Based on Network Pharmacology and Bioinformatics to Explore the Mechanism of Bazhen Decoction in the Treatment of Clear-Cell Renal Cell Carcinoma

Yu Liu
Hospital of Chengdu University of Traditional Chinese Medicine
Mingquan Li (1563540954@qq.com)
Hospital of Chengdu University of Traditional Chinese Medicine https://orcid.org/0000-0002-9594-0577
LaiKuan Teh
Universiti Tunku Abdul Rahman
Liangbin zhao
Hospital of Chengdu University of Traditional Chinese Medicine
Naijing Ye
Hospital of Chengdu University of Traditional Chinese Medicine

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Abstract

Objective: To explore the mechanism of Bazhen Decoction in the treatment of Clear-cell renal cell carcinomaCRCC through the methods of network pharmacology and bioinformatics.

Methods: The active ingredients of Bazhen decoction were identified through TCMSP, Targets of each active ingredients were accessed through TCMSP and Swiss Target Prediction. The target gene by CRCC was obtained from GEO database and interlinked of both active ingredient and cancer gene was performed to obtain drug-disease protein target gene. Cytoscape 3.7.1 software was used to draw the active ingredient-target network. The protein interaction network was drawn using the String database and Cytoscape 3.7.1 software. Gene Ontology (GO) and KEGG pathway analysis on the target were performed using R language.

Results: From analysis, 160 of active ingredients were found from Bazhen Decoction with 5002 of predicted targets; 544 of disease targets and 39 drug-disease protein target genes.Network analysis on drug-disease protein interaction found out the targets were mainly involve mechanisms such as positive regulation of angiogenesis, positive regulation of vasculature development, epithelial cell proliferation,growth factor receptor and growth factor binding activity, as well as prostanoid receptor binding activity. Treatment of clear-cell renal cell carcinoma can be controlled through regulating the signaling pathways in relation to bladder cancer, MicroRNAs in cancer, focal adhesion, Human cytomegalovirus infection, Ovarian steroidogenesis and HIF-1 signaling pathway.

Conclusion: The mechanism of Bazhen Decoction in the treatment of CRCC reflects the characteristics of multiple ingredients, multiple targets, and multiple pathways of traditional Chinese medicine. The findings from this study able to provide a theoretical basis on anti-cancer mechanism.

1. Introduction

Renal cell carcinoma(RCC) is a common malignant tumors of the urinary system. Epidemiology study presented that the incidence of RCC is gradually increasing worldwide, and the incidence in developed countries is higher than in developing countries. Clear-cell renal cell carcinoma (CRCC) encompassed 80% of RCC with the occurrence is closely related to smoking, obesity, hypertension, and inactivation of VHL tumor suppressor genes. Surgery is the most preferred treatment whilst other treatments included with medication such as anti-angiogenesis and mammalian or mechanistic target of rapamycin (mTOR) inhibitors, hypoxia-inducible factor (HIF)-2α inhibitors, glutaminase small molecule inhibitors, immunotherapy, etc[1]. The abovementioned treatments are effective but bring significant toxicity and side effects to patients. Therefore, to look for a treatment method with less side effects plus good efficacy is indeed to treat CRCC. In recent years, traditional Chinese medicine has been widely used in the treatment of tumors because it can alleviate the side effects of drugs and biological treatment, improve patient quality of life, and prolong patient survival time[2].

Bazhen Decoction is one of the famous prescriptions of traditional Chinese medicine. It is widely used clinically in the treatment of tumor and to relieve its complications. Bazhen Decoction was first documented in the "RuiZhuTang Experience Formula" during Yuan Dynasty. It is consisted of eight Chinese herbs [renshen (*Radix Ginseng*), baizhu (*Rhizoma Atractylodis Macrocephalae*), fuling (*Scierotium Poriae Cocos*), zhigancao (*Radix Glycyrrhizae*), shudi (*Radix Rehmanniae*), baishao (*Radix Albus Paeoniae Lactiflorae*), danggui (*Radix Angelicae Sinensis*), chuanxiong (*Rhizoma Ligustici Chuanxiong*)].It is effective in nourishing qi and blood. Clinically, it has been widely used for the treatment of qi and blood deficiency syndrome. According to the Chinese Medicine Guidelines for Kidney Cancer Diagnosis and Treatment (2008 edition), Bazhen decoction is recommended as the first choice for treatment of CRCC with both qi and blood deficiency. Modern research shows that presence of the active ingredients in the eight herbs in Bazhen Decoction exert anti-tumor effects.In which, ginsenosides in *Radix Ginseng*, rehmannia glutinosa polysaccharides in *Radix Rehmanniae*, atractylodes macrocephala in *Rhizoma Atractylodis Macrocephalae*, poria cocos polysaccharides and Poria cocos in *Scierotium Poriae Cocos*, and flavonoids in *Radix Glycyrrhizae*, glycyrrhizic acid, glycyrrhetinic acid,etc.These active ingredients exert anti-tumor properties by inhibiting tumor angiogenesis, tumor invasion and metastasis while promoting the activation of T cells and a series of lymphokines[3].

Although Bazhen decoction is widely used in clinic practice and has gained good effect.However, the characteristics of multiple ingredients, multiple pathways and multiple targets of traditional Chinese medicine, the substance basis and mechanism of action of Bazhen Decoction is yet to be elucidated. With the concept of pharmacology network put forward, it provides theoretical support for exploring the material basis and mechanism of action of traditional Chinese medicine. Network pharmacology is based on multi-disciplinary theories including biology systems, multi-directional pharmacology, and molecular mechanism of drug intervention in diseases from a multi-dimensional perspective. Many studies have shown the mechanism of action of traditional Chinese medicine through network pharmacology is accurate and reliable. This study is to focus on 160 active ingredients of Bazhen Decoction using network pharmacology and bioinformatics prediction to construct an active ingredient-target network. Network topology analysis was performed to explore the multi-component, multi-target and multi-path of Bazhen Decoction. This study will give the fundamental understanding on the mechanism of Bazhen Decoction in the treatment of CRCC.

2. Methods

2.1 Acquisition of chemical and active ingredients

The constituent medicines of Bazhen Decoction was used as keywords to search for all chemical ingredients presence from Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://lsp.nwsuaf.edu.cn/tcmsp.php). Oral bioavailability (OB) greater than 30% and pharmacokinetic value (DL) greater than 0.18 were used as the criterions to screen for the active ingredients from TCMSP.

2.2 Target prediction of the active ingredients

The targets of the active constituents in Bazhen Decoction were queried from TCMSP and SwissTargetPrediction(http://www.swisstargetprediction.ch/), The target protein name was transformed into a gene name by using Perl (http://www.perl.org/) and the UniProt database (http://www.UniProt.org/).

2.3 Target prediction of disease

The gene chip data set GSE16441 was downloaded from GEO database (https://www.ncbi.nlm.nih.gov/geo/). The chip was consisted of 34 samples, including 17 samples in the normal group and 17 samples in the tumor group using the chip platform, GPL6480 Agilent-014850 Whole Human Genome Microarray 4x44K.

Differential gene screening: The matrix file was read and downloaded from R-language software. Limma package was used to obtain the differential genes between normal and tumor group. Adjusted *P*-value < 0.05 and |Log2 (Fold change)|>2 were used as the screening criterions for differentially expressed genes. Hierarchical clustering analysis was performed on the selected differential genes. The "pheatmap" R language package was used to draw volcano maps, differential gene heat maps to intuitively understand the differences in gene expression profiles.

2.4Intersection of active ingredients and disease targets

We downloaded the R package (https://www.rproject.org/) at first and entered the command code to install the toolkit for drawing a Venn diagram in R.Then,by using the files previously prepared that contained the active ingredients of Bazhen Decoction and the disease targets, a specific command code was entered in R, which generated the Venn diagram and a list describing the specific outcomes of the analysis. This “ingredients to disease” list(Table S.Ingredient and disease targets intersection)was used in the following steps.

2.5 Network construction and analysis

The “ingredients to disease” list was imported into Cytoscape 3.7.1 software;followed by introduction of the Bazhen Decoction ingredient names into Cytoscape to construct the model of the ingredients-targets network. In the network construction,nodes were used to represent molecules or target proteins, and edges were used to represent the relationships among ingredients and targets.

2.6 Construction of the protein interaction network

The “ingredients to disease” list was also imported into the Search Tool in Retrieval of Interacting Genes (STRING) database (http://string-db.org) with the operating interface specified to “Homo sapiens” as species. The minimum interaction threshold was adjusted to 0.7 in order to determine the relationships between potential targets of Bazhen Decoction in the treatment of CRCC. Node1, node2 and combined

score information were imported into Cytoscape 3.7.1 software to draw and analyze the interaction network. The node size was set to reflect the size of the degree while the thickness of the edge was set to reflect the combination of size score in the final protein interaction network.

2.7 Gene ontology and pathway enrichment analysis

Bioconductor provided tools (http://www.bioconductor.org/) were used for analysis and interpretation of high-throughput genomic data. The enrichment analysis of Gene Ontology (GO) functions and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were carried out on the genes in the ingredient-targets network, and the results were obtained (P-adjusted value < 0.05). By using the count score, GO with top 5 presentation while pathway enrichment with top 20 presentation were selected, respectively.

3. Result

3.1 Active ingredients of Bazhen Decoction

After setting the filtering criteria mentioned above, 160 active ingredients of Bazhen Decoction were determined.

3.2 Potential targets of the active ingredients

From TCMSP and SwissTargetPrediction, there were 5002 potential targets of Bazhen Decoction active ingredients and their corresponding symbols were collected.

3.3 Potential targets of CRCC

Based on the selection criteria of |Log2 (Fold change) |>2, a total of 544 differentially expressed genes were obtained. A volcano map (Fig. 1) and a differential heat map (Fig. 2) are presented to show the potential targets. Among them, red represents up-regulated genes, green represents down-regulated genes, and black represents undifferentiated genes.

3.4 Ingredient and disease targets intersection

After inputting the potential targets of the ingredients and the disease targets into the R platform, the intersection of the 2 types of targets was determined.The Venn diagram showed that 39 potential targets had r elationships with active ingredients and CRCC (Fig. 3).

3.5 I-T network

As shown in Fig. 4,a ingredients-targets network was generated.

3.6 Protein–protein interaction (PPI) network

In the “ingredients and disease intersection” part of the PPI network, CRCC targets and active ingredient potential targets showed 39 duplicate genes. These genes may become targets for the treatment of CRCC. To study the interaction of the targets *in vivo* and search for the hub genes, PPI network analysis

of the potential target groups was carried out by adjusting the node size and the thickness of the edge(Fig. 5)demonstrates the top 3 proteins involved in the interaction were VEGFA (11), EGF(7) and MMP9 (7).

3.7 GO and KEGG pathway enrichment analysis

The GO enrichment analysis indicated that Bazhen Decoction acted on CRCC by regulating multiple biological processes (BP) ($p < 0.05$, Fig. 6). These processes were response in positive regulation of angiogenesis(GO:0045766), vasculature development（GO:1904018), regulation of vasculature development（GO:1901342）as well as epithelial cell proliferation（GO:0050673）. The main Cellular components (CC) terms ($p < 0.05$,Fig. 7) involved were caveola（GO:0005901）,microvillus（GO:0005902）,actin-based cell projection（GO:0098858）, microvillus membrane（GO:0031528）and plasma membrane raft（GO:0044853）. Whereas, the molecular functions (MF) terms ($p < 0.05$,Fig. 8) were found involving growth factor receptor binding（GO:0070851）,prostaglandin receptor activity（ GO:0004955 ）,heme binding（ GO:0020037 ）growth factor binding（GO:0019838）and prostanoid receptor activity（GO:0004954）.

Meanwhile, a dot plot indicated the gene ratio of the number of target genes involved in one biological process to the number of all annotated genes. The higher the ratio represented higher enrichment level. The size of the dot reflects the number of target genes in the analysis, and the different colors of the dots indicate the different *P*-adjusted value ranges(Fig. 9,10,11).

To elucidate the critical pathways among the 39 potential targets in terms of CRCC therapy, the top 20 pathways were filtered according to a *P*-adjusted value < 0.05 (Fig. 12). Throughout analysis, treating CRCC using Bazhen decoction was found involvement in bladder cancer(hsa05219),microRNAs in cance（hsa05206）focal adhesion（hsa04510）, Human cytomegalovirus infection（hsa05163）,Ovarian steroidogenesis（hsa04913）, HIF-1 signaling pathway（hsa04066）, chemical-induced carcinogenesis（hsa05204）Ras signaling pathway（hsa04014）,PI3K-Akt signaling pathway（hsa04151）Prostate cancer(hsa05215),AGE-RAGE signaling pathway in diabetic complications(hsa04933), MAPK signaling pathway（hsa04010),VEGF signaling pathway(hsa04370),Rap1 signaling pathway（hsa04015）, human papillomavirus infection（hsa05165）,fluid shear stress and atheroscle rosis（hsa05418),Melanoma（hsa05218）,Glioma（hsa05214）,Pancreatic can cer（hsa05212 ）and metabolism of xenobiotics by cytochrome P450（hsa 00980）. By applying an identical analytical method as that used for the GO analysis, a dot plot showing the relevant pathways was obtained that showed the same data as the GO dot plot (Fig. 13).

4. Discussion

Traditional medicine believes that the vital *qi* is kept inside, evil is irrelevant. When the vital *qi* of human body is weak, it will lead to deficiency of *qi* and blood, in which is the root in causing CRCC. Bazhen Decoction able nourish qi and blood. Sufficient *qi* and blood enhance active body mechanism, thereby achieving the goal in treating CRCC .

This article utilized network pharmacology and bioinformatics predictions to explore the mechanism of Bazhen Decoction in the treatment of CRCC. The findings demonstrated that Bazhen decoction composed of variety of anti-tumor properties. Subsequent analysis of the active ingredient-target network of Bazhen Decoction presented that quercetin, kaempferol and myricetin were the three most active ingredients. These three ingredients exhibit anti-tumor by inhibiting cell proliferation, promoting apoptosis, regulating cell cycle progression, affecting autophagy, inhibiting cell invasion and metastasis [4–6]. From the analysis, the targets demonstred the highest moderate value were PTGS2, MMP9, PLA2G4A. PTGS2 is overexpressed in many tumors, including RCC, in promoting tumor formation by inducing proliferation and angiogenesis while reducing apoptosis and immunosuppressive activity, as supported by Mohtarrudin *et al.* Mohtarrudin *et al.* found that 18 patients had a positive immune response when investigating on 36 stained tissue sections from patients with CRCC with PTGS2 monoclonal antibody [7–8]. MMP9 maintain the homeostasis of the extracellular matrix, and its mRNA or protein expression is related to clinicopathological parameters. In addition, MMP9 can also predict disease-free survival in patients with CRCC [9]. From this, the author speculates that quercetin, kaempferol, and myricetin may act on these key targets, PTGS2, MMP9, PLA2G4A as the pharmacolokinetic of Bazhen Decoction.

Protein interaction network analysis shown VEGFA, EGF, MMP9 and other core targets were the target protein for the treatment of CRCC. They are involved in the pathogenesis of CRCC. VEGFA is a member of the PDGF/VEGF growth factor family and can induce the proliferation and migration of vascular endothelial cells which is essential for both physiological and pathological angiogenesis, and thus plays a vital role in the pathogenesis of kidney cancer. [10]. EGF is a growth factor that can stimulate cell growth, proliferation and differentiation by binding to its receptor EGFR. When EGF is up-regulated, it will cause the development of CRCC.In addition, EGF can promote cancer metastasis by affecting tumor lymphangiogenesis and inducing epithelial-mesenchymal transition [11].

The results of GO enrichment analysis showed that the target of Bazhen Decoction 's active ingredients would affect biological processes such as angiogenesis, development, and proliferation of epithelial cells. Cell components involved were microvillus, microvillus membrane, plasma membrane rafts, whereas molecular functions involved were such as prostaglandin receptor activity,growth factor receptor binding and growth factor binding. Angiogenesis is formed by the existing blood vessels through the process of budding. This is used as a hallmark of cancer and in determination on the growth and metastasis of tumors [12]. Growth factor receptors are a type of transmembrane protein with tyrosine kinase activity, which can mediate and regulate various cell signal transductions, including normal and cancer cell differentiation, cell proliferation, and homeostasis [13]. Growth factors are a class of peptides to regulate cell growth and other cell functions by binding to specific, high-affinity cell membrane receptors. The involved growth factors found are epidermal growth factor, fibroblast growth factor and platelet-derived proliferation factor. All these growth factors played an important regulatory role in human immunity, tumorigenesis, inflammation and infection, blood vessel formation, cell differentiation, and apoptosis. Studies have shown that growth factors promote tumor progression and metastasis by regulating autonomous cell growth and clonal expansion, accelerating intraepithelial proliferation,

inducing basement membrane rupture and invasive growth, and inducing angiogenesis [14]. Prostaglandins are naturally occurring lipids, produced by arachidonic acid metabolism mediated by cyclooxygenase, and expressed in large amounts in the kidney. Prostaglandins are upregulated in many malignant tumors in participating of cancer cell metastasis which associated with poor prognosis. PGE2 is a prostaglandin abundantly present in the kidney. Wu *et al.* proposed through research that PGE2 can promote the invasion and metastasis of CRCC by activating EP4 [15].

The enrichment results of KEGG pathway showed that Bazhen Decoction in the treatment of CRCC mainly involves mainly involved signaling pathways such as bladder cancer, microRNAs in cancer, focal adhesion, human cytomegalovirus infection, ovarian steroidogenesis, HIF-1 signaling pathway and so on. MicroRNA (miRNA) is a type of noncoding small RNA with a length of 20–24 nucleotides. It plays an important role in regulating cell homeostasis, differentiation, development, proliferation, and apoptosis. miRNA involved in the pathogenesis of tumors as miRNA able to control cell cycle, apoptosis, neovascularization, and tissue infiltration and metastasis [16]. HIF-1 is a transcription factor that regulates the homeostasis of oxygen. When malignant tumors grow excessively, it will elevate the consumption of oxygen and nutrients in subsequently resulted a hypoxic tumor microenvironment.With this microenvironment, tumor cells need to adapt to the HIF-1 signaling pathway. HIF-1 can mediate tumor adaptation to the hypoxia by participating in the formation of tumor blood vessels, maintaining tumor cell stability, and promoting tumor cell metastasis [17].

5. Conclusion

The network pharmacology and bioinformatics prediction performed in this study found quercetin, kaempferol, myricetin and other active ingredients in Bazhen decoction. These active ingredients may affect angiogenesis, development, epithelial cell proliferation, growth factor receptor binding, and prostaglandin receptor activity. Besides, it also involved in growth factor binding and other mechanisms including regulating some signaling pathways such as bladder cancer formation, MicroRNAs activity in cancer, focal adhesion, human cytomegalovirus infection, Ovarian steroidogenesis, HIF-1 signaling pathway and etc. As the network pharmacology analysis conducted in this study was solely based on findings through bioinformatics predictions, thus, further screening and verification *in vivo* and *in vitro* is encouraged to be carried out based on the findings in order to clearly elucidate the pharmacokinety of Bazhen Decoction in treatment of CRCC.

Abbreviations

RCC
Renal cell carcinoma
CRCC
Clear-cell renal cell carcinoma
TCMSP
Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

manuscript is approved by all authors for publication

Availability of data and materials

The dataset(s) supporting the conclusions of this article are available in the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://lsp.nwsuaf.edu.cn/tcmsp.php)SwissTargetPrediction(http://www.swisstargetprediction.ch/),GEO database (https://www.ncbi.nlm.nih.gov/geo/).

Competing interests

None.

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Authors' contributions

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Acknowledgements

Not applicable

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Figures

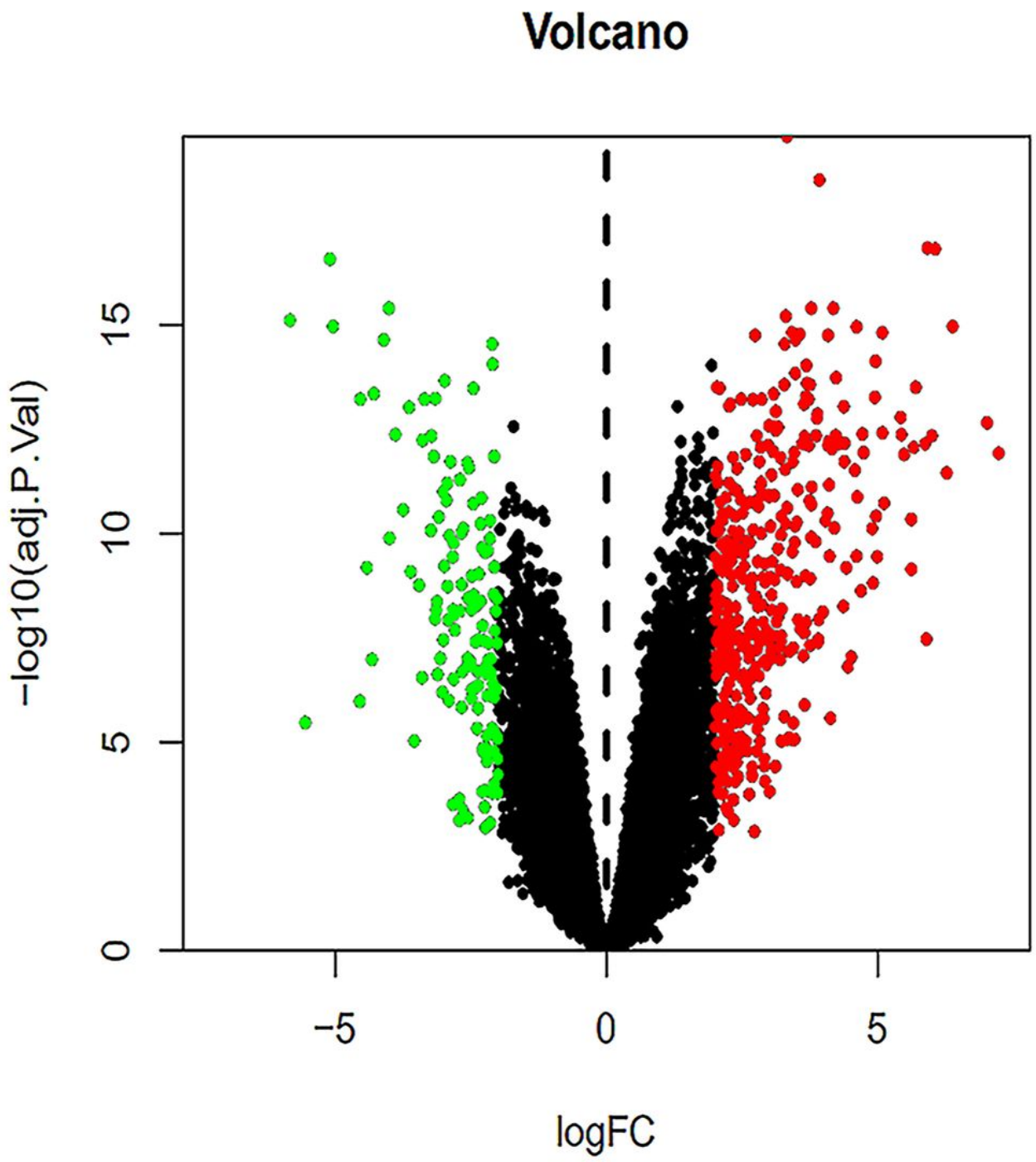

Figure 1

Volcano diagram of differentially expressed genes in kidney tissues between patients with CRCC and healthy people.

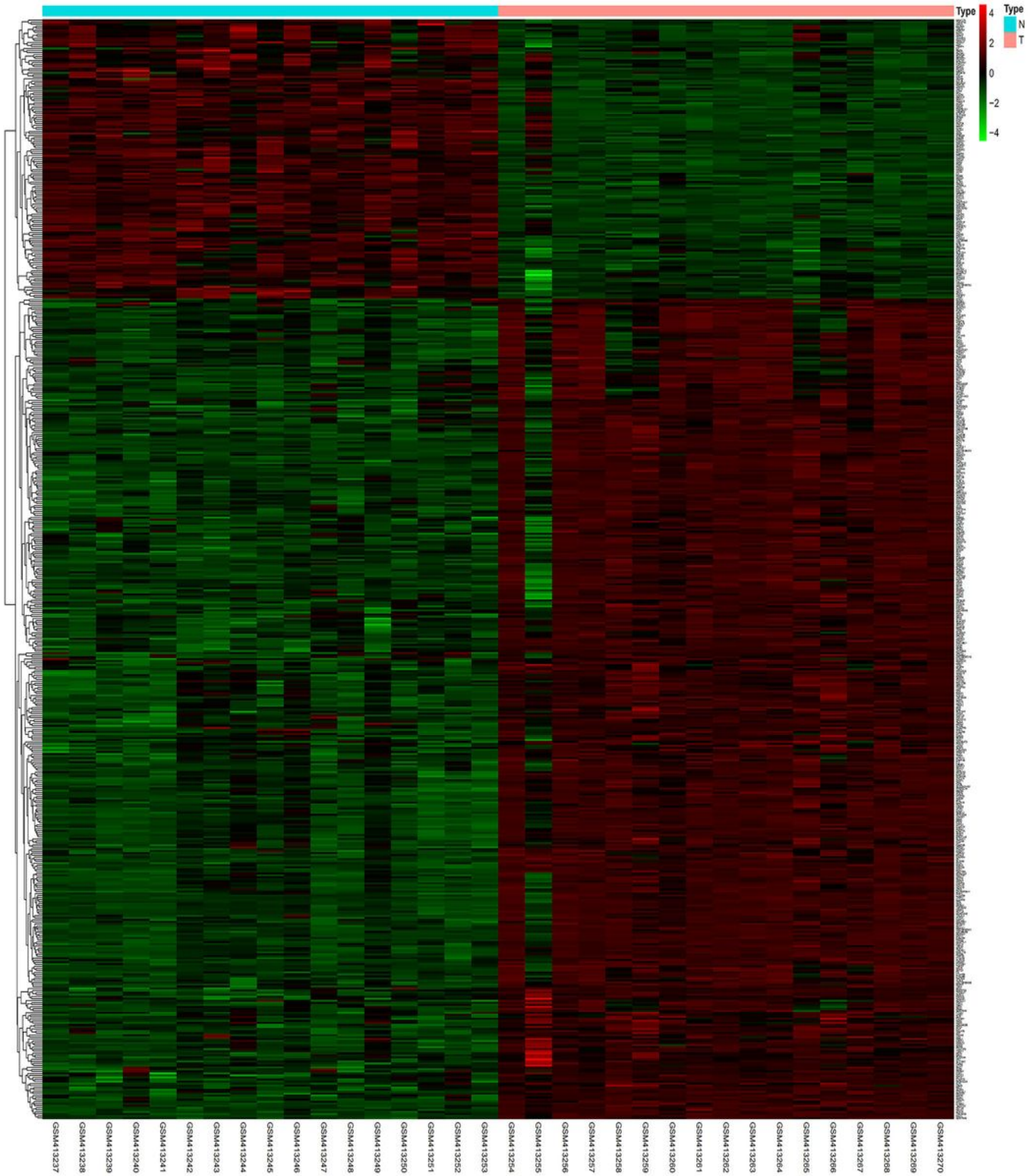

Figure 2

Cluster heat map of renal tissue-specific differentially expressed genes in patients with CRCC and healthy people.

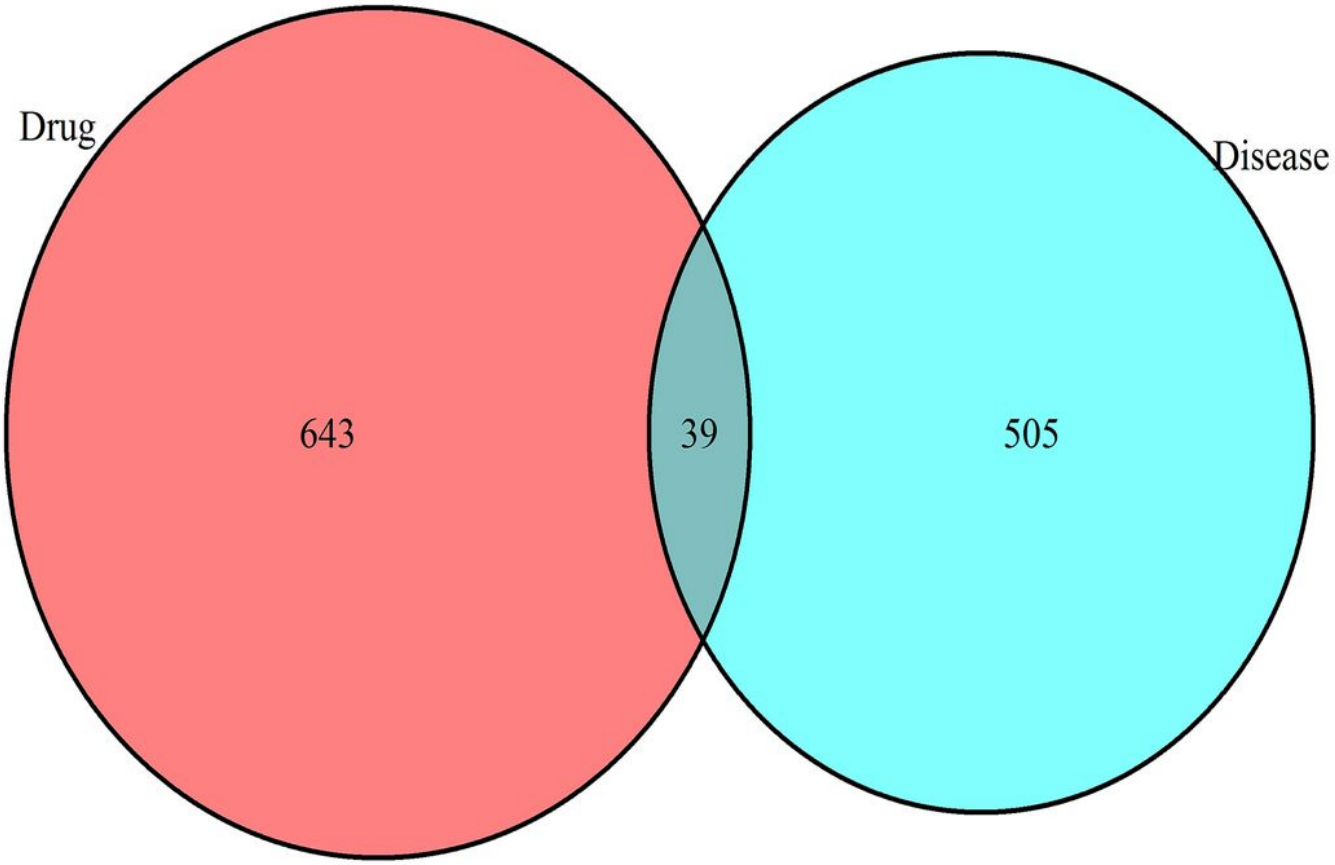

Figure 3

Venn diagram of 39 potential common targets.

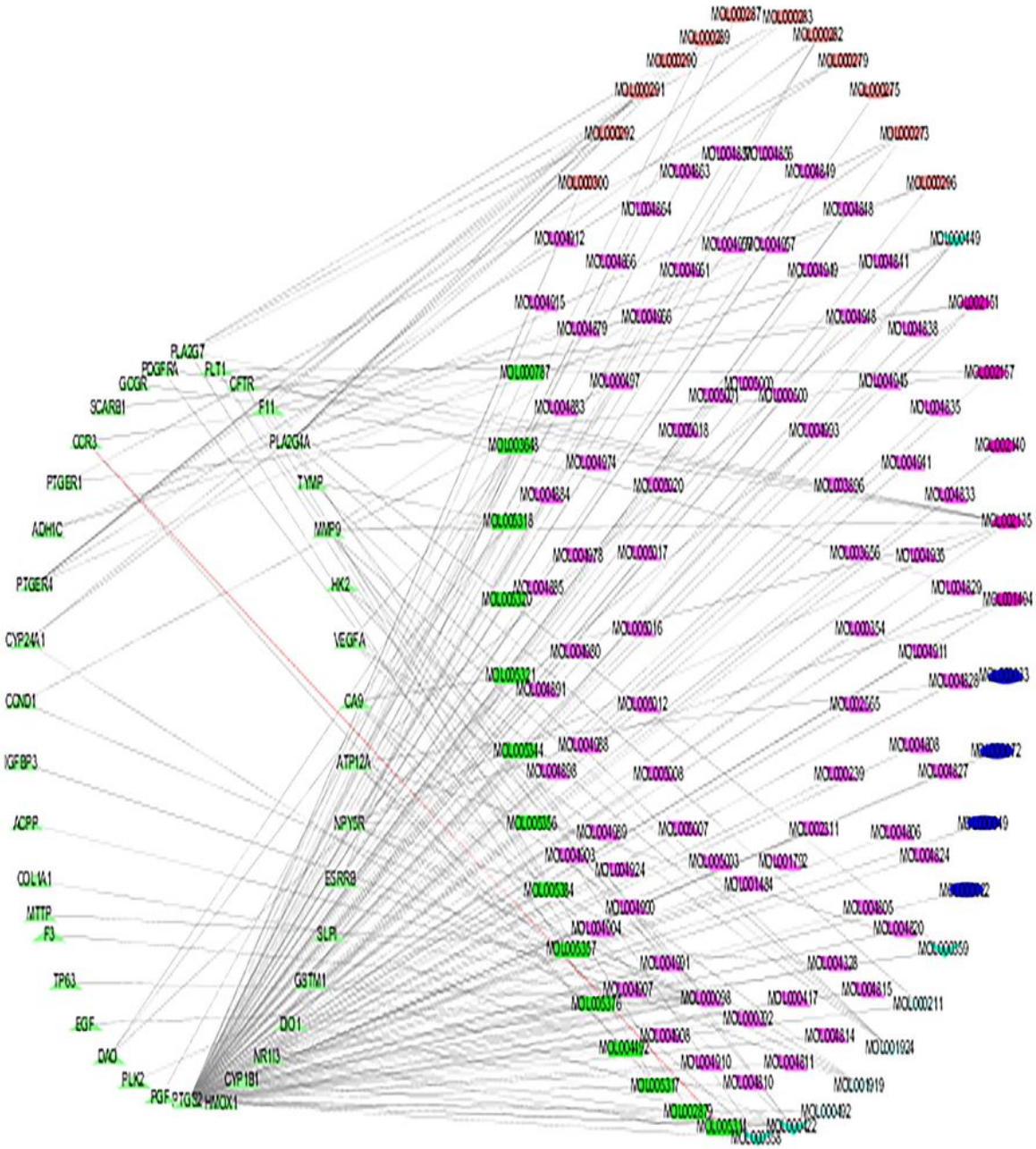

Figure 4

Ingredients-targets network Diamonds represent the active ingredients of Radix Albus Paeoniae Lactiflorae, circles represent the active ingredients of Rhizoma Atractylodis Macrocephalae, hexagons represent the active ingredients of Rhizoma Ligustici Chuanxiong, octagonal represent the active ingredients of Scierotium Poriae Cocos, parallelograms represent the active ingredients of Radix

Glycyrrhizae, Rectangle represent the active ingredients of Radix Ginseng,V represents the interaction drug, and triangle represents the target of drug-disease interaction.

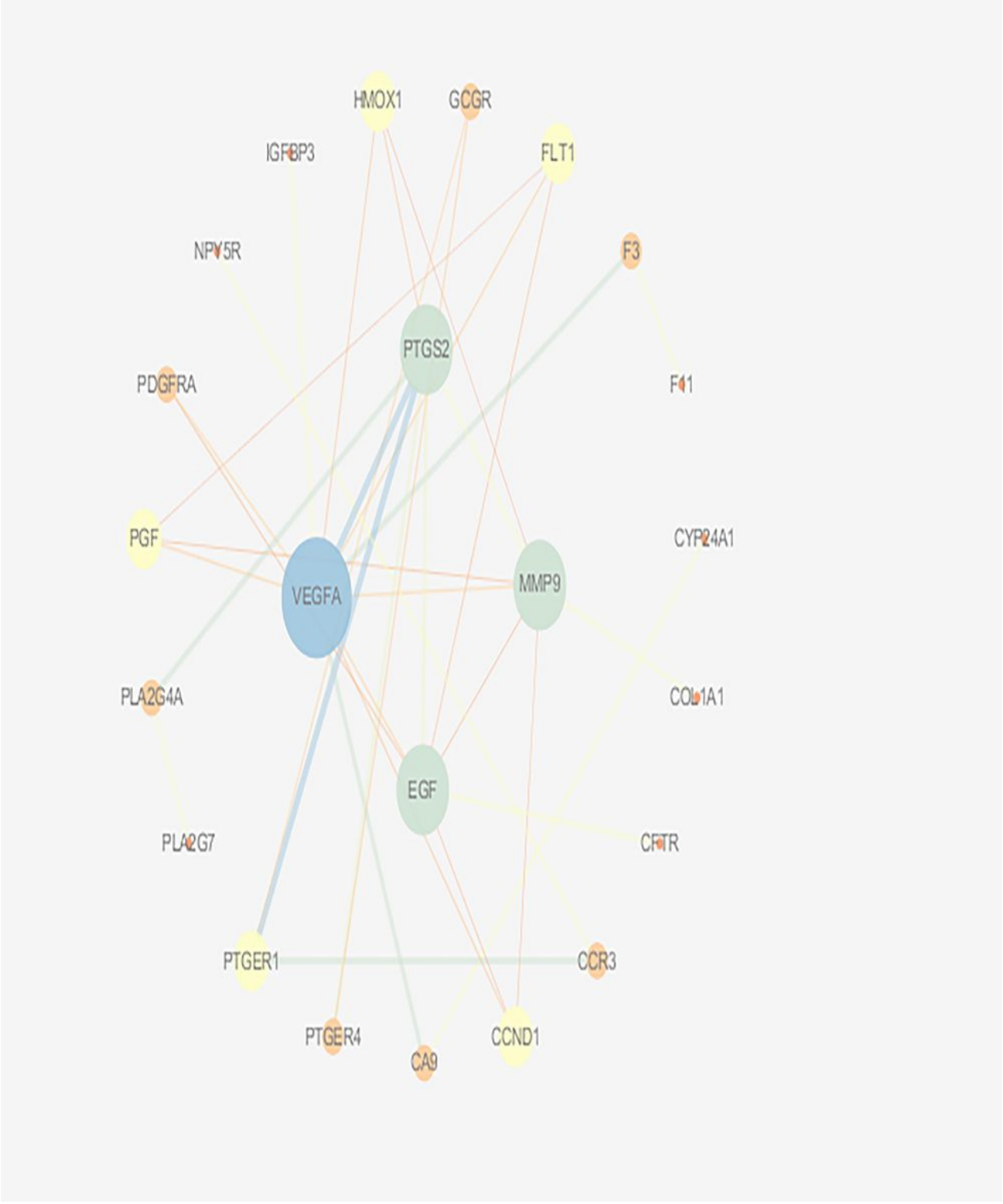

Figure 5

Protein–protein interaction (PPI) network analysis of 39 potential target.

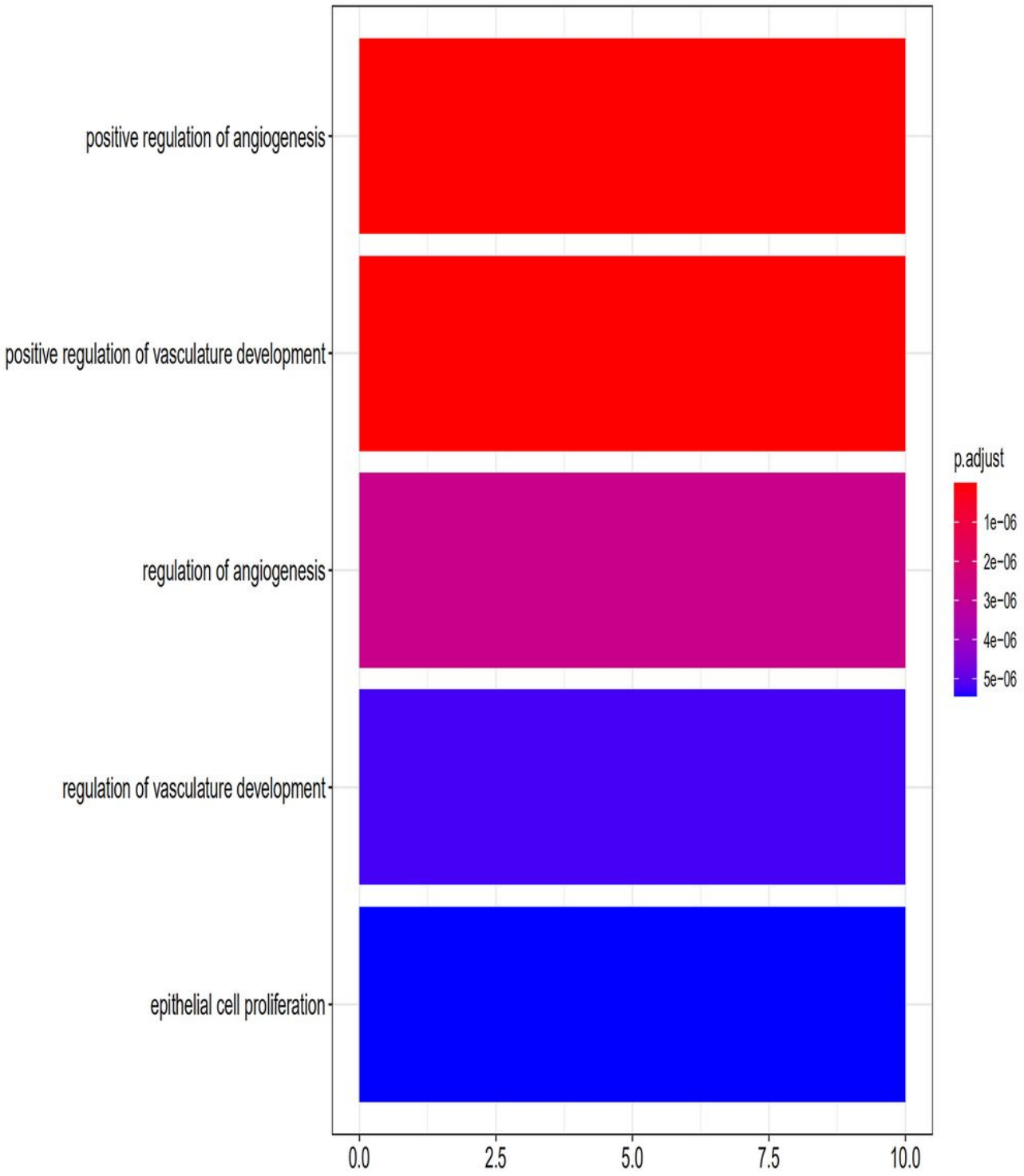

Figure 6

Top 5 enriched Gene Ontology (GO) terms selected from 39 common targets.Biological process (P-adjust value 0.05).

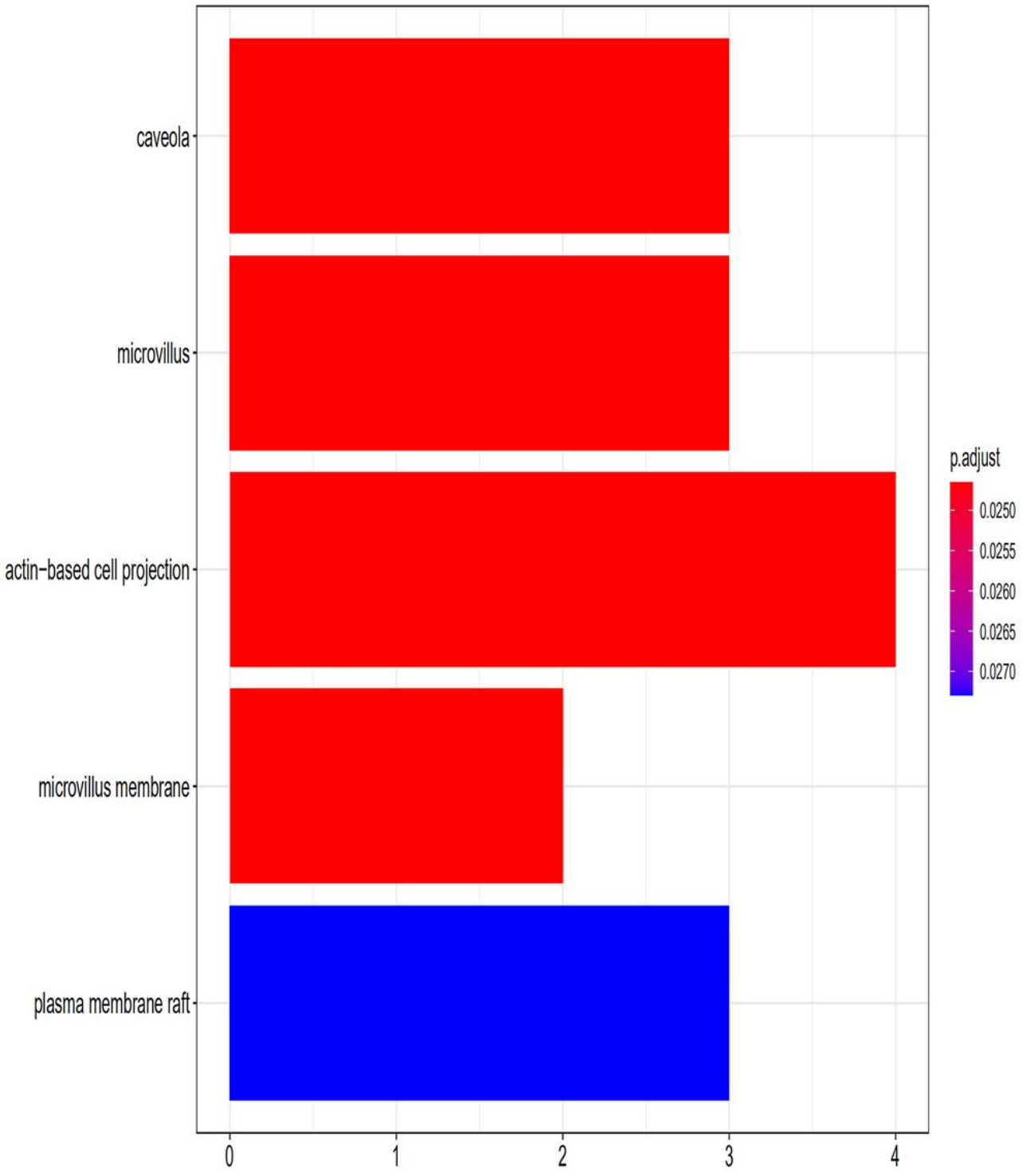

Figure 7

Top 5 enriched Gene Ontology (GO) terms selected from 39 common targets.Cell component (P-adjust value 0.05).

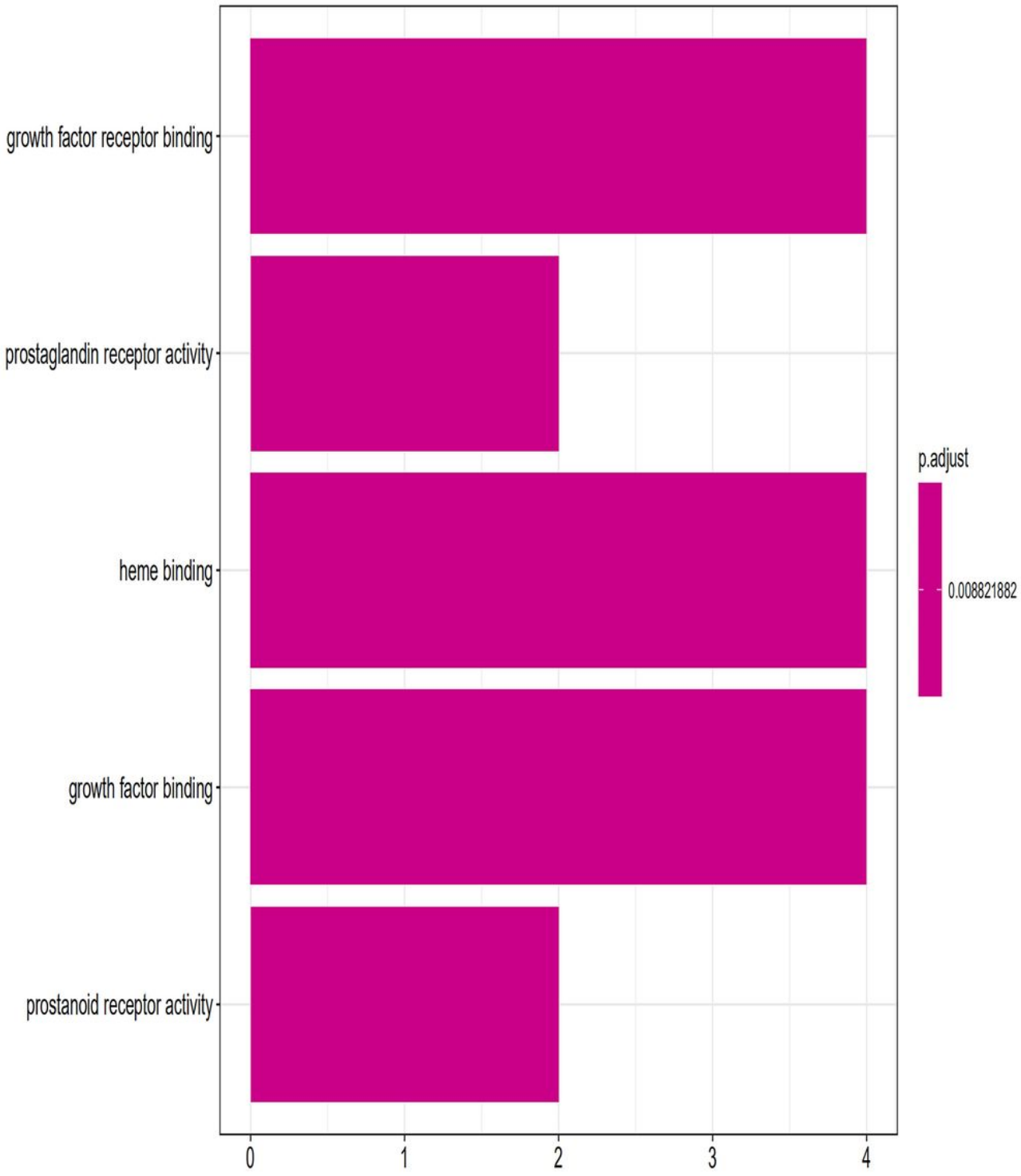

Figure 8

Top 5 enriched Gene Ontology (GO) terms selected from 39 common targets.Molecular function(P-adjust value 0.05).

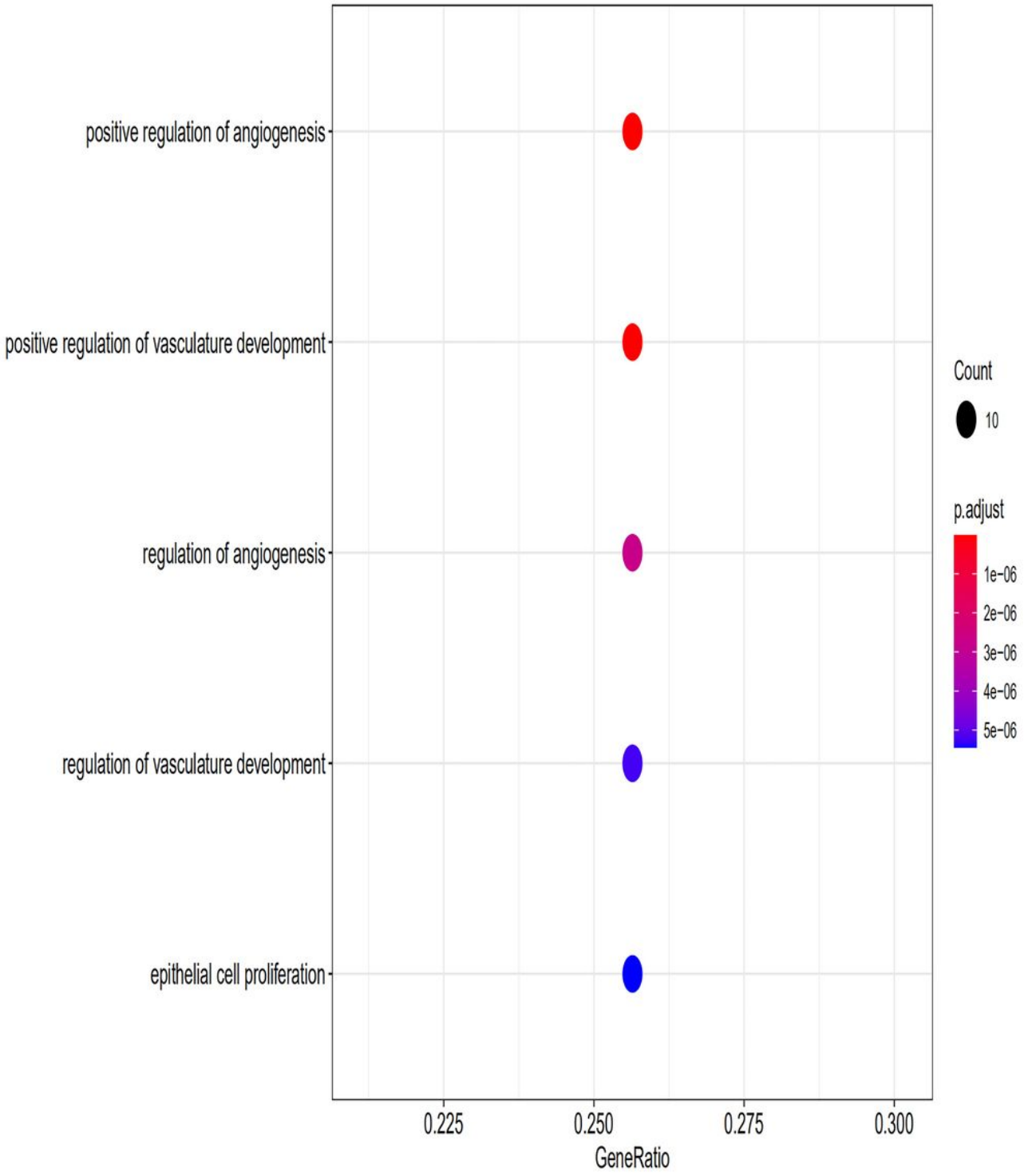

Figure 9

A dot plot to describe P-adjust value range of top 5.Biological process.

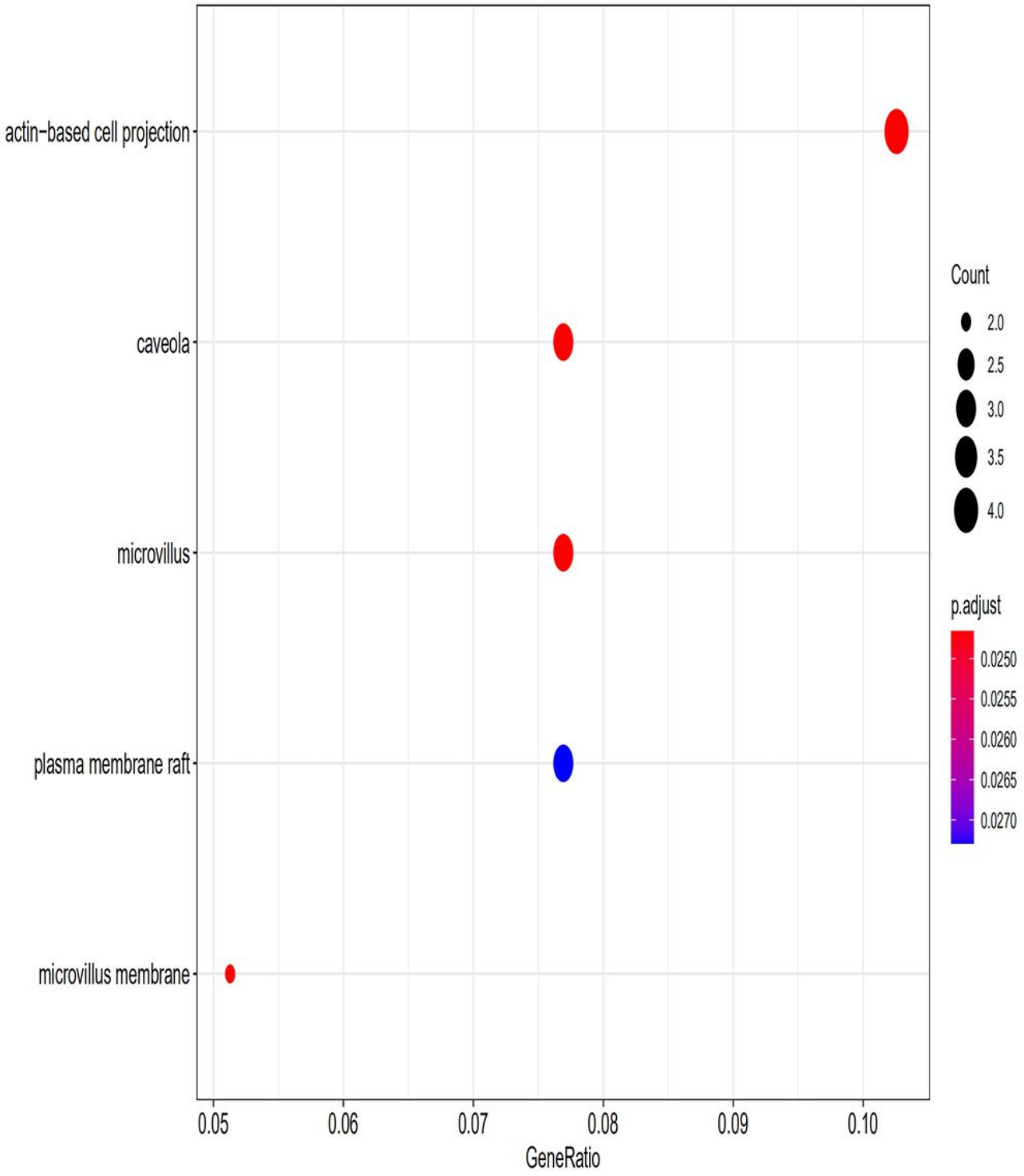

Figure 10

A dot plot to describe P-adjust value range of top 5.Cell component.

Figure 11

A dot plot to describe P-adjust value range of top 5.Molecular function.

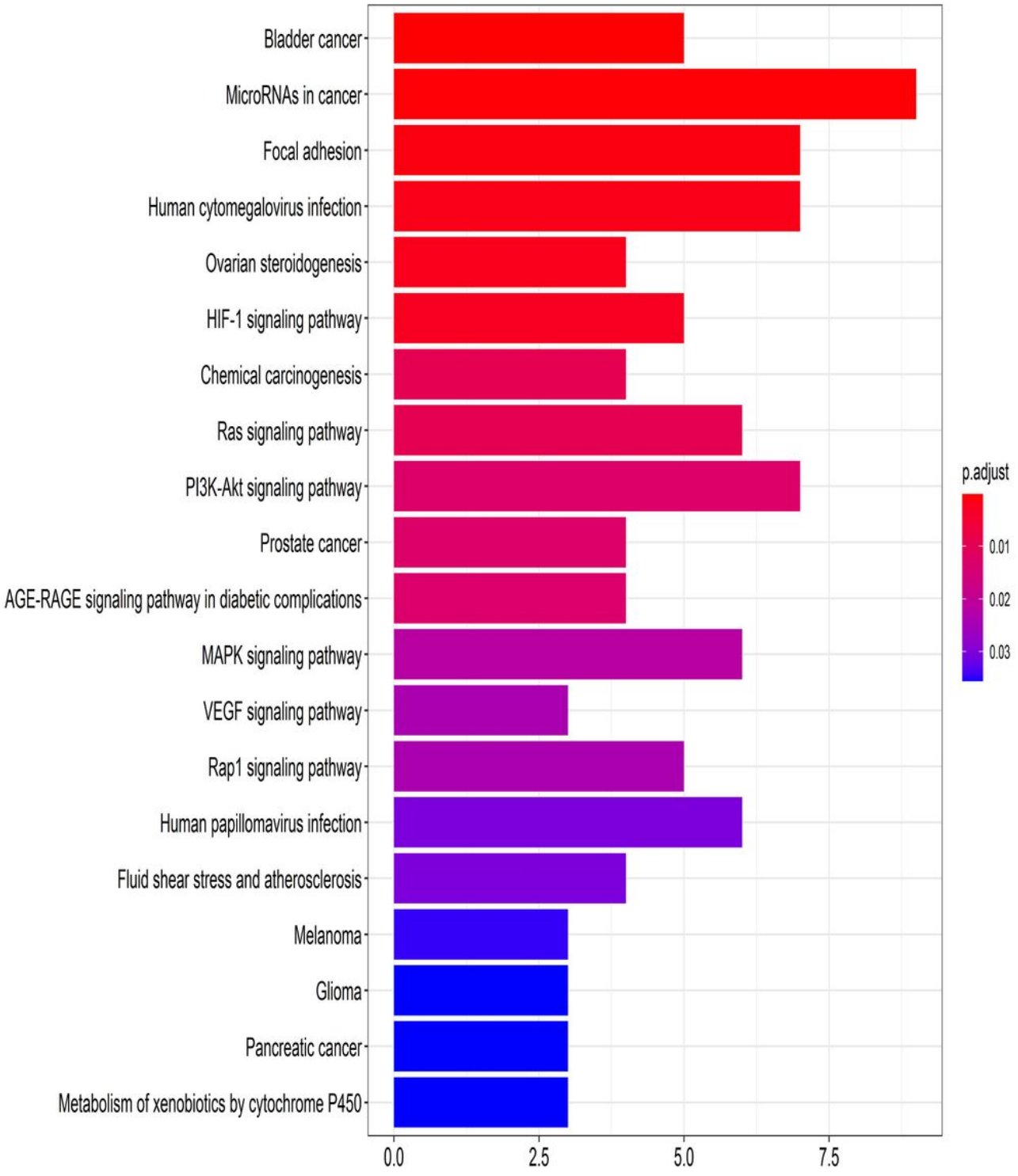

Figure 12

Top 20 pathways from Kyoto Encyclopedia of Genes and Genomes (KEGG).(P-adjust value 0.05).

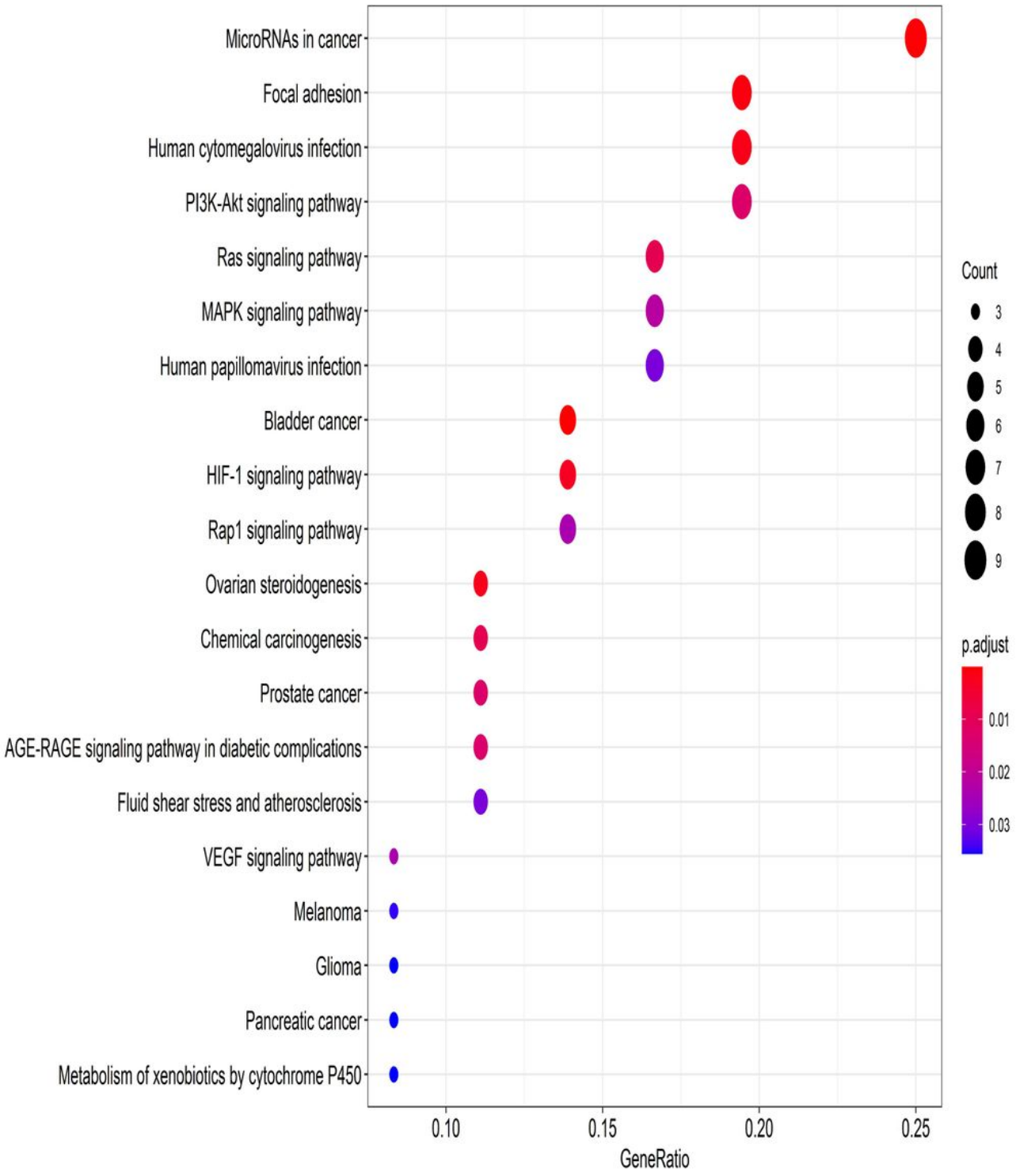

Figure 13

A dot plot to describe P-adjust value range of top 20 pathway.