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·基础研究·

基于数据挖掘、网络药理学和分子对接的中药治疗牙周疾病的用药规律与作用机制

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【摘要】　目的　通过数据挖掘、网络药理学和分子对接探讨中药复方治疗牙周疾病的用药规律及其作用机制。**方法**　首先，数据挖掘搜索治疗牙周疾病的单味药材，并筛选活性成分及其作用靶点。然后，利用疾病靶点数据库下载牙周疾病发病机制相关的靶点，与中药复方的作用靶点去映射，获取被认为中药复方治疗牙周疾病的潜在靶点，并对潜在靶点进行基因本体功能和信号通路分析。潜在靶点再通过筛选获取治疗牙周疾病的关键靶点。最后，将活性成分与关键靶点进行分子对接。**结果**　治疗牙周疾病的中药复方中熟地黄、牡丹皮、当归、茯苓、金银花、山药、知母等药材的出现频率最高，筛选得到43个活性成分及其118个作用靶点，并与856个疾病靶点进行交集得到52个潜在靶点。潜在靶点可能参与的分子功能和生物学过程主要集中在维生素D生物合成过程和对RNA聚合酶Ⅱ调控，并涉及96条信号通路。52个潜在靶点通过网络拓扑参数分析，得到11个关键靶点。分子对接结果表明，活性成分与α-丝氨酸/苏氨酸蛋白激酶(RAC-alpha serine/threonine-protein kinase，AKT1)、细胞肿瘤抗原p53(cellular tumor antigen p53，TP53)和丝裂原活化蛋白激酶-1(mitogen-activated protein kinase-1，MAPK-1)等关键靶点具有较好的结合活性。**结论**　中药复方可能通过抑制牙槽骨吸收、抗菌、抗炎和促进组织修复功能，从而发挥治疗牙周疾病的作用，为中药复方的有效治疗牙周疾病提供更加科学性的参考。

【关键词】　中药复方；　熟地黄；　牡丹皮；　当归；　茯苓；　牙周疾病；　牙周炎；　分子对接；　细胞肿瘤抗原p53；　丝裂原活化蛋白激酶-1；　网络药理学；　数据挖掘；　活性成分；　潜在靶点；　维生素D合成；　“Lipinski”规则
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Exploration of medication rules and mechanisms of traditional Chinese medicine in the treatment of periodontal disease based on data mining, network pharmacology, and molecular docking

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【Abstract】　Objective　To explore the medication law and mechanism of traditional Chinese medicine compounds in the treatment of periodontal disease through data mining, network pharmacology, and molecular docking. **Methods**　First, data mining was used to search single medicinal materials for the treatment of periodontal disease, and the active components and their action targets were screened. Second, the disease target database was employed to download the targets related to the pathogenesis of periodontal disease, map them with the action targets of traditional Chinese medicine, and obtain the targets that are considered potential targets of traditional Chinese medicine in the treatment of peri-

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odontal disease. Potential targets were analyzed for gene ontology function and signaling pathway. They were then screened to obtain the key targets for the treatment of periodontal disease. Finally, the active components were docked with key targets. **Results** Among the traditional Chinese medicine prescriptions for the treatment of periodontal disease, Shudihuang, Mudanpi, Danggui, Fuling, Jinyinhua, Shanyao and Zhimu had the highest frequencies. Forty-three active components and 118 action targets were screened, and 52 potential targets were obtained by intersection with 856 disease targets. The molecular functions and biological processes in which potential targets may participate mainly focus on vitamin D biosynthesis and RNA polymerase Ⅱ regulation and involve 96 signaling pathways. Through the analysis of network topology parameters, 11 key targets were obtained. The results of molecular docking showed that the active components and RAC-alpha serine/threonine-protein kinase (AKT1), cellular tumor antigen p53 (TP53), and mitogen-activated protein kinase-1 (MAPK-1) have good binding activity. **Conclusion** Traditional Chinese medicine compounds may play a role in the treatment of periodontal disease by inhibiting alveolar bone absorption, have antibacterial and anti-inflammatory properties, and promote tissue repair. The effective treatment of periodontal disease by traditional Chinese medicine compounds provides a more scientific reference to the sustainable development of traditional Chinese medicine.

【Key words】 traditional Chinese medicine; Shudihuang; Mudanpi; Danggui; Fuling; periodontal disease; periodontitis; molecular docking; cellular tumor antigen p53; mitogen-activated protein kinase-1; network pharmacology; data mining; active ingredient; potential targets; vitamin D synthesis; "Lipinski" rule

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牙周疾病(periodontal disease)是一种常见的由内分泌紊乱、细菌的感染、假牙的配戴、患者自身心理因素、牙结石的产生和遗传因素等多种因素导致的口腔疾病[1]。牙周疾病会导致牙周组织破坏,造成牙槽骨的吸收,使患牙松动,严重者会出现脱落。牙周疾病的现代治疗方法是基础治疗辅以西药治疗,但西药在治疗过程中存在细菌耐药性和毒副作用等缺点。而中药复方(traditional Chinese medicine)在疾病治疗或预防过程中发挥重要作用[2-3],其疗效持久、副作用少,能够改善患者的症状,还有注重整体平衡,增强和调节机体自身抵抗力,中药复方治疗牙周疾病研究已经获得了较多的研究成果。然而,中药材成分复杂多样,组方成分更加复杂,进入人体之后的作用通路与方式各有差异,发挥多成分、多靶点调节作用。近几年,网络药理学方法在中医药-民族药研究中获得了突破性的发展而引起科研工作者的重视。然而目前中医药作用于牙周疾病的药理作用机制尚未系统阐明。因此本研究拟从多种数据库收载的数据对治疗牙周疾病的中药复方疗效进行初步的筛选,旨在从网络药理学的角度认识中药复方治疗牙周疾病的作用特点,探讨中药复方治疗牙周疾病发病机制,为治疗牙周疾病的药物开发提供一定的依据。

1　资料和方法

1.1　治疗牙周疾病的中药复方来源筛选

通过中国知网、万方、维普等数据库以“牙周炎/牙周疾病”“中医/中药治疗”为关键词,检索时间为2005年至2020年近15年的论文。经过查阅严格按照处方的纳入标准和剔除标准,得到符合纳入标准的58篇论文,并用频数分析法计算每味中药的出现频数,按频数保留频数≥14次的中药进行后续研究。

1.2　ADME和“Lipinski”规则评估

将频次≥14次的“熟地黄、牡丹皮、当归、茯苓、金银花、山药和知母”等7味药材导入TCMSP数据库(http://lsp.nwu.edu.cn/tcmsp.php)的“Herb Name”栏目,整理其相关的成分。以上7味药材再用BANTMAN - TCM (http://bionet.ncpsb.org.cn/batman - tcm/)数据库和TCMIP V2.0(http://www.tcmip.cn/)数据库整理其相关的成分。删除以上3个数据库得

到的重复的成分，并进行ADME筛选。本文用口服生物利用度（oral bioavailability，OB）≥30和类药性（drug-likeness，DL）=0.18为准进行筛选[4]。为了确保化学成分鉴定的准确性和相关性，本文还纳入“Lipinski”规则（MW < 500、AlogP < 5、Hdon < 5、Hacc < 10）为标准进行筛选，得到的成分作为活性成分[5]。

1.3　药物作用靶点的筛选

以上得到的活性成分英文名导入TCMSP和SwissTargetPrediction（http://www.swisstargetprediction.ch/）整理其相关的靶点，并通过Uniprot数据库的Filter By选“Reviewed”和Popular Organism选“Homo sapiens”为关键词，将蛋白名称转换为基因名称。

1.4　牙周疾病靶点的来源以及潜在靶点的筛选

以“Periodontitis或Periodontal disease”作为限定词，CTD（http://ctdbase.org/）数据库、TCMIP数据库和DiSGeNET（http://www.disgenet.org/）获取已知的牙周疾病的靶点，对重复的疾病靶点进行去重。并与“1.3”项下得到的中药复方靶点取交集，获取中药复方治疗牙周疾病的潜在靶点。将中药复方作用靶点与牙周疾病靶点进行映射，通过Veen（http://bioinformatics.psb.ugent.be/webtools/Venn/）工具做交集图，最终获得中药复方治疗牙周疾病的交集靶点。

1.5　基因功能富集分析及通路分析

药物与疾病靶点之间的交集靶点通过Clue GO（https://cytoscape.org）插件进行基因本体（gene ontology，GO）富集分析（BP，MF，CC）和通过DAVIDv6.8（https://david.ncifcrf.gov/）数据库分析信号通路（KEGG）。通过Cytoscape v3.8.2（https://cytoscape.org）软件构建多为网络可视化分析，初步推测中药复方治疗牙周疾病的作用机制。

1.6　药物与疾病靶标的关联性分析

将方法1.2得到的药物与疾病靶点之间的交集靶点输入String数据库中，物种选择人属和Score≥0.9，对蛋白之间相互作用（PPI）进行关联分析，删去孤立节点，得到初始网络。再用CytoNCA（2.1.6）插件对初始网络节点的中介中心性（betweenness centrality，BC）、紧密中心性（closenesscentrality，CC）、特征向量中心性（eigenvector centrality，EC）、度中心性（betweenness centrality，DC）、局部平均连通性（local average connectivity-based method，LAC）、网络中心性（networkcentrality，NC）等拓扑学属性的中位数作为标准进行2步筛选以简化网络，筛选不同的靶点团，排在靠前的靶点团作为中药复方治疗牙周疾病的关键靶点团。

1.7　分子对接验证

通过AutoDock Tools v1.5.6软件构建关键靶点的分子对接模型，进而与中药复方活性成分及牙周疾病阳性药分子进行分子对接并分析对接结果，从而验证拓扑网络分析结果的准确性。中药复方活性成分通过ZINC（http://zinc.docking.org/）和PubMed（https://www.ncbi.nlm.nih.gov/）下载其3D结构，如果找不到其3D结构，用ChemDraw（https://www.chemdraw.com.cn）数据库绘画其3D结构，用PyMol软件以pdb格式保存。并载入AutoDock Tools 1.5.6程序，保存为pdbqt格式，作为对接配体[6]。关键靶点通过PDB（http://www.rcsb.org/）数据库下载获取其蛋白结构，并与以上得到的配体结合，然后运用AutoDock Tools v1.5.6（http://autodock.scripps.edu）进行对接。最后用PyMol（https://pymol.org/）软件作图。

2　结　果

2.1　中药复方的收集与活性成分的筛选结果

通过中国知网、万方、维普等数据库共查到32种治疗牙周疾病的中药方剂，这些方剂共包含438种单位药材，删除重复的药材，得到110味药材。其中提取出现频率≥14次的药材，得到熟地黄、牡丹皮、当归、茯苓、金银花、山药、知母等药材，见表1。7味药材通过TCMSP、BATMAN-TCM

表1　治疗牙周疾病的中药复方单位药材的出现频率

Table 1　Frequency of traditional Chinese medicine prescriptions for periodontitis

Herb name	Frequency（n）	Herb name	Frequency（n）
Shudihuang	24	Gancao	11
Mudanpi	24	Niuxi	10
Danggui	19	Shanzhuyu	9
Fuling	19	Gusuibu	9
Jinyinhua	15	Chuanhuanglian	8
Shanyao	14	Shudihuang	8
Zhimu	14	chuanxiong	7
Shengma	12	Baishao	7
Chuanhuangbo	12	Dangshen	6
Zexie	12	Baishu	6
Huangqin	12	Lianqiao	6
Shengshigao	12	Huangqi	6

Frequency: the frequency includes the frequency of papers from 2005 to 2020

和TCMIP数据库得到1 238个化学成分，见表2。通过ADME和Lipinski规则筛选得到43个活性成分，见表3。

表2　中药复方治疗牙周疾病的单位药材化学成分收集
Table 2　Collection of chemical components of unit medicinal materials of traditional Chinese medicine in the treatment of periodontitis

Herb name	TCMSP	TCMIP	BATMAN-TCM
Shudihuang	76	12	8
Mudanpi	66	15	18
Danggui	125	65	120
Fuling	34	33	21
Jinyinhua	236	47	72
Shanyao	71	45	20
Zhimu	81	41	32

TCMSP: traditional Chinese medicine systems pharmacology database and analysis platform; TCMIP: integrative pharmacology-based research platform of traditional Chinese medicine; BATMAN-TCM: a bioinformatics analysis tool for molecular mechANism of traditional Chinese medicine

2.2　活性成分作用靶点与疾病靶点关联性

32个活性成分有相应的384个靶点，该靶点以蛋白名称形式存在，使用UniProt（https://www.uniprot.org/）数据库进行基因名称转换，得到118个靶点。活性成分中Paeoniflorin_qt、4-O-methylpaeoniflorin_qt、Mudanpioside-h_qt 2、Paeonidanin_qt、Denudatin B、Hancinol、Anemarsaponin E_qt、Phyllanthin、C4、XYLOSTOSIDINE和Pachypodol等11个成分在TCMSP数据库中未收录其成分，若换成其他数据库，存在收载标准和算法的不同的问题，故没有用其他数据库。

2.3　药物与疾病靶点的关联性及其网络分析

通过CTD、TCMIP和DisGeNET数据库分别得到257、27和682个牙周疾病相关的疾病靶点，并删除重复的靶点，得到856个疾病靶点。此疾病靶点与32活性成分作用靶点进行映射得到52个潜在靶点，见图1。将52个潜在靶点连同单味药材及其活性成分，并导入Cytoscape3.8.0软件，绘制单味药材-活性成分-潜在靶点网络图，图中可以看出来，中药复方的各个单味药材通过多成分、多靶点作用于牙周疾病，见图2。

2.4　潜在靶点的基因功能和信号通路富集分析

52个潜在靶点通过ClueGO插件和DAVID数据库进行GO基因功能和KEGG信号通路分析，GO基因功能主要集中在RNA聚合酶Ⅱ启动子转录对缺氧反应的正调控（positive regulation of transcription from RNA polymerase Ⅱ promoter in response to hypoxia）、维生素D生物合成过程的正调控（positive regulation of vitamin D biosynthetic process）、病毒对宿主细胞凋亡过程的抑制作用（suppression by virus of host apoptotic process），和T细胞稳态增殖（T cell homeostatic proliferation），见图3。按照错误发现率（false discovery rate，FDR）大小进一步筛选通路，FDR值表示在富集分析中越小，就代表富集显著程度就越高。以FDR≤0.05为准，筛选得到96条信号通路，并进行排序，前30个结果如图4所示。

2.5　潜在靶点的PPI网络分析

52个潜在靶点String数据库以Score≥0.9为标准进行筛选，初始网络由52个节点和119条边组成的。经CytoNCA插件进行拓扑参数分析，简化得到11个节点与30条边的网络，见图5。靠前的靶点包括肿瘤坏死因子（tumor necrosis factor，TNF）、丝裂原活化蛋白激酶-1（mitogen-activated protein kinase-1，MAPK-1）、丝裂原活化蛋白激酶-14（mitogen-activated protein kinase-14，MAPK-14）、白细胞介素-2（interleukin-2，IL-2）、白细胞介素-6（interleukin-6，IL-6）、细胞肿瘤抗原p53（cellular tumor antigen p53，TP53）、信号转导子和转录激活子1（signal transducer and activator of transcription 1，STAT1）、α-丝氨酸/苏氨酸蛋白激酶（RAC-alpha serine/threonine-protein kinase，AKT1）、维甲酸受体RXRα（retinoic acid receptor RXR-alpha，RXRA）、血管内皮生长因子A（vascular endothelial growth factor A，VEGFA）和转录因子p65（transcription factor p65，RELA）。以上11个靶点作为关键靶点与活性成分进行分子对接。

2.6　分子对接验证

为了确证中药复方活性成分及其治疗牙周疾病的关键靶点筛选结果的准确性，对中药复方43个活性成分与11个关键靶点进行分子对接。按照结合能（binding energy，BE）的大小来判断活性成分与关键靶点的匹配度。当配体和受体的构象稳定时，能量越低，作用的可能性就越大[7]。一般情况下，BE≤−4.25 Kcal/mol表示活性成分与靶标有一定的结合能，BE≤−5.00 Kcal/mol表示活性成分与靶标有良好的结合能，BE≤−7.00 Kcal/mol表示活性成分具有较强的结合能。

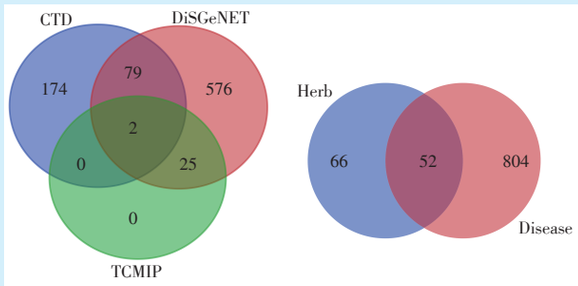
43个活性成分与11个关键靶点进行分子对

表3　中药复方治疗牙周疾病的单位药材活性成分筛选

Table 3　Screening of active components of unit medicinal materials of traditional Chinese medicine in the treatment of periodontitis

Herb name	Mol ID	Molecule Name	MW	AlogP	Hdon	Hacc	OB (%)	DL
Mudanoi, Jinyinhua, Zhimu	MOL000422	Kaempferol	286.25	1.77	4	6	41.88	0.24
Shanyao, Zhimu	MOL000546	Diosgenin	414.69	4.63	1	3	80.88	0.81
Danggui, Fuling	MOL011455	20-Hexadecanoylingenol	418.58	1.91	3	6	32.70	0.65
Mudanpi	MOL002222	Sugiol	300.48	4.99	1	2	36.11	0.28
Mudanpi	MOL001925	Paeoniflorin_qt	318.35	0.46	2	6	68.18	0.40
Mudanpi	MOL007369	4-O-Methylpaeoniflorin_qt	332.38	0.87	1	6	67.24	0.43
Mudanpi	MOL007374	C1	312.30	2.05	2	7	43.44	0.30
Mudanpi	MOL007382	Mudanpioside-h_qt 2	336.37	−0.03	3	7	42.36	0.37
Mudanpi	MOL007384	Paeonidanin_qt	330.41	0.92	1	5	65.31	0.35
Shanyao	MOL000310	Denudatin B	356.45	2.80	0	5	61.47	0.38
Shanyao	MOL000322	Kadsurenone	356.45	2.80	0	5	54.72	0.38
Shanyao	MOL001559	Piperlonguminine	273.36	2.93	1	4	30.71	0.18
Shanyao	MOL004058	Deltoside	260.26	2.17	0	5	33.19	0.19
Shanyao	MOL005430	Hancinone C	400.51	3.34	0	6	59.05	0.39
Shanyao	MOL005429	Hancinol	372.50	2.46	1	5	64.01	0.37
Shanyao	MOL005458	Dioscoreside C_qt	444.72	3.96	2	4	36.38	0.87
Shanyao	MOL005465	AIDS180907	394.45	4.81	3	6	45.33	0.77
Zhimu	MOL000483	C2	313.38	2.86	3	5	118.35	0.26
Zhimu	MOL000631	Coumaroyltyramine	283.35	2.88	3	4	112.90	0.20
Zhimu	MOL001944	Marmesin	246.28	2.03	1	4	50.28	0.18
Zhimu	MOL001677	Asperglaucide	444.57	4.02	2	6	58.02	0.52
Zhimu	MOL004373	Anhydroicaritin	368.41	3.88	3	6	45.41	0.44
Zhimu	MOL004489	Anemarsaponin F_qt	432.71	3.92	2	4	60.06	0.79
Zhimu	MOL004497	Hippeastrine	315.35	1.17	1	6	51.65	0.62
Zhimu	MOL004514	Timosaponin B III_qt	416.71	4.77	2	3	35.26	0.87
Zhimu	MOL004540	Anemarsaponin C_qt	416.71	4.97	2	3	35.50	0.87
Zhimu	MOL004542	Anemarsaponin E_qt	448.76	4.53	2	4	30.67	0.86
Danggui	MOL001956	Cnidilin	300.33	3.64	0	5	32.69	0.28
Danggui	MOL002218	Scopolin	354.34	−0.29	4	9	56.45	0.39
Danggui	MOL001942	Isoimperatorin	270.30	3.65	0	4	45.46	0.23
Danggui	MOL005384	Suchilactone	368.41	3.73	0	6	57.52	0.56
Danggui	MOL006812	Phyllanthin	418.58	4.11	0	6	33.31	0.42
Jinyinhua	MOL000006	Luteolin	286.25	2.07	4	6	36.16	0.25
Jinyinhua	MOL002914	Eriodyctiol (flavanone)	288.27	2.03	4	6	41.35	0.24
Jinyinhua	MOL003006	C3	281.29	−0.96	2	7	87.47	0.23
Jinyinhua	MOL003014	Secologanic Dibutylacetal_qt	384.57	3.58	1	6	53.65	0.29
Jinyinhua	MOL003044	Chrysoeriol	300.28	2.32	3	6	35.85	0.27
Jinyinhua	MOL003044	Chryseriol	300.28	2.32	3	6	35.85	0.27
Jinyinhua	MOL003095	C4	358.37	2.80	1	7	51.96	0.41
Jinyinhua	MOL003111	Centauroside_qt	434.48	0.38	2	9	55.79	0.50
Jinyinhua	MOL003117	Ioniceracetalides B_qt	314.37	1.00	1	7	61.19	0.19
Jinyinhua	MOL003124	XYLOSTOSIDINE	415.51	−1.66	4	9	43.17	0.64
Fuling	MOL005890	Pachypodol	356.40	2.99	1	6	75.06	0.40

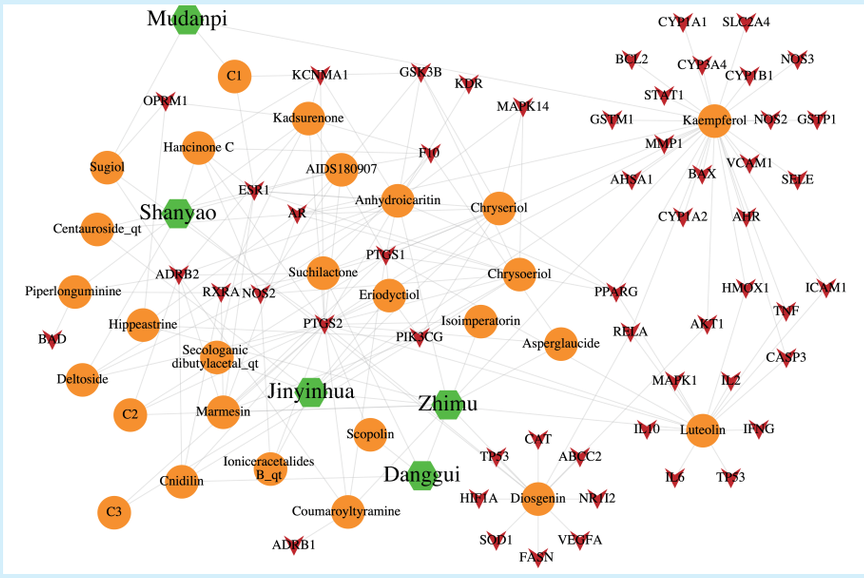
MW: molecular weight; AlogP: value represents the partition coefficient between octanol and water, which is critical for measuring hydrophobicity of molecule; Hdon and Hacc: the Hdon and Hacc are measures of the hydrogen-bonding ability of a molecule expressed in terms of number of possible hydrogen-bond donors and acceptors, respectively; OB: oral bioavailability; DL: drug-likeness; C1: 5-[[5-(4-methoxyphenyl)-2-furyl] methylene] barbituric acid; C2: (Z)-3-(4-hydroxy-3-methoxy-phenyl)-N-[2-(4-hydroxyphenyl)ethyl] acrylamide; C3: (-)-(3R, 8S, 9R, 9aS, 10aS)-9-ethenyl-8-(beta-D-glucopyranosyloxy)-2, 3, 9, 9a, 10, 10a-hexahydro-5-oxo-5H, 8H-pyrano[4, 3-d] oxazolo[3, 2-a] pyridine-3-carboxylic acid_qt; C4: 5-hydroxy-7-methoxy-2-(3, 4, 5-trimethoxyphenyl) chromone



CTD: comparative toxicogenomics database; TCMIP: integrative pharmacology - based research platform of traditional Chinese medicine; disGeNet: a database of gene - disease associations; herb: traditional Chinese medicine; disease: periodontal disease

Figure 1　Relationship between periodontal disease and traditional Chinese medicine targets

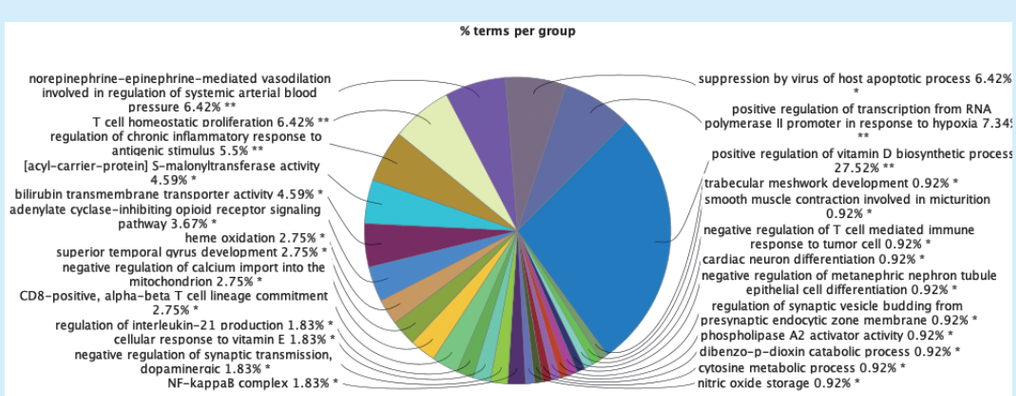
图1　牙周疾病与中药复方药物靶点的关联性



Green polygon: traditional Chinese medicine; yellow circle: active ingredient; red diamond: potential target

Figure 2　Network diagram of single medicinal material - active ingredient - potential target of traditional Chinese medicine in the treatment of periodontitis

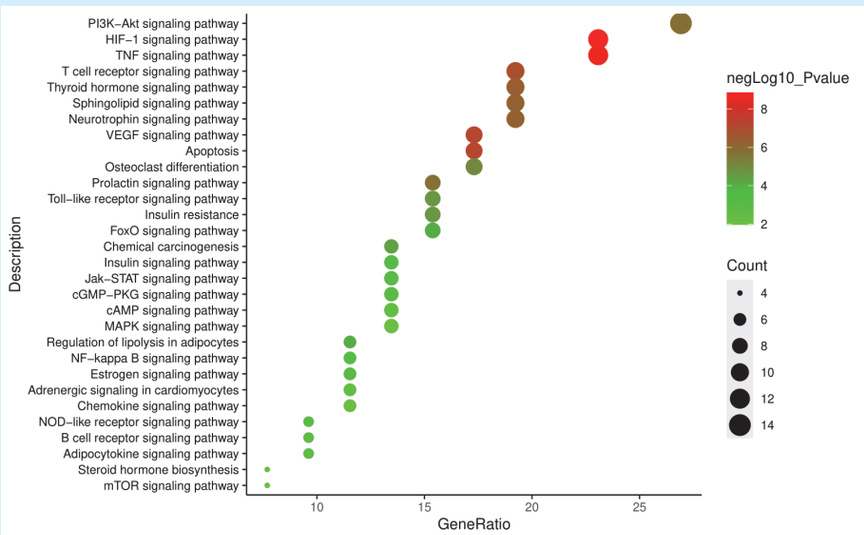
图2　中药复方治疗牙周疾病的单味药材-活性成分-潜在靶点网络图



Only the most significant item labels are displayed in each group, and the functional related components overlap; different biological processes are distinguished by different colors, and the related molecular functions are connected with each other by line segments

Figure 3　Gene ontology analysis of potential targets of traditional Chinese medicine in the treatment of periodontitis

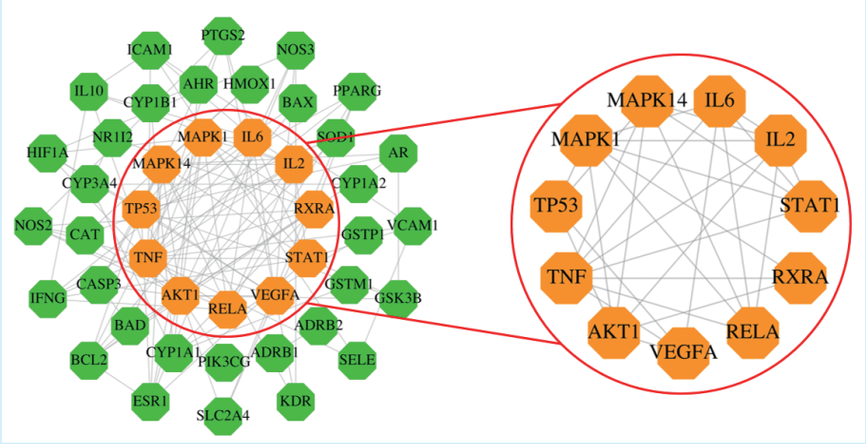
图3　中药复方治疗牙周疾病潜在靶点的基因本体分析



Color represents the degree of enrichment; the circular size represents the number of participating targets

Figure 4　Analysis of KEGG signal pathway of potential target of traditional Chinese medicine in the treatment of periodontitis

图4　中药复方治疗牙周疾病潜在靶点的KEGG信号通路分析

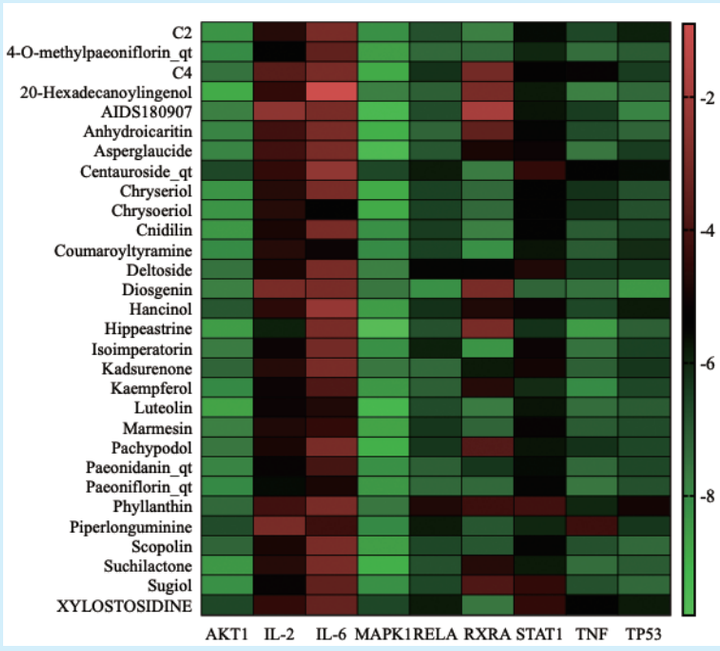


TNF: tumor necrosis factor; MAPK-1: mitogen-activated protein kinase-1; MAPK-14: mitogen-activated protein kinase-14; IL-2: interleukin-2; IL-6: interleukin-6; TP53: cellular tumor antigen p53; STAT1: signal transducer and activator of transcription 1; AKT1: RAC-alpha serine/threonine-protein kinase; RXRA: retinoic acid receptor RXR-alpha; RELA: transcription factor p65

Figure 5　Identification of core target clusters and hub targets

图5　关键靶点团与关键靶点的识别

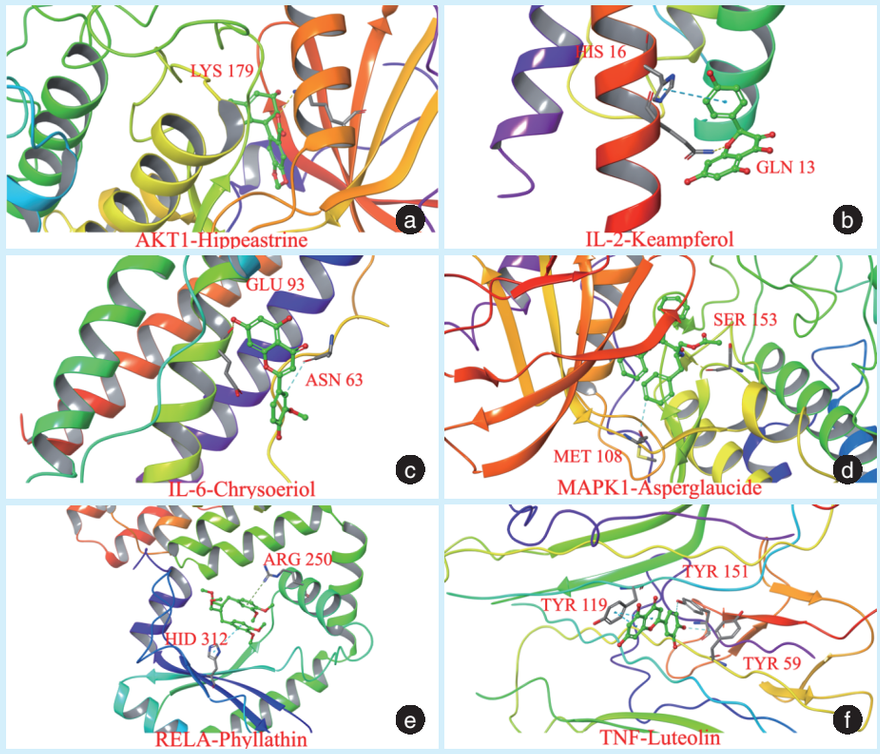
接，11个关键靶点的PDB ID分别是MAPK-14（PDB ID=5ETC）、MAPK-1（PDB ID=4FMQ）、IL-6（PDB ID=1ALU）、STAT1（PDB ID=1YVL）、IL-2（PDB ID=4MEN）、RXRA（PDB ID=7B88）、RELA（PDB ID=1VJ7）、VEGFA（PDB ID=4KZN）、AKT1（PDB ID=2UVM）、TNF（PDB ID=2AZ5）和TP53（PDB ID=7BVM）。43个活性成分中C1、Mudanpioside-h_qt 2、Denudatin B、Hancinone C、Dioscoreside C_qt、Anemarsaponin F_qt、Timosaponin B III_qt、Anemarsaponin C_qt、Anemarsaponin E_qt、Eriodyctiol（flavanone）、C3、Secologanic dibutylacetal_qt、Ioniceracetalides B_qt等活性成分与11个活性成分未能对接，因此被删除。30个中药复方活性成分与VEGFA和MAPK-14关键靶点没能对接，因此中药复方30个活性成分与剩余9个关键靶点进行分子对接，结果如图6所示。其中部分活性成分与关键靶点的结合模式如图7所示，中药复方活性成分与关键靶点的氨基酸残基形成氢键（稳定氢键和芳



TNF: tumor necrosis factor; MAPK-1: mitogen-activated protein kinase-1; MAPK-14: mitogen-activated protein kinase-14; IL-2: interleukin-2; IL-6: interleukin-6; TP53: cellular tumor antigen p53; STAT1: signal transducer and activator of transcription 1; AKT1: RAC-alpha serine/threonine-protein kinase; RXRA: retinoic acid receptor RXR-alpha; RELA: transcription factor p65

Figure 6　Docking results of active components with core targets of traditional Chinese medicine in the treatment of periodontitis

图6　中药复方治疗牙周疾病的活性成分与核心靶点对接结果



a: Hippeastrine and AKT1 (BE=−8.5); b: Kaempferol and IL-2 (BE=−5.1); c: Chrysoeriol and IL-6 (BE=−5.4); d: Asperglaucide and MAPK-1 (BE=−9.5); e: Luteolin and TNF (BE=−7.4); f: Phyllanthin and LELA (BE=−4.8); AKT1: RAC-alpha serine/threonine-protein kinase; IL-2: interleukin-2; IL-6: interleukin-6; MAPK-1: mitogen-activated protein kinase-1; RELA: transcription factor p65; TNF: tumor necrosis factor

Figure 7　Docking results mode of active components with core targets of traditional Chinese medicine in the treatment of periodontitis

图7　中药复方治疗牙周疾病的活性成分与核心靶点对接结果模式

香烃氢键）和π-π相互作用而稳定地结合。氢键和π-π相互作用对小分子和蛋白质的识别和稳定性起着关键作用。

3　讨　论

本研究通过聚类分析和多维网络可视化分析，在治疗牙周疾病中，牡丹皮、当归、金银花、山药和知母的出现频率和网络拓扑参数均较高。其中牡丹皮具有抑菌效果，而丹皮酚是牡丹皮的主要活性成分，对牙龈卟啉单胞菌具有较佳的体外抗菌效果，它是一种牙周疾病的致病菌[8]。金银花具有清热解毒、抑菌和抗炎的作用。金银花对变异链球菌和牙龈卟啉单胞菌抑制作用[9]。知母具有清热泻火以及滋阴润燥的功能[10]。知母须根中芒果苷、异芒果苷的含量最高，其中成功制备缓释芒果苷的聚乳酸-羟基乙酸[poly(lactic-co-glycolic acid)，PLGA]支架可促进糖尿病大鼠牙槽骨缺损再生，可能为治疗糖尿病状态牙槽骨缺损愈合障碍提供新方法[11]。山药具有抗炎、抗氧化和抗肿瘤等作用[12]。研究表明，山药总皂苷能抑制人口腔癌细胞HSC-3的迁移能力和金属基质蛋白酶-2（matrix metallo proteinase-2，MMP-2）的活性[13]。当归具有活血补血、抗炎、抗肿瘤和抗氧化等药理作用[14]。牙周疾病牙周组织中环氧化酶-2（cyclooxygenase-2，COX-2）高表达，而当归的二聚酞化合物对COX-2酶的具有抑制活性[15]。综上，单味中药对牙周疾病的网络具有较强的扰动作用，可推测以上中药具有更高的开发价值。然而，知母、山药和当归等药材对牙周疾病的研究报道较少，需要进一步研究。

在充分考虑中药复方成分的ADME（OB≥30、DL≥0.18）和“Lipinski”规则（MW < 500、AlogP < 5、Hdon < 5、Hacc < 10）得到的活性成分，结合该中药复方包含的各个单位药材的法定质量控制指标或文献报道的有效成分进行对接验证。通过筛选得到43个活性成分，通过聚类分析山奈酚（Kaempferol）、薯蓣皂苷（Diosgenin）和20-Hexadecanoylingenol等活性成分存在于2个或2个以上的单位药材，可以作为中药复方作用于牙周疾病的重要成分。牙槽骨吸收异常会导致局部口腔牙菌斑的积聚，能增加牙周疾病发生发展的易感性。COX-2是在牙槽骨吸收中起到重要作用，在牙周疾病组牙槽骨成骨细胞上清液中高表达[16]。研究报道，薯蓣皂苷通过糖皮质激素受体下调COX-2和微粒体前列腺素E2合酶-1（microsomal prostaglandin E synthase-1，mPGES-1），并抑制脂多糖诱导的小鼠急性肝损伤巨噬细胞中的COX-2和mPGES-1[17]。研究表明，山奈酚也能够抑制COX-2的表达，从而发挥抗炎作用[18]。还有研究表明，异欧前胡素（Isoimperatorin）具有双重COX-2选择性/5-脂氧合酶抑制活性[19]。本研究的单味药材-活性成分-潜在靶点网络图的多为网络可视化分析中可以看出来，作用于前列腺素内过氧化物合酶（prostaglandin-endoperoxide synthase，PTGS2）的成分包括牡丹皮、金银花和知母的山奈酚、山药和知母的薯蓣皂苷和当归的异欧前胡素等。因此，可以推测，以上中药复方的活性成分可能通过抑制牙槽骨成骨细胞上清液中COX-2的表达，调节牙槽骨吸收，从而发挥治疗牙周疾病的作用。

GO富集分析结果表明，中药复方干预牙周疾病可能参与的分子功能和生物学过程主要集中在维生素D生物合成过程和RNA聚合酶Ⅱ调控。研究结果表明，维生素D抑制牙龈上皮细胞和人类牙周韧带细胞中牙龈卟啉单胞菌的黏附和感染[20]。牙龈卟啉单胞菌是革兰氏阴性黑色素厌氧菌，是一种牙周疾病的致病菌。可以推测，抗菌作用是在中药复方治疗牙周疾病过程中发挥起到主要作用。RNA聚合酶Ⅱ的相互作用因子是Che-1，可参与细胞周期调节和维持核糖体RNA转录。RNA聚合酶Ⅱ与Che-1之间的相互作用在细胞的增殖和氧化应激反应中发挥重要作用[21]。而氧化应激会导致线粒体质量控制失衡，在牙周炎发展中起关键作用。因此，可以说这种蛋白质转运也是在中药复方治疗牙周疾病过程中发挥起到主要作用。GO富集分析结果表明，52个潜在靶点中参与维生素D生物合成过程的潜在靶点包括γ-干扰素（interferon gamma，IFNG）和TNF，参与RNA聚合酶Ⅱ调控的潜在靶点包括缺氧诱导因子1-α（hypoxia-inducible factor 1-alpha，HIF1A）、核因子红系2相关因子2（Nuclear factor erythroid 2-related factor 2，NFE2L2）、NOTCH1、RBPJ、TP53和VEGFA。结合富集分析和网络可视化分析结果可以推测，牡丹皮、金银花和知母的山奈酚作用于TNF以及金银花的木犀草素（Luteolin）作用于TNF和IFNG，从而调控维生素D生物合成过程；山药和知母的薯蓣皂苷作用于HIF1A、金银花的木犀草素和山药和知母的薯蓣皂苷作用于TP53、山药和知母的薯蓣皂苷作用于VEGFA，从而调节RNA聚合酶Ⅱ的

活性。

KEGG信号通路富集分析结果表明，PI3K-Akt信号通路参与的靶点最多，包括糖原合成酶激酶-3β（glycogen synthase kinase-3 beta，GSK3B）、内皮型一氧化氮合酶（endothelial nitric oxide synthase，eNOS）、血清淀粉样蛋白A（serum amyloid A protein，BAD）、IL-2、磷脂酰肌醇-4，5-二磷酸3-激酶催化亚单位，γ亚型（phosphatidylinositol-4，5-bisphosphate 3-kinase catalytic subunit，gamma isoform，PIK3CG）、RELA、VEGFA、IL-6、RXRA、血管内皮生长因子受体2（vascular endothelial growth factor receptor 2，KDR）、凋亡调节因子Bcl-2（apoptosis regulator Bcl-2，BCL2）、AKT1、MAPK-1、TP53。牙龈卟啉单胞菌可通过激活PI3K-Akt信号通路诱导中性粒细胞和巨噬细胞向病变部位的聚集，还有研究表明，PI3K-Akt信号通路在中性粒细胞引发的组织炎症损伤调控过程起到主要作用[22]。低强度脉冲超声（low intensity pulsed ultrasound，LIPUS）应用于结扎诱导的小鼠模型，作为牙周疾病的一种新的治疗选择。LIPUS治疗通过PI3K-Akt/NRF2信号下调氧化应激，减轻牙周炎患者的牙槽骨稳态[23]。缺氧诱导因子1（hypoxia-inducible factor 1，HIF-1）信号通路的FDR最小，就代表富集显著程度就越高。参与HIF-1信号通路的潜在靶点包括IL-6、IFNG、NOS2、NOS3、BCL2、MAPK-1、血红素加氧酶1（heme oxygenase 1，HMOX1）、AKT1、HIF1A、RELA、PIK3CG和VEGFA。研究报道，牙周炎组织中HIF-1表达上调，起到牙周组织破坏作用[24]。另外，可诱导牙周膜细胞自吞噬以及其过表达有利于牙周组织和骨组织的再生[25-26]。本研究的多维网络图和分子对接结果结合显示，山奈酚和薯蓣皂苷作用于AKT1和RELA；木犀草素作用于IL-2、IL-6、MAPK-1和RELA；山药的海风酮（Kadsurenone）、荜拨明宁碱（Piperlonguminine）、三角叶薯蓣混甙（Deltoside），知母的Marmesin和Anhydroicaritin以及当归的Cnidilin和荜茇明宁碱（Suchilactone）作用于维甲酸受体RXRα（Retinoic acid receptor RXR-alpha，RXRA）。可以推测，以上中药复方的活性成分可能通过PI3K-Akt和HIF-1信号通路，作用于以上的潜在靶点，从而达到治疗牙周疾病的作用。

综上，本研究主要通过数据挖掘和网络药理学方面侧面证明了中药复方治疗牙周疾病可以从抑制牙槽骨吸收、抗菌、抗炎和促进组织修复功能的等方面入手，从网络药理学视角重新认识了牙周疾病的治疗。

【Author contributions】 Li XS, Niu QL analyzed the data and wrote the article. Zhao J revised the article. All authors read and approved the final manuscript as submitted.

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