Observational Study

Medicine

OPEN

Regulatory mechanism of immune-related genes in patients with hypertension

Linhu Zhang, MD[a], Wei Zhang, MD[a], Jianling Chen, MD[a,*]

Abstract

Hypertension (HT) is among the most common cardiovascular diseases in the world and is an important risk factor for stroke, myocardial infarction, heart failure, and kidney failure. Recent studies have demonstrated that activation of the immune system plays an important role in the occurrence and maintenance of HT. Thus, this research aimed to determine the immune-related biomarkers in HT. In this study, RNA sequencing data of the gene expression profiling datasets (GSE74144) were downloaded from the Gene Expression Omnibus database. Differentially expressed genes between HT and normal samples were identified using the software limma. The immune-related genes associated with HT were screened. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analyses were performed using the program "clusterProfiler" of the R package. The protein-protein interaction network of these differentially expressed immune-related genes (DEIRGs) was constructed based on the information from the STRING database. Finally, the TF-hub and miRNA-hub gene regulatory networks were predicted and constructed using the miRNet software. Fifty-nine DEIRGs were observed in HT. The Gene Ontology analysis indicated that DEIRGs were mainly enriched in the positive regulation of cytosolic calcium ions, peptide hormones, protein kinase B signaling, and lymphocyte differentiation. The Kyoto Encyclopedia of Genes and Genomes enrichment analysis indicated that these DEIRGs were significantly involved in the intestinal immune network for IgA production, autoimmune thyroid disease, JAK-STAT signaling pathway, hepatocellular carcinoma, and Kaposi sarcoma-associated herpesvirus infection, among others. From the protein-protein interaction network, 5 hub genes (insulin-like growth factor 2, cytokine-inducible Src homology 2-containing protein, suppressor of cytokine signaling 1, cyclin-dependent kinase inhibitor 2A, and epidermal growth factor receptor) were identified. The receiver operating characteristic curve analysis was performed in GSE74144, and all genes with an area under the curve of > 0.7 were identified as the diagnostic genes. Moreover, miRNA-mRNA and TF-mRNA regulatory networks were constructed. Our study identified 5 immune-related hub genes in patients with HT and demonstrated that they were potential diagnostic biomarkers for HT.

Abbreviations: AUC = area under the curve, CDKN2A = cyclin-dependent kinase inhibitor 2A, CISH = cytokine-inducible Src homology 2-containing protein, DEGs = differentially expressed genes, DEIRGs = differentially expressed immune-related genes, EGFR = epidermal growth factor receptor, HT = hypertension, IGF2 = insulin-like growth factor 2, KEGG = Kyoto Encyclopedia of Genes and Genomes, PPI = protein-protein interaction, ROC = receiver operating characteristic, SOCS1 = suppressor of cytokine signaling 1.

Keywords: cardiovascular disease, genomics, hub genes, hypertension, immune-related

1. Introduction

Hypertension (HT) is a cardiovascular clinical syndrome. The main clinical manifestation of HT is elevated systemic arterial pressure. According to the guidelines of ESH/ESC (European Society of HT/European Society of Cardiology), the diagnosis of HT is confirmed when systolic blood pressure is ≥ 140 mm Hg and/or diastolic blood pressure ≥ 90 mm Hg.[1] With the growth of the global population, the prevalence of HT has been increasing, and blood pressure increases with age in almost all regions of the world. The level of blood pressure is positively correlated with the risk of cardiovascular disease. High blood pressure, the main cause of cardiovascular disease and mortality worldwide, has seriously endangered the health of the global population.[2,3] Therefore, effective control of the rise in blood pressure is an important challenge for researchers. At present, the treatment of HT is mainly based on drug therapy.[3] Although there are several drugs for the treatment of HT, it remains uncontrollable for most patients, with a control rate of only 35 to 50%.[4,5] Therefore, we need to further develop diagnostic markers and therapeutic targets for HT and provide novel methods for the treatment and intervention of hypertensive patients.

The authors have no funding to disclose.

The authors have no conflicts of interest to disclose.

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

[a] Department of Cardiology, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou, People's Republic of China.

** Correspondence: Jianling Chen, Department of Cardiology, Affiliated Hospital of Zunyi Medical University, Zunyi, Guizhou 563000, People's Republic of China (e-mail: 2871766504@qq.com).*

How to cite this article: Zhang L, Zhang W, Chen J. Regulatory mechanism of immune-related genes in patients with hypertension. Medicine 2023;102:9(e32627).

Received: 23 November 2022 / Received in final form: 20 December 2022 / Accepted: 21 December 2022

http://dx.doi.org/10.1097/MD.0000000000032627

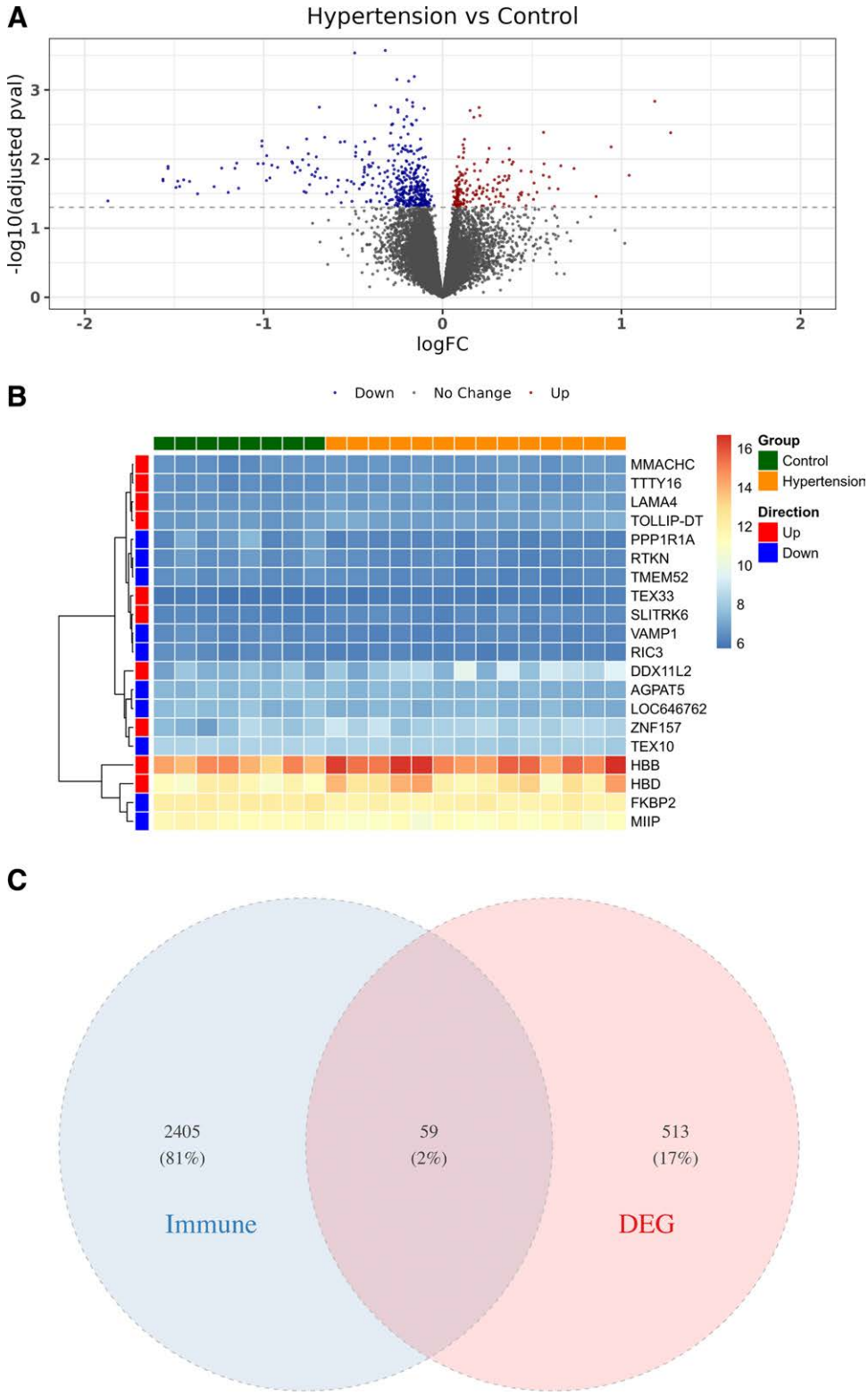

Figure 1. (A) Volcano plot for the differential expression analysis. (B) Heatmap for DEGs. (C) Immune-related DEGs. DEGs = differentially expressed genes.

Several recent studies have demonstrated that immune activation and inflammation also play important roles in the occurrence and maintenance of HT.[6] Elevated blood pressure promotes vascular and renal damage, cytokine release, and immune cell infiltration, which exacerbate tissue damage. Tissue damage may stimulate the formation or presentation of antigens or neoantigens, which is followed by an immune response, the infiltration of macrophages, and T lymphocytes, which stimulates the release of inflammatory mediators.[7] In humans and experimental models of HT, cells of the innate and adaptive immune systems enter the target tissues, including blood vessels and kidneys, and release powerful mediators such as cytokines, matrix metalloproteinases, and reactive oxygen species, resulting in tissue damage, fibrosis, and dysfunction.[8]

The above studies have shown that immunity plays an important role in HT, although its specific mechanism remains unclear.

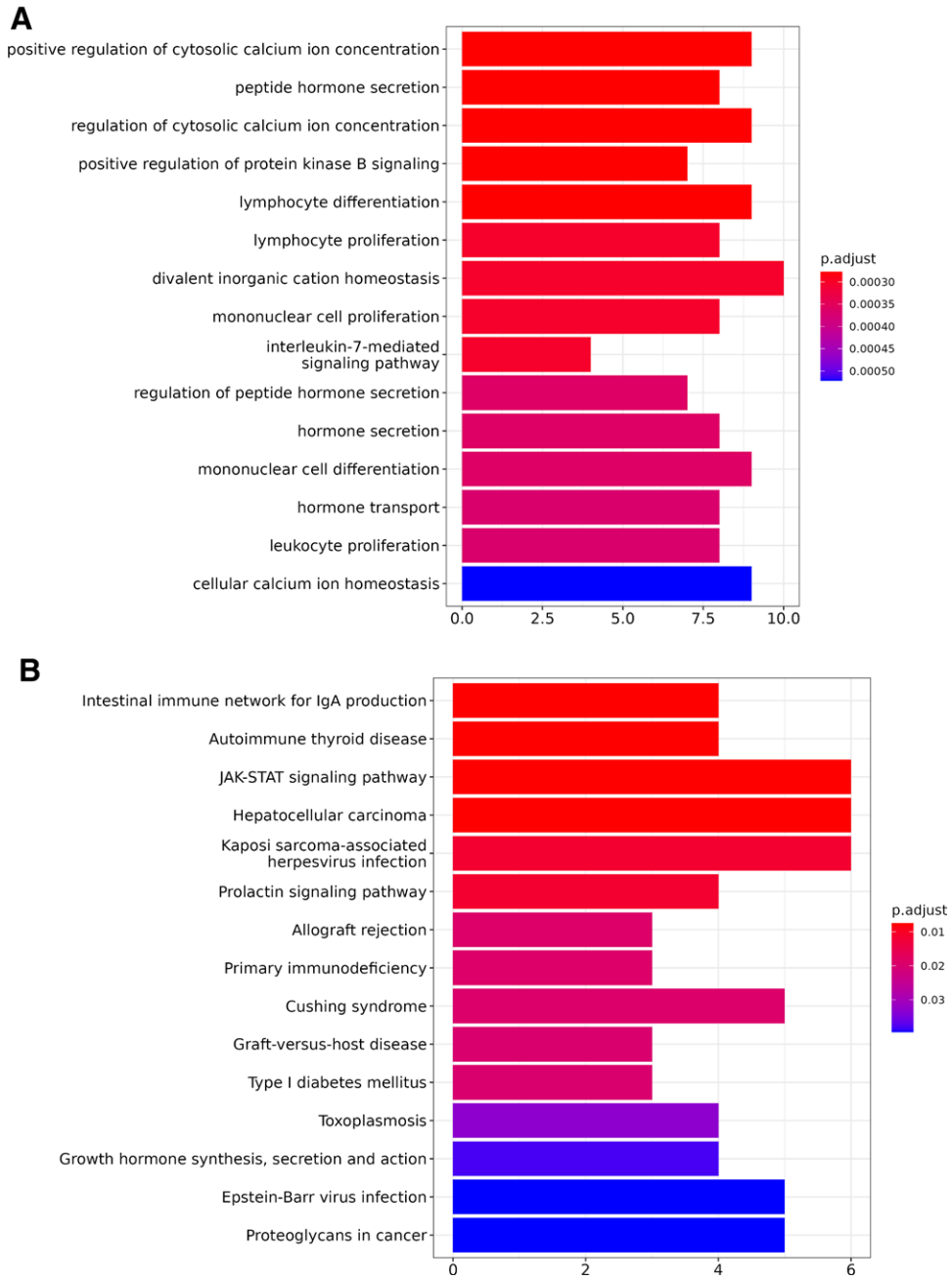

Figure 2. (A) GO enrichment of immune-related DEGs. (B) KEGG enrichment of immune-related DEGs. DEGs = differentially expressed genes, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

Therefore, this study used bioinformatics to analyze the genetic differences between normal people and hypertensive patients, correlated them with immune-related genes, and screened hub genes with good specificity and sensitivity. This study will not only help advance the research on immunity in the pathogenesis of HT but also assist in identifying potential therapeutic targets for the treatment of HT.

2. Materials and methods

2.1. Data source

The microarray RNA expression dataset (GSE74144) was downloaded from the Gene Expression Omnibus of NCBI. Expression profiles from 14 hypertensives and 8 normal samples were based on the platform GPL13497 (Agilent-026652 Whole Human Genome Microarray 4×44K v2). We obtained 2464 immune-related genes from ImmPort,[9] TISIDB,[10] and InnateDB.[11]

2.2. Identification of differentially expressed genes (DEGs)

Differential expression was analyzed using the package "limma."[12] DEGs were identified if the P value was < 0.05. Pheatmap package and ggplot2 package were used to prepare the heatmaps and volcano maps.

2.3. Functional enrichment analysis

To identify the key pathways involved in the progression of HT, the GO term (including biological processes, molecular

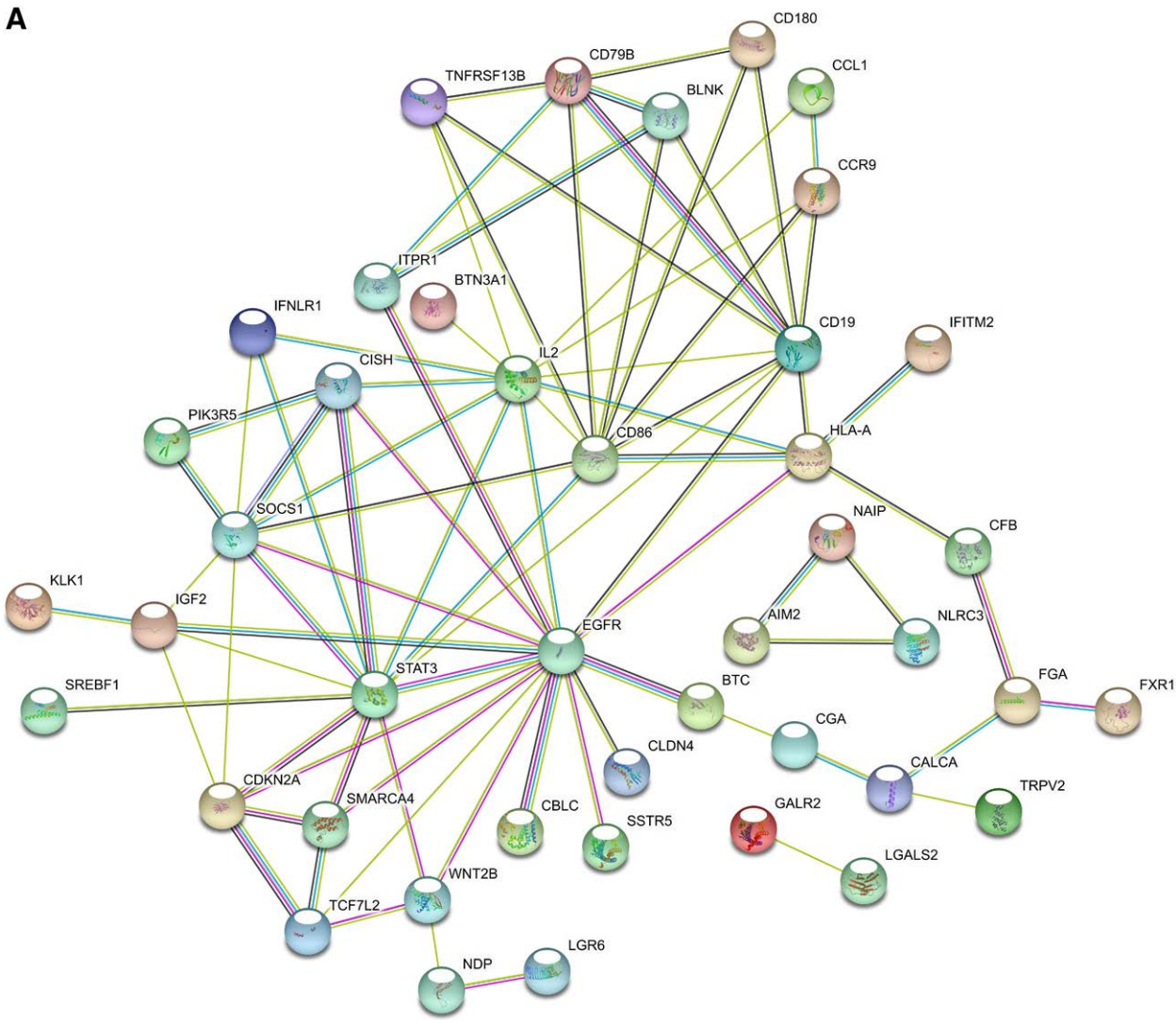

Figure 3. (A) PPI network. (B) PPI sub-network of hub genes. (C) Expression correlation of hub genes. PPI = protein-protein interaction.

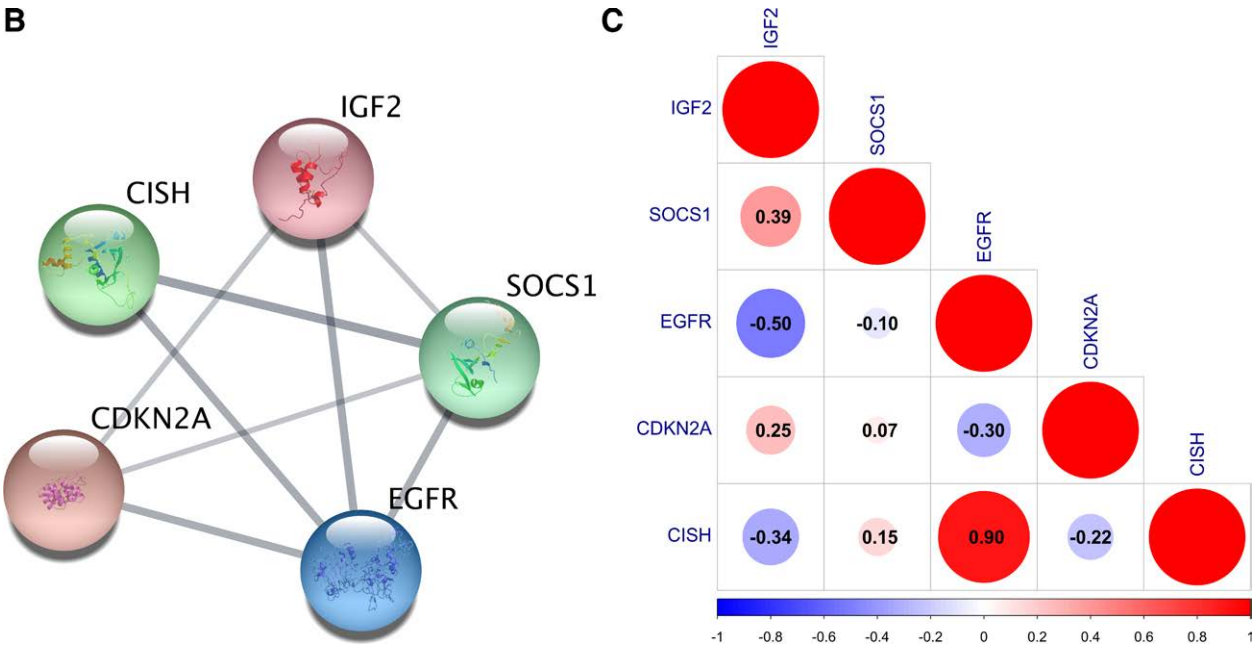

Figure 3. Continued

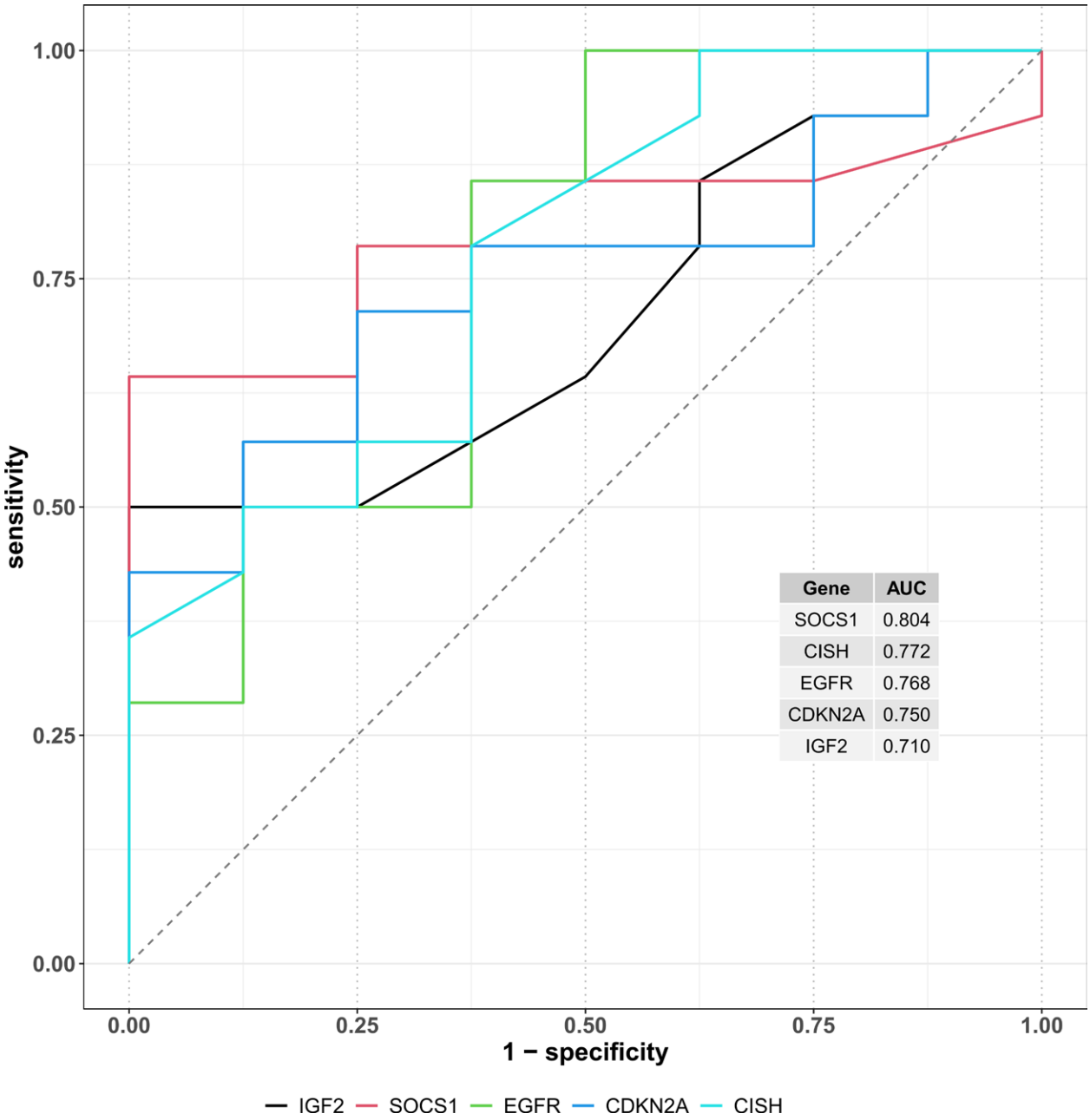

Figure 4. ROC analysis of hub genes in differentiating patients from the normal control. ROC = receiver operating characteristic.

functions, and cellular components) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed based using the "clusterProfiler" function of the package R.[13] GO/KEGG-pathway terms with adjusted *P* values of <0.05 were statistically significant.

2.4. Construction of the protein-protein interaction (PPI) network

The PPI network for differentially expressed immune-related genes (DEIRGs) was constructed using the STRING database. The software Cytoscape was then used to construct the interaction network map, and the MCODE plug-in was used to screen the key gene modules in the network map. The key module genes were defined as the hub genes. The package "Corrplot" was used to analyze the correlation of hub genes.

2.5. Construction and analysis of hub gene-miRNA and hub gene-TF networks

In this study, miRNet (a miRNA-centric network visual analytics platform) was used to identify the miRNAs and TFs targeting hub genes. miRNAs and TFs were predicted using the database miRNet.[14] The miRNA-hub gene and TF-hub gene networks were generated using Cytoscape.

2.6. Analysis of receiver operating characteristic (ROC) curve

In the GSE74144 dataset, 14 HT samples and 8 normal samples were utilized to plot the ROC curves, from which their areas under the curve (AUCs) were computed using the package "pROC" in the R software.[15] The hub genes with an AUC of >0.7 were useful for disease diagnosis. For further verification,

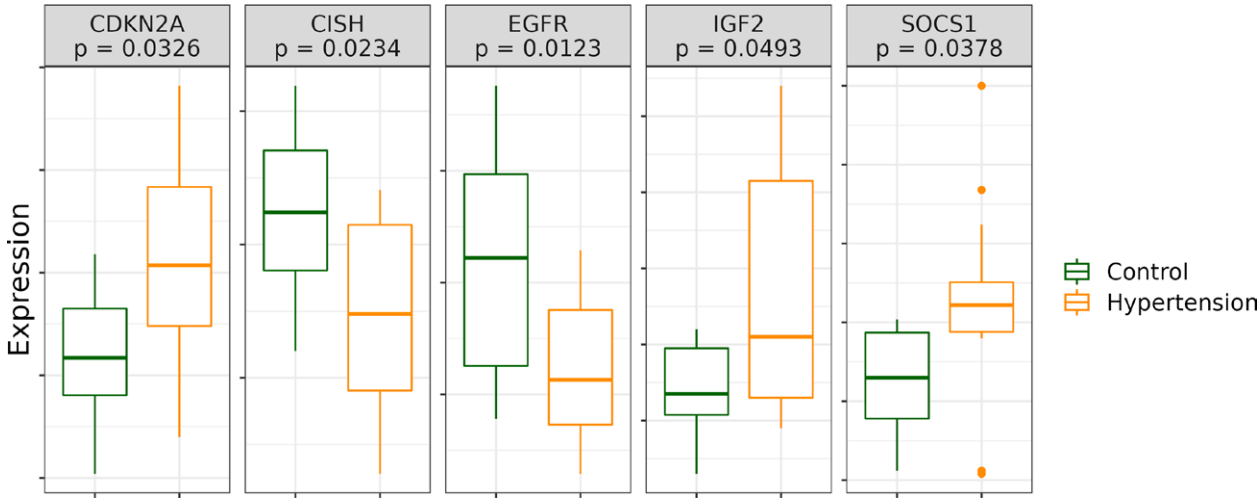

Figure 5. Expressions of hub genes.

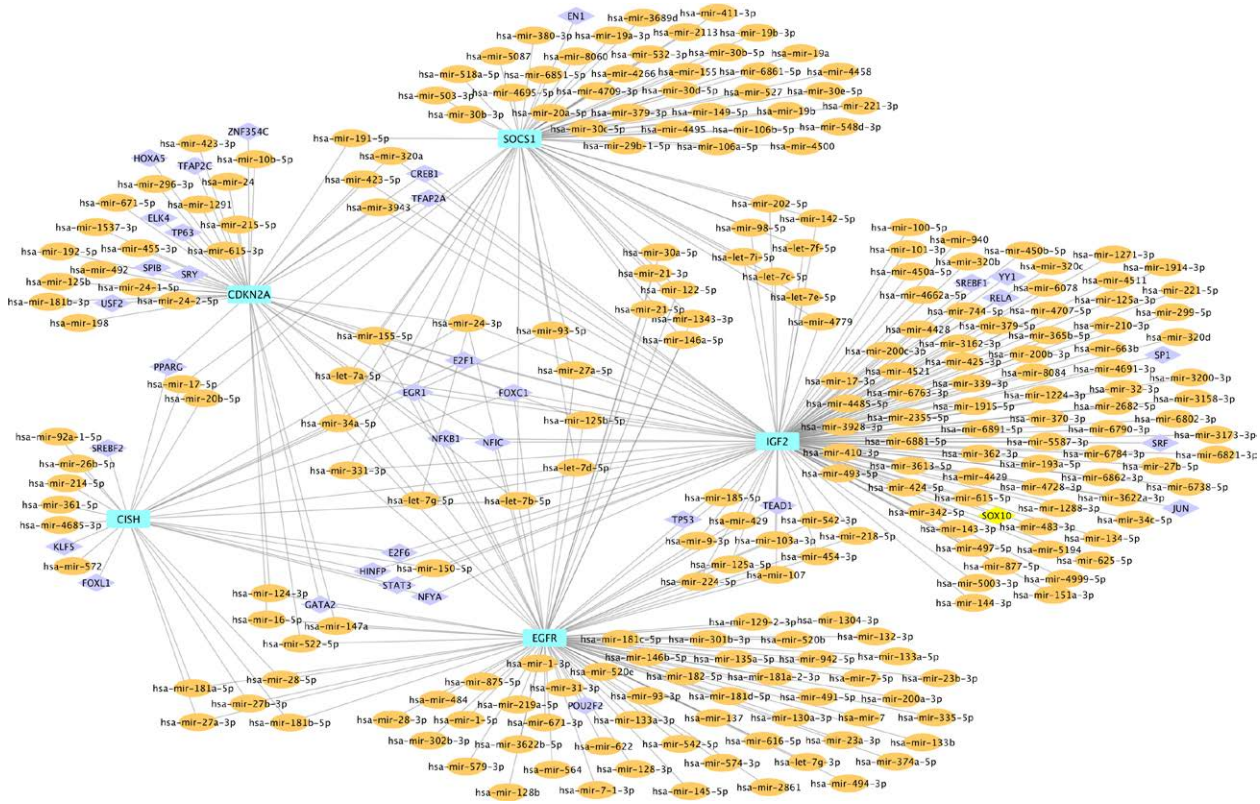

Figure 6. Regulation network.

the GSE74144 database was used to analyze the expression level of the hub genes. The boxplot of the expressed hub genes was drawn using the "ggplot2" in the R package.

3. Results

3.1. Identification of DEGs in HT

We compared the gene expressions between normal and HT samples by analyzing the GSE74144. A total of 168 upregulated and 404 downregulated genes were identified. The DEGs are presented in the volcano plot and the heatmap (Fig. 1A and B). It was observed that 59 genes overlapped between DEGs and immune-related genes (Fig. 1C).

3.2. Functional enrichment analysis

The results of the GO functional analysis are presented in Figure 2A. The top 10 enriched GO terms of DEIRGs were the positive regulation of cytosolic calcium ion concentration, peptide hormone secretion, positive regulation of protein kinase B signaling, lymphocyte differentiation, lymphocyte proliferation, divalent inorganic cation homeostasis, mononuclear cell proliferation, interleukin-7-mediated signaling pathway, and regulation of peptide hormone secretion.

The results of KEGG-pathway enrichment are presented in Figure 2B. The significant KEGG pathways of DEIRGs included the intestinal immune network for IgA production, autoimmune thyroid disease, JAK-STAT signaling pathway, hepatocellular carcinoma, Kaposi sarcoma-associated

herpesvirus infection, prolactin signaling pathway, allograft rejection, primary immunodeficiency, Cushing syndrome, and graft-versus-host disease.

3.3. PPI network and hub gene identification

The PPI network of the DEIRGs was constructed using STRING tools. DEIRGs were uploaded onto Cytoscape software to further construct the sub-network and identify hub genes. The results of the PPI network analysis are presented in Figure 3A. Five hub genes (insulin-like growth factor 2 [IGF2], cytokine-inducible Src homology 2-containing protein [CISH], suppressor of cytokine signaling 1 [SOCS1], cyclin-dependent kinase inhibitor 2A [CDKN2A], and epidermal growth factor receptor [EGFR]) were identified by the MCODE plug-in of Cytoscape. The PPI network of these 5 hub genes is presented in Figure 3B. The correlation between the hub genes was analyzed, and it was observed that CISH and EGFR were the most strongly correlated (R = 0.90) (Fig. 3C).

3.4. The ROC curve analysis

The ROC curves of 5 hub genes in the GSE74144 dataset are presented in Figure 4. The AUCs of SOCS1, CISH, EGFR, CDKN2A, and IGF2 were 0.804, 0.772, 0.768, 0.750, and 0.710, respectively. The AUC of all genes were >0.7, which suggested their potential diagnostic significance.

3.5. Analysis of the expression of hub genes

To explore the expression of these genes in the HT, the expressions of the 5 hub genes between the HT and normal groups in the GSE74144 dataset were determined (Fig. 5). The results indicated that the expressions of EGFR and CISH in HT patients were downregulated in HT patients, while those of CDKN2A, IGF2, and SOCS1 were upregulated.

3.6. Prediction of the key miRNAs and TF

The miRNA and TF regulatory network of the 5 hub genes was constructed using the program miRNet. The single gene had multiple relevant miRNAs and TFs, which formed a complex communication network. The interaction network consisted of 5 hub genes and 305 miRNA (Fig. 6). Subsequently, 110 miRNAs targeting IGF2, including hsa-let-7a-5p, hsa-mir-125b-5p, and hsa-mir-9-3p, 83 miRNAs targeting EGFR, including hsa-mir-27a-3p, hsa-mir-30a-5p, and hsa-mir-7-5p, 62 miRNAs targeting SOCS1, including hsa-let-7a-5p, hsa-let-7b-5p, and hsa-mir-155, 31 miRNAs targeting CDKN2A, including hsa-mir-24, hsa-mir-125b, and hsa-mir-147a, and 19 miRNAs targeting CISH, including hsa-let-7b-5p, hsa-let-7d-5p, and hsa-mir-17-5p were identified.

The interaction network consisted of 5 hub genes and 56 TFs. We observed that 20 TFs, including JUN and FOXC1, could regulate IGF2, 16 TFs, including FOXC1 and CREB1, could regulate CDKN2A, 10 TFs, including FOXC1 and NFYA, could regulate CISH, 8 TFs, including NFKB1 and TP53, could regulate EGFR, and 2 TFs, including EGR1 and EN1, could regulate SOCS1.

4. Discussion

HT is currently among the most common cardiovascular diseases in the world and is an important risk factor for diseases such as stroke, myocardial infarction, heart failure, and kidney failure.[16] Interestingly, increasing evidence indicates that immunity is involved in the pathogenesis of HT, including tissue damage, fibrosis, and dysfunction.[6–8,17–19] In this study, we used bioinformatics to identify the potential DEIRGs in HT, which provides the relevant theoretical basis for subsequent studies on HT.

In this study, we compared the DEIRGs between HT and normal and constructed a PPI network for them. Five key immune genes, SOCS1, CISH, EGFR, CDKN2A, and IGF2, were obtained.

The SOCS1 is the prototype molecule of the SOCS family. It contains a central SH2 domain that interacts with the JAK kinases and inhibits their phosphorylation.[20,21] Increasing studies have shown that SOCS1 plays a protective role in the development of cardiovascular disease, and changes in its expression are closely related to the cardiovascular risk of patients.[22,23] Satou et al reported that interferon-γ increased the expression of angiotensinogen in juxtaglomerular cells during the induction of inflammation which may lead to increased levels of angiotensin II, mediate the increase in blood pressure, and damage the kidney. Interestingly, SOCS1 can limit the increase in the angiotensinogen levels in the kidney by activating the JAK-STAT pathway in this process.[24] In contrast, deletion of SOCS1 can exacerbate immune and inflammatory response-mediated tissue cell damage.

CISH belongs to the SOCS family, which negatively regulates cytokine induction, especially inflammatory cytokine responses. It plays a key role in the occurrence and development of several inflammatory diseases.[25,26]

The EGFR belongs to the ErbB receptor tyrosine kinase family, which consists of an extracellular ligand-binding domain, an α-helical transmembrane domain, an intracellular tyrosine kinase domain, and a carboxyl-terminal region containing autophosphorylation sites. EGFR can act on a vast majority of cells in the body and plays an important role in cardiac development.[27] Moreover, Zeboudj et al reported that EGFR could limit the development of arteriosclerosis[28] and play a role in vasodilation in the pathogenesis of HT.[29]

CDKN2A is an inhibitor of cyclin-dependent kinase 4 and cyclin-dependent kinase 6. As a significant marker of cellular aging,[30] it exhibits tumor suppressor function.[31,32]

IGF2 is a polypeptide that is produced by the liver. It travels throughout the body through different modes of transport and participates in life activities such as cell growth, differentiation, and metabolism.[33] Besides, Wang et al reported that IGF2 was an epicardial mitogenic factor that was closely related to the proliferation of ventricular wall cardiomyocytes.[34]

However, no studies have yet confirmed whether there is a link between these 5 genes and high blood pressure. These genes are possibly novel genes that are associated with HT and can also be hotspots for future research.

Next, we identified 5 hub genes as biomarkers for the diagnosis of HT based on the ROC results, constructed and analyzed the target gene-miRNA regulatory network and target gene-TF regulatory network of the 5 hub genes using miRNet, and screened the closely-related target genes, miRNAs and TFs, thus providing new directions for subsequent research.

A major limitation of our research is the lack of independent experimental verification. Although 5 hub genes were identified, their roles in the pathogenesis of HT were not experimentally verified, and their specific mechanisms remain unclear. Further studies are needed to confirm this hypothesis and explore the specific mechanism of each gene. In conclusion, this study has identified 5 immune-related feature genes, and further exploration of these biomarkers may provide novel diagnostic and therapeutic targets for hypertensive patients.

5. Conclusion

Our study identified 5 immune-related hub genes in the HT and demonstrated that they were potential diagnostic biomarkers for HT.

Author contributions

Data curation: Wei Zhang.
Formal analysis: Wei Zhang, Linhu Zhang.
Writing – original draft: Linhu Zhang.
Writing – review & editing: Jianling Chen.

References

[1] Bakris G, Ali W, Parati G. ACC/AHA versus ESC/ESH on hypertension guidelines: JACC guideline comparison. J Am Coll Cardiol. 2019;73:3018–26.
[2] Poulter N, Prabhakaran D, Caulfield M. Hypertension. Lancet. 2015;386:801–12.
[3] Al-Makki A, DiPette D, Whelton PK, et al. Hypertension pharmacological treatment in adults: a World Health Organization guideline executive summary. Hypertension. 2022;79:293–301.
[4] Messerli FH, Williams B, Ritz E. Essential hypertension. Lancet. 2007;370:591–603.
[5] Kara SP, Ozkan G, Yılmaz A, et al. MicroRNA 21 and microRNA 155 levels in resistant hypertension, and their relationships with aldosterone. Ren Fail. 2021;43:676–83.
[6] Perrotta M, Carnevale D. Brain areas involved in modulating the immune response participating in hypertension and its target organ damage. Antioxid Redox Signal. 2021;35:1515–30.
[7] Hall JE, Mouton AJ, Da Silva AA, et al. Obesity, kidney dysfunction, and inflammation: interactions in hypertension. Cardiovasc Res. 2021;117:1859–76.
[8] Van Beusecum JP, Moreno H, Harrison DG. Innate immunity and clinical hypertension. J Hum Hypertens. 2021;1:7.
[9] Bhattacharya S, Dunn P, Thomas CG, et al. ImmPort, toward repurposing of open access immunological assay data for translational and clinical research. Sci Data. 2018;5:1–9.
[10] Ru B, Wong CN, Tong Y, et al. TISIDB: an integrated repository portal for tumor–immune system interactions. Bioinformatics. 2019;35:4200–2.
[11] Breuer K, Foroushani AK, Laird MR, et al. InnateDB: systems biology of innate immunity and beyond—recent updates and continuing curation. Nucleic Acids Res. 2013;41:D1228–33.
[12] Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43:e47–e47.
[13] Yu GC, Wang LG, Han YY, et al. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16:284–7.
[14] Fan Y, Xia J. miRNet-functional analysis and visual exploration of miRNA-target interactions in a network context. Methods Mol Biol. 2018;1819:215–33.
[15] Robin X, Turck N, Hainard A, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinform. 2011;12:1–8.
[16] Daugherty SL, Powers JD, Magid DJ, et al. Incidence and prognosis of resistant hypertension in hypertensive patients. Circulation. 2012;125:1635–42.
[17] Jafri S, Ormiston ML. Immune regulation of systemic hypertension, pulmonary arterial hypertension, and preeclampsia: shared disease mechanisms and translational opportunities. Am J Physiol Regul Integr Comp Physiol. 2017;313:R693–705.
[18] Rodriguez-Iturbe B, Pons H, Johnson RJ. Role of the immune system in hypertension. Physiol Rev. 2017;97:1127–64.
[19] Zhang RM, McNerney KP, Riek AE, et al. Immunity and hypertension. Acta Physiol. 2021;231:e13487.
[20] Ilangumaran S, Bobbala D, Ramanathan S. SOCS1: regulator of T cells in autoimmunity and cancer. Curr Top Microbiol Immunol. 2017;410:159–89.
[21] Zhang J, Li H, Yu JP, et al. Role of SOCS1 in tumor progression and therapeutic application. Int J Cancer. 2012;130:1971–80.
[22] Cittadini A, Monti MG, Iaccarino G, et al. SOCS1 gene transfer accelerates the transition to heart failure through the inhibition of the gp130/JAK/STAT pathway. Cardiovasc Res. 2012;96:381–90.
[23] Zhuang Y, Li T, Xiao H, et al. LncRNA-H19 drives cardiomyocyte senescence by targeting miR-19a/socs1/p53 axis. Front Pharmacol. 2021;12:631835.
[24] Satou R, Miyata K, Gonzalez-Villalobos RA, et al. Interferon-γ biphasically regulates angiotensinogen expression via a JAK-STAT pathway and suppressor of cytokine signaling 1 (SOCS1) in renal proximal tubular cells. FASEB J. 2012;26:1821–30.
[25] Peng HY, Hsiao JR, Chou ST, et al. MiR-944/CISH mediated inflammation via STAT3 is involved in oral cancer malignance by cigarette smoking. Neoplasia. 2020;22:554–65.
[26] Takeshima H, Horie M, Mikami Y, et al. CISH is a negative regulator of IL-13-induced CCL26 production in lung fibroblasts. Allergol Int. 2019;68:101–9.
[27] Makki N, Thiel KW, Miller F Jr. The epidermal growth factor receptor and its ligands in cardiovascular disease. Int J Mol Sci. 2013;14:20597–613.
[28] Zeboudj L, Giraud A, Guyonnet L, et al. Selective EGFR (Epidermal Growth Factor Receptor) deletion in myeloid cells limits atherosclerosis—brief report. Arterioscler Thromb Vasc Biol. 2018;38:114–9.
[29] Flamant M, Tharaux PL, Placier S, et al. Epidermal growth factor receptor transactivation mediates the tonic and fibrogenic effects of endothelin in the aortic wall of transgenic mice. FASEB J. 2003;17:327–9.
[30] Uyar B, Palmer D, Kowald A, et al. Single-cell analyses of aging, inflammation and senescence. Ageing Res Rev. 2020;64:101156.
[31] Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. Cell. 2007;130:223–33.
[32] Cottone L, Eden N, Usher I, et al. Frequent alterations in p16/CDKN2A identified by immunohistochemistry and FISH in chordoma. J Pathol Clin Res. 2020;6:113–23.
[33] Livingstone C. IGF2 and cancer. Endocr Relat Cancer. 2013;20:R321–39.
[34] Wang K, Shen H, Gan P, et al. Differential roles of insulin like growth factor 1 receptor and insulin receptor during embryonic heart development. BMC Dev Biol. 2019;19:1–8.