

A Network Pharmacology Study on the Qiju Dihuang Pills for Treatment of Dry Eye Disease

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Research

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Abstract

Background

Dry eye disease(DED) is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. DED has a higher incidence in old age and is seen predominantly in females worldwide. Treatments that replenish deficient tears include artificial tears, gels and ointments in mild to moderate disease. Qiju Dihuang Pills is a typical herb decoction applied to the treatment of ocular disease. This study is to identify potentially active compounds and the underlying mechanisms of Qiju Dihuang Pills in the treatment of DED.

Method

Compounds in Qiju Dihuang Pills were collected from Traditional Chinese Medicine Systems Pharmacology database and analysis platform (TCMSP) online databases and then screened by bioavailability and drug likeness parameters. Related targets of DED obtained from GeneCards database, thereby obtaining the targets of Qiju Dihuang Pills against DED. DED-related genes were conducted for Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. Protein-protein interaction (PPI) networks of the genes were constructed using Cytoscape software.

Result

In total, 121 compounds from Qiju Dihuang Pills were screened out with 219 putative target genes. Hence, 219 potential target genes of Qiju Dihuang Pills were selected for further study. After overlap analysis of the 219 genes of Qiju Dihuang Pills and 3533 DED-associated genes, we finally achieved 162 Qiju Dihuang Pills-DED target genes. Network analysis indicated that main targets of main active components of Qiju Dihuang Pills were target genes such as Proline-rich AKT1 substrate 1 (AKT1), interleukin 6(IL-6), Mitogen-activated protein kinase 3(MAPK3), Vascular endothelial growth factor A(VEGFA), and Caspase-3(CASP3), which are involved in the regulation of cancer pathway, Tumor necrosis factor(TNF) signal pathway, hepatitis B pathway, The interleukin 17(IL-17) signaling pathway and so forth.

Conclusion

Our data provide deep understanding of the pharmacological mechanisms of Qiju Dihuang Pills in attenuating dry eye syndrome. The discovery shed new light on the development of active compounds of Qiju Dihuang Pills for the treatment of DED. Qiju Dihuang Pills alleviated dry eye disease through multi-component, and multi-pathway.

Background

Dry eye disease(DED) is a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability with potential damage to the ocular surface. [1]

It is accompanied by increased osmolarity of the tear film and inflammation of the ocular surface. The prevalence of DED is currently between 5% and 50% globally and particularly high in Asian countries [2,3,4,5]. The causes of DED are very complex, including age, systemic immune disease, visual display terminal, and meibomian gland dysfunction (MGD) [6]. MGD is one of the most common causes of DED overall and is also the main underlying factor leading to evaporative dry eye (EDE).

A variety of methods are utilized for the treatment of MGD-related DED, including physical therapies such as meibomian gland massage, intraductal meibomian gland probing, lipiflow, intense pulsed light, and local anti-inflammatory drugs. Although these approaches are all effective to a certain extent, problems remain including the inconvenience of application, high financial costs, insignificant effects, and some side effects [7,8].

In China, traditional medicine is often used in clinical treatment of DED, and a large amount of literature supports this approach with positive therapeutic results. Animal experiments and clinical studies have confirmed that Qiju Dihuang Pills has the effect of treating DED, but the specific mechanism is still unclear.

Traditional Chinese medicine (TCM) network pharmacology is a preferred method to study herb-compounds-diseases-targets because of its capacity of describing complex interactions between drugs and biological systems and the “multi-component, multi-target, and multi-pathway” characteristics of TCM. Therefore, in the present study, we are committed to screen the active compounds in Qiju Dihuang Pills that may modulate DED-related genes. Besides, the underlying mechanism of Qiju Dihuang Pills-induced DED relief was investigated.

Methods

1. Bioactive ingredient and target identification for Qiju Dihuang Pills

The Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) is a platform for integrating pharmacokinetics, medicinal chemistry, and drug-target-disease networks [9]. According to the TCMSP platform (<http://lsp.nwu.edu.cn/tcmsp.php>), the bioactive ingredients and targets of Qiju Dihuang Pills were obtained. Bioactive ingredients (OB) refers to the rate and extent that a drug is absorbed into the body's circulation. Drug-like properties (DL) reflects the nature of a drug which has a specific functional group or contains the same or similar physical characteristics. Bioactive ingredients were collected based on the condition that $OB \geq 30\%$, $DL \geq 0.18$. Then the corresponding molecular targets of these collected active compounds were obtained by the same database. Gene targets of potential targets of active components were obtained through UniProt. (<https://www.uniprot.org/>) database, species "Homo sapiens (Human)" was selected.

2. Target prediction of Qiju Dihuang Pills in the treatment of DED

Search for DED related targets with “dry eye” “dry eye disease” as a search term using GeneCards database (<https://www.genecards.org/>). The overlapping targets from DED treatment and from bioactive ingredients of Qiju Dihuang Pills then allowed identification of targets of Qiju Dihuang Pills in the treatment of DED. Venn diagram was drawn for overlap analysis to obtain potential DED-associated target genes of active compounds.

3. PPI network construction

The STRING database (<https://string-db.org/>) can be used to analyze the interaction between proteins and proteins. In our study, the species was limited to “Homo sapiens”, the lowest interaction score was set to medium confidence (0.900), then discrete targets were hidden and the remaining parameters remained the default settings. Subsequently, the PPI network obtained in the STRING database was visualized.[10] The networks were generated using Cytoscape (version 3.7.2) to further illustrate scientific interpretation of the complicated relationships among genes.

4. Enrichment analysis

Gene Ontology (GO) Enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) Pathway Enrichment Analysis were carried out using R software package.

Results

1. Bioactive ingredients and target genes collection

From TCMSP database, we obtained 11 compounds in Danpi, 16 compounds in Fuling, 45 compounds in Gouqizi, 20 compounds in Juhua, 16 compounds in Shanyao, 20 compounds in Shanzhuyu, 2 compounds in Shudihuang and 10 compounds in Zexie according to the OB, DL. Then 121 bioactive components of Qiju Dihuang Pills were obtained by removing duplicates, as presented in Table 1. 155 target genes in Danpi, 20 target genes in Fuling, 185 targets in Gouqizi, 193 target genes in Juhua, 64 in Shanyao, 58 targets in Shanzhuyu, 30 in Shudihuang and 5 target genes in Zexie. Then 219 target genes of Qiju Dihuang Pills were obtained by removing duplicates.

2. Network construction

The compound-target interaction network was constructed by Cytoscape 3.7.2 software. The network was found to have 272 nodes, including 45 bio-active molecules, 219 common target genes, 8 drug, and 765 edges, as shown in Fig. 1. (The genes in the figure are presented in the form of diamond, with the ellipse as the drug and the hexagon as the drug component. The larger the figure is, the more the type connection points and the more the action points are).

3. Target prediction of Qiju Dihuang Pills in the treatment of Dry Eye Disease(DED)

Genecards to screen the DED-related genes. In total, 3533 DED-related genes were obtained. After overlapping analysis, 162 frequently affected therapeutic target genes for DED in active compounds of Qiju Dihuang Pills were discovered. Fig 2a. PPI network reflects the spatiotemporal relationship of molecules within the cell and provides valuable information about molecular mechanisms in the physiological and pathological condition. The 162 common targets were then inputted into the STRING database for PPI network analysis Fig 2b, then visualized by Cytoscape. Fig 3a. There were 162 nodes and 649 edges in the PPI network, as shown in Fig 2b. The average node degree is 8.01, and the average aggregation coefficient is 0.531. The key targets were identified via the degree Cytoscape. The top 10 targets were presented in Fig 3b. Besides, among all the core targets, the darker the red, the more important it was. It suggests that target genes such as AKT1, IL-6, VEGFA, MAPK3, TNF may play crucial roles in the treatment of DED.

4. GO enrichment and KEGG enrichment

To further explore the multiple mechanisms of Qiju Dihuang Pills as a therapy drug against DED, GO enrichment analysis of 162 target genes shared by Qiju Dihuang Pills and DED was performed using R 4.3.0. The top 20 significantly enriched terms including biological process (BP), molecular function (MF), and cellular component (CC) are presented (p -value < 0.05) in Fig. 4a-c. The top 20 MF pathways included: G protein-coupled amine receptor activity, nuclear receptor activity, ligand-activated transcription factor activity, drug binding, cytokine activity, cytokine receptor binding, catecholamine binding, DNA-binding transcription factor binding, neurotransmitter receptor activity, G protein-coupled serotonin receptor activity etc. which are all classical pathways involved in DED inducing and relieving. BP and CC terms analysis indicate that Qiju Dihuang Pills are mainly involved in response to drug, reactive oxygen species metabolic process, and vascular process in circulatory system. What's more, Qiju Dihuang Pills are strongly related to the membrane raft, membrane microdomain, and membrane region.

The KEGG enrichment analysis of 162 target genes was performed to explore the potential biological pathways. We obtained 20 pathways in total which belong to several categories, including AGE-RAGE signaling pathway in diabetic complications, Fluid shear stress and atherosclerosis, Hepatitis B, TNF signaling pathway, IL-17 signaling pathway, etc. (Fig. 5).

Discussion

Dry eye disease is a multifactorial disorder of the ocular surface. Dry eye is one of the most frequent ocular disorders, affecting 5% to 50% of the entire population at all ages. DED is associated with a long list of causes which can be divided into primary and secondary.[11] Dry eye may develop secondary to inflammatory disease, environmental conditions (e.g. allergens, cigarette smoke, dry climate) and contact lens wear. [12]The pathogenesis of DED is complex, and it is difficult for single-target drugs to achieve good efficacy. Therefore, multi-component, multi-target drugs have become the trend of treatment of DED in the future.

As Traditional Chinese Medicine (TCM) has been widely accepted around the world, there are still several problems to be addressed, among which, the active components and target genes have always been the issue and key point for TCM modernization [13,14]. While existing methods mainly concerned the indicative ingredients and their potential pharmacological effects, network pharmacology study emerged as a more powerful method to identify active compounds and target genes due to multi-component and multi-target mode of TCM [15]. In the present study, resulting in 11 compounds in Danpi, 16 compounds in Fuling, 45 compounds in Gouqizi, 20 compounds in Juhua, 16 compounds in Shanyao, 20 compounds in Shanzhuyu, 2 compounds in Shudihuang and 10 compounds in Zexie. In total, 121 compounds were obtained from Qiju Dihuang Pills, which provided more compounds for further analysis. Normally, $OB \geq 30\%$ and $DL \geq 0.18$ are considered chemically suitable for drug development, they are used as the included criteria of bioactive compounds in most literatures [16]. Quercetin (MOL000098), as a common component of Juhua, Gouqizi and Danpi, is the main active component of Qiju Rehmannia pills, with 116 gene targets. Quercetin is categorized as a posposol, one of the six subclasses of posoid Company. Studies have shown that the anti-inflammatory effect of Quercetin on DED-experimental models suggesting their topical applications could be used for DED treatment. This is consistent with the results of this study.

Studies suggest that Qiju Dihuang Pills can better relieve discomfort symptoms in patients with dry eyes, has better clinical curative effect. This is because the Gouqizi and Juhua in Qiju Dihuang Pills can reduce corneal epithelial injury, protect lacrimal cells, maintain lacrimal membrane stability and lacrimal gland basic secretion. Dihuang, Shanyao and Zexie can improve the immune function of the body and have some anti-inflammatory effects. Qiju Dihuang Pills can reduce the expression levels of IL-1, IL-8 and MMP-9 in tears of patients with DED, and reduce local inflammatory response.

Evidence suggests that inflammation and hyperosmolarity are considered core mechanisms in the development of dry eye. Dry eye is accompanied by changes in tear composition including enhanced hyperosmolarity and secretion of pro-inflammatory mediators. [17] Eventually these processes lead to corneal and conjunctival epithelial cells death as well as conjunctival goblet cell dysfunction and death. At the molecular level, dry eye induction leads to a persistent increase in corneal expression of IL-1 α and TNF- α . These cytokines are important mediators. Produced constitutively in the corneal epithelium and on release by injury or death,[18] IL-1 α can up-regulate TNF- α release and its own autocrine production. Tumor necrosis factor α has been implicated as an important mediator of pathogenesis in DED. Elevated gene expression of IL-1, IL-6, IL-8, and TNF- α in the conjunctival epithelium[19] and a higher tear concentration of IL-1 has been reported in patients with DED. In one study, a short-term desiccation (0-30 min) of human corneal epithelial cell line induced an increase in the expression of IL-6 and TNF- α as well as an increase cell death. All these factors may act as mediators of tissue damage leading to lysis of cell membranes and tight junctions in epithelial cells. Cell death was partially suppressed by the addition of anti-IL-6 antibody.[20]

The regulatory immune and anti-inflammatory effects of Qiju Dihuang Pills are very similar to the treatment strategy for DED. Qiju Dihuang Pills is also commonly used clinically to treat DED, but the

specific mechanism is still unclear. This study is based on the network pharmacology technology, relying on the corresponding database and software, to construct the "drug-component-key target- pathway" network, and scientifically and systematically analyze the mechanism of action of Qiju Dihuang Pills on DED.

As shown in PPI network of Qiju Dihuang Pills, the most frequently targeted genes are IL-6, AKT1, VEGFA, CASP3, TNF, PTGS2, MAPK3. Combined with network topology analysis and relevant literature reports, this study predicts that PTGS2, AKT1, CASP3, TNF, IL-6, MAPK3, and VEGF are potential key targets for treatment of DED with Qiju Dihuang Pills. Amongst them, the target genes associated with inflammation mostly, indicating that Qiju Dihuang Pills plays an important role in inflammation. IL6 with a wide variety of biological functions in immunity, tissue regeneration, and metabolism, which is a potent inducer of the acute phase response. AKT1 is one of 3 closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3) called the AKT kinase, and which regulate many processes including metabolism, proliferation, cell survival, growth and angiogenesis. It is mainly secreted by macrophages and can induce cell death of certain tumor cell lines. It is potent pyrogen causing fever by direct action or by stimulation of interleukin-1 secretion and is implicated in the induction of cachexia, Under certain conditions it can stimulate cell proliferation and induce cell differentiation. In our study, Qiju Dihuang pills inhibits the cell injury and apoptosis of corneal epithelium by regulating the expression of VEGF, AKT1 and IL-6, so as to treat DED. The results of pathway enrichment analysis indicate that the effect of Qiju Dihuang Pill in treating DED may be related to TNF signaling pathway and IL-17 signaling pathway. TNF, as a critical cytokine, can induce a wide range of intracellular signal pathways including apoptosis and cell survival as well as inflammation and immunity. The results of this study indicate that the active ingredients in Qiju Dihuang Pill may further regulate TNF signaling pathways by acting on AKT1, CASP3, CXCL8, IL-6 and other key target proteins, so as to treat DED. The interleukin 17 (IL-17) family, a subset of cytokines consisting of IL-17A-F, plays crucial roles in both acute and chronic inflammatory responses. The results of this study suggest that the active ingredients of Qiju Dihuang Pill may further regulate IL-17 signaling pathway and prevent apoptosis by acting on CASP3 and other key target proteins, so as to treat DED.

Conclusions

In this paper, network pharmacology was used to explore the active components, potential targets and mechanisms of Qiju Dihuang Pills in treating DED. The results showed that 121 ingredients of Qiju Dihuang Pills may play important roles in biological processes of anti-angiogenesis, anti-apoptosis, anti-inflammatory and immune regulation by acting on AKT1, IL-6, VEGF, and MAPK3, through IL-17 and TNF signaling pathway. More importantly, the potential targets and biological processes mentioned above may provide valuable information for further investigation into the mechanism of Qiju Dihuang Pills for treating DED.

Abbreviations

Not applicable

Declarations

Ethics approval and consent to participate:Not applicable

Consent to publish:Not applicable

Availability of data and materials:All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests:The authors declare that they have no competing interests

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Authors' Contributions: Qiuyi SNG, Jiawei Yang, Siquan Zhu conceived and designed the study. Mingxu Zhang analyzed the transcriptional data and performed network analysis. Qiuyi Song, Jiawei Yang wrote the manuscript. All the authors read and approved the final manuscript.

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Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

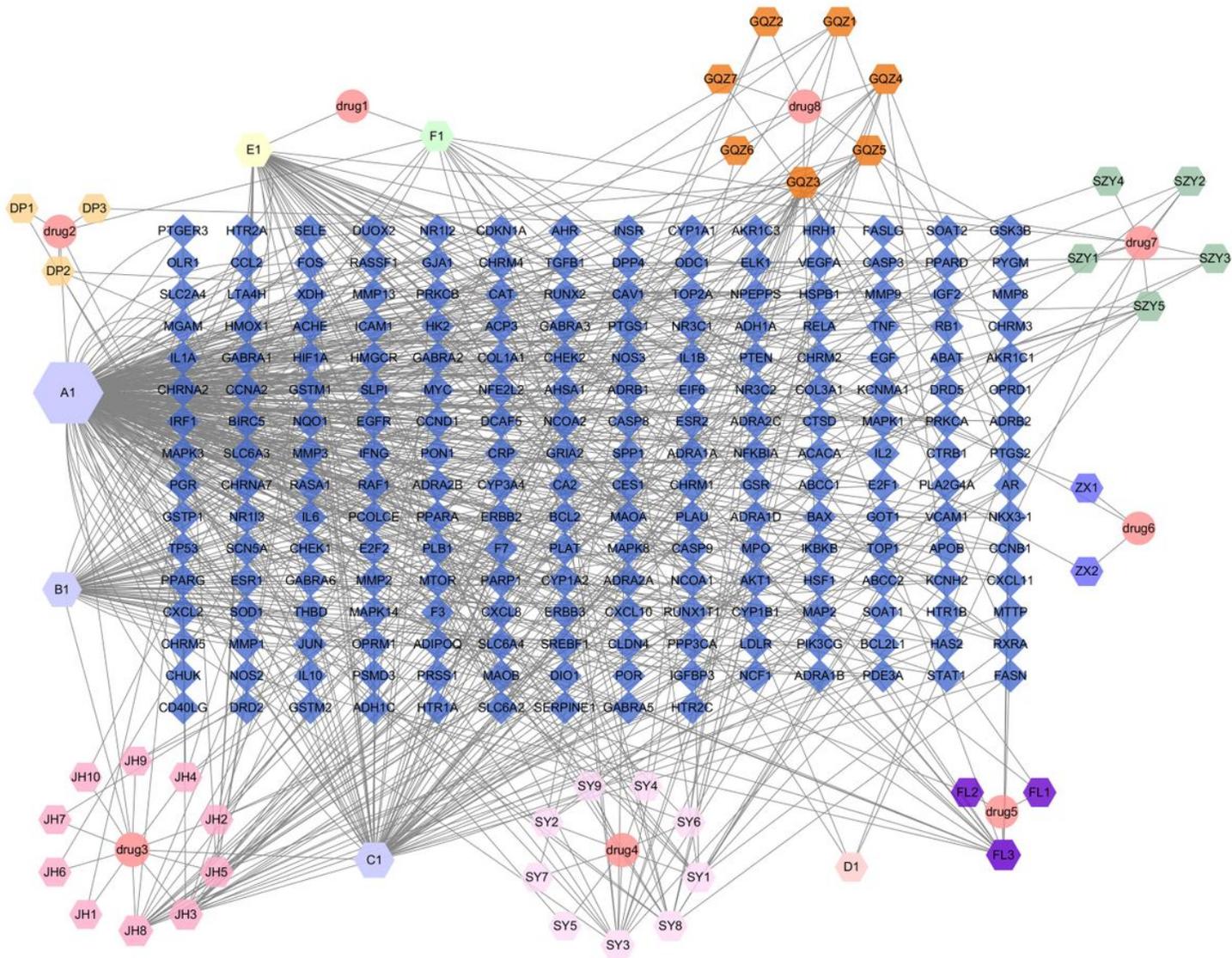


Figure 1

Compound-targets interaction network

The GO enrichment analysis of 162 target genes

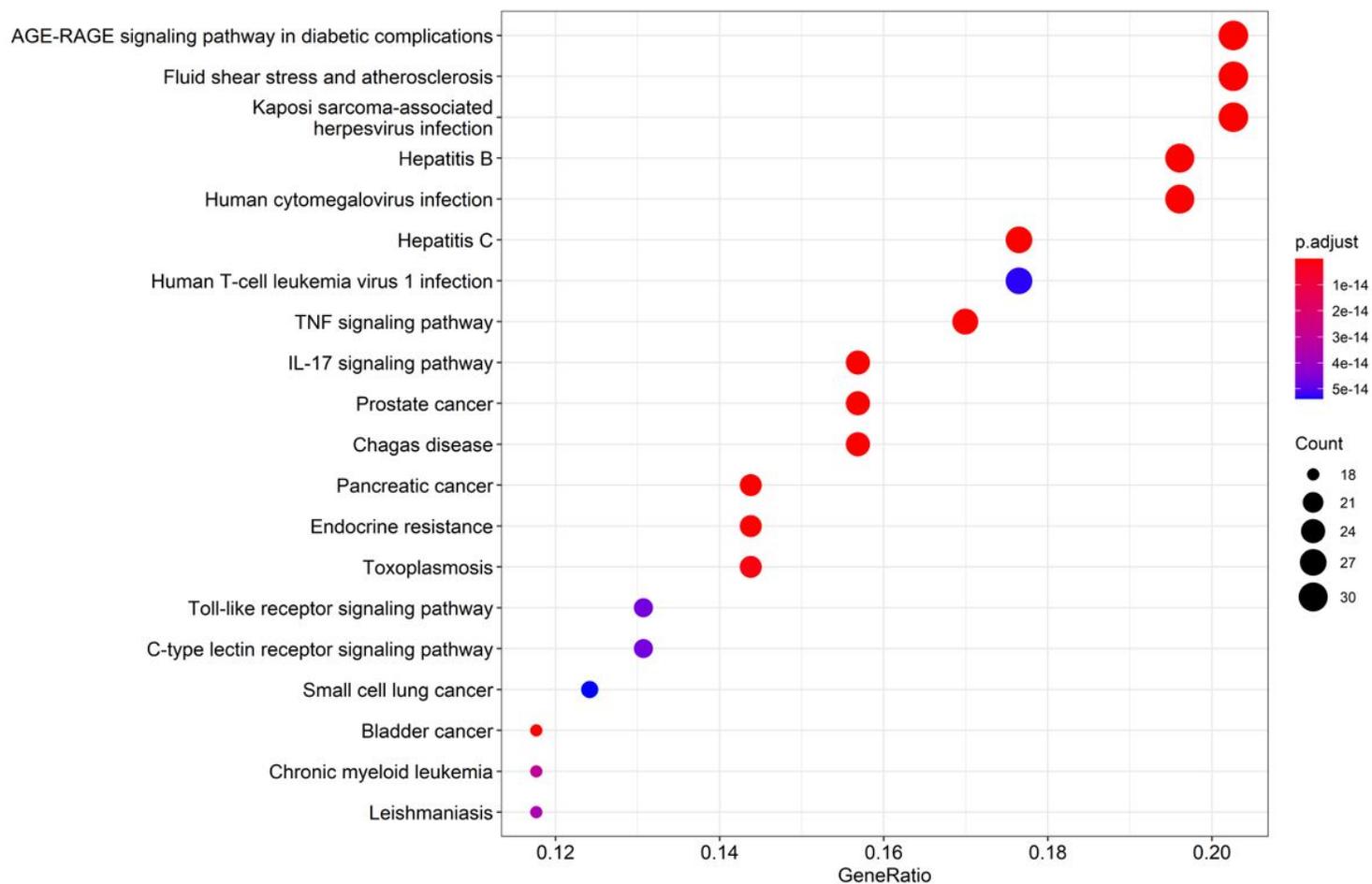


Figure 5

The KEGG enrichment analysis

Supplementary Files

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- [Table1.xls](#)