• 药理与临床 •

基于网络药理学的桂枝茯苓胶囊治疗痛经、子宫肌瘤和盆腔炎的分子作用机制研究

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摘 要：目的 探索桂枝茯苓胶囊治疗痛经、子宫肌瘤和盆腔炎的分子作用机制和潜在活性成分群。**方法** 通过文献挖掘、多个数据库联用检索与痛经、盆腔炎以及子宫肌瘤疾病相关的 130 个靶蛋白基础上，利用分子计算结合网络特征分析获得桂枝茯苓胶囊的主要活性成分和潜在的靶点蛋白，并将其投射到 KEGG 数据库阐释桂枝茯苓胶囊的分子作用机制。**结果** 数据分析结果表明，与蛋白相互作用较强的 115 个活性分子主要分布在桂枝和茯苓中，进一步的网络特征分析发现高网络度和介数的分子主要为五环三萜类和甾醇类化合物；与化合物作用的绝大部分靶蛋白（78.57%）分布在与痛经、盆腔炎及子宫肌瘤密切相关的 15 条生物通路中，而这些通路涉及到子宫平滑肌的增殖和收缩，子宫内的血管形成和血液循环，雌激素和黄体酮等多种激素的分泌，以及前列腺素等炎症因子合成或释放和相关钙通道的调控等。**结论** 桂枝茯苓胶囊主要是由其所含的五环三萜类和甾醇类化合物与多个靶点蛋白作用，调控多条生物通路（如 arachidonic acid metabolism，calcium signaling pathway，GnRH signaling pathway，complement and coagulation cascades，progesterone-mediated oocyte maturation）来抑制子宫平滑肌收缩和增殖、改善微循环、降低激素分泌和炎症反应（如 PGE_2，$PGF_{2\alpha}$，leukotriene B4），从而起到缓解痛经和盆腔炎引起的疼痛、改善子宫肌瘤患者的生活质量的作用。

关键词： 网络药理学；桂枝茯苓胶囊；分子计算；靶蛋白；痛经；盆腔炎；子宫肌瘤

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Molecular mechanism of Guizhi Fuling Capsule for treatment of dysmenorrhea, pelvic inflammatory disease, and hysteromyoma via network pharmacology

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Abstract: Objective To investigate the molecular maechanism and potential active constituents population of Guizhi Fuling Capsule (GFC) for the treatment of dysmenorrhea, pelvic inflammatory disease (PID), and hysteromyoma. **Methods** One handred and thirty target proteins related with dysmenorrhea, PID, and hysteromyoma were selected through mining literature, retrieving in DrugBank and TTD database, the main active constituents and potential target proteins from GFC were computed and analyzed by DOVIS2.0 and Cytoscape 3.0. The potential target proteins were then projected into the KEGG databases to illustrate the molecular mechanism of GFC. **Results** The results of data analysis showed that the 115 active molecules with stronger interaction with protein were distributed in *Cinnamomi Ramulus* and *Poria*. The High network degree and betweenness of molecules were found to be pentacyclic triterpenes and steroids by further analysis of network characteristics. The most of the potential target proteins (78.57%)

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interacted wth the compounds in GFC were from 15 biological pathways closely related with dysmenorrhea, PID, and hysteromyoma in KEGG database, which was involved in cell proliferation, angiogenesis, coagulation, dysregulation of inflammatory process, uterine contractions, and release of estrogen or progesterone in uterus. As well as the synthesis or release of inflammatory factors such as prostaglandin and the regulation of calcium channels and so on. **Conclusion** GFC has the function by the interaction of pentacyclic triterpenes and steroids with multi target proteins, such as arachidonic acid metabolism, calcium signaling pathway, GnRH signaling pathway, complement and coagulation cascades, and progesterone-mediated oocyte maturation, to alleviate the pain of dysmenorrhea and PID, or improve the quality of life for the patients with hysteromyoma through inhibiting uterine contractions, improving microcirculation, and reducing the release of estrogen or promegestone and inflammatory response (such as PGE_2, $PGF_{2\alpha}$, and leukotriene B4).

Key words: network pharmacology; Guizhi Fuling Capsule; molecular calculation; target protein; dysmenorrhea; pelvic inflammatory disease; hysteromyoma

痛经（dysmenorrhea）、盆腔炎（pelvic inflammatory disease, PID）和子宫肌瘤（hysteromyoma）是妇科常见疾病，多见于月经期妇女，疼痛是其主要的临床表现，严重时会影响到患者的工作、学习以及生活质量[1-4]。目前用于痛经和盆腔炎治疗的药物多是一些非甾体抗炎药（NSAIDS）[5]，用于治疗子宫肌瘤的药物主要有孕激素类、纤溶药物、NSAIDS、促性腺激素类似物/拮抗剂（GnRH analogs/antagonists）[6]等。

由于化学药的不良反应或疗效不理想等问题，近年来从中药中寻找治疗妇科疾病的药物日渐受到重视[5,7-9]。桂枝茯苓胶囊是由江苏康缘药业股份有限公司根据经典方桂枝茯苓丸研发的现代中药制剂，其君药桂枝、臣药桃仁、佐以牡丹皮、白芍和茯苓而成，具有活血、化瘀、消癥之功效。临床主要用于妇女瘀血阻络所致癥块、经闭、痛经、产后恶露不尽以及子宫肌瘤、慢性盆腔炎等[10-11]。虽然前期对桂枝茯苓胶囊的作用机制和化学成分有一些研究[12-13]，但由于中药成分的多样性和作用机制的多靶点、多通路，桂枝茯苓胶囊的主要活性成分和作用机制的实验研究仍面临着诸多挑战。

网络药理学是基于网络观点从系统水平分析药物作用机制[14]、发现先导化合物或新适应症[15]、识别新药靶[16]等一种新的药物研发策略，其与中药整体调控机体的理念相吻合[17]。目前网络药理学研究思路也逐渐被引入到中药中来发现药效物质基础、探索分子作用机制以及阐释其科学内涵[18-22]，同时也能大大降低中药后续实验研究的工作量。因此，本研究将网络药理学研究思路与中药桂枝茯苓胶囊相结合来阐释其治疗痛经、盆腔炎和子宫肌瘤的药效活性成分和可能的分子机制，旨在为后续的实验研究方向选择提供一定的信息支撑。

1 方法

1.1 分子和靶蛋白数据集的建立与处理

在前期对桂枝茯苓胶囊中 164 个化学成分分离确认的基础上[23-24]，根据 OpenBabel 2.3.2 软件产生的化合物 InChiKey 字符串从 UNPD 数据库（http://pkuxxj.pku.edu.cn/UNPD/）共计筛选出 172 个分子（表 1），经 Open Babel 加氢后，选用 MMFF94 力场进行构象优化，优化时能量阈值设定为 1×10^{-3} J/mol。

根据桂枝茯苓胶囊的临床主要用于痛经、盆腔炎、子宫肌瘤等妇科疾病的治疗情况，利用 Therapeutic Target Database（TTD）、DrugBank 数据库结合文献综述分析[6, 8-9, 25-31]，以 dysmenorrhea、hysteromyoma、uterine fibroids（子宫平滑肌瘤）、PID 为检索词共筛选与上述疾病密切相关的 130 个靶点，并从 RCSB 数据库（www.pdb.org）获取人源的且含有原配体的晶体结构（Resulation＜0.25 nm，表 2），导入 Discovery Studio 2.5 通过 Clean Protein 和 Forcefiled 中 CHARMm 力场完成靶蛋白的加氢、去水、蛋白修饰以及自动分配原子类型和确定活性位点的中心坐标。

1.2 分子对接

根据上述确定的分子 UNPD 与靶蛋白的 Uniprot，从 TCMN 数据库[32]中抽提出分子与靶蛋白相互作用数据对。在 TCMN 数据库中，分子与靶蛋白相互作用通过 DOVIS2.0 平台中 AutoDock4.0 内核来完成分子与靶蛋白对接计算[33]。具体对接参数如下：以靶蛋白中原配体的坐标为活性位点的中心，盒子大小为 4 nm×4 nm×4 nm，格点间隔为 0.037 5 nm，采用拉马克遗传算法采集分子构象，初始种群为 150，旋转步长为 50°，平移步长为 0.2 nm，交叉率为 0.8，突变率为 0.02，局部搜索频率为 0.06，其余均为默认设置。

表 1 桂枝茯苓胶囊中筛选出的化合物

Table 1 Compounds separated from GFC

ID	化合物	ID	化合物
UNPD10084	3-epi-dehydrotumulosic acid	UNPD187407	suffrupaeonidanin A
UNPD101050	pachymic acid	UNPD188804	hyperoside
UNPD101054	poricoic acid AM	UNPD191345	hederagenin
UNPD102502	α-amyrin	UNPD191562	lactoflorin
UNPD102661	3-epi-dehydropachymic acid	UNPD194734	α-amyrin
UNPD102760	poricoic acid C	UNPD195952	sodium paeoniflorin sulfonate
UNPD105334	guanosine	UNPD20150	hyperoside
UNPD105452	betulinic acid	UNPD20394	arginine
UNPD106083	poricoic acid C	UNPD21132	coumarin
UNPD108574	catechins	UNPD23348	poricoic acid DM
UNPD109023	cinnamic acid	UNPD25688	α-amyrin
UNPD109974	trehalose	UNPD28633	cinnamyl alcohol
UNPD11020	β-sitosterol	UNPD28717	4-hydroxybenzoic acid
UNPD111541	sucrose	UNPD30980	hyperoside
UNPD115852	polyporenic acid C	UNPD31327	α-amyrin
UNPD117238	kaempferol-3-*O*-glucosylside	UNPD31682	dehydropachymic acid
UNPD118360	α-amyrin	UNPD32715	sucrose
UNPD120575	β-*L*-arabinose	UNPD33147	5,7-dimethyl-3′,4′-di-*O*-methylene-(±)-epicatec
UNPD120855	hyperoside	UNPD35192	α-*D*-galatose
UNPD121048	caffeic acid	UNPD35358	poricoic acid A
UNPD12116	oleanolic acid	UNPD3627	poricoic acid D
UNPD121313	suffrupaeonidanin A	UNPD36336	mudanpioside-F
UNPD123516	citrostadienol	UNPD36636	poricoic acid DM
UNPD126066	mudanpioside-B	UNPD37924	eburicoic acid
UNPD127168	hyperoside	UNPD38191	campesterol
UNPD128526	mudanpioside-A	UNPD38563	citrostadienol
UNPD12870	trehalose	UNPD41421	alanine
UNPD128750	sucrose	UNPD41767	benzoyloxypaeoniflorin
UNPD129222	dehydroeburicoicacid	UNPD45635	daucosterol
UNPD129797	hyperoside	UNPD46148	β-sitosterol
UNPD130468	α-amyrin	UNPD46267	trehalose
UNPD13342	α-amyrin	UNPD48168	mannitol
UNPD133665	caffeic acid	UNPD48910	apigenin
UNPD135747	lactoflorin	UNPD48939	oleic acid
UNPD136822	α-amyrin	UNPD49205	quercetin
UNPD137580	α-amyrin	UNPD49664	cinnamic aldehyde
UNPD138219	hyperoside	UNPD49764	4-hydroxybenzyl alcohol
UNPD140050	acetoguaiacon	UNPD50991	poricoic acid AM
UNPD140430	ergosta-4,6,8(14),22-tetraen-3-one	UNPD51284	benzoic acid
UNPD140949	gallic acid	UNPD52427	caffeic acid
UNPD143140	dehydroeburicoicacid	UNPD52570	β-sitosterol
UNPD143725	paeoniflorin	UNPD53479	benzoylpaeoniflorin
UNPD14414	α-amyrin	UNPD55450	trehalose
UNPD145255	adenosine	UNPD57689	epicatechin
UNPD147172	mudanpioside-F	UNPD57841	proline
UNPD147436	mudanpioside-D	UNPD58928	hyperoside
UNPD147757	15a-hydroxydehydrotumulosic acid	UNPD60430	protocatechuic acid
UNPD14867	polyporenic acid C	UNPD61258	leucine
UNPD148744	2,5-dihydroxy-4-methylacetophenone	UNPD6300	β-sitosterol

续表 1

ID	化合物	ID	化合物
UNPD148754	lactoflorin	UNPD64073	paeonol
UNPD149531	5, 7, 3′-Trimethyl-(-)-epicatechin	UNPD64969	suffrupaeonidanin B
UNPD152742	citrostadienol	UNPD65144	mudanpioside-B
UNPD152846	citrostadienol	UNPD65702	dehydroeburicoicacid
UNPD15360	α-amyrin	UNPD66565	β-amyrin acetate
UNPD153802	suffrupaeonidanin B	UNPD66644	poricoic acid B
UNPD155042	taxifolin	UNPD6707	daucosterol
UNPD16064	7-hydroxycoumarin	UNPD68611	guanosine
UNPD161634	kaempferol	UNPD70084	sucrose
UNPD161812	trans-*O*-methoxy cinnamic acid	UNPD70549	oxypaeoniflora
UNPD16201	albiflorin	UNPD72055	dehydrotumulosic acid
UNPD162992	taxifolin	UNPD72395	amygdalin
UNPD164160	α-amyrin	UNPD72621	glucose
UNPD165682	2-methoxybenzoic acid	UNPD73147	α-amyrin
UNPD166501	α-amyrin	UNPD73469	hyperoside
UNPD167245	sucrose	UNPD75597	hyperoside
UNPD167251	syringaresinol	UNPD79460	citrostadienol
UNPD167512	dehydropachymic acid	UNPD80387	catechins
UNPD170534	suffrupaeonidanin C	UNPD80675	citrostadienol
UNPD172175	poricoic acid D	UNPD81316	pachymic acid
UNPD172347	taxifolin	UNPD82149	betulinic acid
UNPD172457	5-HMF	UNPD83717	glucose
UNPD173098	poricoic acid DM	UNPD83955	poricoic acid E
UNPD173899	prunasin	UNPD85202	poricoic acid CM
UNPD176049	taxifolin	UNPD86062	desbenzoylpaeoniflorin
UNPD17684	syringic acid	UNPD86478	poricoic acid A
UNPD177395	3,3′-di-*O*-methyl ether ellagic acid	UNPD89095	ergosterol
UNPD180657	paeoniflorin	UNPD95458	cinnamyl alcohol
UNPD181583	poricoic acid D	UNPD95544	methyl gallate
UNPD182597	α-amyrin	UNPD96215	α-amyrin
UNPD183645	3,3′-di-*O*-methyl ether ellagic acid	UNPD99873	dulcitol
UNPD184720	poricoic acid C	GZFL_1	3-hydroxy-tirucallon-(7)-oic acid
UNPD184770	tumulosic acid	GZFL_2	galloylpaeoniflorin
UNPD186476	α-amyrin	GZFL_3	mudanpioside-E
UNPD186668	poricoic acid F	GZFL_4	mudanpioside-C
UNPD187258	3β-*p*-hydroxybenzoyl-dehydrotumulosic acid	UNPD59312	5a,8a-peroxydehydrotumulosic acid
UNPD77610	1,2,3,4,6-penta-*O*-galloyl-beta-*D*-glucopyranose	UNPD134433	β-*D*-glucopyranoside, phenylmethyl 6-*O*-β-galactopyranosyl

表 2 与痛经、盆腔炎和子宫肌瘤相关的靶蛋白

Table 2 Target proteins related with dysmenorrhea, PID, and hysteromyoma

Uniprot	PDB	蛋白名称	Uniprot	PDB	蛋白名称
Q8IU85	2JC6	calcium/calmodulin-dependent protein kinase type 1D	Q8NBQ5	1YB1	estradiol 17-beta-dehydrogenase 11
Q96NX5	2JAM	calcium/calmodulin-dependent protein kinase type 1G	P62508	2P7A	estrogen-related receptor gamma
Q16566	2W4O	calcium/calmodulin-dependent protein kinase type IV	P05230	3K1X	fibroblast growth factor 1
P27815	3I8V	cAMP-specific 3',5'-cyclic phosphodiesterase 4A	P09038	1BFB	fibroblast growth factor 2
Q07343	1XLX	cAMP-specific 3',5'-cyclic phosphodiesterase 4B	P11362	2FGI	fibroblast growth factor receptor 1
Q08499	3G4K	cAMP-specific 3',5'-cyclic phosphodiesterase 4D	P21802	3B2T	fibroblast growth factor receptor 2
P22680	3SN5	cholesterol 7-alpha-monooxygenase	Q9BVM4	3JUC	gamma-glutamylaminecyclotransferase
P61073	3ODU	C-X-C chemokine receptor type 4	P49841	3I4B	glycogen synthase kinase-3 beta
P00533	1XKK	epidermal growth factor receptor	P62993	2H5K	growth factor receptor-bound protein 2
P14061	1I5R	estradiol 17-beta-dehydrogenase 1	P62826	3GJ0	GTP-binding nuclear protein Ran

续表 2

Uniprot	PDB	蛋白名称	Uniprot	PDB	蛋白名称
P04629	4AOJ	high affinity nerve growth factor receptor	P29466	1RWX	caspase-1
Q14643	1N4K	inositol 1,4,5-trisphosphate receptor type 1	P00740	3LC3	coagulation factor IX
P23677	1W2C	inositol-trisphosphate 3-kinase A	P12259	3P70	coagulation factor V
Q96DU7	2A98	inositol-trisphosphate 3-kinase C	P08709	2FLR	coagulation factor VII
P08069	3i81	insulin-like growth factor 1 receptor	P00451	3HNB	coagulation factor VIII
Q9NWZ3	2NRU	interleukin-1 receptor-associated kinase 4	P00742	3M36	coagulation factor X
P29460	1F42	interleukin-12 subunit beta	P03951	1ZOM	coagulation factor XI
P07333	3DPK	macrophage colony-stimulating factor 1 receptor	P45452	3ELM	collagenase 3
P14174	3IJG	macrophage migration inhibitory factor	P00746	1DIC	complement factor D
P49137	3R2B	MAP kinase-activated protein kinase 2	P11511	3EQM	cytochrome P450 19A1
P28482	3I5Z	mitogen-activated protein kinase 1	P47712	1CJY	cytosolic phospholipase A2
A6NG28	3TTI	mitogen-activated protein kinase 10	P14416	2HLB	d(2) dopamine receptor
P53779	3TTI	mitogen-activated protein kinase 10	P00374	2W3A	dihydrofolate reductase
Q15759	3GC9	mitogen-activated protein kinase 11	P03372	2QXS	estrogen receptor
P53778	1CM8	mitogen-activated protein kinase 12	Q92731	2QTU	estrogen receptor beta
Q16539	1KV1	mitogen-activated protein kinase 14	P49888	1G3M	estrogen sulfotransferase
P27361	2ZOQ	mitogen-activated protein kinase 3	P02751	2OCF	fibronectin
Q13164	4B99	mitogen-activated protein kinase 7	P04150	3H52	glucocorticoid receptor
P45983	3PZE	mitogen-activated protein kinase 8	Q9BY41	1T69	histone deacetylase 8
P45984	3NPC	mitogen-activated protein kinase 9	P09960	3FH5	leukotriene A-4 hydrolase
Q99558	4DN5	mitogen-activated protein kinase kinase kinase 14	Q16873	2UUH	leukotriene C4 synthase
Q99683	2CLQ	mitogen-activated protein kinase kinase kinase 5	P09237	1MMP	matrilysin
O43318	2YIY	mitogen-activated protein kinase kinase kinase 7	P51512	1RM8	matrix metalloproteinase-16
P80192	3DTC	mitogen-activated protein kinase kinase kinase 9	O60882	2JSD	matrix metalloproteinase-20
Q9HBL8	2WM3	nmrA-like family domain-containing protein 1	P14780	1GKC	matrix metalloproteinase-9
O75469	1M13	nuclear receptor subfamily 1 group i member 2	P08235	2OAX	mineralocorticoid receptor
Q8NEB9	3LS8	phosphatidylinositol 3-kinase catalytic subunit type 3	P29474	1M9J	nitric oxide synthase, endothelial
P27986	4A55	phosphatidylinositol 3-kinase regulatory subunit alpha	P35228	4NOS	nitric oxide synthase, inducible
P14555	1J1A	phospholipase A2, membrane associated	P51575	4DW1	P2X purinoceptor 1
P04626	3RCD	receptor tyrosine-protein kinase erbB-2	O75051	3Q3J	plexin-A2
P23921	2WGH	ribonucleoside-diphosphate reductase large subunit	P06401	2W8Y	progesterone receptor
O76054	1OLM	SEC14-like protein 2	O14684	3DWW	prostaglandin E synthase
P42345	1FAP	serine/threonine-protein kinase mTOR	P23219	3N8X	prostaglandin G/H synthase 1
Q9NQU5	2ODB	serine/threonine-protein kinase PAK 6	P35354	3LN1	prostaglandin G/H synthase 2
P05093	3RUK	steroid 17-alpha-hydroxylase/17,20 lyase	Q14914	2Y05	prostaglandin reductase 1
P11474	3K6P	steroid hormone receptor ERR1	Q8N8N7	2W4Q	prostaglandin reductase 2
P48061	2NWG	stromal cell-derived factor 1	P41222	3O19	prostaglandin-H2 D-isomerase
P36897	2X7O	TGF-beta receptor type-1	P17252	3IW4	protein kinase C alpha type
P21731	1LBN	thromboxane A2 receptor	P05771	2I0E	protein kinase C beta type
P01375	2AZ5	tumor necrosis factor	P24723	3TXO	protein kinase C eta type
P23458	3EYG	tyrosine-protein kinase JAK1	P41743	1ZRZ	protein kinase C iota type
P17948	3HNG	vascular endothelial growth factor receptor 1	Q04759	1XJD	protein kinase C theta type
P35968	2QU5	vascular endothelial growth factor receptor 2	P57735	3TSO	ras-related protein Rab-25
P22303	4EY5	acetylcholinesterase	P13501	1U4L	C-C motif chemokine 5
P10275	3L3X	androgen receptor	Q00722	2ZKM	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-2
P01008	3EVJ	antithrombin-III	P16885	2W2W	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase
P10415	2W3L	apoptosis regulator Bcl-2	Q9UQM7	3SOA	calcium/calmodulin-dependent protein kinase type II subunit alpha
P09917	3V99	arachidonate 5-lipoxygenase	Q13554	3BHH	calcium/calmodulin-dependent protein kinase type II subunit beta
Q96GD4	4AF3	aurora kinase B	Q13557	2VN9	calcium/calmodulin-dependent protein kinase type II subunit delta
P07550	3NY8	beta-2 adrenergic receptor	Q13555	2V7O	calcium/calmodulin-dependent protein kinase type II subunit gamma
P62158	1CTR	calmodulin	P36507	1S9I	dual specificity mitogen-activated protein kinase kinase 2
P42336	3HHM	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform	Q13946	1ZKL	high affinity cAMP-specific 3',5'-cyclic phosphodiesterase 7A
			P52564	3FME	dual specificity mitogen-activated protein kinase kinase 6
P48736	3SD5	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit gamma isoform	P67775	2IE4	serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform
Q02750	3DY7	dual specificity mitogen-activated protein kinase kinase 1	P19438	1FT4	tumor necrosis factor receptor superfamily member 1A

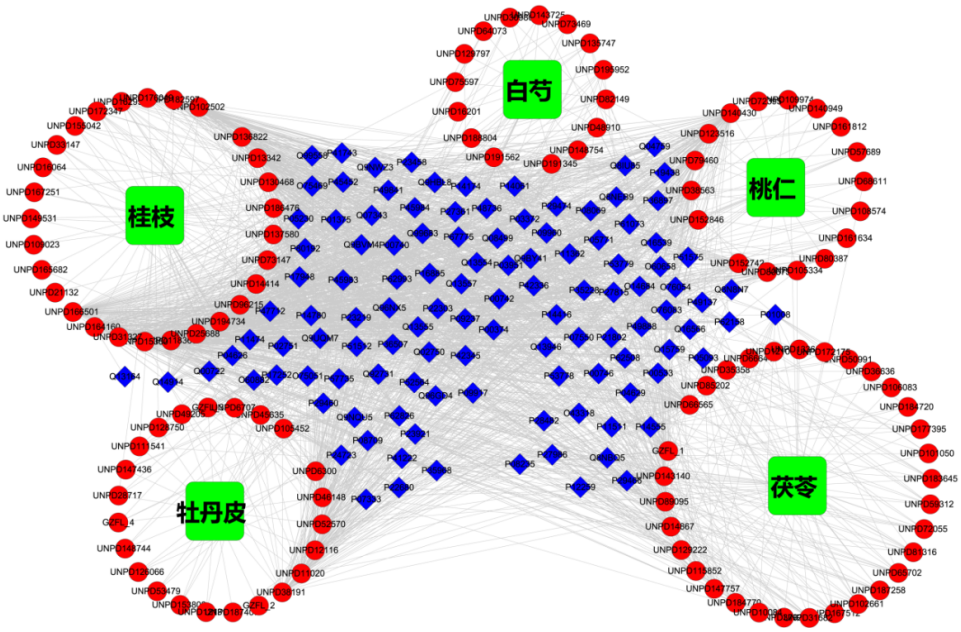
1.3　网络构建与靶蛋白生物通路分析

根据分子与蛋白的对接分值，以原配体对接得分为阈值，抽取对接得分较高（score≥5.00）的分子-靶蛋白相互作用数据对导入 Cytoscape 3.0[34]构建桂枝茯苓胶囊中小分子与靶蛋白相互作用网络，并将网络中小分子进一步投射到相应的药材构建出药材-分子-靶标网络（herb-drug-target network，HDTN）。同时，针对网络中的靶蛋白，进一步投射到 KEGG 数据库（http://www.genome.jp/kegg/pathway. html）构建出靶蛋白-生物通路网络（target-pathway network，TPN），从而获得桂枝茯苓胶囊对生物通路的影响。上述网络的构建及网络特征的分析均采用 Cytoscape 3.0 及其插件 NetworkAnalyzer 来计算。

2　结果

2.1　网络特征分析

在构建的 HDTN 网络（图 1）中，包含 115 个分子，116 个靶蛋白以及 5 味中药，平均每个分子靶向 17.90 个靶蛋白，每个蛋白有 17.75 个分子，然后利用 Cytoscape 3.0 中插件 NetworkAnalyzer[35]进一步对 HDTN 网络中分子与靶蛋白间的相互关系网的分析发现，此关系网的平均最短路径为 17.827，远小于整个节点数（231），提示分子与靶标的关系网为典型的小世界网络（small world），而从分子与靶标关系网中各节点的度分布服从幂律分布［$P(\kappa)=28.96\times\kappa^{-0.73}$（$r=0.865$）］来看，说明分子与靶标构成的关系网具有良好的稳定性[36-37]，提示桂枝茯苓胶囊中这些活性成分能纠正疾病状态下的机体平衡网络促使其形成新的机体平衡，进而可能起到调控机体的治疗作用[38]。同时，在分子与药材的相互关系分析发现，46 个分子来自于君药桂枝、46 个分子来自于茯苓，而来自白芍和牡丹皮的分子各有 27 个，此外还有桃仁的 28 个分子，其中桃仁与桂枝之间的活性成分有较大的重叠，这也印证了桂枝茯苓胶囊组方的合理性（图 2）。



圆形代表从茯苓桂枝胶囊中筛选的化合物；菱形代表蛋白靶点；圆角矩形代表组方药材
Ellipse represents small molecules from GFC; Diamond represents target proteins; Round rectangle represents herbs

图 1　药材-分子-靶点网络

Fig. 1　Herb-drug-target network (HDTN)

2.2　主要活性化合物和潜在靶蛋白分析

在网络分析中，除了整体网络特征分析，还提出了度（degree）、介数（betweenness）、聚类系数（clustering coefficient）等一系列可定量刻画网络内部结构的度量[39]，并已被应用到生物网络中来鉴别可药的靶点[40]、发现药物新适应症[15]、解析药物作用新机制以及活性化合物的发现[14]等，尤其是度、介数常被用于研究分子网络中各节点的重要性。因此，在对 HDTN 网络的整体拓扑学特征分析的基础上，进一步对网络中各节点的度和介数进行了详细分析，以确定桂枝茯苓胶囊中主要活性化合物和潜在的靶蛋白。

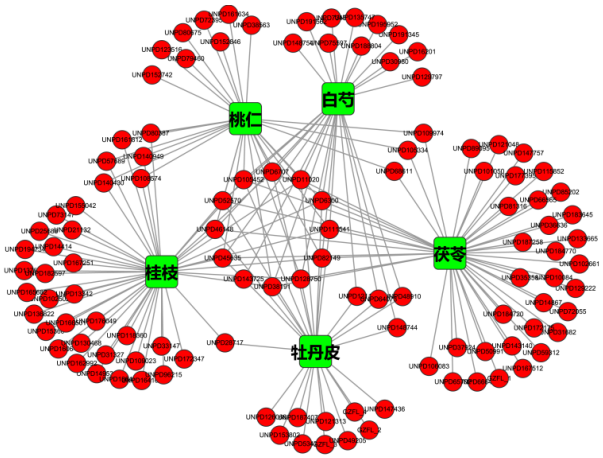


图 2 桂枝茯苓胶囊中活性成分在药味归属中的分布

Fig. 2 Distribution of active constituents from GFC in herbal attribution

从 HDTN 网络中分子节点的网络度和介数来看（表 3、4），67 个分子（35 个化合物）可与 6 个以上的靶蛋白相互作用，其中网络度和介数较高的分子多数为五环三萜类化合物和甾醇类化合物，如柠檬甾二烯醇（citrostadienol）、菜油甾醇（campesterol）、麦角甾醇（ergosterol）、去氢茯苓酸（dehydropachymic acid）、茯苓酸（pachymic acid）、去氢土莫酸（dehydrotumulosic acid）、ergosta-4,6,8(14),22-tetraen-3-one、齐墩果酸（oleanolic acid），还有部分黄酮类化合物如槲皮素（quercetin）、芹菜素（apigenin）。而文献分析也发现，ergosta-4,6,8(14),22-tetraen-3-one、oleanolic acid、polyporenicacid C、eburicoic acid 和 dehydroeburicoic acid 能明显抑制 NO、IL-1β 和 TNF-α[41-44]，β-amyrin acetate 具有

表 3 HDTN 网络中部分节点（化合物）的网络特征

Table 3 Network features of nodes (compounds from GFC) with high degree in HDTN

编码	名称	度	介数	编码	名称	度	介数
UNPD166501	α-amyrin	76	0.048 5	UNPD80675	citrostadienol	25	0.003 4
UNPD164160	α-amyrin	74	0.055 0	UNPD14867	polyporenic acid C	24	0.011 9
UNPD31327	α-amyrin	73	0.061 3	UNPD191345	hederagenin	17	0.004 0
UNPD15360	α-amyrin	72	0.043 9	UNPD129222	dehydroeburicoic acid	16	0.005 5
UNPD140430	ergosta-4,6,8(14),22-tetraen-3-one	65	0.061 0	UNPD105452	betulinic acid	15	0.001 4
UNPD118360	α-amyrin	62	0.030 0	UNPD115852	polyporenic acid C	14	0.002 7
UNPD25688	α-amyrin	60	0.023 6	UNPD148754	lactoflorin	14	0.001 9
UNPD194734	α-amyrin	59	0.029 6	UNPD184770	tumulosic acid	12	0.001 5
UNPD96215	α-amyrin	58	0.022 9	UNPD147757	15α-hydroxydehydrotumulosic acid	12	0.001 3
UNPD14414	α-amyrin	58	0.019 7	UNPD176049	taxifolin	11	0.001 2
UNPD73147	α-amyrin	57	0.029 6	UNPD45635	daucosterol	11	0.000 7
UNPD66565	β-amyrin acetate	57	0.024 7	UNPD48910	apigenin	10	0.004 1
UNPD137580	α-amyrin	55	0.022 9	UNPD162992	taxifolin	10	0.003 2
UNPD130468	α-amyrin	55	0.021 5	UNPD10084	3-*epi*-dehydrotumulosic acid	10	0.001 1
UNPD186476	α-amyrin	55	0.019 5	UNPD37924	eburicoic acid	9	0.002 5
UNPD13342	α-amyrin	53	0.020 6	UNPD167512	dehydropachymic acid	9	0.002 4
UNPD136822	α-amyrin	52	0.022 6	UNPD31682	dehydropachymic acid	9	0.000 8
UNPD102502	α-amyrin	43	0.012 0	UNPD105334	guanosine	9	0.000 6
UNPD182597	α-amyrin	41	0.024 7	UNPD65702	dehydroeburicoicacid	8	0.001 4
UNPD123516	citrostadienol	41	0.014 5	UNPD81316	pachymic acid	8	0.000 5
UNPD38191	campesterol	39	0.011 4	UNPD102661	3-*epi*-dehydropachymic acid	8	0.000 5
UNPD12116	oleanolic acid	38	0.033 3	UNPD187258	3β-*p*-hydroxybenzoyl-dehydrotumulos ic acid	8	0.000 5
UNPD79460	citrostadienol	38	0.008 0	GZFL_3	mudanpioside-E	8	0.000 4
UNPD11020	β-sitosterol	36	0.017 7	UNPD80387	catechins	7	0.001 3
UNPD52570	β-sitosterol	35	0.009 9	UNPD49205	quercetin	7	0.000 7
UNPD38563	citrostadienol	35	0.005 9	UNPD72055	dehydrotumulosic acid	7	0.000 5
UNPD46148	β-sitosterol	34	0.017 8	UNPD195952	sodium paeoniflorin sulfonate	7	0.000 4
UNPD6300	β-sitosterol	33	0.009 2	UNPD172347	taxifolin	7	0.000 3
UNPD152846	citrostadienol	31	0.007 1	UNPD161634	kaempferol	6	0.002 5
GZFL_1	3-hydroxy-tirucallon-(7)-oic acid	28	0.014 0	UNPD183645	3,3'-di-*O*-methyl ether ellagic acid	6	0.000 3
UNPD191562	lactoflorin	27	0.004 9	UNPD6707	daucosterol	6	0.000 2
UNPD143140	dehydroeburicoic acid	26	0.008 1	UNPD59312	5a,8a-peroxydehydrotumulosic acid	6	0.000 1
UNPD152742	citrostadienol	26	0.004 9	UNPD135747	lactoflorin	6	0.000 1
UNPD89095	ergosterol	25	0.010 2				

表 4　HDTN 网络中部分节点（靶蛋白）的网络特征

Table 4　Network features of nodes (target proteins) with high degree in HDTN

Uniprot	名称	度	介数
P29474	nitric oxide synthase, endothelial	36	0.033 6
P14416	D(2) dopamine receptor	35	0.027 1
Q13557	calcium/calmodulin-dependent protein kinase type II subunit δ	32	0.017 2
P45452	collagenase 3	32	0.013 1
Q14914	prostaglandin reductase 1	32	0.009 4
P07550	beta-2 adrenergic receptor	32	0.009 0
P14061	estradiol 17-beta-dehydrogenase 1	31	0.015 3
P49841	glycogen synthase kinase-3 beta	31	0.010 9
O75469	nuclear receptor subfamily 1 group I member 2	30	0.020 0
P14555	phospholipase A2, membrane associated	30	0.018 5
Q02750	dual specificity mitogen-activated protein kinase kinase 1	29	0.010 6
P41743	protein kinase C iota type	29	0.007 6
P49888	estrogen sulfotransferase	28	0.013 0
P42345	serine/threonine-protein kinase mTOR	28	0.010 2
P35228	nitric oxide synthase, inducible	28	0.007 4
Q9UQM7	calcium/calmodulin-dependent protein kinase type II subunit α	27	0.019 7
Q16539	mitogen-activated protein kinase 14	27	0.018 0
P29466	caspase-1	27	0.008 4
Q13555	calcium/calmodulin-dependent protein kinase type II subunit γ	27	0.008 0
Q9HBL8	nmrA-like family domain-containing protein 1	25	0.017 5
P62826	GTP-binding nuclear protein Ran	25	0.017 1
Q96NX5	calcium/calmodulin-dependent protein kinase type 1G	25	0.013 8
Q99683	mitogen-activated protein kinase kinase kinase 5	25	0.007 4
P23921	ribonucleoside-diphosphate reductase large subunit	24	0.015 1
P41222	prostaglandin-H2 D-isomerase	24	0.013 8
O76054	SEC14-like protein 2	24	0.009 3
P53778	mitogen-activated protein kinase 12	24	0.006 8
Q16566	calcium/calmodulin-dependent protein kinase type IV	24	0.005 7
Q8IU85	calcium/calmodulin-dependent protein kinase type 1D	24	0.004 8
P27815	cAMP-specific 3',5'-cyclic phosphodiesterase 4A	24	0.003 5
O76083	high affinity cGMP-specific 3',5'-cyclic phosphodiesterase 9A	24	0.003 3
P00533	epidermal growth factor receptor	23	0.005 0
Q9NWZ3	interleukin-1 receptor-associated kinase 4	22	0.006 5
Q15759	mitogen-activated protein kinase 11	22	0.005 8
O60658	high affinity cAMP-specific and IBMX-insensitive 3',5'-cyclic phosphodiesterase 8A	22	0.004 7
P00374	dihydrofolate reductase	22	0.004 0
P61073	C-X-C chemokine receptor type 4	22	0.003 8
P05771	protein kinase C beta type	22	0.002 6
P14780	matrix metalloproteinase-9	21	0.018 5
P11362	fibroblast growth factor receptor 1	21	0.006 5
P48736	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit γ	21	0.005 4
P03951	coagulation factor XI	21	0.004 5
P08069	insulin-like growth factor 1 receptor	21	0.003 3
P00742	coagulation factor X	20	0.007 8
P52564	dual specificity mitogen-activated protein kinase kinase 6	20	0.005 9
P01375	tumor necrosis factor	20	0.005 7
P53779	mitogen-activated protein kinase 10	20	0.005 1
P23219	prostaglandin G/H synthase 1	20	0.004 7
Q08499	cAMP-specific 3',5'-cyclic phosphodiesterase 4D	20	0.004 3
P09237	matrilysin	20	0.003 0
P45983	mitogen-activated protein kinase 8	19	0.025 5
P23458	tyrosine-protein kinase JAK1	19	0.012 9
O14684	prostaglandin E synthase	19	0.007 8
P45984	mitogen-activated protein kinase 9	19	0.007 1
P05093	steroid 17-alpha-hydroxylase/17,20 lyase	19	0.005 5
P22680	cholesterol 7-alpha-monooxygenase	19	0.003 2

续表 4

Uniprot	名称	度	介数
P00746	complement factor D	19	0.002 5
P01008	antithrombin-III	18	0.013 2
Q07343	cAMP-specific 3',5'-cyclic phosphodiesterase 4B	18	0.006 1
P09917	arachidonate 5-lipoxygenase	18	0.005 1
Q13946	high affinity cAMP-specific 3',5'-cyclic phosphodiesterase 7A	18	0.003 4
Q04759	protein kinase C theta type	18	0.001 6
P80192	mitogen-activated protein kinase kinase kinase 9	17	0.010 5
P00740	coagulation factor IX	17	0.007 8
P17948	vascular endothelial growth factor receptor 1	17	0.007 4
P36897	TGF-beta receptor type-1	17	0.003 3
Q13554	calcium/calmodulin-dependent protein kinase type II subunit β	17	0.003 0
P27361	mitogen-activated protein kinase 3	17	0.002 3
P62158	calmodulin	17	0.002 1
P07333	macrophage colony-stimulating factor 1 receptor	16	0.007 7
P04626	receptor tyrosine-protein kinase erbB-2	16	0.007 4
Q8NBQ5	estradiol 17-beta-dehydrogenase 11	16	0.007 0
P35968	vascular endothelial growth factor receptor 2	15	0.002 8
Q8NEB9	phosphatidylinositol 3-kinase catalytic subunit type 3	15	0.001 2
Q9BVM4	gamma-glutamylaminecyclotransferase	14	0.007 7
P67775	serine/threonine-protein phosphatase 2A catalytic subunit α	14	0.004 0
P02751	fibronectin	14	0.002 6
O75051	plexin-A2	13	0.032 2
P62993	growth factor receptor-bound protein 2	13	0.008 2
P03372	estrogen receptor	13	0.004 6
Q00722	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase β2	13	0.004 5
P21802	fibroblast growth factor receptor 2	13	0.001 6
P62508	estrogen-related receptor gamma	13	0.000 8
P08709	coagulation factor VII	12	0.003 1
P04629	high affinity nerve growth factor receptor	12	0.002 5
Q92731	estrogen receptor beta	12	0.002 5
O60882	matrix metalloproteinase-20	12	0.002 1
P49137	MAP kinase-activated protein kinase 2	12	0.001 1
Q9BY41	histone deacetylase 8	12	0.000 7
P14174	macrophage migration inhibitory factor	11	0.036 8
P17252	protein kinase C alpha type	11	0.001 8
P24723	protein kinase C eta type	11	0.000 9
P22303	acetylcholinesterase	11	0.000 6
Q9NQU5	serine/threonine-protein kinase PAK 6	10	0.011 2
P51512	matrix metalloproteinase-16	10	0.004 6
P57735	ras-related protein Rab-25	10	0.004 5
Q96GD4	aurora kinase B	10	0.001 1
P36507	dual specificity mitogen-activated protein kinase kinase 2	9	0.003 1
P09960	leukotriene A-4 hydrolase	9	0.000 9
Q99558	mitogen-activated protein kinase kinase kinase 14	8	0.003 6
P12259	coagulation factor V	7	0.018 4
P08235	mineralocorticoid receptor	7	0.001 3
P19438	tumor necrosis factor receptor superfamily member 1A	7	0.001 3
P51575	P2X purinoceptor 1	7	0.000 7
O43318	mitogen-activated protein kinase kinase kinase 7	7	0.000 3
P42336	phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit α	7	0.000 3
P05230	fibroblast growth factor 1	6	0.000 4
P28482	mitogen-activated protein kinase 1	6	0.000 4

镇痛和抗炎活性[45-46]，campesterol 可抑制血管形成[47]，与用于治疗子宫肌瘤的 Progesterone 受体激动剂和调节剂药物有类似的结构的甾醇类化合物也被报道治疗多种癌症[6,8,48]，黄酮类化合物与用于子宫肌瘤治疗的 genistein、isoliquiritigenin 等化合物有类似的结构，且槲皮素已被作为有可能治疗子宫肌瘤的多酚类化合物

被研究，儿茶素类化合物用于癌症的治疗[9,49]。

在对 HDTN5 网络中 116 个靶蛋白节点的网络特征分析发现（表 5），有 109 个靶蛋白可与 6 个以上的活性分子存在较强相互作用，其中 NOS、collagenase 3、coagulation factor X、GSK、PLA_2、FGFR、MMP-9、EGFR、COX 等 50 个靶点可与 20 个以上的活性分子存在较强相互作用，在这些靶蛋白中，即包括可与 NO、PGE_2、TNF-α、leukotriene 炎症因子的合成与释放炎症因子密切相关的 NOS、COX、PLA2、PGES、5-LOX、caspase-1 等关键酶、与凝血过程相关的 antithrombin-III、coagulation factor XI、coagulation factor VII、cogulation factor X、cogulation factor IX、coagulation factor V、antithrombin-III 酶以及与血管形成和激素作用密切相关的 GFR、TGFR、FGR、VEGFR、estrogen receptor、P2X purinoceptor 1 受体[9]。同时桂枝茯苓胶囊中化合物还可与 PI3K、GSK3、IRAK4、ERK、PKC、mTOR、MMP-9 等靶蛋白相互作用来调控 PI3K、PKC、MAPKs 等多条信号通路和钙离子通道，进而来影响子宫平滑肌收缩和细胞增殖。

2.3　潜在生物通路分析

生物通路通过其构成的不同靶蛋白间的相互作用来执行特定的生物学功能，是理解疾病临床表现的生理学基础[50-51]。因此药物的作用不仅与靶蛋白有关，而且也受靶蛋白所在的生物通路的影响，多靶点的中药更是如此，故在上述靶蛋白分析的基础上，又进一步将 116 个潜在靶点投射到 KEGG 数据库[52]，其中 98 个靶蛋白可投射到 KEGG 上的 157 条人源生物通路上（图 3）。进一步的网络分析发现（表 6），网络度最高的 3 条生物通路分别为 proteoglycans in cancer、pathways in cancer 和 MAPK signaling pathway，均是与肿瘤、炎症等密切相关，介数最高的生物通路为 complement and coagulation cascades，是与凝血系统密切相关。

表 5　TPN 中部分节点（生物通路）的网络特征

Table 5　Network features of nodes (pathway from KEGG database) with high degree in TPN

ID	名称	度	介数	ID	名称	度	介数
hsa05205	proteoglycans in cancer	30	0.039 4	hsa04730	long-term depression	10	0.007 9
hsa05200	pathways in cancer	29	0.034 6	hsa04666	Fc gamma R-mediated phagocytosis	10	0.005 6
hsa04010	MAPK signaling pathway	28	0.042 0	hsa05132	salmonella infection	10	0.004 4
hsa04722	neurotrophin signaling pathway	27	0.021 9	hsa04713	circadian entrainment	10	0.002 6
hsa04151	PI3K-Akt signaling pathway	23	0.023 0	hsa04930	type II diabetes mellitus	10	0.002 3
hsa05164	influenza A	23	0.021 3	hsa04726	serotonergic synapse	9	0.012 3
hsa04912	GnRH signaling pathway	23	0.020 0	hsa04070	phosphatidylinositol signaling system	9	0.008 9
hsa04380	osteoclast differentiation	23	0.016 4	hsa05203	viral carcinogenesis	9	0.005 8
hsa04012	ErbB signaling pathway	23	0.013 9	hsa04060	cytokine-cytokine receptor interaction	9	0.004 1
hsa04066	HIF-1 signaling pathway	21	0.017 2	hsa04150	mTOR signaling pathway	9	0.003 7
hsa05152	tuberculosis	20	0.025 0	hsa05162	measles	9	0.002 3
hsa04664	Fc epsilon RI signaling pathway	20	0.018 9	hsa05120	epithelial cell signaling in helicobacter pylori infection	9	0.001 7
hsa05145	toxoplasmosis	20	0.017 3				
hsa04510	focal adhesion	20	0.014 8	hsa04622	RIG-I-like receptor signaling pathway	9	0.001 0
hsa05142	chagas disease (American trypanosomiasis)	19	0.016 4	hsa05211	renal cell carcinoma	9	0.001 0
hsa05214	glioma	19	0.008 6	hsa05220	chronic myeloid leukemia	9	0.000 7
hsa01100	metabolic pathways	18	0.150 6	hsa05221	acute myeloid leukemia	9	0.000 6
hsa04660	T cell receptor signaling pathway	18	0.011 1	hsa04960	aldosterone-regulated sodium reabsorption	8	0.009 3
hsa05160	hepatitis C	18	0.008 4	hsa05034	alcoholism	8	0.007 8
hsa04620	toll-like receptor signaling pathway	18	0.006 1	hsa04064	NF-κB signaling pathway	8	0.004 6
hsa04728	dopaminergic synapse	17	0.018 7	hsa04210	apoptosis	8	0.002 4
hsa04370	VEGF signaling pathway	17	0.015 3	hsa05014	amyotrophic lateral sclerosis (ALS)	8	0.001 9
hsa05161	hepatitis B	17	0.009 4	hsa04520	adherens junction	8	0.001 4
hsa04020	calcium signaling pathway	16	0.022 8	hsa04971	gastric acid secretion	8	0.001 2

续表 5

ID	名称	度	介数	ID	名称	度	介数
hsa05169	epstein-barr virus infection	16	0.019 0	hsa05168	herpes simplex infection	8	0.000 9
hsa04725	cholinergic synapse	15	0.013 4	hsa05131	shigellosis	8	0.000 4
hsa04910	insulin signaling pathway	15	0.008 3	hsa04610	complement and coagulation cascades	7	0.964 3
hsa05215	prostate cancer	15	0.004 2	hsa05032	morphine addiction	7	0.047 0
hsa05133	pertussis	14	0.009 8	hsa00230	purine metabolism	7	0.012 6
hsa05166	HTLV-I infection	13	0.014 6	hsa05010	alzheimer's disease	7	0.004 8
hsa04310	wnt signaling pathway	13	0.013 5	hsa00590	arachidonic acid metabolism	7	0.003 4
hsa04916	melanogenesis	13	0.005 3	hsa04920	adipocytokine signaling pathway	7	0.001 5
hsa04720	long-term potentiation	13	0.004 4	hsa04911	insulin secretion	7	0.000 8
hsa04810	regulation of actin cytoskeleton	13	0.003 9	hsa05219	bladder cancer	7	0.000 8
hsa05223	non-small cell lung cancer	13	0.003 5	hsa04913	ovarian steroidogenesis	6	0.010 7
hsa04914	progesterone-mediated oocyte maturation	13	0.002 2	hsa04360	axon guidance	6	0.009 9
hsa05212	pancreatic cancer	13	0.002 2	hsa04530	tight junction	6	0.004 6
hsa04270	vascular smooth muscle contraction	12	0.025 2	hsa04724	glutamatergic synapse	6	0.002 8
hsa05140	leishmaniasis	12	0.006 3	hsa04630	jak-STAT signaling pathway	6	0.000 4
hsa04650	natural killer cell mediated cytotoxicity	12	0.005 9	hsa00140	steroid hormone biosynthesis	5	0.016 8
hsa04540	gap junction	11	0.010 6	hsa05143	african trypanosomiasis	5	0.003 2
hsa04062	chemokine signaling pathway	11	0.007 3	hsa00562	inositol phosphate metabolism	5	0.003 2
hsa04670	leukocyte transendothelial migration	11	0.006 4	hsa04970	salivary secretion	5	0.002 9
hsa04144	endocytosis	11	0.006 3	hsa04350	TGF-beta signaling pathway	5	0.001 7
hsa04621	NOD-like receptor signaling pathway	11	0.003 8	hsa05222	small cell lung cancer	5	0.001 2
hsa04723	retrograde endocannabinoid signaling	11	0.003 6	hsa04390	hippo signaling pathway	5	0.001 0
hsa04114	oocyte meiosis	11	0.003 2	hsa04973	carbohydrate digestion and absorption	5	0.000 9
hsa04662	B cell receptor signaling pathway	11	0.003 0	hsa05202	transcriptional misregulation in cancer	5	0.000 5
hsa05218	melanoma	11	0.002 0	hsa05216	thyroid cancer	5	0.000 4
hsa05210	colorectal cancer	11	0.001 8	hsa04320	dorso-ventral axis formation	5	0.000 1
hsa05213	endometrial cancer	11	0.001 6	hsa04740	olfactory transduction	5	0.000 1
hsa05146	amoebiasis	10	0.009 6				

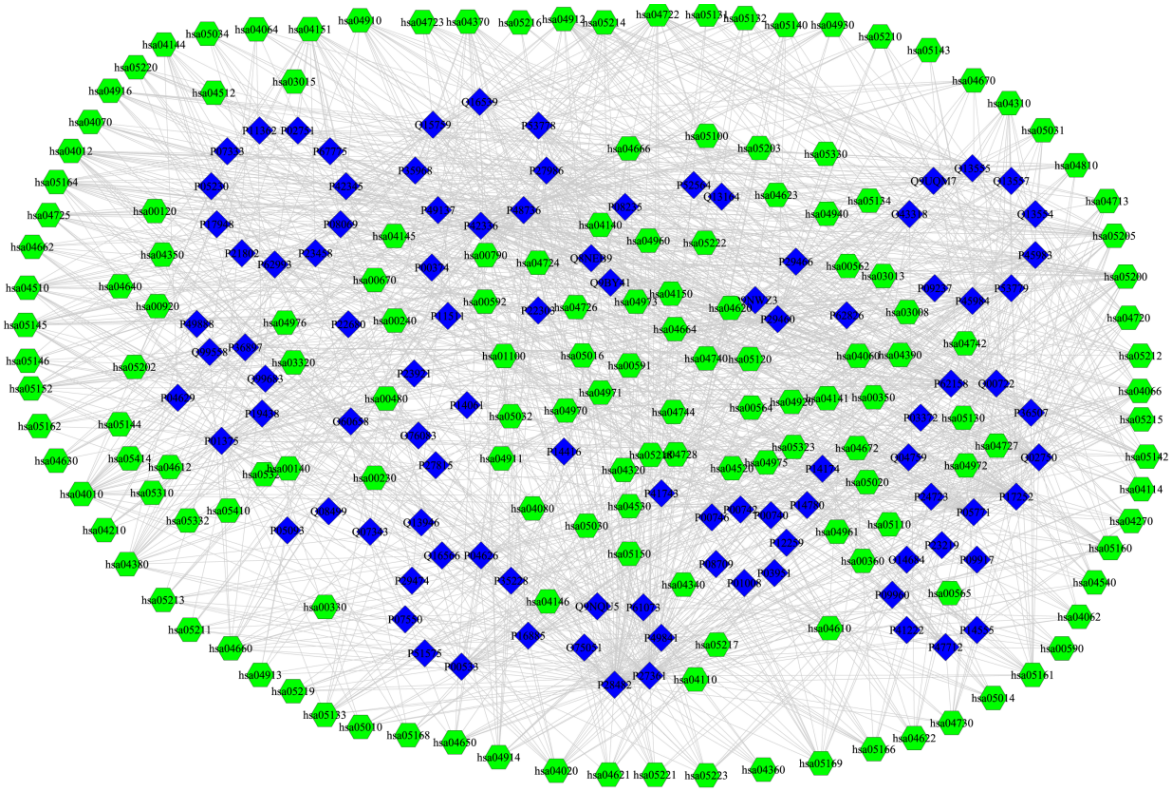


图 3 靶蛋白-生物通路网络

Fig. 3 Potential target proteins –biological pathway network

为了更好地理解桂枝茯苓胶囊发挥治疗痛经、盆腔炎和子宫肌瘤的作用机制，从上述的靶点-通路网络中抽提了与痛经、盆腔炎、子宫肌瘤密切相关的 15 条生物通路（表 6）。在这 15 条生物通路中，GnRH signaling pathway 和 progesterone-mediated oocyte maturation 分别涉及到雌激素对子宫平滑肌细胞的生理作用、子宫内膜增生、月经出血[6,8]；VEGF signaling pathway 和 arachidonic acid metabolism 分别涉及到子宫内膜内血管形成、PGE_2、$PGF_{2\alpha}$、leukotriene B4 生成以及子宫内膜内腺体增生等[28,31,53-55]；TGF-beta signaling pathway 涉及到调控细胞生长、纤维化以及炎症反应等[9]；而 complement and coagulation cascades 和 steroid hormone biosynthesis 则分别与凝血系统以及类固醇激素合成密切相关[56]；calcium signaling pathway 和 vascular smooth muscle contraction 与子宫平滑肌的松弛有密切关系[57]。而从 target-pathway 中节点网络特征分析来看，这 15 条生物通路多数均有较高的网络度和或较高的介数，且包含了 98 个潜在蛋白中 77 个，占 78.57%（图 4）。因此，推测桂枝茯苓胶囊可能是通过多个成分对多条生物通路的作用来抑制子宫平滑肌的增殖、改善子宫内膜的血管形成和血液循环、降低/减弱雌激素和黄体酮的分泌、子宫平滑肌收缩以及前列腺素等炎症因子合成或释放，从而起到缓解痛经、盆腔炎和子宫肌瘤引起的疼痛、降低炎症反应、改善患者生活质量的作用。在上述分析结果的基础上，对前期的实验报道分析也发现桂枝茯苓胶囊的确能明显降低子宫肌瘤组织中 progesterone receptor 和大鼠血清中 progesterone 水平[58-59]，能下调盆腔炎大鼠血清中 TGF-β1 的水平[60]。同时，整体动物实验也发现桂枝茯苓胶囊的醋酸乙酯部位能显著抑制缩宫素诱导的子宫收缩[61]，能显著降低痛经大鼠血清中 $PGF_{2\alpha}$ 的量[62]。

表 6　TPN 网络中与痛经、盆腔炎和子宫肌瘤密切相关生物通路的网络特征

Table 6　Network features of biological pathways related to dysmenorrhea, PID, and hysteromyoma in TPN

ID	通路	度	介数
hsa05200	pathways in cancer	29	0.034 6
hsa04010	MAPK signaling pathway	28	0.042 0
hsa04151	PI3K-Akt signaling pathway	23	0.023 0
hsa04912	GnRH signaling pathway	23	0.020 0
hsa04370	VEGF signaling pathway	17	0.015 3
hsa04020	calcium signaling pathway	16	0.022 8
hsa04310	wnt signaling pathway	13	0.013 5
hsa04914	progesterone-mediated oocyte maturation	13	0.002 2
hsa04270	vascular smooth muscle contraction	12	0.025 2
hsa04064	NF-kappa B signaling pathway	8	0.004 6
hsa04210	apoptosis	8	0.002 4
hsa04610	complement and coagulation cascades	7	0.964 3
hsa00590	arachidonic acid metabolism	7	0.003 4
hsa00140	steroid hormone biosynthesis	5	0.016 8
hsa04350	TGF-beta signaling pathway	5	0.001 7

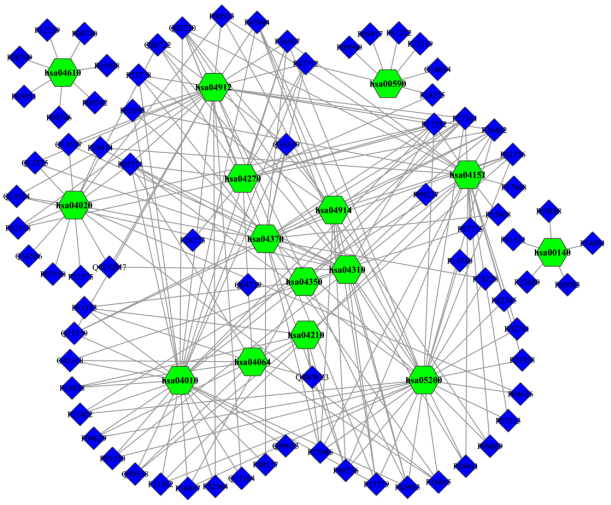


图 4　靶蛋白在与痛经、盆腔炎及子宫肌瘤密切相关的 15 条生物通路中的分布

Fig. 4　Potential target proteins in 15 biological pathway related to dysmenorrheal, PID, and mysteromyoma

3　讨论

通过分子对接、分子-靶蛋白网络特征分析以及生物通路的信息挖掘对桂枝茯苓胶囊治疗痛经、盆腔炎以及子宫肌瘤疾病的主要活性成分和可能的分子进行了分析，明确了 67 个分子（35 化合物），主要为五环三萜类、甾醇类和黄酮类，进一步的药材归属发现这些分子在各单味中药中的分布与桂枝茯苓方的君臣佐使组方相一致，这也从侧面印证了该处方的合理性。生物通路分析发现桂枝茯苓胶囊中的活性化合物通过与 arachidonic acid metabolism、complement and coagulation cascades、calcium signaling pathway、VEGF signaling pathway、GnRH signaling pathway 等 15 条生物通路作用来抑制子宫平滑肌的增殖、改善子宫内膜的血管形成和血液循环、降低/减弱雌激素和黄体酮的分泌、子宫平滑肌收缩以及 NO、PGE_2、$PGF_{2\alpha}$、TNF-α、leukotriene 等炎症因子合成或释放。本研究通过分

子计算、网络分析和信息挖掘的组合应用，初步解析了桂枝茯苓胶囊治疗痛经、盆腔炎及子宫肌瘤的药效物质基础、可能的分子机制和相关的生物通路，这将为后续桂枝茯苓胶囊药效成分分析和作用机制探索等实验研究选择提供一定的参考方向。

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