[DOI]10.12016/j.issn.2096-1456.2021.12.003

·基础研究·

网络药理学探索雷公藤治疗口腔扁平苔藓的药理特性和治疗机制

吴泽钰[1,2]，赵今[1,2]，王琛[1]，龚怡[1]，薛瑞[2,3]
1. 新疆医科大学第一附属医院(附属口腔医院)牙体牙髓病科，新疆维吾尔自治区 乌鲁木齐(830054)；2. 新疆维吾尔自治区口腔医学研究所，新疆维吾尔自治区 乌鲁木齐(830054)；3. 新疆医科大学第一附属医院(附属口腔医院)口腔黏膜中西医结合科，新疆维吾尔自治区 乌鲁木齐(830054)

【摘要】 目的 基于网络药理学方法研究雷公藤治疗口腔扁平苔藓(oral lichen planus，OLP)的主要活性成分及其潜在的药理特性和作用机制。**方法** 基于中药系统药理学分析平台等获取雷公藤化学成分及靶点，Gene Cards等数据库获取OLP靶点，将药物与疾病靶点进行韦恩分析，得到雷公藤治疗OLP的潜在靶点。采用Cytoscape 3.7.2构建化学成分-靶点网络，STRING构建蛋白质-蛋白质相互作用网络，Network Analyzer计算网络拓扑属性，利用Cluster Profiler软件包进行GO、KEGG富集分析，并构建雷公藤化学成分-靶点-通路网络。**结果** 获得雷公藤23个成分和150个靶点，OLP靶点472个，交集靶点44个。雷公藤治疗OLP的核心活性成分有雷公藤内酯、山奈酚、川陈皮素等，关键靶点包括肿瘤坏死因子、丝氨酸/苏氨酸蛋白激酶1等。GO富集分析得到白细胞分化、对脂多糖的反应等63个GO条目。KEGG富集分析得到TNF信号通路、IL17信号通路等111条通路。**结论** 应用网络药理学方法初步揭示雷公藤治疗OLP的主要成分、作用靶点和作用途径，为将来从雷公藤中开发出高活性、低毒性的治疗OLP的药物的深入研究提供了理论依据。

【关键词】 雷公藤；雷公藤内酯；山奈酚；网络药理学；药理特性；活性成分；口腔扁平苔藓；靶点；治疗机制
【中图分类号】 R78　**【文献标志码】** A　**【文章编号】** 2096-1456(2021)12-0809-11
开放科学(资源服务)标识码(OSID)
【引用著录格式】 吴泽钰, 赵今, 王琛, 等. 网络药理学探索雷公藤治疗口腔扁平苔藓的药理特性和治疗机制[J]. 口腔疾病防治, 2021, 29(12): 809-819. doi: 10.12016/j.issn.2096-1456.2021.12.003.

Prediction of the pharmacological characteristics and therapeutic mechanism of *Tripterygium wilfordii* in treating oral lichen planus based on network pharmacology

WU Zeyu[1,2], ZHAO Jin[1,2], WANG Chen[1], GONG Yi[1], XUE Rui[2,3]. 1. Department of Cariology and Endodontics, The First Affiliated Hospital of Xinjiang Medical University (The Affiliated Stomatology Hospital of Xinjiang Medical University), Urumqi 830054, China; 2. Stomatology Disease Institute of Xinjiang Uyghur Autonomous Region, Urumqi 830054, China; 3. Department of Integrated Traditional Chinese Medicine and Oral Medicine, The First Affiliated Hospital of Xinjiang Medical University (The Affiliated Stomatology Hospital of Xinjiang Medical University), Urumqi 830054, China
Corresponding author: XUE Rui, Email: xuerui322@126.com, Tel: 86-991-4362923

【Abstract】 Objective To explore the potential mechanism of the main active component *Tripterygium wilfordii* in the treatment of oral lichen planus based on network pharmacology. **Methods** The components of *Tripterygium wilfordii* and targets were searched through the Traditional Chinese Medicine system pharmacology database and analysis platform (TCMSP) and the Traditional Chinese Medicine integrated database (TCMID) databases. The related targets of oral lichen planus (OLP) were obtained through databases such as Gene Cards. The OLP targets were mapped by Venn analysis to the targets of *Tripterygium wilfordii* to screen out the common targets as the treatment of OLP targets of

【收稿日期】 2020-12-05；**【修回日期】** 2021-06-07
【基金项目】 新疆维吾尔自治区自然科学基金-联合基金(2016D01C248)
【作者简介】 吴泽钰，主治医师，博士，Email：wuzeyuyu@hotmail.com
【通信作者】 薛瑞，副主任医师，硕士，Email：xuerui322@126.com，Tel：86-991-4362923

Tripterygium wilfordii. The Cytoscape software and STRING were used to construct a chemical component-target network and protein-protein interaction network, a network analyzer was used to compute the network topology properties, a cluster profiler software was used to analyze the GO classification enrichment analysis and KEGG signal path analysis, and a *Tripterygium wilfordii* chemical components-targets-pathway network diagram was constructed. **Results** Twenty-three components and 44 OLP treatmenttargets of *Tripterygium wilfordii* were obtained. The key active ingredients of *Tripterygium wilfordii* in the treatment of OLP are triptolide, kaempferol, and tangerine peel. The key targets include TNF and AKT1. The GO classification enrichment analysis obtained 63 GO terms, which are mainly involved in the leukocyte differentiation and reaction to lipopolysaccharides. The KEGG analysis identified 111 signaling pathways, which are mainly related to the TNF signaling pathway and IL17 signaling pathway. **Conclusion** Based on the network pharmacology, this study preliminarily revealed themain components, targets and pathways of *Tripterygium wilfordii* in the treatment of OLP. This study can provide a theoretical basis for further research to explore drugs with high activity and low toxicity to treat OLP from *Tripterygium wilfordii*.

【Key words】 *Tripterygium wilfordii*; triptolide; kaempferol; network pharmacology; pharmacological characteristics; active ingredient; OLP; target; therapeutic mechanism

J Prev Treat Stomatol Dis, 2021, 29(12): 809-819.

【Competing interests】 The authors declare no competing interests.

This study was supported by the grants from Natural Science Foundation of Xinjiang Province (No. 2016D01C248).

口腔扁平苔藓(oral lichen planus,OLP)是口腔黏膜疾病中一种常见的慢性炎性疾病,WHO将其列入癌前状态的范畴。其病因不明确,可能与免疫因素[1]、感染因素、内分泌因素等有关,所以其治疗也多以免疫抑制剂为主[2],如糖皮质激素、羟氯喹[3]等。研究发现,卫矛科植物雷公藤(*Tripterygium wilfordii Hook. f.*)有很强的抗炎作用,可抑制体液免疫,同时对细胞免疫有双向调节作用,口服雷公藤总苷片是一种有效的OLP治疗方法[4]。然而雷公藤的毒副作用大[5]、药效成分和作用机制不明确又使许多医师及患者望而却步。因此探寻雷公藤治疗OLP的主要成分及其作用机制为雷公藤制剂应用于临床的必经之路。

近年来,研究发现人类疾病的复杂程度远比最初预想的要复杂得多,它们往往是由多个分子异常引起的,而不是由单一缺陷引起。利用网络药理学,可以在靶蛋白、生物功能和生物活性物质的基础上形成复杂的相互作用网络,能够在分子水平上从系统的角度阐明中药的作用机理,成为中药研究的一种新方法。该方法已被应用于指导和协助药物重新定位,发挥治疗作用的药物可以直接作用于疾病相关蛋白,也可以调节病理过程中涉及的通路[6]。本研究运用网络药理学的思路和方法筛选雷公藤治疗OLP活性成分,探寻其作用靶点,以经济有效的方式从计算生物信息学角度阐明雷公藤治疗OLP的药效物质基础,以期为雷公藤治疗OLP的机制探究及临床应用提供理论依据和药效保障。

1　资料和方法

1.1　筛选雷公藤活性组分和靶点

在中药系统药理学数据库及分析平台(Traditional Chinese Medicine systems pharmacology database and analysis platform,TCMSP)、中药综合数据库(Traditional Chinese Medicine integrated database,TCMID)中检索“雷公藤”,得到雷公藤的成分及作用靶点。利用PubChem和ChemBL确定成分的三维结构,建立雷公藤组分数据库。

1.1.1　雷公藤活性组分的筛选　本研究筛选口服生物利用度(oral bioavailability,OB)≥30%,化合物类药性(drug likeness,DL)≥0.18,及该活性化合物在雷公藤中的占比,及OLP防治潜力作为筛选条件。

1.1.2　OLP靶点基因的筛选　通过在Gene Cards、DisGeNET、DrugBank和OMIM中输入“oral lichen planus”等关键词等搜索相关基因,得到的疾病靶点与雷公藤治疗靶点筛选出共同靶点,作为雷公藤治疗OLP的潜在靶点。

1.2　化学成分-靶点网络构建与分析

利用Cytoscape 3.7.2软件将雷公藤筛选出的化学成分、治疗靶点导入,构建“化学成分-靶点”网络,分析拓扑属性,节点越多,度值越大,节点的中介中心度(betweenness centrality,BC)、接近中心性(closeness centrality,CC)以及拓扑系数(topological

coefficient，TC）等信息通过该软件分析计算得出[7]，筛选网络拓扑中的关键药物成分，分析主要作用靶点及潜在作用机制。

1.3　构建蛋白质-蛋白质相互作用（protein-protein interaction，PPI）网络

在STRING 11.0[8]数据库中会对每个蛋白质的相互作用信息打分，其分值越高蛋白质的互作置信度越高。输入靶点，得到分值＞0.4的高置信度蛋白相作数据，构建PPI网络，利用Network Analyzer分析拓扑属性。

1.4　基因本体（gene ontology，GO）和京都基因与基因组百科全书（Kyoto Encyclopedia of Genes and Genomes，KEGG）富集分析

使用Cluster Profiler软件包[9]，找出显著富集的生物学注释，对靶点进行GO富集分析及KEGG富集分析，筛选$P < 0.01$，结果利用R 3.6.1可视化。

1.5　雷公藤化学成分-靶点-通路网络的构建

将代谢通路信息、核心靶点和雷公藤活性成分导入Cytoscape3.7.2中，利用“Merge”功能将其融合，构建“雷公藤化学成分-靶点-通路”网络图。

2　结　果

2.1　雷公藤活性组分的筛选

中药系统药理学数据库分析平台（TCMSP）、中医药综合数据库（TCMID）获得雷公藤活性组分51个，根据筛选条件共筛选出活性组分23个，详见表1。

表1　雷公藤主要活性组分及药理参数

Table 1　The main active components and pharmacological parameters of *Tripterygium wilfordii*

NO.	ID	Ingredient	OB (%)	DL
1	MOL000296	Hederagenin	36.91	0.75
2	MOL003182	(+)-medioresinol di-O-beta-D-glucopyranoside_qt	60.69	0.62
3	MOL003184	Neotriptophenolide	45.42	0.53
4	MOL003185	(1R, 4aR, 10aS)-5-hydroxy-1-(hydroxymethyl)-7-isopropyl-8-methoxy-1, 4a-dimethyl-4, 9, 10, 10a-tetrahydro-3H-phenanthren-2-one	48.84	0.38
5	MOL003187	Triptolide	51.29	0.68
6	MOL003196	Tryptophenolide	48.50	0.44
7	MOL003199	5, 8-Dihydroxy-7-(4-hydroxy-5-methyl-coumarin-3)-coumarin	61.85	0.54
8	MOL003217	Isoxanthohumol	56.81	0.39
9	MOL003225	Hypodiolide A	76.13	0.49
10	MOL003229	Triptinin B	34.73	0.32
11	MOL003231	Triptoditerpenic acid B	40.02	0.36
12	MOL003245	Triptonoditerpenic acid	42.56	0.39
13	MOL003248	Triptonoterpene	48.57	0.28
14	MOL003280	Triptonolide	49.51	0.49
15	MOL000358	Beta-sitosterol	36.91	0.75
16	MOL007415	[(2S)-2-[[(2S)-2-(benzoylamino)-3-phenylpropanoyl]amino]-3-phenylpropyl] acetate	58.02	0.52
17	MOL000449	Stigmasterol	43.83	0.76
18	MOL002058	Medioresil	57.20	0.62
19	MOL003283	(2R, 3R, 4S)-4-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2, 3-dimethylol-tetralin-6-ol	66.51	0.39
20	MOL004443	Zhebeiresinol	58.72	0.19
21	MOL005828	Nobiletin	61.67	0.52
22	MOL000422	Kaempferol	41.88	0.24
23	MOL009386	3, 3′-bis-(3, 4-dihydro-4-hydroxy-6-methoxy)-2H-1-benzopyran	52.11	0.54

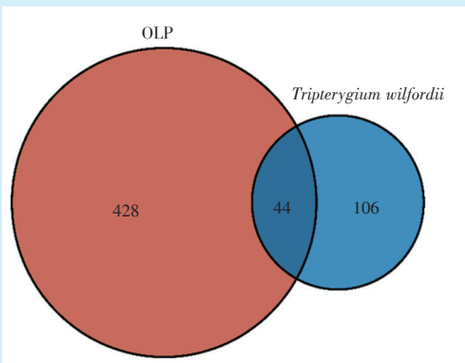
OB: oral bioavailability; DL: drug likeness

2.2　雷公藤和OLP相关靶点

雷公藤的23个活性组分在TCMSP、TCMID数据库中获得去重后靶点150个。通过GeneCards、DisGeNET、DrugBank和OMIM数据库得到去重后靶点472个。药物作用靶点与疾病靶点韦恩分析，得到44个雷公藤治疗OLP的潜在靶点（图1、表2）。

2.3　构建与分析化合成分-靶点网络

如图2所示，雷公藤化学成分-靶点网络运用Cytoscape 3.7.2软件构建。



44 common targets between OLP and *Tripterygium wilfordii*, OLP: oral lichen planus

Figure 1　Venn diagram

图1　韦恩图

表2　雷公藤治疗OLP的潜在活性成分及作用靶标

Table 2　Potential active ingredients and targets of *Tripterygium wilfordii* in the OLP treatment

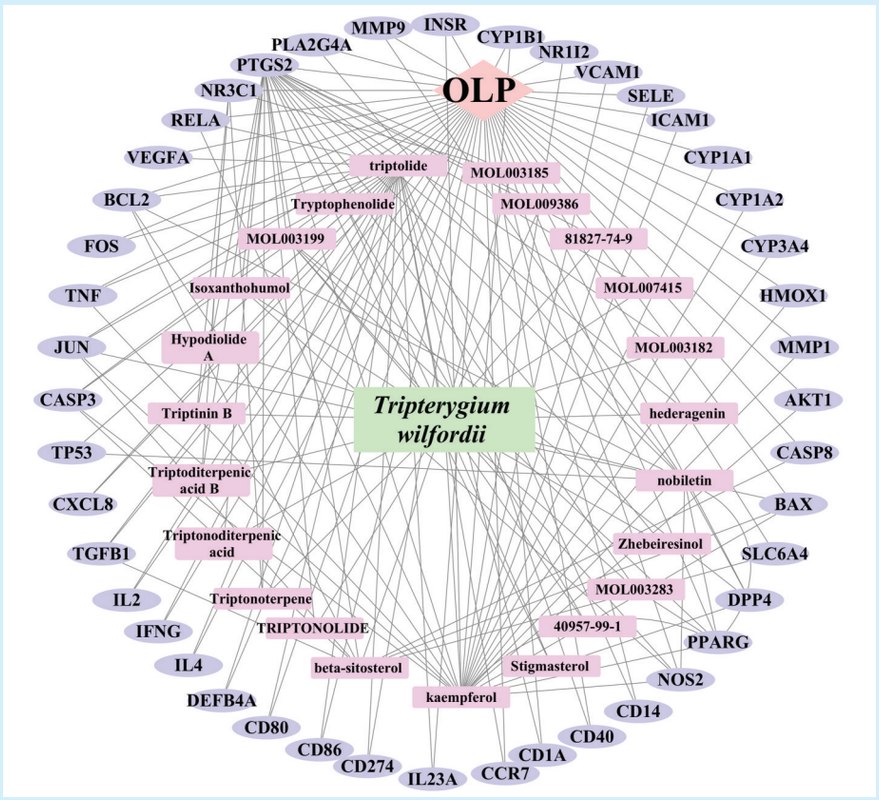
NO.	Target	Entrez ID	NO.	Target	Entrez ID	NO.	Target	Entrez ID	NO.	Target	Entrez ID
1	CYP3A4	1576	12	TGFB1	7040	23	INSR	3643	34	CCR7	1236
2	TNF	7124	13	VCAM1	7412	24	DPP4	1803	35	CD86	942
3	CYP1B1	1545	14	AKT1	207	25	IL4	3565	36	NR1I2	8856
4	PTGS2	5743	15	FOS	2353	26	ICAM1	3383	37	CD80	941
5	MMP9	4318	16	CASP3	836	27	CYP1A1	1543	38	JUN	3725
6	SLC6A4	6532	17	IL23A	51561	28	RELA	5970	39	BAX	581
7	PPARG	5468	18	BCL2	596	29	TP53	7157	40	CD274	29126
8	CXCL8	3576	19	HMOX1	3162	30	CD1A	909	41	VEGFA	7422
9	NR3C1	2908	20	CASP8	841	31	CD40	958	42	SELE	6401
10	DEFB4A	1673	21	IFNG	3458	32	CYP1A2	1544	43	CD14	929
11	MMP1	4312	22	NOS2	4843	33	PLA2G4A	5321	44	IL2	3558

OLP: oral lichen planus; CYP3A4: cytochrome P450 3A4; TNF: tumor necrosis factor; CYP1B1: cytochrome P450 1B1; PTGS2: prostaglandin G/H synthase 2; MMP9: matrix metalloproteinase 9; PPARG: peroxisome proliferator-activated receptor gamma; DEFB4A: beta-defensin 4A; MMP1: matrix metalloproteinase 1; TGFB1: transforming growth factor beta 1; VCAM1: vascular cell adhesion protein 1; AKT1: RAC-alpha serine/threonine-protein kinase; FOS: proto-oncogene c-Fos; CASP3: caspase 3; IL23A: interleukin 23 subunit alpha; HMOX1: heme oxygenase 1; CASP8: caspase 8; IFNG: interferon gamma; NOS2: nitric oxide synthase 2; INSR: insulin receptor; DPP4: dipeptidyl peptidase 4; IL4: interleukin 4; ICAM1: intercellular adhesion molecule 1; CYP1A1: cytochrome P450 1A1; RELA: transcription factor p65; TP53: cellular tumor antigen p53; CYP1A2: cytochrome P450 1A2; CCR7: C-C chemokine receptor type 7; NR1I2: nuclear receptor subfamily 1 group I member 2; VEGFA: vascular endothelial growth factor A; SELE: E selectin; IL2: interleukin 2

图中有69个节点(23个化学成分,44个目标)和320个边。网络拓扑分析表明,平均度数为4.64,说明雷公藤多靶点治疗OLP具有多靶点特性,其拓扑学参数见表3。同时,可以看出雷公藤中发挥主要作用的成分雷公藤内酯、山奈酚、川陈皮素等。许多靶点与多种成分相关,这表明雷公藤发挥药效过程中,不同成分之间存在的协同作用。

2.4　蛋白质-蛋白质相互作用网络分析

STRING数据库获得44个靶点的PPI网络,共44个节点和214个边缘,网络的平均度值为10.4(图3a)。节点反映网络中连接"度"的大小,按照度值降序排序筛选出核心靶蛋白30个,其中以边缘数排序前10位的有:肿瘤坏死因子(tumor necrosis factor, TNF)、丝氨酸/苏氨酸蛋白激酶1(AKT serine/threonine kinase 1, AKT1)、转录因子AP-1(transcription factor AP-1, JUN)、肿瘤蛋白p53(tumor protein p53, TP53)、白细胞介素4(interleukin 4, IL4)、白细胞介素8(interleukin 8/C-X-C motif chemokine ligand 8, IL8/CXCL8)、细胞间黏附分子1(intercellular cell adhesion molecule 1, ICAM1)、转录因子p65(Transcription factor p65, RELA)、血管内皮



CYP3A4: cytochrome P450 3A4; TNF: tumor necrosis factor; CYP1B1: cytochrome P450 1B1; PTGS2: prostaglandin G/H synthase 2; MMP9: matrix metalloproteinase 9; PPARG: peroxisome proliferator-activated receptor gamma; DEFB4A: beta-defensin 4A; MMP1: matrix metalloproteinase 1; TGFB1: transforming growth factor beta 1; VCAM1: vascular cell adhesion protein 1; AKT1: RAC-alpha serine/threonine-protein kinase; FOS: proto-oncogene c-Fos; CASP3: caspase 3; IL23A: interleukin 23 subunit alpha; HMOX1: heme oxygenase 1; CASP8: caspase 8; IFNG: interferon gamma; NOS2: nitric oxide synthase 2; INSR: insulin receptor; DPP4: dipeptidyl peptidase 4; IL4: interleukin 4; ICAM1: intercellular adhesion molecule 1; CYP1A1: cytochrome P450 1A1; RELA: transcription factor p65; TP53: cellular tumor antigen p53; CYP1A2: cytochrome P450 1A2; CCR7: C-C chemokine receptor type 7; NR1I2: nuclear receptor subfamily 1 group I member 2; VEGFA: vascular endothelial growth factor A; SELE: E selectin; IL2: interleukin 2

Figure 2　*Tripterygium wilfordii* chemical components-targets network diagram

图2　雷公藤化合成分-靶点网络拓扑学参数

生长因子A（vascular endothelial growth factor A，VEGFA）、γ干扰素（interferon gamma，IFNG）（图3b、表4）。

2.5　GO和KEGG富集分析

对上述44个靶点进行GO富集分析，根据$P<0.01$确定了63个GO条目（表5）。

雷公藤治疗OLP的潜在靶点GO富集分析结果如表5所示，生物过程（biological process，BP）相关条目19个，涉及白细胞分化、对脂多糖的反应、对细菌起源分子的反应等方面（图4）；分子功能（molecular function，MF）相关条目33个，涉及细胞因子受体结合、蛋白质异构化活性、血红素结合等方面（图5）；细胞成分（cellular component，CC）相关条目11个，涉及膜筏、膜微结构域、膜区等方面（图6）。

KEGG分析得到111条信号通路，根据$P<0.01$筛选出66条通路，绘制气泡图（表6、图7）。包括IL17信号通路、AGE-RAGE信号通路在糖尿病并发症、乙型肝炎、卡波西肉瘤相关疱疹病毒感染、TNF信号通路、肺结核、美洲锥虫病、Toll样受体信号通路等通路。

2.6　雷公藤化学成分-靶点-通路网络

由Cytoscape3.7.2的“Merge”功能构建的“雷公藤化学成分-靶点-通路”网络图，存在83个节点，

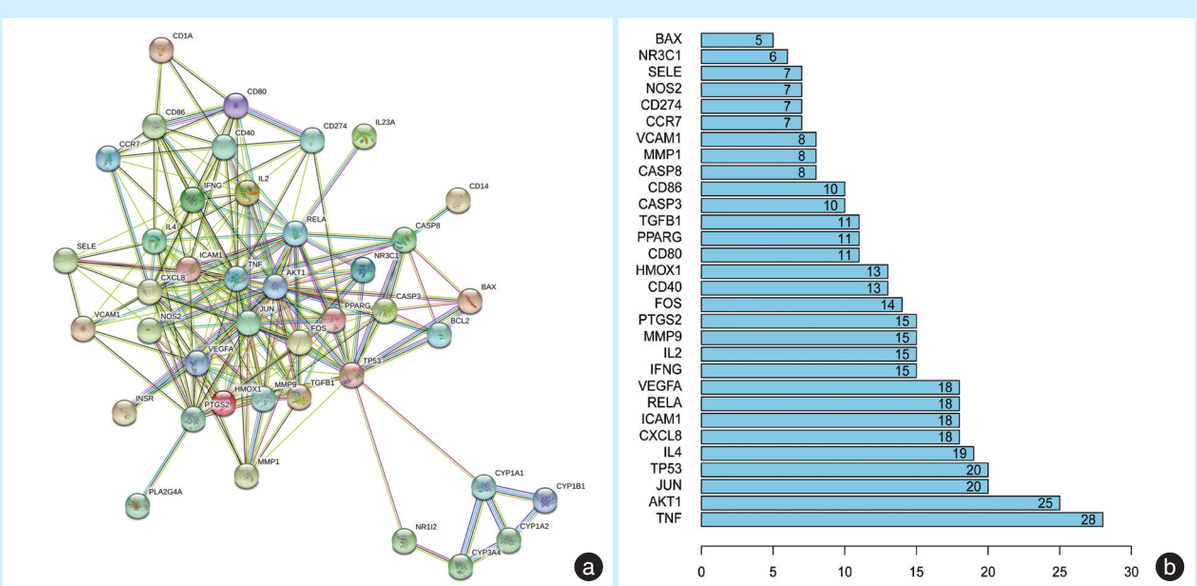
584条边缘，平均“度”值为7.06。由图8可见KEGG富集到的前16个信号通路、44个核心靶点、23个活性成分间关系密切。这可能说明雷公藤中的多种化合物可以通过多个靶点调节多条信号通路来达到潜在治疗OLP的作用。

表3　雷公藤化学成分的拓扑学参数分析

Table 3　Topological parameter analysis of *Tripterygium wilfordii* chemical component

NO.	Ingredient ID	Degree	BC	CC	TC	NO.	Ingredient ID	Degree	BC	CC	TC
1	MOL003187	24	0.160	0.511	0.141	13	MOL003182	2	0.000	0.384	0.957
2	MOL000422	24	0.143	0.504	0.161	14	MOL003184	2	0.000	0.384	0.957
3	MOL005828	12	0.036	0.428	0.277	15	MOL003196	2	0.000	0.384	0.957
4	MOL000358	9	0.028	0.417	0.300	16	MOL003225	2	0.000	0.384	0.587
5	MOL003199	5	0.006	0.398	0.478	17	MOL003245	2	0.000	0.384	0.957
6	MOL003185	3	0.001	0.389	0.710	18	MOL003280	2	0.000	0.384	0.957
7	MOL003217	3	0.001	0.389	0.696	19	MOL000449	2	0.000	0.384	0.957
8	MOL003229	3	0.001	0.389	0.710	20	MOL002058	2	0.000	0.384	0.957
9	MOL003231	3	0.001	0.389	0.710	21	MOL004443	2	0.000	0.384	0.957
10	MOL003248	3	0.002	0.389	0.710	22	MOL007415	2	0.000	0.384	0.957
11	MOL003283	3	0.002	0.389	0.696	23	MOL009386	2	0.000	0.384	0.957
12	MOL000296	2	0.000	0.384	0.957						

BC: betweenness centrality; CC: closeness centrality; TC: topological coefficient. Following bioinformatics analysis, the core components of *Tripterygium wilfordii* treated OLP were identified accordingly, including triptolide, kaempferol, nobiletin, et al; OLP: oral lichen planus



a: protein-protein interactions network; b: ranking of gene edge number in network; OLP: oral lichen planus; TNF: tumor necrosis factor; AKT1: RAC-alpha serine/threonine-protein kinase; JUN: transcription factor AP-1; TP53: cellular tumor antigen p53; IL4: interleukin 4; CXCL8: C-X-C motif chemokine ligand 8; ICAM1: intercellular adhesion molecule 1; RELA: transcription factor p65; VEGFA: vascular endothelial growth factor A; IFNG: interferon gamma; IL2: interleukin 2; MMP9: matrix metalloproteinase 9; PTGS2: prostaglandin G/H synthase 2; FOS: proto-oncogene c-Fos; HMOX1: heme oxygenase 1; PPARG: peroxisome proliferator-activated receptor gamma; TGFB1: transforming growth factor beta 1; CASP3: caspase 3; CASP8: caspase 8; MMP1: matrix metalloproteinase 1; VCAM1: vascular cell adhesion protein 1; CCR7: C-C chemokine receptor type 7; NOS2: nitric oxide synthase 2; SELE: E-selectin; NR3C1: glucocorticoid receptor

Figure 3　Active components of the *Tripterygium wilfordii* treated OLP protein-protein interaction

图3　雷公藤活性组分治疗OLP的蛋白质-蛋白质相互作用

表4　雷公藤治疗OLP核心靶点的拓扑学参数分析

Table 4　Analysis of the topological parameters of the core target of *Tripterygium wilfordii* in the OLP treatment

NO.	Gene symbol	Gene name	BC	CC	TC
1	TNF	tumor necrosis factor	0.001	0.442	0.667
2	AKT1	AKT serine/threonine kinase 1	0.000	0.436	0.739
3	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	0.006	0.453	0.482
4	TP53	tumor protein p53	0.002	0.442	0.576
5	IL4	Interleukin 4	0.000	0.436	0.750
6	CXCL8	C-X-C motif chemokine ligand 8	0.000	0.436	0.750
7	ICAM1	intercellular cell adhesion molecule 1	0.000	0.436	0.739
8	RELA	Transcription factor p65	0.001	0.442	0.667
9	VEGFA	vascular endothelial growth factor A	0.000	0.436	0.750
10	IFNG	Interferon gamma	0.000	0.436	0.750

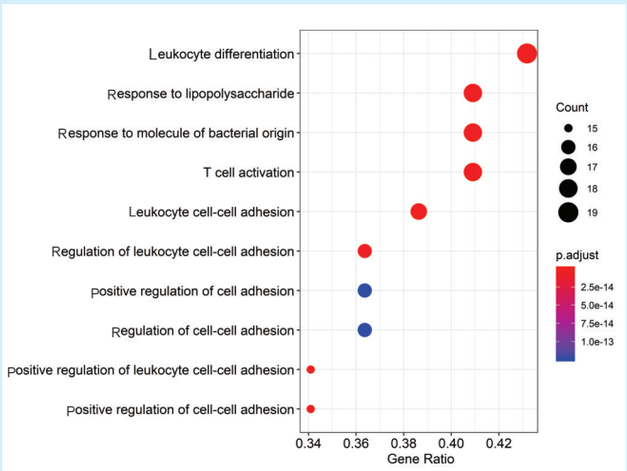
BC: betweenness centrality; CC: closeness centrality; TC: topological coefficient; OLP: oral lichen planus

表5　雷公藤治疗OLP的潜在靶点GO富集分析结果（各前10条）

Table 5　GO enrichment analysis results of potential targets of *Tripterygium wilfordii* for the OLP treatment (top ten of each items)

GO-ID	GO-term	Type	*P*	FDR value	Gene ratio	Gene
GO: 0002521	Leukocyte differentiation	BP	< 0.001	< 0.001	0.431	TNF, MMP9, PPARG, etc.
GO: 0032496	Response to lipopolysaccharide	BP	< 0.001	< 0.001	0.409	TNF, PTGS2, CXCL8, etc.
GO: 0002237	Response to molecule of bacterial origin	BP	< 0.001	< 0.001	0.409	TNF, PTGS2, CXCL8, etc.
GO: 0042110	T cell activation	BP	< 0.001	< 0.001	0.409	TGFB1, VCAM1, AKT1, etc.
GO: 0007159	Leukocyte cell-cell adhesion	BP	< 0.001	< 0.001	0.386	TNF, TGFB1, VCAM1, etc.
GO: 1903037	Regulation of leukocyte cell-cell adhesion	BP	< 0.001	< 0.001	0.363	TNF, TGFB1, VCAM1, etc.
GO: 0045785	Positive regulation of cell-cell adhesion	BP	< 0.001	< 0.001	0.363	TNF, TGFB1, VCAM1, etc.
GO: 0022407	Regulation of cell-cell adhesion	BP	< 0.001	< 0.001	0.363	TNF, TGFB1, VCAM1, etc.
GO: 1903039	Positive regulation of leukocyte cell-cell adhesion	BP	< 0.001	< 0.001	0.340	TNF, TGFB1, VCAM1, etc.
GO: 0022409	Positive regulation of cell-cell adhesion	BP	< 0.001	< 0.001	0.340	TNF, TGFB1, VCAM1, etc.
GO: 0005126	Cytokine receptor binding	MF	< 0.001	< 0.001	0.250	TNF, CXCL8, DEFB4A, etc.
GO: 0046982	Protein heterodimerization activity	MF	< 0.001	< 0.001	0.205	PPARG, TGFB1, FOS, etc.
GO: 0020037	Heme binding	MF	< 0.001	< 0.001	0.159	CYP3A4, CYP1B1, PTGS2, etc.
GO: 0046906	Tetrapyrrole binding	MF	< 0.001	< 0.001	0.159	CYP3A4, CYP1B1, PTGS2, etc.
GO: 0016705	Oxidoreductase activity、acting on paired Donors, with incorporation or reduction of molecular oxygen	MF	< 0.001	< 0.001	0.159	CYP3A4, CYP1B1, PTGS2, etc.
GO: 0005125	Cytokine activity	MF	< 0.001	< 0.001	0.159	TNF, CXCL8, TGFB1, etc.
GO: 0004497	Monooxygenase activity	MF	< 0.001	< 0.001	0.114	CYP3A4, CYP1B1, NOS2, etc.
GO: 0002020	Protease binding	MF	< 0.001	< 0.001	0.114	TNF, CASP3, BCL2, etc.
GO: 0016712	Oxidoreductase activity、acting on paired donors, with incorporation or reduction of Molecular oxygen、reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen	MF	< 0.001	< 0.001	0.091	CYP3A4, CYP1B1, CYP1A1, etc.
GO: 0070330	Aromatase activity	MF	< 0.001	< 0.001	0.068	CYP1B1, CYP1A1, CYP1A2, etc.
GO: 0045121	Membrane raft	CC	< 0.001	< 0.001	0.273	TNF, PTGS2, SLC6A4, etc.
GO: 0098857	Membrane microdomain	CC	< 0.001	< 0.001	0.273	TNF, PTGS2, SLC6A4, etc.
GO: 0098589	Membrane region	CC	< 0.001	< 0.001	0.273	TNF, PTGS2, SLC6A4, etc.
GO: 0009897	External side of plasma membrane	CC	< 0.001	< 0.001	0.136	TNF, VCAM1, INSR, etc.
GO: 0098552	Side of membrane	CC	< 0.001	< 0.001	0.136	TNF, VCAM1, INSR, etc.
GO: 0005667	Transcription factor complex	CC	< 0.001	< 0.001	0.114	PPARG, FOS, RELA, etc.
GO: 0005901	Caveola	CC	< 0.001	< 0.001	0.091	PTGS2, HMOX1, INSR, etc.
GO: 0044853	Plasma membrane raft	CC	< 0.001	< 0.001	0.091	PTGS2, HMOX1, INSR, etc.
GO: 0090575	Polymerase Ⅱ transcription factor complex	CC	< 0.001	< 0.001	0.091	PPARG, FOS, TP53, etc.
GO: 0044798	Nuclear transcription factor complex	CC	< 0.001	< 0.001	0.091	PPARG, FOS, TP53, etc.

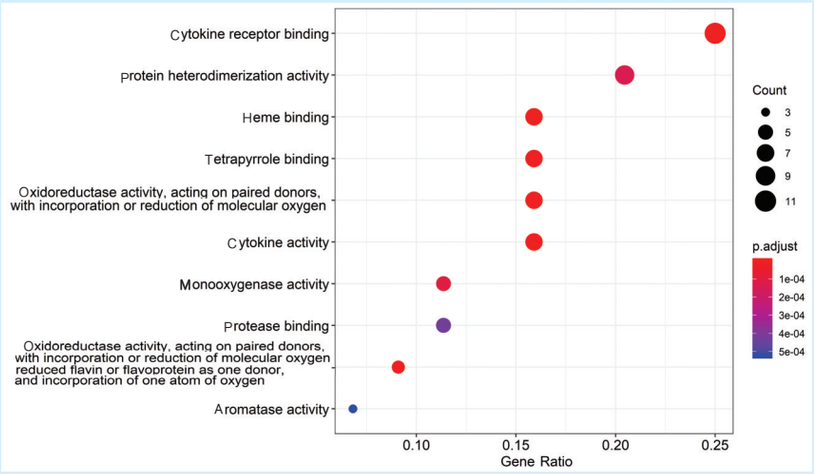
OLP: oral lichen planus; GO: gene ontology; FDR: false discovery rate



GO: gene ontology; OLP: oral lichen planus

Figure 4　Enrichment and analysis of GO biological processes in potential targets of *Tripterygium wilfordii* for the OLP treatment

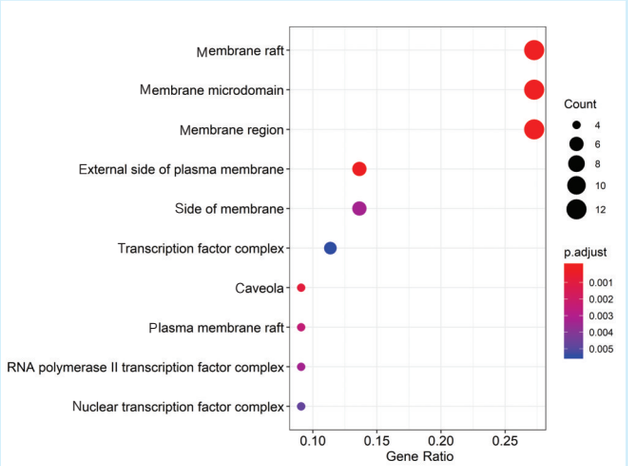
图4　雷公藤治疗OLP潜在靶点的GO生物学过程富集分析



GO: gene ontology; OLP: oral lichen planus

Figure 5　Enrichment and analysis of GO molecular functions in potential targets of *Tripterygium wilfordii* for the OLP treatment

图5　雷公藤治疗OLP潜在靶点的GO分子功能富集分析



GO: gene ontology; OLP: oral lichen planus

Figure 6　Enrichment and analysis of GO cellular components in potential targets of *Tripterygium wilfordii* for the OLP treatment

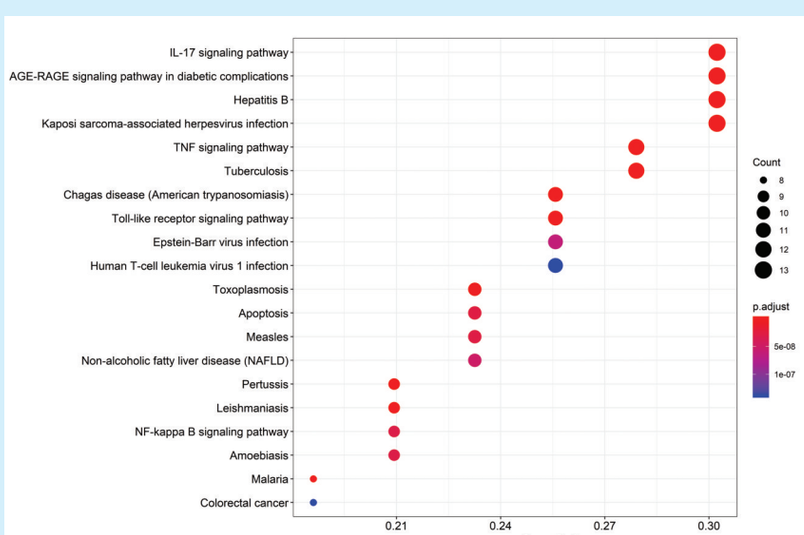
图6　雷公藤治疗OLP潜在靶点的GO细胞成分富集分析

表6　雷公藤治疗OLP的潜在靶点KEGG富集分析(前10条)

Table 6　KEGG enrichment analysis results of potential targets of *Tripterygium wilfordii* for OLP treatment (top 10 of each)

KEGG-ID	KEGG-term	*P*	Gene ratio	Gene
hsa04657	IL17 signaling pathway	< 0.001	0.302	TNF, PTGS2, MMP9, etc.
hsa04933	AGE-RAGE signaling pathway in diabetic complications	< 0.001	0.302	TNF, CXCL8, TGFβ1, etc.
hsa05161	Hepatitis B	< 0.001	0.302	TNF, MMP9, CXCL8, etc.
hsa05167	Kaposi sarcoma-associated herpesvirus infection	< 0.001	0.302	PTGS2, CXCL8, AKT1, etc.
hsa04668	TNF signaling pathway	< 0.001	0.279	TNF, PTGS2, MMP9, etc.
hsa05152	Tuberculosis	< 0.001	0.279	TNF, TGFβ1, AKT1, etc.
hsa05142	Chagas disease (American trypanosomiasis)	< 0.001	0.256	TNF, CXCL8, TGFβ1, etc.
hsa04620	Toll-like receptor signaling pathway	< 0.001	0.256	TNF, CXCL8, AKT1, etc.
hsa05169	Epstein-Barr virus infection	< 0.001	0.256	TNF, AKT1, CASP3, etc.
hsa05166	Human T-cell leukemia virus 1 infection	< 0.001	0.256	TNF, TGFβ1, AKT1, etc.

TNF: tumor necrosis factor; PTGS2: prostaglandin G/H synthase 2; MMP9: matrix metalloproteinase 9; CXCL8: chemokine (C-X-C motif) ligand 8; TGFB1: transforming growth factor beta 1; AKT1: RAC-alpha serine/threonine-protein kinase; CASP3: caspase 3



KEGG: Kyoto Encyclopedia of Genes and Genomes; OLP: oral lichen planus

Figure 7　KEGG enrichment analysis of potential targets of *Tripterygium wilfordii* for the OLP treatment

图7　雷公藤治疗OLP潜在靶点的KEGG富集分析

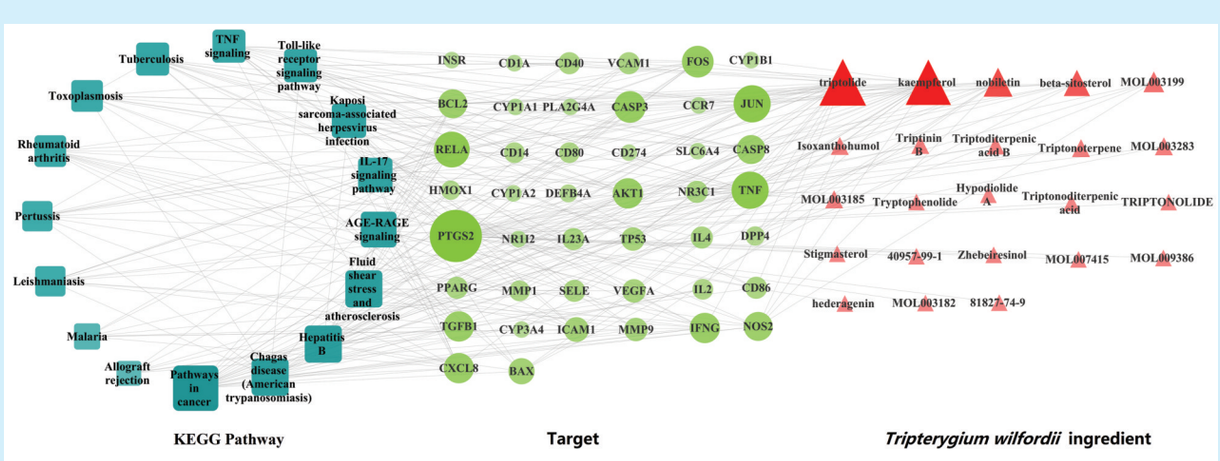


Figure 8　*Tripterygium wilfordii* chemical component-target-pathway network diagram

图8　雷公藤化学成分-靶点-通路网络

3　讨　论

雷公藤作为中药，在我国有悠久的应用历史，现代医学也证实雷公藤及其制剂有广泛的药理学作用，对慢性荨麻疹、类风湿性关节炎等难治的自身免疫性疾病有较好疗效；对各类肿瘤如乳腺癌、胰腺癌均有显著疗效，并有保护神经的作用，被用于治疗阿尔兹海默症、帕金森综合征等疾病[10]。OLP是临床发病率仅次于复发性阿弗他口腔溃疡的口腔黏膜及皮肤慢性疾病，并且属于口腔黏膜潜在恶性疾患，其发病机制不明，可能与精神、免疫、感染等多因素相关。OLP上皮固有层内有大量以T淋巴细胞为主的淋巴细胞呈密集带状浸润，无论在病损早期、后期均有T细胞介导的细胞免疫反应存在[2]。

本研究运用网络药理学方法构建“雷公藤化学成分-靶点-通路”的作用网络，系统分析雷公藤的化学成分多通路、多靶点治疗OLP的潜在机制。最终得到23个活性组分和44个潜在靶标。在PPI网络中，TNF、AKT1、JUN、TP53、IL4、CXCL8、ICAM1、RELA、VEGFA、IFN-γ等蛋白靶点与雷公藤活性成分及OLP相关性更高，它们可能是雷公藤调控OLP网络的潜在核心调控因子。

本研究结果中的调控网络显示雷公藤内酯、山奈酚等匹配多个靶点，是雷公藤治疗OLP的主要成分。雷公藤内酯对炎症、纤维化、癌症、病毒感染、氧化应激和骨质疏松症具有强大的药理活性[10]。Wang等[11]证明在类风湿性关节炎模型小鼠中，雷公藤内酯可通过降低TNF-α水平，促进破骨细胞前体细胞凋亡，并且其肝毒性小于甲氨蝶呤，半胱氨酸天冬氨酸蛋白酶3（Caspase 3，CASP3）是TNF信号通路的下游分子，位于细胞凋亡途径的关键节点，在暴露于雷公藤内酯的终末分化肝细胞活力显著下降，CASP3的活性形式增加，抑制细胞增殖，诱导细胞凋亡[12]；雷公藤内酯通过激活抑癌基因p53，抑制肝癌细胞活力，诱导细胞凋亡[13]。这也与本研究所富集到的靶点及通路一致，也说明雷公藤可能通过细胞凋亡途径、TNF信号通路等多通路共同调控以上靶蛋白，对细胞免疫进行调控，发挥其抗炎抗癌作用，从而对OLP产生治疗作用，但仍需进一步的体内、外研究证实。

KEGG富集分析中IL17信号通路占有很大的基因比例。不同种类的IL17可激活CASP3，诱导细胞凋亡。研究表明雷公藤内酯可导致辅助性T细胞17（T helper cell 17，Th17）与调节性T细胞（Regulatory cells，Tregs）比例失衡，Th17细胞扩增增强，Tregs产生抑制，特别是辅助Th17细胞分泌的IL17显著增加[14]。贾沛茹等[15]检测到OLP患者外周血中Th17和Treg细胞比率增加，Th17/Treg平衡被打破，这种平衡紊乱的结果与临床上OLP患者病情的严重程度有关。IFN-γ水平上调，IL4水平下调，细胞免疫增强，这是OLP的易患因素[2]，IL4作为辅助型T细胞2（T helper 2 cell，Th2）分泌的细胞因子，可抑制Th17的应答[16]。本研究结果与其具有一致性，说明雷公藤内酯调节IL17信号通路可能为其治疗OLP的一个方式。但在OLP的治疗中雷公藤内酯的作用还有待进一步研究证明。

在癌细胞的增殖过程中，有氧代谢有着非常重要的地位，山奈酚通过直接作用于抗氧化酶，作为一种抗氧化剂清除活性氧（reactive oxygen species，ROS）的产生发挥抗氧化作用[17]，并且其对未突变的细胞具有保护作用，而在突变的细胞中则会触发细胞凋亡[18]，这对治疗属于口腔黏膜潜在恶性疾患的OLP有很大意义，但鲜有研究阐述其肝、肾等毒性，仍需进一步研究证明。

综上所述，本研究应用网络药理学方法初步揭示雷公藤治疗OLP的主要成分、作用靶点和作用途径，为将来从雷公藤中开发出高活性、低毒性的治疗OLP的药物的深入研究提供了理论依据，但仍需进一步的科学验证。

【Author contributions】 Wu ZY, Wang C, Gong Y analyzed the data and wrote the article, Zhao J, Xue R revised the article. All authors read and approved the final manuscript as submitted.

参考文献

[1] 王安训. 口腔黏膜相关疾病免疫功能检测及意义[J]. 口腔疾病防治, 2021, 29(2): 73-80. doi: 10.12016/j.issn.2096-1456.2021.02.001.
Wang AX. Detection and significance of immune function in oral mucosa-associated diseases[J]. J Prev Treatment Stomatol Dis, 2021, 29(2): 73-80. doi: 10.12016/j.issn.2096-1456.2021.02.001.

[2] Carrozzo M, Porter S, Mercadante V, et al. Oral lichen planus: a disease or a spectrum of tissue reactions? Types, causes, diagnostic algorhythms, prognosis, management strategies[J]. Periodontol 2000, 2019, 80(1): 105-125. doi: 10.1111/prd.12260.

[3] 林琳, 吴开慧, 王文梅. 氯喹/羟氯喹的作用机制及其在口腔疾病中的应用[J]. 口腔疾病防治, 2021, 29(3): 198-201. doi: 10.12016/j.issn.2096-1456.2021.03.010.
Lin L, Wu KH, Wang WM. The mechanism of chloroquine/hydroxychloroquine and its application in stomatological diseases[J].

J Prev Treatment Stomatol Dis, 2021, 29(3): 198 - 201. doi: 10.12016/j.issn.2096-1456.2021.03.010.

[4] Song C, Xu Y, Lu Y. Use of *Tripterygium wilfordii Hook F* for immune-mediated inflammatory diseases: progress and future prospects[J]. J Zhejiang Univ Sci B, 2020, 21(4): 280 - 290. doi: 10.1631/jzus.B1900607.

[5] 田雅格, 苏晓慧, 刘立玲, 等. 近20年来雷公藤肝毒性研究概述[J]. 中国中药杂志, 2019, 44(16): 3399-3405. doi: 10.19540/j.cnki.cjcmm.20190527.408.
Tian YG, Su XH, Liu LL, et al. Overview of hepatotoxicity studies on *Tripterygium wilfordii* in recent 20 years[J]. Chin J Chin Mater Med, 2019, 44(16): 3399 - 3405. doi: 10.19540/j.cnki.cjcmm.20190527.408.

[6] Ye H, Wei J, Tang K, et al. Drug repositioning through network pharmacology[J]. Curr Top Med Chem, 2016, 16(30): 3646-3656. doi: 10.2174/1568026616666160530181328.

[7] Hu W, Fu W, Wei X, et al. A network pharmacology study on the active ingredients and potential targets of *Tripterygium wilfordii Hook* for treatment of rheumatoid arthritis[J]. Evid Based Complement Alternat Med, 2019: 5276865. doi: 10.1155/2019/5276865.

[8] Szklarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality - controlled protein - protein association networks, made broadly accessible[J]. Nucleic Acids Res, 2017, 45(D1): D362-D368. doi: 10.1093/nar/gkw937. D1

[9] Yu G. Cluster profiler: an universal enrichment tool for functional and comparative study[J]. Bio Rxiv, 2018: 256784. doi: 10.1101/256784.

[10] Chen SR, Dai Y, Zhao J, et al. A mechanistic overview of triptolide and celastrol, natural products from *Tripterygium wilfordii Hook F*[J]. Front Pharmacol, 2018, 9: 104. doi: 10.3389/fphar.2018.00104.

[11] Wang S, Zuo S, Liu Z, et al. Study on the efficacy and mechanism of triptolide on treating TNF transgenic mice with rheumatoid arthritis[J]. Biomed Pharmacother, 2018, 106: 813-820. doi: 10.1016/j.biopha.2018.07.021.

[12] You L, Dong X, Ni B, et al. Triptolide induces apoptosis through Fas death and mitochondrial pathways in HepaRG cell line[J]. Front Pharmacol, 2018, 9: 813. doi: 10.3389/fphar.2018.00813.

[13] Sun YY, Xiao L, Wang D, et al. Triptolide inhibits viability and induces apoptosis in liver cancer cells through activation of the tumor suppressor gene p53[J]. Int J Oncol, 2017, 50(3): 847-852. doi: 10.3892/ijo.2017.3850.

[14] Xu YY, Wang DM, Liang HS, et al. The role of Th17/Treg axis in the Traditional Chinese Medicine intervention on immune-mediated inflammatory diseases: a systematic review[J]. Am J Chin Med, 2020, 48(3): 535-558. doi: 10.1142/S0192415X20500275.

[15] 贾沛茹, 黄韵颖, 汪鹰, 等. 口腔扁平苔藓外周血中辅助性T细胞/调节性T细胞平衡与临床特征相关性分析[J]. 华西口腔医学杂志, 2018, 36(4): 384-388. doi: 10.7518/hxkq.2018.04.007.
Jia PR, Huang YY, Wang Y, et al. Correlations between the T helper cell 17/regulatory T cells balance in peripheral blood of patients with oral lichen planus and clinical characteristics[J]. West Chin J Stomatol, 2018, 36(4): 384 - 388. doi: 10.7518/hxkq.2018.04.007.

[16] Faust HJ, Zhang H, Han J, et al. IL-17 and immunologically induced senescence regulate response to injury in osteoarthritis[J]. J Clin Investig, 2020, 130(10): 5493-5507. doi: 10.1172/JCI134091.

[17] Salehi B, Martorell M, Arbiser JL, et al. Antioxidants: positive or negative actors?[J]. Biomolecules, 2018, 8(4): 124. doi: 10.3390/biom8040124.

[18] Ashrafizadeh M, Tavakol S, Ahmadi Z, et al. Therapeutic effects of kaempferol affecting autophagy and endoplasmic reticulum stress[J]. Phytother Res, 2020, 34(5): 911 - 923. doi: 10.1002/ptr.6577.

（编辑　张琳，孟文霞）