Mechanism of Tripterygium Wilfordii for the Treatment of Idiopathic Membranous Nephropathy Based on Network Pharmacology

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Research

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Abstract

Background

Tripterygium wilfordii has been widely used for idiopathic membranous nephropathy (IMN), while the pharmacological mechanisms are still unclear. This study is aimed at revealing potential therapeutic targets and pharmacological mechanism of tripterygium wilfordii for the treatment of IMN based on network pharmacology.

Methods

Active components of tripterygium wilfordii were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform. IMN-associated target genes were collected from GeneCards database, DisGeNET database, and OMIMI database. VENNY 2.1 was used to identify the overlapping genes between active compounds of tripterygium wilfordii and IMN target genes. Using STRING database and Cytoscape 3.7.2 software to analyze interactions among overlapping genes. The Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment of the targets were analyzed using the Rx64 4.0.2 software, colorspace, stringi, DOSE, clusterProfiler, and enrichplot packages.

Results

A total of 153 compounds-related genes, 1485 IMN-related genes were obtained, and 77 overlapping genes between them were identified. The protein–protein interactions network indicated that the targets AKT1, TNF, VEGFA, TP53, PTGS2, CXCL8, MAPK8, STAT3, JUN, and CASP3 play an important role in the treatment effect of tripterygium wilfordii for IMN. The enrichment analysis showed that the main pathways of targets were AGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, Toll-like receptor signaling pathway.

Conclusion

This study reveals potential mechanisms of multi-component and multi-target of tripterygium wilfordii for the treatment of IMN based on network pharmacologic approach, and provide a scientific basis for further experimental studies.

Introduction

Idiopathic membranous glomerulonephritis (IMN) is an immune-mediated primary glomerular disease that usually manifested as nephrotic syndrome[1]. Although 30% of patients undergo spontaneous remission, persistent proteinuria are occur in 30%-40% of patients, and even develop end-stage renal

disease[2-3]. Although the optional treatment options for IMN include rituximab, glucocorticoids plus cyclophosphamide and glucocorticoids plus calcineurin inhibitors, there are some adverse events. The adverse events of rituximab include infusion reaction, opportunistic infections, allergic reaction and hypogammaglobulinemia, in addition, have some defects including slower effect and expensive. Cyclophosphamide is associated with a variety of side effects, such as gonad injury, malignancy, bone marrow suppression and hemorrhagic cystitis. The patients who use calcineurin inhibitors have the risk of renal insufficiency, and a high rate of relapse after drug withdrawal.

Tripterygium wilfordii, as a well-known traditional Chinese medicine, has been widely used for the treatment of various autoimmune diseases, such as nephritic syndrome, inflammatory bowel disease, systemic lupus, erythematosus, and rheumatoid arthritis[4]. Clinical trials have demonstrated that Tripterygium wilfordii was effective in the treatment of IMN[5-6]. However, we know little about the molecular mechanisms of the effect of tripterygium wilfordii on IMN.

The incorporation of traditional Chinese medicine into clinical therapy via network pharmacology can provide insights into the possible mechanism and enhance the specificity and effectiveness of the treatment scheme[7]. In our study, we explored the possible molecular mechanism based on network pharmacology.

Materials And Methods

Identification and screening of Active Compounds and Targets for Tripterygium Wilfordii and Therapeutic Targets for IMN

The active compounds and potential target proteins of tripterygium wilfordii were obtained from the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP) (http://tcmspw.com)[8] according to the parameters of ADME (absorption, distribution, metabolism, excretion)[9-10], which contained criteria of drug likeness (DL) of ≥ 0.18, oral bioavailability (OB) ≥ 30%, hydrogen bond donors (Hdon≤ 5, and hydrogen bond acceptors (Hacc≤ 10. The gene names of targets were further obtained from the UniProt Knowledgebase (UniProtKB) (http://www.uniprot.org).

IMN-associated target genes were gathered from GeneCards database (https://www.genecards.org/), DisGeNET database (https://www.disgenet.org/home/) and OMIMI database (http://www.mvforge.com/). And the overlapping genes between compounds of tripterygium wilfordii and IMN target genes were identified and visualized by VENNY 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

Construction of Drug-disease Target Protein-Protein Interactions (PPI) Network

The overlapping genes between compounds of tripterygium wilfordii and IMN target genes were imported into STRING[11] (https://string-db.org/cgi/input.pl) to construct and visualize the PPI network. And

Cytoscape 3.7.2 software[12] was used to further analyze and visualize the PPI network. The node size and color reflected the number of combined targets (degree), and the larger node and the redder color indicates the higher degree value. The thickness of the side indicates the value of the combine score, and the thicker side indicates the higher combine score.

Gene Ontology (GO) and Kyoto Encyclopedia of Gene, Genomes (KEGG) Pathway Enrichment Analysis of Tripterygium Wilfordii for IMN and the Drug-Compound-Target-Signaling Pathway Network

The GO enrichment analysis and KEGG pathway enrichment of the targets were analyzed using the Rx64 4.0.2 software, colorspace, stringi, DOSE, clusterProfiler, and enrichplot packages[13-14], and the screening conditions were adjusted P value < 0.05 and q < 0.05, and the result of GO enrichment analysis included 3 different levels: biological processe (BP), molecular function (MF), and cellular component (CC)[15]. We used Cytoscape 3.7.2 to construct and visualize the drug- compound-target-signaling pathway network.

Results

Identification and screening of Active Compounds and Targets for Tripterygium Wilfordii and Therapeutic Targets for IMN

A total of 42 active compounds of tripterygium wilfordii were obtained by searching the TCMSP database, and using ADME parameters such as OB, DL, Hdon and Hacc (Additional file 1). After finding the target data from TCMSP database, and deleting the repeated items, 153 targets were finally obtained. Then, we used UniprotKB database normalized the protein targets collected in TCMSP database. A total of 1485 therapeutic targets of IMN were acquired from GeneCards, DisGeNET and OMIMI databases after removing duplicates.

Drug-Disease Target PPI Network

The result of Venn diagram showed that 77 overlapping genes were identified between 153 genes related to tripterygium wilfordii and the 1485 genes related to IMN (Fig. 1, Table 1). To predicate the relationship between the overlapping genes, we uploaded the overlapping genes to the STRING database. A PPI network was built, with 77 nodes and 1009 edges (Fig. 2). The average node degree of was 26.20, and the average local clustering coefficient was 0.69. The result of PPI network indicated that there was a complex relationship between these genes. The results were used for further analysis through Cytoscape software, and the network was constructed as Fig. 3. The top ten targets AKT1, TNF, VEGFA, TP53, PTGS2, CXCL8, MAPK8, STAT3, JUN, and CASP3 have higher degree in this process, which explained their significance in the network.

Table 1: The overlapping genes between the IMN and tripterygium wilfordii.

Overlapping gnens name						
HTR2A	CASP8	PON1	SLC6A4	AR	SLPI	BAX
BCL2	ALOX5	CASP3	CYP1A1	CYP1A2	CYP1B1	CYP3A4
DPP4	SELE	GSTM1	GSTP1	HMOX1	IKBKB	INSR
ICAM1	MMP1	MAPK8	NOS2	NOS3	PPARG	PIK3CG
AKT1	STAT1	SLC2A4	JUN	RELA	TNF	VCAM1
XDH	AKR1B1	ADRB1	XIAP	CCR7	C3	CXCR4
CDKN1A	IFNG	IL2	IL4	CXCL8	CD14	CD274
FOS	STAT3	CD80	CD86	TGFB1	CD40	PLAU
VEGFA	VTCN1	KCNMA1	KDR	NR3C2	ACHE	CA2
NR3C1	ESR1	ESR2	MAPK14	ADRB2	HSP90AA1	PTGS1
PTGS2	CASP9	TP53	CREB1	PLA2G4A	MMP9	TIMP1

GO Enrichment Analysis of Tripterygium Wilfordii for the Treatment of IMN.

A total of 1782 BP terms, 119 MF, and 25 CC terms were enriched for the 77 target genes. We selected the top 10 according to the *P* value as shown in Fig. 4. The results showed that tripterygium wilfordii treats IMN through various BPs, including response to lipopolysaccharide, response to molecule of bacterial origin, response to antibiotic, response to oxygen levels, positive regulation of cytokine production, regulation of reactive oxygen species metabolic process, positive regulation of reactive oxygen species metabolic process, response to hypoxia, response to metal ion. In the MF classification, the effect of tripterygium wilfordii for the treatment of IMN was mainly manifested in the following aspects: heme binding, tetrapyrrole binding, oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, cytokine receptor binding, nuclear receptor activity, steroid hormone receptor activity, virus receptor activity, exogenous protein binding, ubiquitin-like protein ligase binding. According to the enrichment results of CC, membrane was the main classification of the target proteins.

KEGG Pathway Enrichment Analysis of Tripterygium Wilfordii for the Treatment of IMN.

The result of of KEGG pathway enrichment analysis demonstrated that 75 genes were related to 144 signaling pathways. The details of 144 signaling pathways were shown in Additional file 2. We selected the top 10 according to the *P* value as shown in Fig. 5 and Additional file 3-6, which revealed that main processes of tripterygium wilfordii in treating IMN included AGE signaling pathway in diabetic complications, IL-17 signaling pathway, TNF signaling pathway, Toll-like receptor signaling pathway. In addition, there were some other pathways such as Kaposi sarcoma-associated herpesvirus infection, fluid shear stress and atherosclerosis, hepatitis B, toxoplasmosis, Epstein-Barr virus infection, Chagas disease, which revealed that tripterygium wilfordii has a potential application in other related diseases.

The Drug-Compound-Target-Signaling Pathway Network

In our research, a drug-compound-target-pathway network of of tripterygium wilfordii for IMN treatment was constructed (Fig. 6). The network consisted of 93nodes (1 for drug, 10 for components, 1 for disease, 77 for targets, and 4 for pathways). The integrative network showed that the therapeutic effect of tripterygium wilfordii on IMN might be attributed to the active components (triptolide, kaempferol, beta-sitosterol) acting on targets (AKT1, TNF, VEGFA, TP63, PTGS2, CXCL8, MAPK8, and STAT3 etc.) that regulate key pathways (AGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway).

Discussion

In the present study, we undertook a network pharmacologic approach to identify potential therapeutic targets and significant pathways of tripterygium wilfordii for IMN. Finally, we identified that the main active ingredient of tripterygium wilfordii, triptolide, kaempferol, beta-sitosterol play an important role in the treatment of IMN. Triptolide, the main component tripterygium wilfordii has the functions of reducing established proteinuria and podocyte injuries in IMN[16]. The PPI network revealed that there were complex relationships between these proteins. The proteins AKT1, TNF, VEGFA, TP63, PTGS2, CXCL8, MAPK8, and STAT3 etc. were in the core network of PPI, which may be the important direct targets of tripterygium wilfordii in the treatment of IMN. Previous studies have demonstrated that AKT1, TNF, VEGFA, PTGS2, CXCL8, MAPK8 and STAT3 were over-expressed in renal tissue or serum of MN animals or patients[17-23].

Based on the GO terms, the activity of tripterygium wilfordii was associated with numerous BPs (response to lipopolysaccharide, response to oxygen levels, positive regulation of cytokine production, response to hypoxia, etc.), a variety of MFs (oxidoreductase activity, cytokine receptor binding, nuclear receptor activity, steroid hormone receptor activity, exogenous protein binding, ubiquitin-like protein ligase binding, etc.), and diversified CCs (membrane raft, membrane microdomain, membrane region, external side of plasma membrane etc.), which might be basis for tripterygium wilfordii treatment in IMN.

The KEGG enrichment analysis revealed that pharmacological effects of tripterygium wilfordii on IMN were closely related to well-known IMN-associated pathways, such as AGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway. This indicates that tripterygium wilfordii may through multiple pathways to treat IMN. AGE signaling pathway was related to the autoimmune disorder, inflammation, and tissue damage in the MN rats, and AGE inhibition could reduce the inflammatory reactions and oxidative lesions in MN[24]. The IL-17 plays crucial roles in the development of inflammatory autoimmune diseases. Ifuku M demonstrated that IL-17 mRNA were over-expressed in kidney biopsy specimens of MN patients[25]. TNF-αwas demonstrated directly cytotoxic to many glomerular cell types and can promote procoagulant activity with formation of microthrombi that could contribute to renal vein thrombosis associated with MN[26]. Anti-TNF-α therapy attenuated renal immune cell infiltration in experimental MN[27]. Toll-like receptor, as classic example of pattern

recognition receptors, participate in autoimmune disorders. Signals generated by Toll-like receptor are transduced through NF-κB signaling and MAP kinases pathway to recruit pro-inflammatory cytokines and co-stimulatory molecules, which promote inflammatory responses[28]. Many studies have highlighted the importance of Toll-like receptor signaling pathway in the podocyte apoptosis in IMN patients[29-30].

Our study explored the potential molecular mechanism of tripterygium wilfordii in the treatment of IMN from an integrity and systematic perspective, which provided theoretical a basis for further understanding and experimental studies. It is worth nothing that our results can only give hints, and it is necessary to be verified by real experimental data.

Conclusion

In conclusion, the potential molecular mechanism of tripterygium wilfordii in treating IMN has the characteristics of multi-component, multi-target, and multi-pathway. AKT1, TNF, VEGFA, TP63, PTGS2, CXCL8, MAPK8, and STAT3 may be the important and direct targets of tripterygium wilfordii in the treatment of IMN. It may be related to the AGE signaling pathway, IL-17 signaling pathway, TNF signaling pathway, and Toll-like receptor signaling pathway. Our study provides a research basis for further studies of tripterygium wilfordii in the treatment of IMN.

Declarations

Ethics approval and consent to participate

No applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data are available in the manuscript and the Additional files.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

SHH and WLH: study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; statistical analysis. HYJ, SXL and QJ: acquisition of data; analysis and interpretation of data, and revision of the manuscript and study supervision. WQ, GXJ and GZH: material support; analysis and interpretation of data. The authors have read and approved the final manuscript.

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References

1. Debiec H, Ronco P. Immunopathogenesis of membranous nephropathy: an update. Semin Immunopathol. 2014;36(4):381-97.
2. Polanco N, Gutiérrez E, Covarsí A, Ariza F, Carreño A, Vigil A, et al. Spontaneous remission of nephrotic syndrome in idiopathic membranous nephropathy. J Am Soc Nephrol. 2010;21(4):697-704.
3. Polanco N, Gutiérrez E, Rivera F, Castellanos I, Baltar J, Lorenzo D, et al. Spontaneous remission of nephrotic syndrome in membranous nephropathy with chronic renal impairment. Nephrol Dial Transplant. 2012;27(1):231-4.
4. Luo D, Zuo Z, Zhao H, Tan Y, Xiao C. Immunoregulatory effects of Tripterygium wilfordii Hook F and its extracts in clinical practice. Front Med. 2019;13(5):556-563.
5. Liu S, Li X, Li H, Liang Q, Chen J, Chen J. Comparison of tripterygium wilfordii multiglycosides and tacrolimus in the treatment of idiopathic membranous nephropathy: a prospective cohort study. BMC Nephrol. 2015;16:200.
6. Shang SL, Cai GY, Duan SW, Li P, Li QG, Chen XM. Retrospective analysis of tacrolimus combined with Tripterygium wilfordii polyglycoside for treating idiopathic membranous nephropathy. BMC Nephrol. 2018;19(1):182.
7. Boezio B, Audouze K, Ducrot P, Taboureau O. Network-based Approaches in Pharmacology. Mol Inform. 2017;36(10).
8. Ru J, Li P, Wang J, Zhou W, Li B, Huang C, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines. J Cheminform. 2014;6:13.
9. Liu H, Wang J, Zhou W, Wang Y, Yang L. Systems approaches and polypharmacology for drug discovery from herbal medicines: an example using licorice. J Ethnopharmacol. 2013;146(3):773-93.
10. Xu X, Zhang W, Huang C, Li Y, Yu H, Wang Y, et al. A novel chemometric method for the prediction of human oral bioavailability. Int J Mol Sci. 2012;13(6):6964-82.
11. Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45(D1):D362-D368.

12. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13(11):2498-504.
13. Kanehisa M, Goto S. KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27-30.
14. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284-7.
15. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet. 2000;25(1):25-9.
16. Chen ZH, Qin WS, Zeng CH, Zheng CX, Hong YM, Lu YZ, et al. Triptolide reduces proteinuria in experimental membranous nephropathy and protects against C5b-9-induced podocyte injury in vitro. Kidney Int. 2010;77(11):974-88.
17. Feng Z, Tang L, Wu L, Cui S, Hong Q, Cai G, et al. Na+/H+ exchanger-1 reduces podocyte injury caused by endoplasmic reticulum stress via autophagy activation. Lab Invest. 2014;94(4):439-54.
18. Thibaudin D, Thibaudin L, Berthoux P, Mariat C, Filippis JP, Laurent B, et al. TNFA2 and d2 alleles of the tumor necrosis factor alpha gene polymorphism are associated with onset/occurrence of idiopathic membranous nephropathy. Kidney Int. 2007;71(5):431-7.
19. Morita M, Mii A, Shimizu A, Yasuda F, Shoji J, Masuda Y, et al. Glomerular endothelial cell injury and focal segmental glomerulosclerosis lesion in idiopathic membranous nephropathy. PLoS One. 2015;10(4):e0116700.
20. Segerer S, Henger A, Schmid H, Kretzler M, Draganovici D, Brandt U, et al. Expression of the chemokine receptor CXCR1 in human glomerular diseases. Kidney Int. 2006;69(10):1765-73.
21. Heise G, Grabensee B, Schrör K, Heering P. Different actions of the cyclooxygenase 2 selective inhibitor flosulide in rats with passive Heymann nephritis. Nephron. 1998;80(2):220-6. doi: 10.1159/000045171.
22. Sha W, Shen L, Zhou L, Xu D, Yang J, Lu G. Silencing of CXCL12 performs a protective effect on C5b-9-induced injury in podocytes. Int Urol Nephrol. 2018;50(8):1535-1544.
23. Zheng R, Deng Y, Chen Y, Fan J, Zhang M, Zhong Y, et al. Astragaloside IV attenuates complement membranous attack complex induced podocyte injury through the MAPK pathway. Phytother Res. 2012;26(6):892-8.
24. Wu J, Liu B, Liang C, Ouyang H, Lin J, Zhong Y, et al. Zhen-wu-tang attenuates cationic bovine serum albumin-induced inflammatory response in membranous glomerulonephritis rat through inhibiting AGEs/RAGE/NF-κB pathway activation. Int Immunopharmacol. 2016;33:33-41.
25. Ifuku M, Miyake K, Watanebe M, Ito K, Abe Y, Sasatomi Y, et al. Various roles of Th cytokine mRNA expression in different forms of glomerulonephritis. Am J Nephrol. 2013;38(2):115-23.
26. Neale TJ, Rüger BM, Macaulay H, Dunbar PR, Hasan Q, Bourke A, et al. Murray-McIntosh RP, Kitching AR. Tumor necrosis factor-alpha is expressed by glomerular visceral epithelial cells in human membranous nephropathy. Am J Pathol. 1995;146(6):1444-54.

27. Huang YS, Fu SH, Lu KC, Chen JS, Hsieh HY, Sytwu HK, et al. Inhibition of tumor necrosis factor signaling attenuates renal immune cell infiltration in experimental membranous nephropathy. Oncotarget. 2017;8(67):111631-111641.
28. Vidya MK, Kumar VG, Sejian V, Bagath M, Krishnan G, Bhatta R. Toll-like receptors: Significance, ligands, signaling pathways, and functions in mammals. Int Rev Immunol. 2018;37(1):20-36.
29. Jin LW, Pan M, Ye HY, Zheng Y, Chen Y, Huang WW, et al. Down-regulation of the long non-coding RNA XIST ameliorates podocyte apoptosis in membranous nephropathy via the miR-217-TLR4 pathway. Exp Physiol. 2019;104(2):220-230.
30. Sha WG, Shen L, Zhou L, Xu DY, Lu GY. Down-regulation of miR-186 contributes to podocytes apoptosis in membranous nephropathy. Biomed Pharmacother. 2015;75:179-84.

Figures

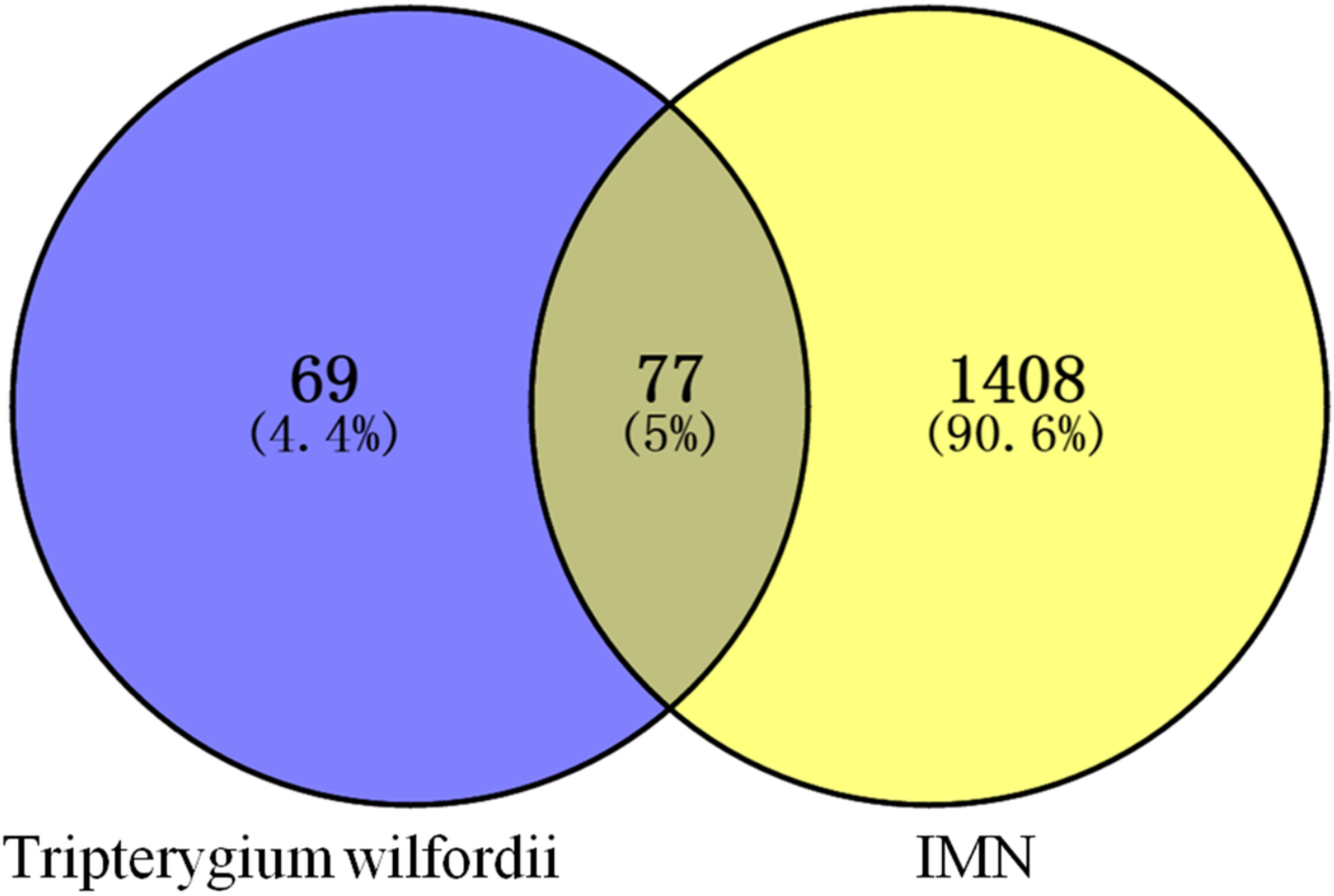


Figure 1

The 77 overlapping genes between the IMN and tripterygium wilfordii.

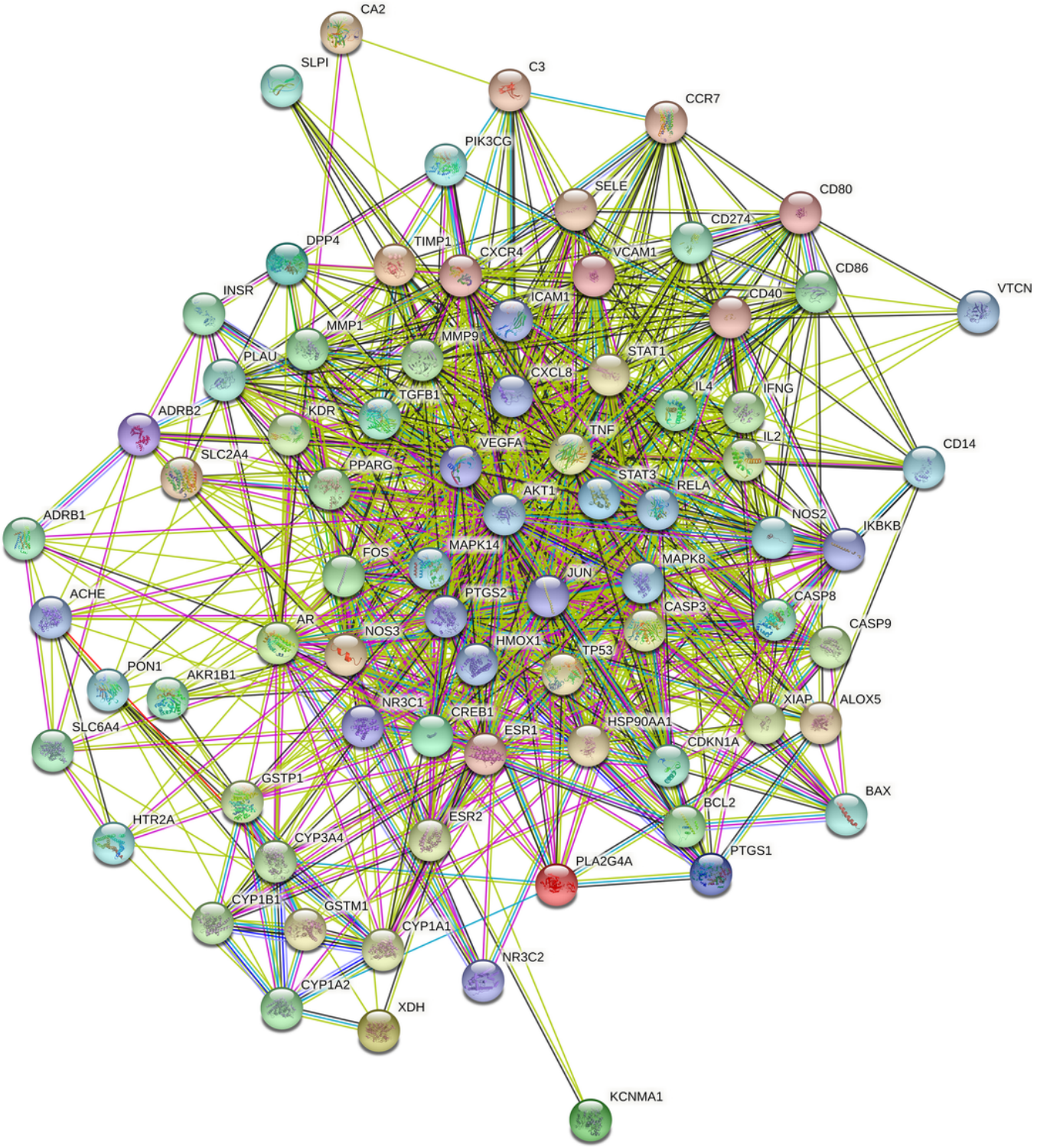


Figure 2

The PPI network of 77 overlapping genes.

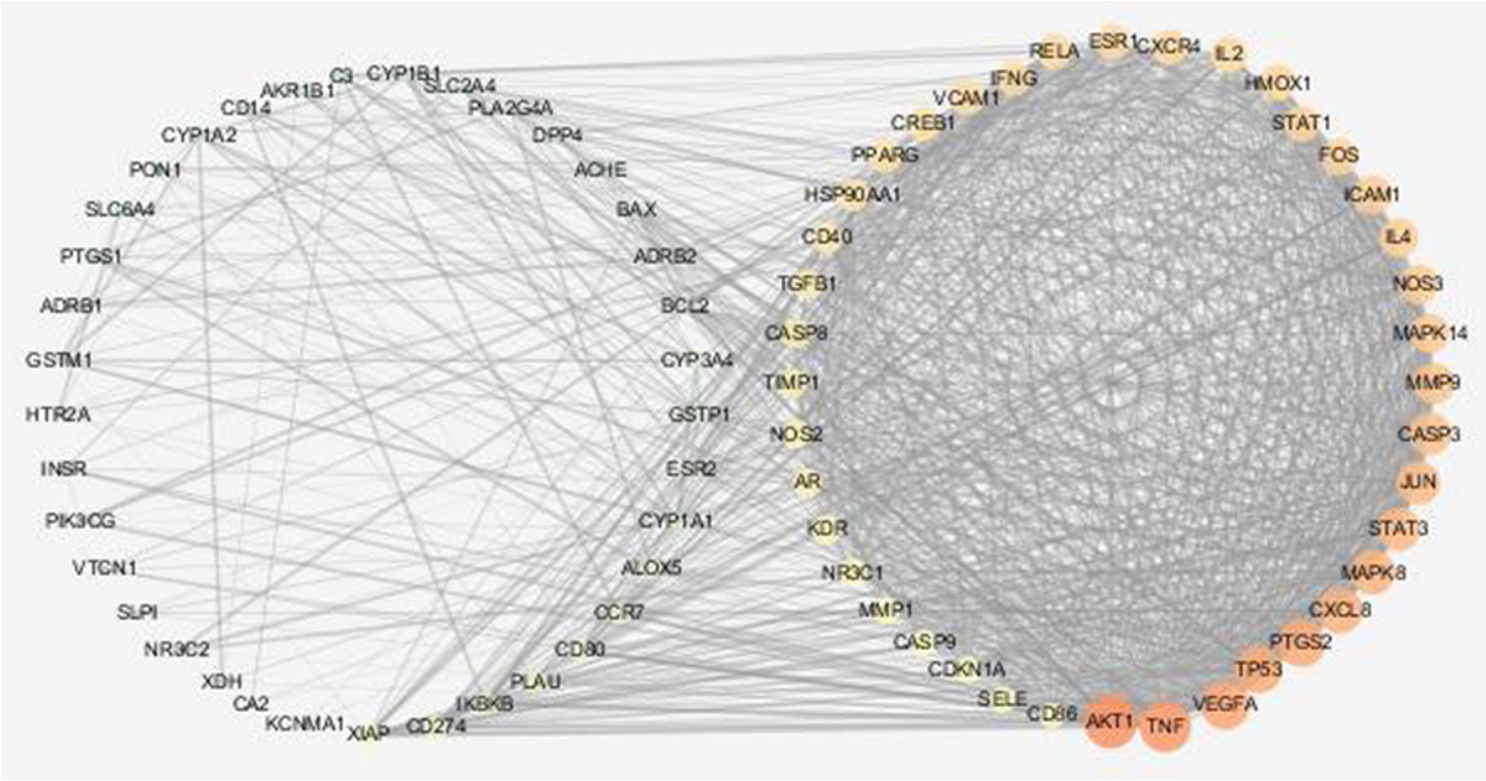
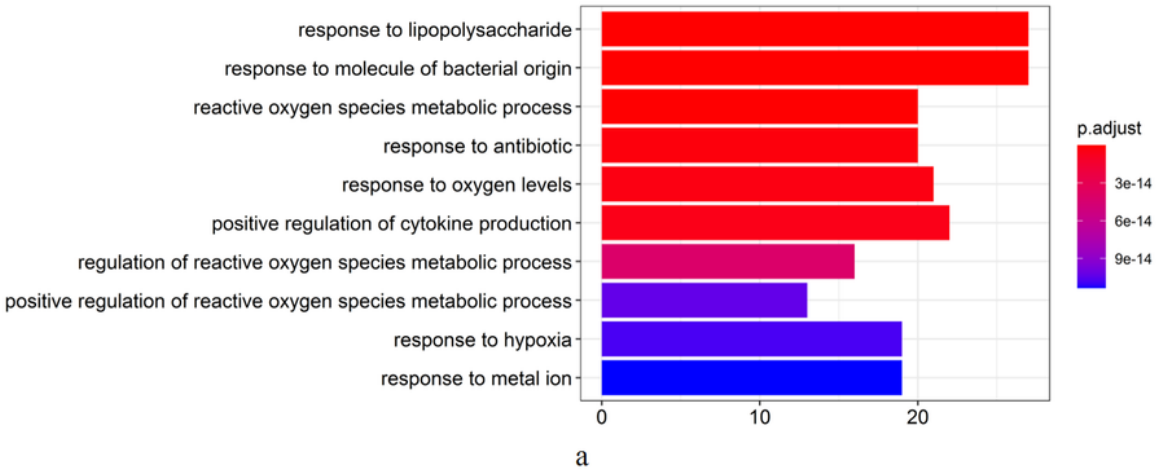


Figure 3

The PPI network of 77 overlapping genes. The size and the color of the node represents the value of the degree (The larger node and the redder color indicates the higher degree value), the thickness of the side indicates the value of the combine score (The thicker side indicates the higher combine score).



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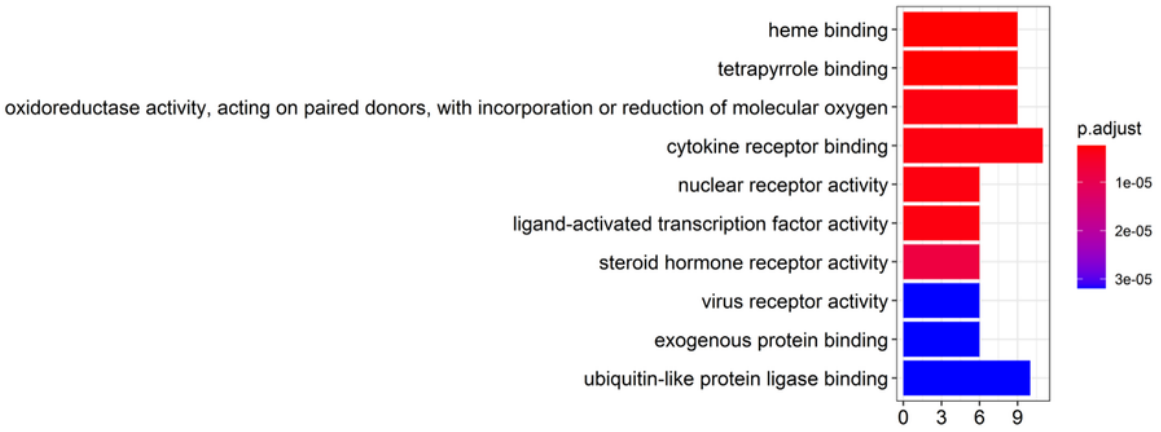

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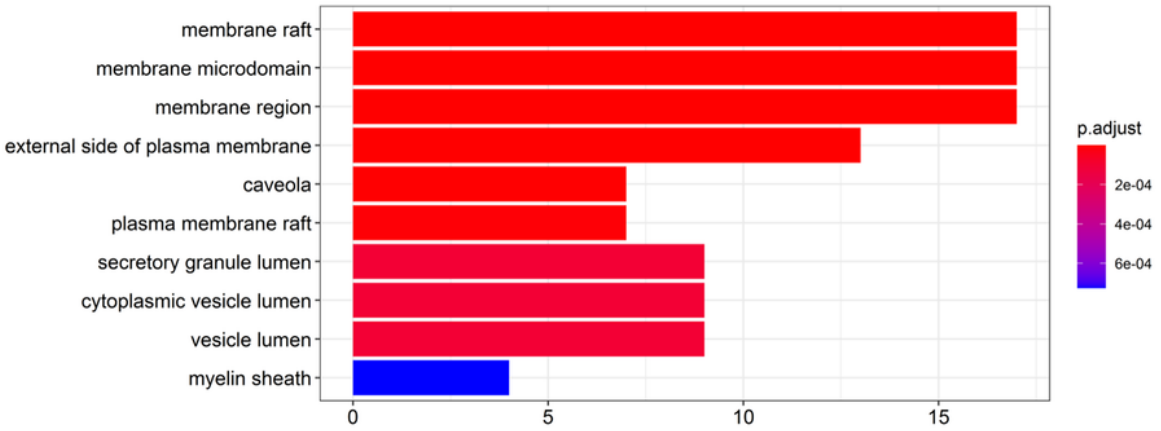

c

Figure 4

Gene ontology analyses of the therapeutic target genes of tripterygium wilfordii for the treatment of IMN. (a) BP, (b) MF, and (c) CC. Each bar represents a GO term on the vertical axis. The number of genes enriched in each term is recorded on the horizontal axis. Color of each bar represents the adjusted p value of each GO term. More red the color of the term is, smaller its adjusted p value is. Abbreviations: GO, Gene Ontology; BP, biological process; MF, molecular function.

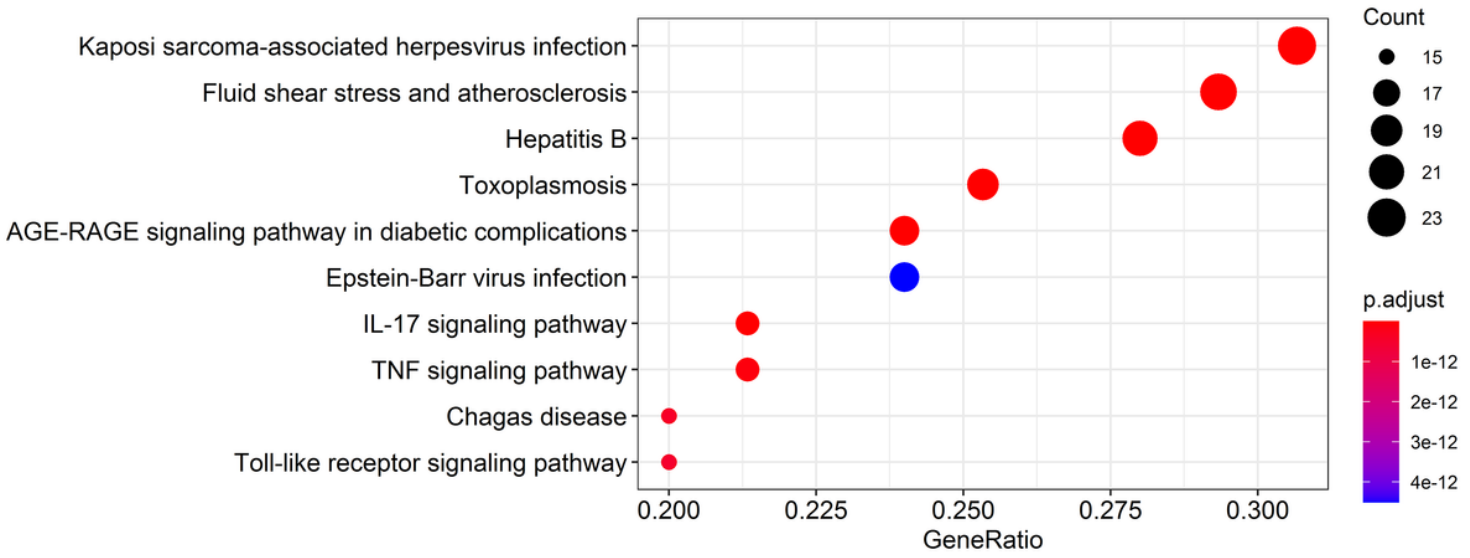

Figure 5

Gene ontology analyses of the therapeutic target genes of tripterygium wilfordii for the treatment of IMN. Each bubble represents a KEGG pathway on the vertical axis. The gene ratio is recorded on the horizontal axis. The size of each bubble indicates the number of genes enriched in each KEGG pathway. Larger the bubble is, more number of genes is involved in the pathway. Color of each bubble represents the adjusted P value of each KEGG pathway. More red the color of the bubble is, smaller its adjusted P value is.

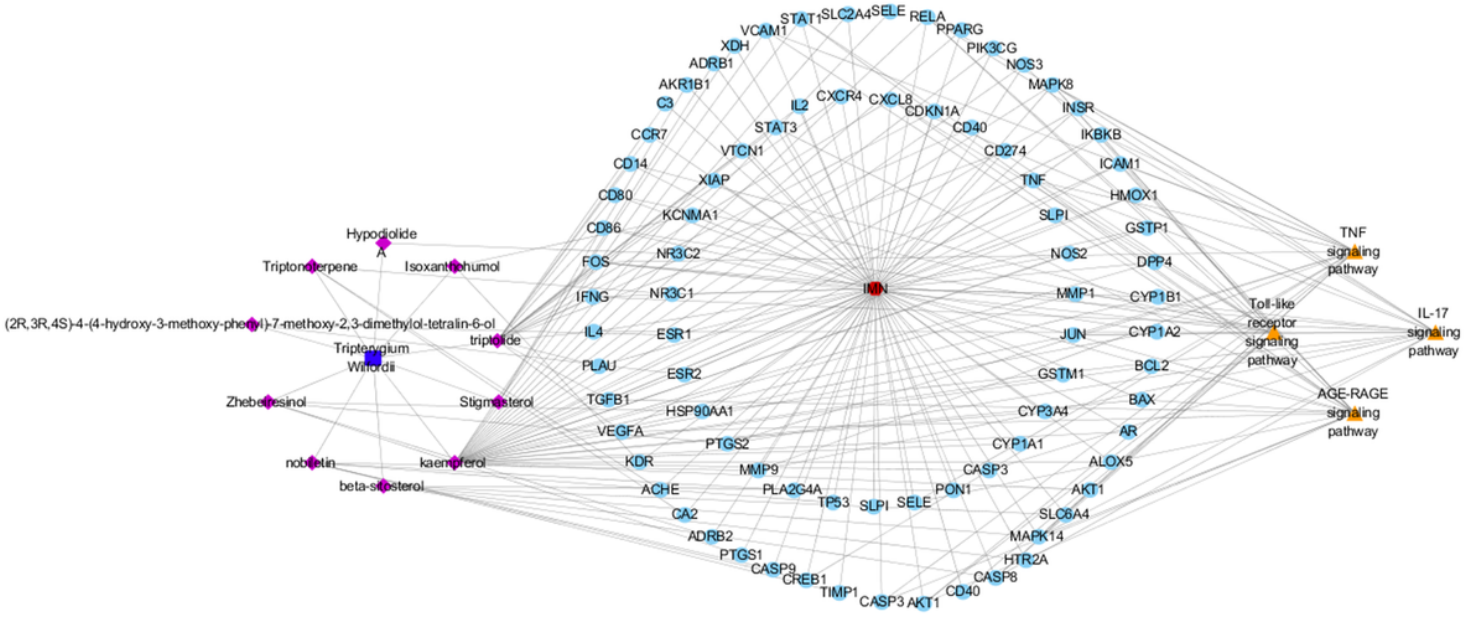

Figure 6

The relationship between KEGG pathways and active compounds, targets of tripterygium wilfordii for the treatment of IMN.

Supplementary Files

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