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Bioinformatics analysis of common differential genes of coronary artery disease and ischemic cardiomyopathy

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Abstract. – OBJECTIVE: This paper aims at screening the common differential genes of coronary atherosclerotic heart disease (CAD) and ischemic cardiomyopathy (ICM), and to conduct pathway analysis and protein-protein interaction (PPI) network analysis for the differential genes.

MATERIALS AND METHODS: The CAD and ICM datasets were collected from the Gene Expression Omnibus (GEO) database for human tumors to extract data components of peripheral blood RNA of patients and normal people in GSE71226 and GSE9128 chips; "limma" package of "R" software was used to screen the differential genes, and "pheatmap" package was applied to construct heat maps for the differential genes; Cytoscape, Database for Annotation, Visualization and Integration Discovery (DAVID) and String platforms were utilized for PPI network analysis, Genome Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis on the selected differential genes.

RESULTS: A total of 575 differential genes were screened from GSE71226, including 350 genes with up-regulated expression and 225 with down-regulated expression, which was statistically significant ($p<0.05$, fold change >1). 75 differential genes were screened from GSE9128, including 47 genes with up-regulated expression and 28 with down-regulated expression. By virtue of String, DAVID and Cytoscape software, the PPI network diagram was constructed, and GO and KEGG analyses were performed successfully.

CONCLUSIONS: A total of 8 common differential genes are screened, and functional annotation and pathway analysis are conducted, which is conducive to further studying the interactions between the differentially expressed genes.

Key Words
Coronary artery disease, Ischemic cardiomyopathy, Differential gene, Bioinformatics, KEGG.

Introduction

Coronary atherosclerotic heart disease (CAD) is one of the diseases with the highest mortality in the world, which is the first cause of death in the United States and other developed countries; the number of death from CAD in China ranks second in the world[1,2]. The causes of both CAD and ischemic cardiomyopathy (ICM) are related to atherosclerosis, and there is a certain association between the two diseases; as the incidence rate of CAD is increasing continuously, ICM is posing a more serious threat to human health[3,4]. Finding out more effective diagnostic methods, and fully understanding the similarities in the pathogenesis of CAD and ICM, through the association between the two diseases, can prevent the occurrence of CAD and ICM in a more effective manner. The data of gene expression profiles have been increased rapidly in recent years, and it has become a new research hot issue to deeply explore the data of gene expression profiles by taking advantage of bioinformatics methods. However, joint studies on the CAD and ICM mainly concentrate on the treatment, and there are few studies on the gene bioinformatics of both diseases at the same time although some studies on the gene bioinformatics of a single disease are conducted. In this research, bioinformatics methods were used to carry out a series of analyses on the data of gene expression profiles, and the analysis results were utilized to investigate the bioinformatics significance of the gene expression differences in patients with CAD and ICM, thus further expanding the studies on genes for molecular pathogenesis of CAD and ICM.

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Materials and Methods

Acquisition of Data of Gene Expression Profiles

The datasets of gene expression profiles with sequence numbers of GSE71226 and GSE9128 were acquired by logging in the Gene Expression Omnibus (GEO) database affiliated to National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov)[5] and the retrieval strategies of CAD and ICM were edited. In GSE71226, there were 3 cases of samples from CAD patients and 3 cases of samples from normal people; in GSE9128, the number of cases from ICM patients and normal people was 4 and 3, respectively. Both of the two chips were peripheral blood specimens, of which the detailed information is shown in Table I.

Preprocessing of Raw Data, Screening of Differential Genes and Drawing of Heat Maps

BRB-ArrayTools Version 4.3.2 Beta software was used to perform statistical analysis on the chip data; as for data processing method, the chip data were preprocessed with JustRMA algorithm, and the data were filtered and normalized by means of the median. Requirements for filtering gene: (1) Genes of the two types of samples with a greater than 2-fold change in the median, of which such a change occurred in at least 20% of the samples; (2) genes with a missing data rate of less than 50%. Independent-samples *t*-test was performed for genes meeting the filtering criteria. Class comparison tool was used for sample classification and comparison of the dataset, from which the differentially expressed genes of CAD, ICM and normal sample ($p<0.05$) were found. Finally, the "pheatmap" package of "R" software was applied to construct heat maps, and the regions in which the differential genes were mainly concentrated were highlighted.

Screening of Common Differential Genes

The differential genes selected from the two chips underwent Venn diagram analysis and a minority of genes in differential genes of the two was defined as common differential genes, whose expression levels in the chips were stored for subsequent analysis.

Genome Ontology (GO) Enrichment Analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Analysis

Database for Annotation, Visualization and Integration Discovery (DAVID) and "Bingo" (plug-in of Cytoscape software) were applied for GO enrichment analysis and KEGG pathway analysis for integrated differential genes. First, by logging in the DAVID database (https://david.ncifcrf.gov/), the Gene ID Conversion was selected, followed by submission of differential gene lists. Then, the options "Select Identifier" and "OFFICIAL_GENE_SYMBOL" were selected, followed by "List Type" and "Gene List". Finally, "Submit List" was clicked.

DAVID: DAVID software integrates all the major public bioinformatics resources, and can interpret gene-related biological mechanisms by providing enrichment analysis with standardized genetic terminologies. DAVID knowledge base aims to accelerate high-throughput gene function analysis; as for given gene lists, it provides rapid accessibility of extensive heterogeneous annotation data from the enriched area and enhances biological information levels of the individual gene.

Cytoscape: It is a type of software with graphical display network which can conduct analysis and edition, and it can perform GO enrichment analysis after the "Bingo" plug-in is downloaded.

Protein-Protein Interaction (PPI) Network Analysis

PPI network analysis was performed for differential genes by using String software. PPI refers to the generation process of protein complex combined with two or more protein molecules non-covalently. By logging in the String website (https://string-db.org/), the differential gene lists were submitted under the option "Multiple proteins", followed by a selection of options "Organism" and "Homo sapiens"; after that, "SEARCH" was clicked.

Results

Screening of Differential Genes and Preparation of Heat Maps

Screening of differential genes in GSE71226 and preparation of heat map

A total of 575 differential genes were selected from GSE64217, including 350 and 225 genes with up-regulated and down-regulated expressions, respectively, which were statistically significant (p<0.05, fold change >1). A part of major differential genes is presented in Table II. Based on the p-value, the former 50 differential genes with the lowest p-values were analyzed in the heat map (Figure 1).

Screening of differential genes in GSE9128 and preparation of heat map

A total of 75 differential genes were selected based on GSE9128, including 47 genes with up-regulated expression and 28 genes with down-regulated expression, which were statistically significant (p<0.05, fold change >1). A part of major differential genes is presented in Table III. Based on the p value, the former 50 differential genes with the lowest p-values were analyzed in the heat map (Figure 2).

Screening of common differential genes

A total of 8 common differential genes were screened from GSE71226 and GSE9128 (Table IV).

GO Enrichment Analysis

GO enrichment analysis of GSE71226

The differential genes of GSE71226 chip were mainly enriched in biological process (BP) and molecular function (MF), especially in "nucleic acid binding" (GO-ID: 3676, p=4.03E-05), "transition metal ion binding" (GO-ID: 46914, p=5.24E-05) and "DNA binding" (GO-ID: 3677, p=6.38E-05), which mainly influenced the synthesis of DNA and nucleic acid and ion binding function (Figure 3).

GO enrichment analysis of GSE9128

The differential genes of GSE9128 were mainly enriched in "regulation of nitrogen compound metabolic process" (GO-ID: 0051171, p=9.07E-07) and "cell migration" (GO-ID: 0016477, p=3.78E-06) of BP, influencing the cell migration and nitrogen compound metabolic process (Figure 4).

GO enrichment analysis of common differential genes

The GO enrichment analysis results of the 8 common differential genes of the two chips were mainly concentrated on the BP of EGR1 and PTGS2 genes, which were enriched in "regulation of synaptic plasticity" and "response to monosaccharide stimulus", mainly influencing the regulation of synaptic plasticity and the response to monosaccharide stimulus (Table V).

KEGG Pathway Analysis

KEGG pathway analysis of GSE71226

Three key pathways were found through the KEGG pathway analysis of differential genes via DAVID software, which were "Ubiquitin-mediated proteolysis", "Ribosome biogenesis in eukaryotes" and "Malaria" (Table VI).

KEGG pathway analysis of GSE9128

A total of ten key pathways were found through the KEGG pathway analysis of GSE9128, mainly including "Influenza A", "Legionellosis" and "Epstein-Barr virus infection" (Table VII).

PPI Network Analysis

PPI network analysis of GSE71226

30 key proteins were found via String software analysis, with topoisomerase (DNA) II beta (TOP2β) and ubiquitin A-52 residue ribosomal protein fusion product 1 (UBA52) as the most important, which connected 38 and 35 nodes, respectively (Figures 5 and 6).

PPI network analysis of GSE9128

3 major proteins were discovered in the PPI network analysis of GSE9128, with JUN connecting 15 nodes, early growth response protein 1 (EGR1) connecting 13 nodes and interleukin-8 (IL-8) connecting 13 nodes (Figures 7 and 8).

PPI network analysis of common differential genes

In the PPI network analysis of common differential genes of the two chips, EGR1 and prostaglandin-endoperoxide synthase 2 (PTGS2) proteins were the most important, which influenced each other; the remaining 6 proteins had no significant influencing characteristics (Figure 9).

Table I. Detailed data of GSE71226 and GSE9128.

Sequence number of chip	GSE71226	GSE9128
Platform	GPL570	GPL96
Disease	CAD	ICM
Chip provider	Cardiology Department of China-Japan Union Hospital of Jilin University	Immunological Laboratory of Regina Elena National Cancer Institute, Rome
Address	No. 126, Xiantai Street, Changchun, Jilin	Via delle Messi d'Oro 156
Research object	Human	Human
Sample type	Peripheral blood	Peripheral blood
Number of chip samples (used/total)	6/6	7/11
Time of uploading chip	Public on Jul. 23, 2015	Public on Sep. 22, 2007

Table II. A part of major differential genes in GSE64217.

Gene	Log [fold change (FC)]	*p*-value	Adjusted *p*-value (adj. *p*-value)
Rab-interacting lysosomal protein (RILP)	-2.147095722	2.39E-05	0.519622402
Spindling family member 2B (SPIN2B)	1.661030583	0.00015407	0.580694256
Myb/SANT DNA binding domain containing 2 (MSANTD2)	2.488876083	0.000173428	0.580694256
DROSHA	2.185495167	0.000226862	0.580694256
High mobility group nucleosome-binding domain-containing protein 3 (HMGN3)	1.699501333	0.000268061	0.580694256
Tubulin epsilon 1 (TUBE1)	1.555862278	0.000380039	0.580694256
Protein kinase, X-linked, pseudogene 1 (PRKXP1)	1.402863056	0.000419211	0.580694256
Fc receptor-like protein 1 (FCRL1)	1.564744667	0.000479077	0.580694256
Mitochondrial calcium uptake family, member 3 (MICU3)	1.583186583	0.000501088	0.580694256
Leucine rich repeat containing 37B (LRRC37B)	1.61102	0.000533859	0.580694256

Table III. A part of major differential genes in GSE9128.

Gene	LogFC	*p*-value	Adj. *p*-value
Retinoic acid receptor-related orphan receptor alpha (RORA)	1.5142365	1.13E-05	0.148124982
Modulator of apoptosis 1 (MOAP1)	1.47661056	4.10E-05	0.26810863
Heat shock protein family A (Hsp70) member 1 like (HSPA1L)	-1.065330929	8.93E-05	0.284855477
Tumor necrosis factor, alpha-induced protein 3 (TNFAIP3)	1.618240083	0.000124435	0.284855477
C-X3-C motif chemokine receptor 1 (CX3CR1)	-2.14302519	0.000132368	0.284855477
Zinc finger protein 331 (ZNF331)	1.685025048	0.000140332	0.284855477
Tribbles homolog 1 (TRIB1)	-1.486817298	0.000256554	0.284855477
LOC643549	1.004895286	0.000321581	0.284855477
JUN	1.074778405	0.000384447	0.284855477
Protein phosphatase 1 regulatory subunit 16B (PPP1R16B)	1.070037	0.000393124	0.284855477

Table IV. Common differential genes.

Gene	GSE71226			GSE9128		
	LogFC	*p*-value	Adj. p-value	LogFC	*p*-value	Adj. p-value
HAUS augmin-like complex subunit 3 (HAUS3)	1.288698167	0.008594697	0.580694256	1.573242202	0.000438314	0.28687629
Cluster of differentiation 69 (CD69)	1.278809556	0.006387396	0.580694256	1.705543196	0.001306	0.349257947
Formyl peptide receptor 2 (FPR2)	-2.119608722	0.011907638	0.580694256	-1.08499781	0.003579677	0.480971822
Prostaglandin-endoperoxide synthase 2 (PTGS2)	-1.602670667	0.02767191	0.622268496	-2.019087929	0.005876628	0.508553289
T-cell leukemia/ lymphoma 1A (TCL1A)	1.560834611	0.008745679	0.580694256	-1.06131219	0.00905957	0.516235805
G protein-coupled receptor associated sorting protein 1 (GPRASP1)	2.110098444	0.001530409	0.580694256	1.117531298	0.009229789	0.516235805
SMAD specific E3 ubiquitin protein ligase 2 (SMURF2)	1.271235167	0.012808126	0.580694256	1.101842905	0.010575545	0.516235805
Early growth response 1 (EGR1)	1.402198278	0.007568938	0.580694256	-1.334624202	0.018584697	0.58696371

Table V. GO enrichment analysis of common differential genes.

GO-ID	Description	*p*-value	Gene in test set
48167	Regulation of synaptic plasticity	2.79E-04	EGR1, PTGS2
34284	Response to monosaccharide stimulus	3.74E-04	EGR1, PTGS2
9746	Response to hexose stimulus	3.74E-04	EGR1, PTGS2
90271	Positive regulation of fibroblast growth factor production	4.50E-04	PTGS2
90270	Regulation of fibroblast growth factor production	4.50E-04	PTGS2
9743	Response to carbohydrate stimulus	5.22E-04	EGR1, PTGS2
31915	Positive regulation of synaptic plasticity	8.99E-04	PTGS2
4666	Prostaglandin-endoperoxide synthase activity	8.99E-04	PTGS2
71505	Response to mycophenolic acid	8.99E-04	EGR1
71504	Cellular response to heparin	8.99E-04	EGR1

Table VI. KEGG pathway analysis of GSE71226.

Term	Count	*p*-value	FDR
hsa04120: Ubiquitin mediated proteolysis	8	3.01E-02	31.23727048
hsa03008: Ribosome biogenesis in eukaryotes	6	4.12E-02	40.32156025
hsa05144: Malaria	4	9.11E-02	68.98225994

Term: enriched KEGG; Count: number of differential genes within Term; *p*-value: *p*-value of enrichment analysis; FDR: adjusted *p*-value.

Table VII. KEGG pathway analysis of GSE9128.

Term	Count	p-value	FDR
hsa05164: Influenza A	6	1.68E-03	1.880208757
hsa05134: Legionellosis	4	2.47E-03	2.755741792
hsa05169: Epstein-Barr virus infection	6	2.48E-03	2.756455026
hsa05162: Measles	5	4.33E-03	4.771844839
hsa05161: Hepatitis B	5	5.88E-03	6.431553444
hsa05132: Salmonella infection	4	8.29E-03	8.965010771
hsa04350: TGF-beta signaling pathway	4	8.57E-03	9.251985553
hsa05166: HTLV-I infection	6	8.77E-03	9.455377258
hsa04062: Chemokine signaling pathway	5	1.39E-02	14.57685928
hsa04668: TNF signaling pathway	4	1.61E-02	16.7176025

Term: enriched KEGG; Count: number of differential genes within Term; *p*-value: *p*-value of enrichment analysis; FDR: adjusted *p*-value.

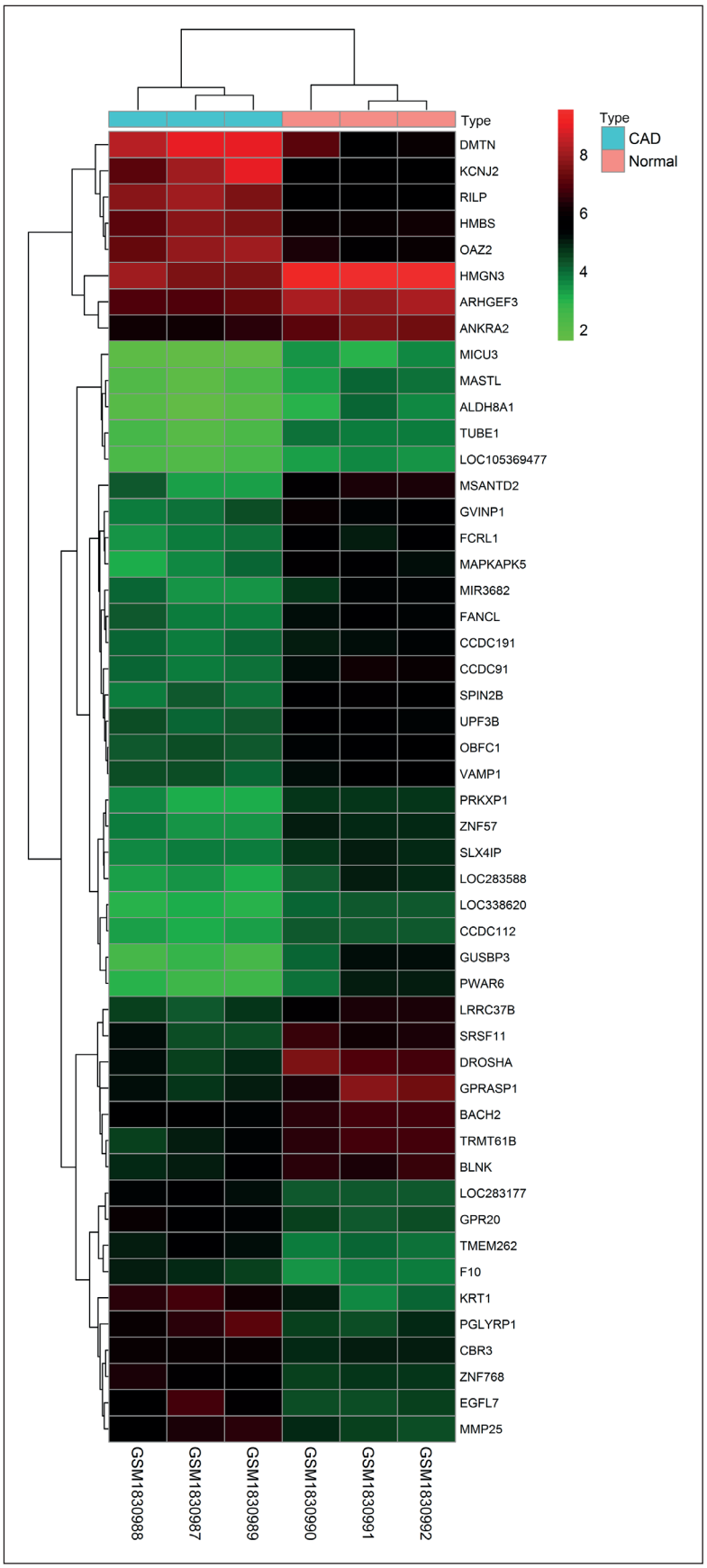

Figure 1. Heat map of GSE71226 differential genes. Heat map analysis is conducted for the former 50 differential genes by using "pheatmap" package of "R" software, and the regions in which the differential genes are mainly concentrated are highlighted.

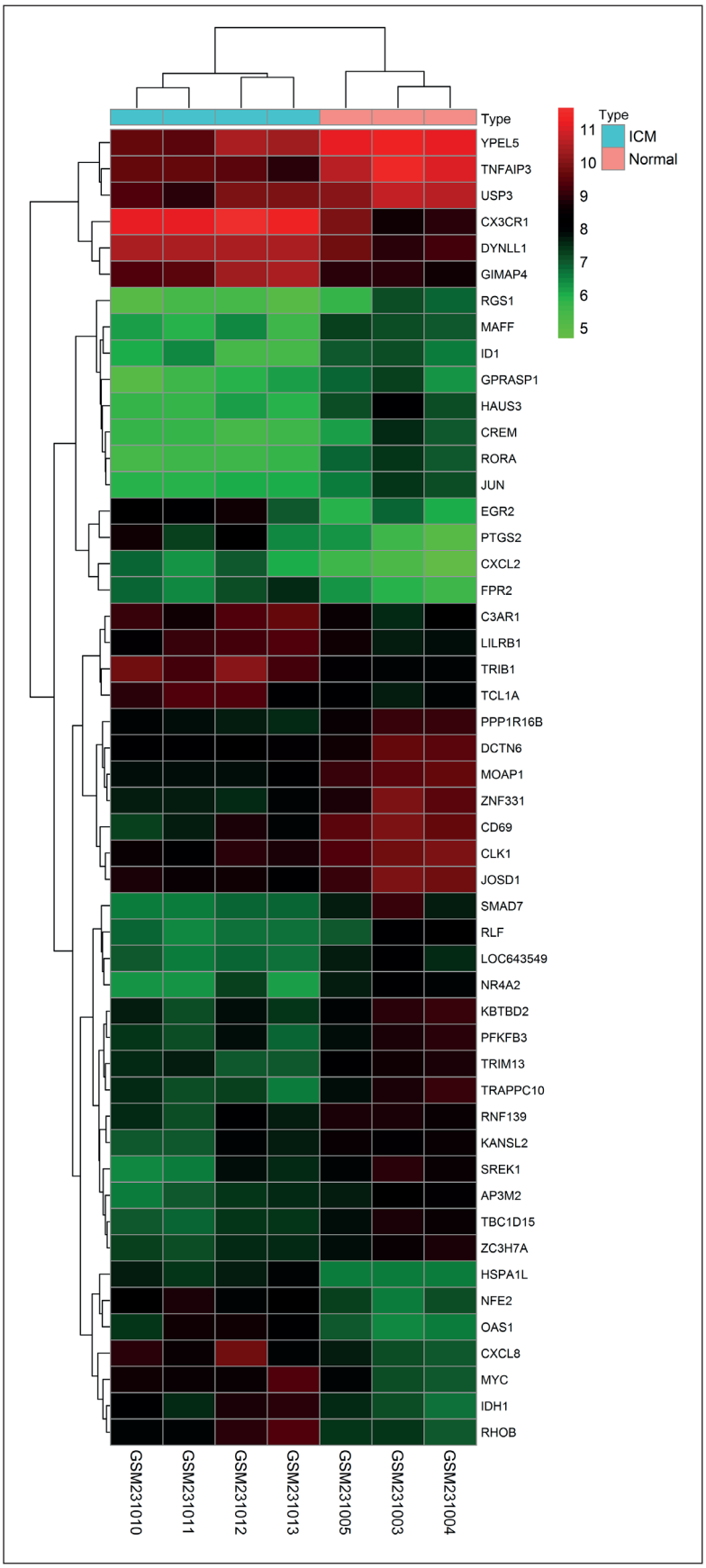

Figure 2. Heat map of GSE9128 differential genes. Heat map analysis is conducted for the former 50 differential genes by using "pheatmap" package of "R" software, and the regions in which the differential genes are mainly concentrated are highlighted.

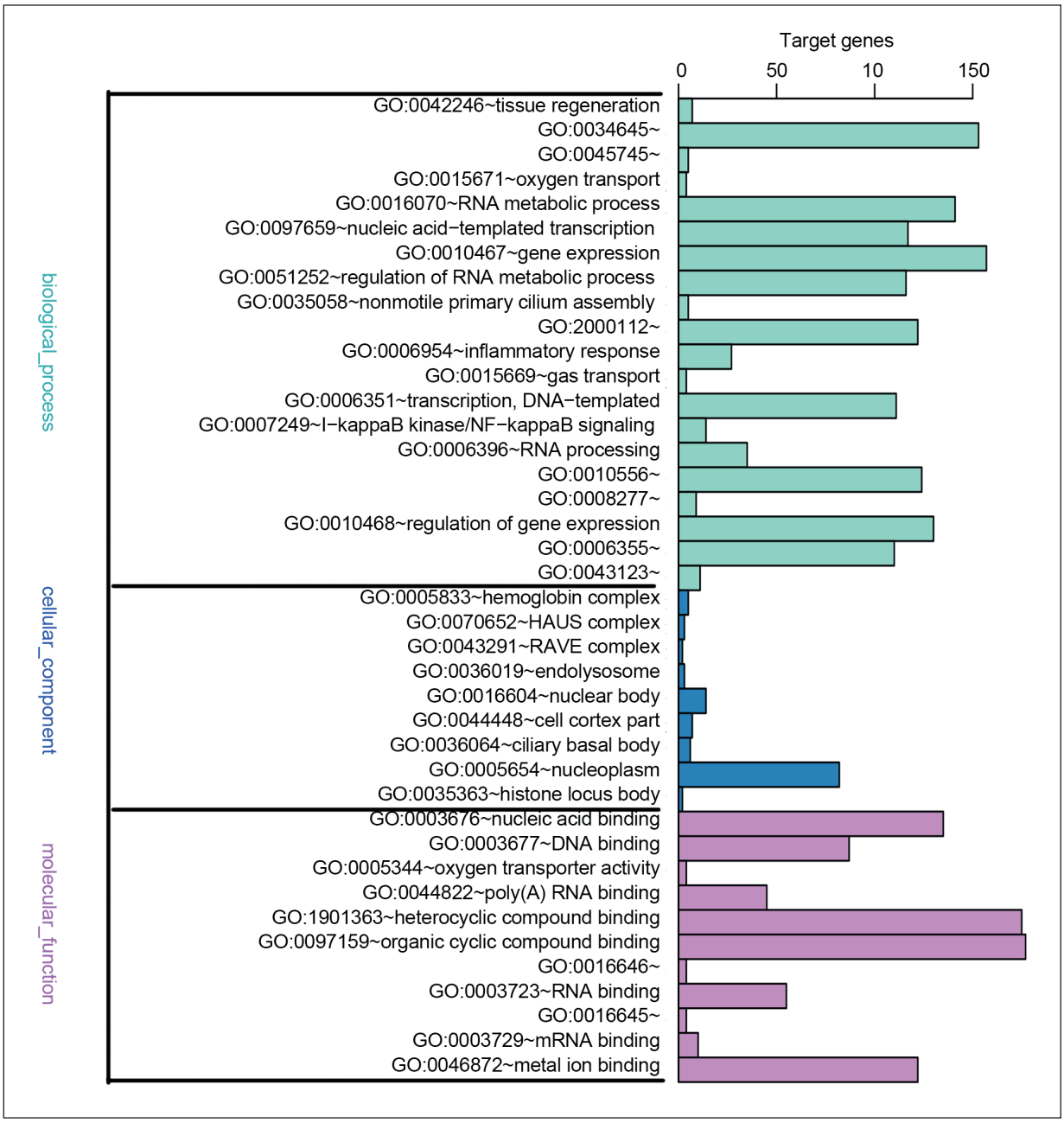

Figure 3. Results of GO enrichment analysis of GSE71226 differential genes. Abscissa represents enriched GO, and ordinate represents number and ratio of differential genes. Different colors stand for different GO classifications.

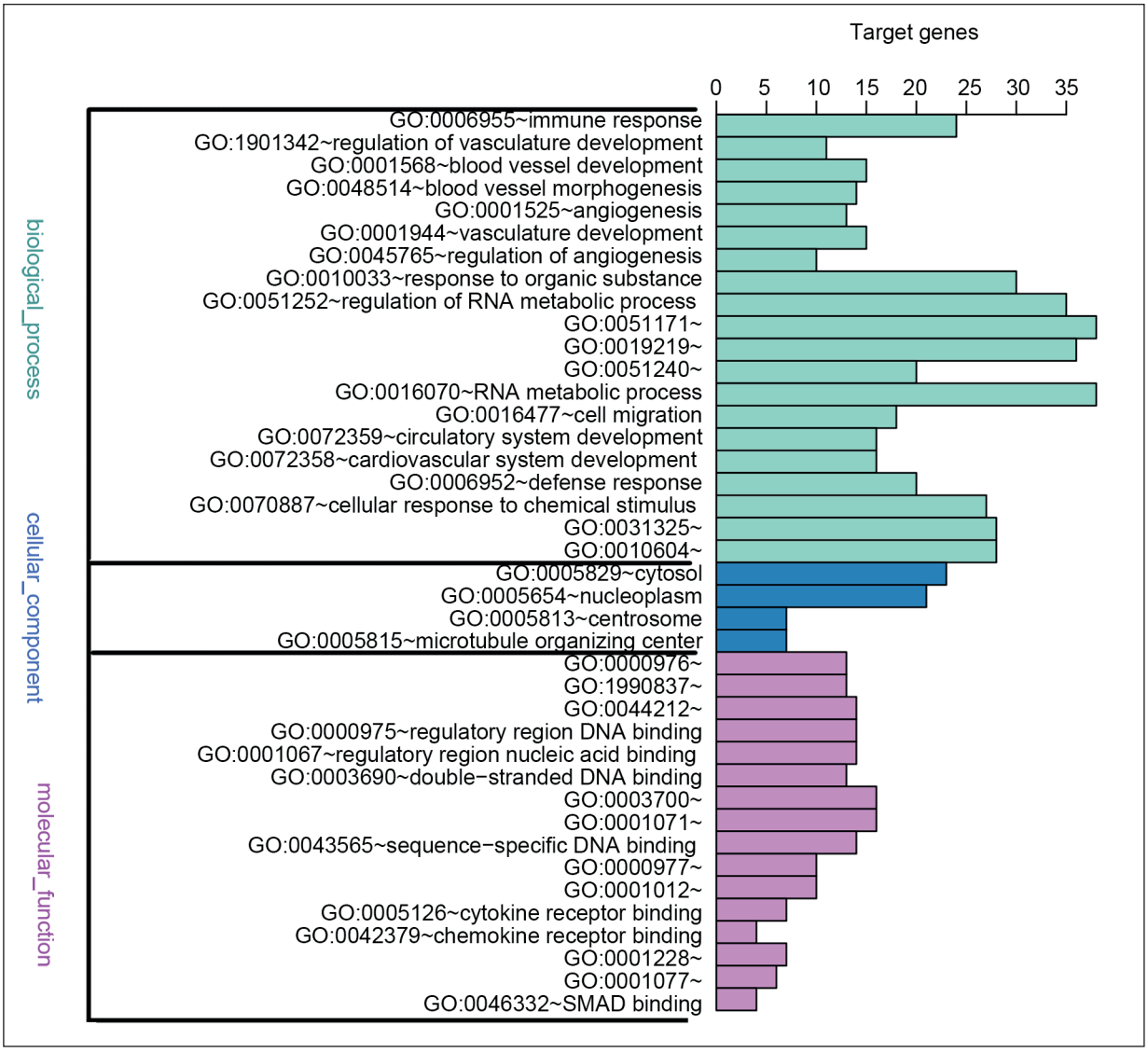

Figure 4. Results of GO enrichment analysis of GSE9128 differential genes. Abscissa represents enriched GO, and ordinate represents number and ratio of differential genes. Different colors stand for different GO classifications.

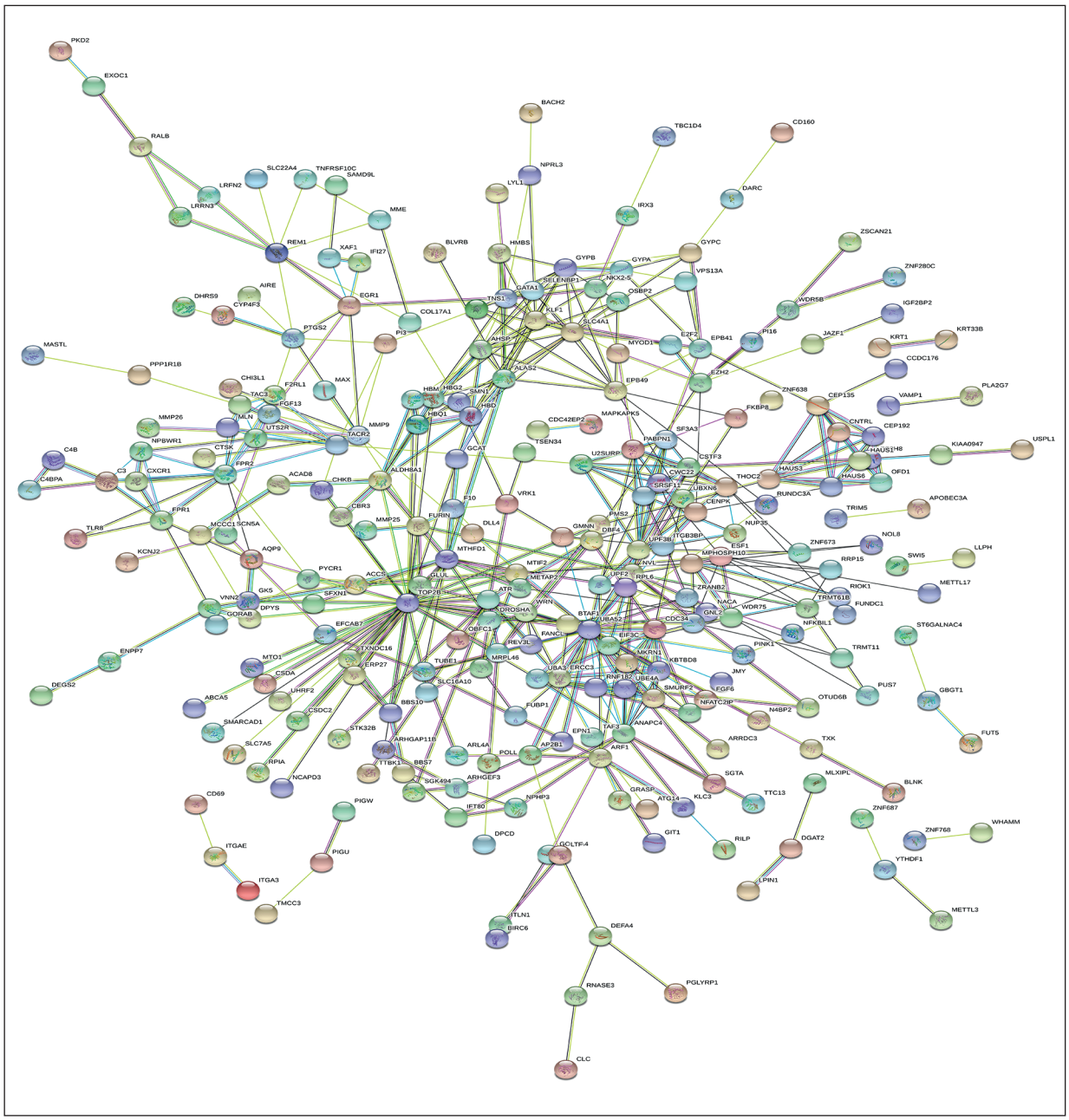

Figure 5. Results of PPI network analysis of GSE71226 differential genes. Circle represents gene; line represents PPI between genes, and results inside the circle represent protein structure. Line colors stand for the evidence of PPI.

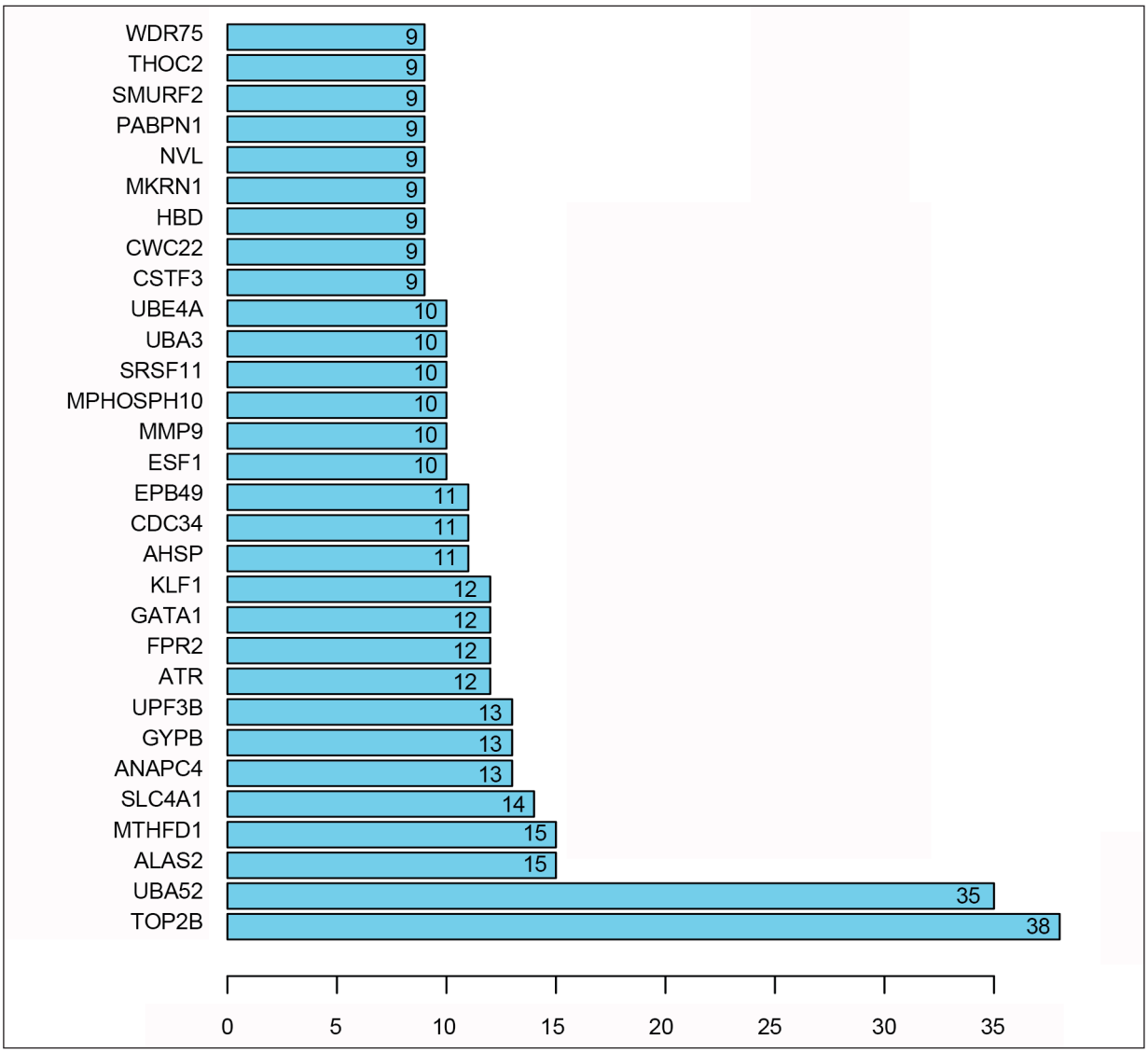

Figure 6. Histograms of core proteins of GSE71226 differential genes. Abscissa indicates gene name; ordinate represents number of contiguous gene, and height stands for number of gene connection.

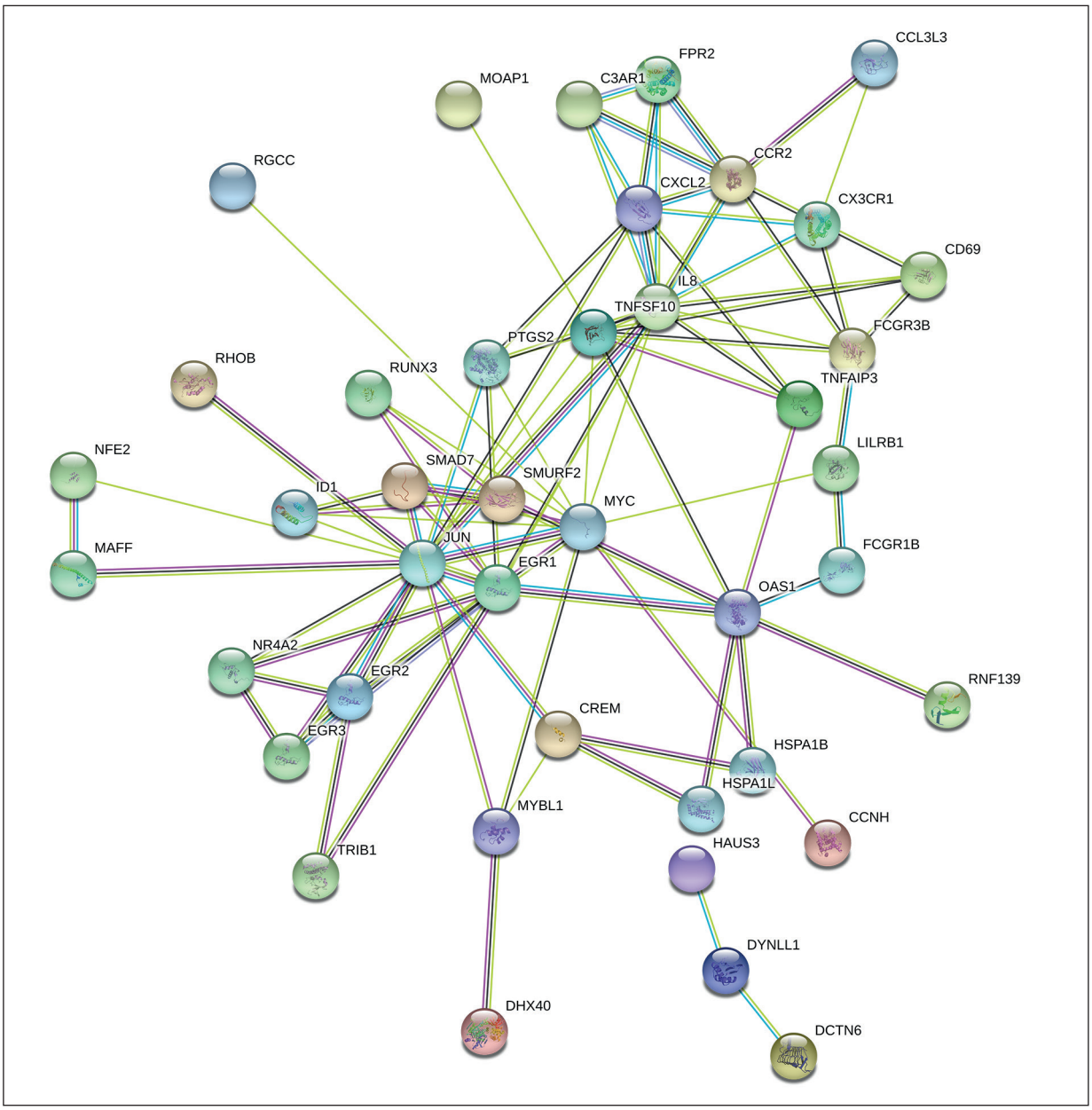

Figure 7. Results of PPI network analysis of GSE9128 differential genes. Circle represents gene; line represents PPI between genes, and results inside the circle represent protein structure. Line colors stand for the evidence of PPI.

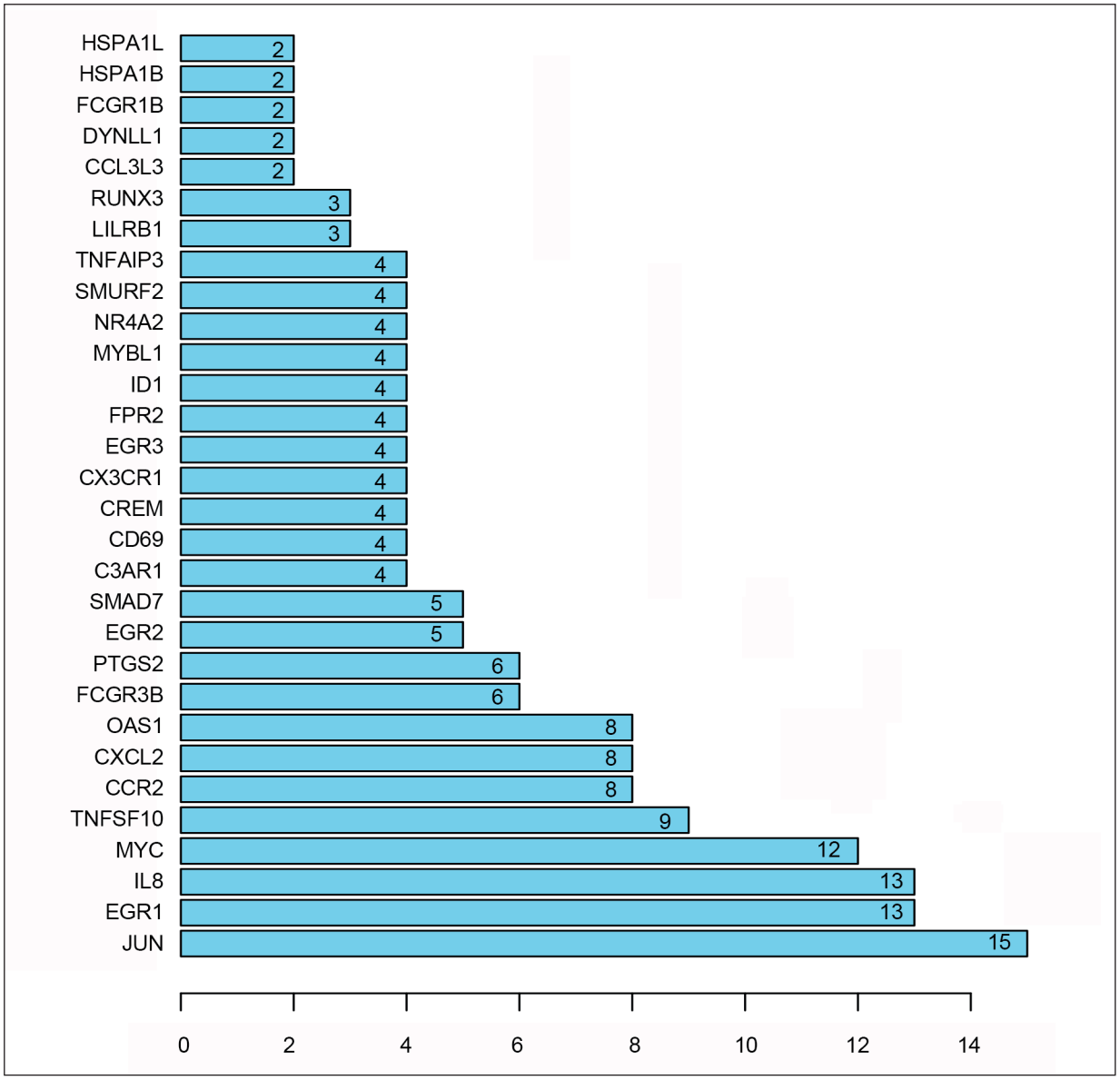

Figure 8. Histograms of core proteins of GSE9128 differential genes. Abscissa indicates gene name; ordinate represents number of contiguous gene, and height stands for number of gene connection.

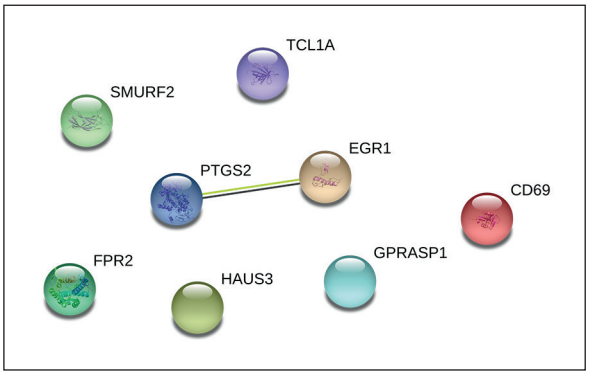

Figure 9. Diagram of PPI network analysis of common differential genes. Circle represents gene; line represents PPI between genes, and results inside the circle represent protein structure. Line colors stand for the evidence of PPI.

Discussion

As a disease with very high mortality, CAD is always a matter of concern, and the close association between ICM onset and CAD has attracted much attention. At present, China is in a period of rapid development, and people's material living standard is improving; the intake of a large amount of fatty food every day may aggravate cardiovascular diseases[6,7]. Although the mortalities of CAD and ICM have been controlled to some extent, there is no obvious decrease in the incidence rates, and the diseases are prone to occur in more young and middle-aged people[8]. LeBlanc et al[9] conducted a gene expression data analysis for CAD the other day, and it was assumed that CAD, and ICM may have a correlation at the genetic level; however, the correlation between the two diseases at the genetic level has not been reported in current studies yet.

The gene chip data of the two diseases were selected to find a common breakthrough point, by means of gene expression profile data, enrichment analysis, PPI network analysis and other bioinformatics approaches, so as to analyze the differences between CAD and ICM patients and normal people, and investigate the molecular pathogenesis of CAD and ICM. By virtue of gene chip data in the public database, the utilization of human, financial and material resources necessary for previous research on these aspects becomes more efficient; according to the exclusion and inclusion criteria, the most favorable and reliable chips for our study were selected from large quantities of gene chips; using multiple sample genes and a large amount of microarray data made the experimental results more reliable, and reduced the error rate, thus providing some valuable clinical references for treatment and prevention of CAD and ICM.

In our study, a total of 8 common differential genes of CAD and ICM were selected, including, HAUS3, CD69, FPR2, PTGS2, TCL1A, GPRASP1, SMURF2 and EGR1; the PPI analysis results suggested that PTGS2 and EGR1 were the most influential factors. PTGS2 is a prostaglandin-endoperoxide synthase, whose variant may increase the risk of prostatic hyperplasia[10]; moreover, the study of Vogel et al[11] proved that PTGS2 also affects the occurrence of colorectal cancer at a certain level; it is associated with the increased risk of tumor recurrence and poor colorectal cancer-specific survival in patients with the disease. EGR1 has an important relation with myopia and non–small-cell lung cancer all the time, and it is a crucial impact factor for inhibiting the synthesis of nucleoli and ribosomes; however, its correlation with eye growth and myopia still remains controversial at present[12]. PTGS2 and EGR1 were mentioned in the studies on CAD and ICM conducted by Deloukas et al[13] and Herrer et al[14]. However, not much attention has been paid to these genes in the two diseases due to some data. In this experiment, these two factors were regarded as the most important common differential genes of CAD and ICM, on which more investigations and experiments should be conducted; however, our limited experimental conditions could not satisfy the requirements for in-depth study on the molecular mechanisms of the factors; therefore, it is expected that scientific researchers can carry out systematic studies on the factors as well as CAD and ICM. The GO enrichment analysis of the 8 selected common differential genes showed that "regulation of synaptic plasticity", "response to monosaccharide stimulus", "response to hexose stimulus" and other BPs played vital roles in the pathogenesis of CAD and ICM. Alicea et al[15] indicated that the pathway for regulation of synaptic plasticity may be conducive to solving brain diseases, and that treatment strategies set for the pathway may treat the intracranial injury in a safe and effective way. The stimulus responses of monosaccharide and hexose are related to nutrient metabolism in the human body[16,17]; in CAD and ICM, changes in energy metabolisms of heart and myocardial ischemia have a close correlation with the two BPs; however, the intake of high-lipid energy seriously affects the changes, thus leading to abnormal BPs, which can easily induce CAD and ICM, and even accelerate the development of the diseases. In terms of KEGG pathway analysis, since there were too few common differential genes screened from the two diseases, the KEGG pathway analysis on the common differential genes failed, and no experimental results were obtained. However, the KEGG pathway analysis results of a single disease were collected, and "Ubiquitin-mediated proteolysis" and "Ribosome biogenesis in eukaryotes" of protein hydrolysis and ribosome synthesis were the major pathways influencing CAD; it was consistent with the results of GO analysis on the common differential genes, which was involved in human body metabolism as well[18]. The results of the KEGG pathway analysis on ICM were related to other diseases and viruses, of which "Influenza A" and "Le-

gionellosis" belong to the respiratory virus[19-21]. It suggested that the lack of antigens of such viruses may trigger ICM easily, and the results need to be further verified via basic experiments. It was indicated by Yu et al[22] that the main pathway influencing ICM is the interaction between drug metabolism and extracellular matrix receptor, which is inconsistent with our study results. The reason for such inconsistence may be the differences in selected chips, sample type and utilized analysis software.

In this experiment, the publishing time of the chips and number of samples might be the biggest defects; as time goes by, the differential genes of the two diseases may change due to variations of some reason. It was the greatest pity that the conclusions obtained from this paper could not be further investigated due to the limited experimental condition. It is expected that other scientific researchers can continue exploring the association between the two diseases at the genetic level.

Conclusions

There are 8 common differential genes of CAD and ICM in total, with PTGS2 and EGR1 as the most important, and the nutrient metabolism function in the human body has the potential to become an effective breakthrough point for preventing and treating the two diseases; however, the association between them needs to be explored by in-depth studies, and strengthening the research on genes of CAD and ICM will have an important impact on the diagnosis and treatment of the diseases.

Conflict of interest

The authors have no conflicts of interest to declare.

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