells infiltrate key organs, such as the blood vessel walls, heart, and kidney. These infiltrating immune cells, such as T lymphocytes and macrophages, can secrete a variety of cytokines and growth factors, which cause vasoconstriction, smooth muscle cell proliferation and inflammation and subsequently induce hypertension [31] [32] [33]. However, the association between immune infiltration and hypertension has not been fully elucidated. In the present study, the Ecm1, Mecp2, Sparc, Sphk1, and Thbs1 genes were found to be significantly correlated with the level of immune cell infiltration in patients with hypertension. Furthermore, the correlation of this feature with the response to immunotherapy was evaluated. The analysis of the significantly differentially expressed DEGs showed that Ecm1, Mecp2, Sparc, Sphk1, and Thbs1 were related to autophagy. Autophagy plays an important role in the development of cardiovascular diseases such as atherosclerosis, cardiac ischemia/reperfusion, cardiomyopathy, heart failure and hypertension, and generated reactive oxygen species interact to prevent hypertension [34] [35]. This finding is consistent with the results obtained from our DEGs dataset.

5. Summary

In summary, this study performed bioinformatics analysis of data from the GEO database and established a model of angiogenesis-related genes and the prognosis of patients with hypertension. Sparc, Sphk1 and Thbs1 could be used to predict the prognosis and immune response in patients with hypertension. The genes we identified can be used in the clinically stratified management of patients and can also lay the foundation for future studies of angiogenesis as a therapeutic target for hypertension. This study has several limitations. First, although new biomarkers associated with hypertension have been predicted, their mechanism of action remains unclear, and further study is needed. Second, the results need to be verified by experimental studies.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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