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基于网络药理学方法预测扶正抑瘤方作用于PD-1和CTLA-4免疫检查点潜在信号转导途径

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摘　要

目的：运用网络药理学方法预测扶正抑瘤方作用于PD-1和CTLA-4免疫检查点潜在信号转导途径。方法：ETCM、TCMSP数据库筛选扶正抑瘤方中各味药物活性成分，利用STITCH、Swiss Target Prediction等数据库对各活性成分进行作用靶点预测，构建成分－靶点集，与MalaCards、DisGeNET等数据库中PD-1和CTLA-4免疫检查点相关核心靶点取交集。利用STRING数据库进行蛋白互作网络构建，DAVID数据库进行富集分析，利用Cytoscape构建“药物－成分－靶点－通路”网络图，对数据进行可视化。结果：从扶正抑瘤方种筛选得到195个活性化合物，涉及相关作用靶标676个；筛选核心靶标44个，映射交集16个。蛋白互作网络筛选得到5个关键靶标HIF1A、STAT1、STAT3、CD80、CD86，主要通过PD-L1表达于PD-1检查点通路及CTLA4介导的T细胞受体信号转导。结论：扶正抑瘤方可通过多成分多靶点发挥肿瘤免疫杀伤作用，与T细胞受体信号通路CTLA-4和PD-1检查点通路等高度相关。

关键词

扶正抑瘤方，网络药理学，免疫检查点，信号转导途径

Research on Potential Signal Transduction Pathways of Fuzheng Yiliu Prescription on PD-1 and CTLA-4 Immune Checkpoints Based on Network Pharmacology

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Abstract

Objective: Using network pharmacology methods to predict the potential signal transduction pathways of Fuzheng Yiliu Prescription on PD-1 and CTLA-4 immune checkpoints. Methods: ETCM, TCMSP databases screened the active ingredients of various medicines in Fuzheng Yiliu Prescription, and used STITCH, Swiss Target Prediction and other databases to predict the action target of each active ingredient, and constructed the ingredient-target set, which was combined with MalaCards, DisGeNET and other databases Intersection of relevant targets for PD-1 and CTLA-4 immune checkpoints. Use STRING database for protein-protein interaction network construction, DAVID database for enrichment analysis, use Cytoscape to construct a "drug-component-target-pathway" network diagram to visualize the data. Result: 195 active compounds were screened from Fuzheng Yiliu Prescription, involving 676 related targets; 44 liver cancer-related key targets were screened, and 16 were mapped. The protein interaction network screened 5 key targets HIF1A, STAT1, STAT3, CD80, and CD86, mainly through PD-L1 expression PD-1 checkpoint pathway and CTLA4 mediated T cell receptor signal transduction. Conclusion: Fuzheng Yiliu Prescription can play a role in tumor immune killing through multiple components and multiple targets, which is highly related to T cell receptor signaling pathway CTLA-4 and PD-1 checkpoint pathway.

Keywords

Fuzheng Yiliu Prescription, Network Pharmacology, Immune Checkpoints, Signal Transduction Pathway

1. 引言

扶正抑瘤方是“全国名中医”杜建教授依据扶正培本法则拟定而来的经验方[1]，由黄芪、女贞子、灵芝、山药组成，具有健脾益气，滋补肝肾、养心安神、扶正固本之功效。在长期的临床实践中已证实其具有降低术后癌症复发、延长患者生存时间的作用[2] [3]。目前，对于扶正抑瘤方抗癌的完整药效机制尚未完全阐明。

网络药理学由生物信息学、系统生物学等学科整合而成，其分析方式注重系统性、整体性，可较好的阐明多成分，多靶点之间的协同作用[4]，与中医药整体观念相符。本研究通过运用网络药理学，构建“药物－成份－靶标－通路”作用网络，探讨扶正抑瘤方作用于 PD-1 和 CTLA-4 免疫检查点潜在信号转导途径，为中医药抗癌后续研究提供方向和思路。

2. 方法

2.1. 扶正抑瘤方活性化合物与靶标获取

分别以“Astragali Radix”、“Ganoderma”、“Dioscoreae Rhizoma”、“Ligustri Lucidi Fructus”为关键词在中药系统药理学分析平台(TCMSP, https://tcmspw.com/tcmsp.php)与中医药百科全书(ETCM, http://www.tcmip.cn/ETCM/index.php/Home/Index/)中进行搜索，汇总得扶正抑瘤方所含活性化学成分。

通过 PubChem (https://pubchem.ncbi.nlm.nih.gov)查找并获取上述活性化学成分的 SMILE 结构式，运用 STITCH (http://stitch.embl.de/)、Swiss Target Prediction (http://swisstargetprediction.ch)进行对应的生物作用靶点预测。利用 UniProt (https://www.uniprot.org)将各靶点信息归一化为 Gene Symbol。

2.2. 免疫检查点核心靶点的收集

以“PD-1”、“CTLA-4”、“immune checkpoint”等为关键词，在 MalaCards (https://www.malacards.org)、DisGeNET (https://www.disgenet.org)等数据库中进行搜索，汇总取交集后得到免疫检查点相关核心靶点。

2.3. 基因映射与网络构建

将筛选所得的扶正抑瘤方活性成分对应靶点与免疫检查点相关核心靶点进行映射，得到交集基因并利用 Venny (https://bioinfogp.cnb.csic.es/tools/venny)绘制花瓣图。将上述所得映射基因通过 STRING (https://string-db.org)建立蛋白质－蛋白质相互作用(PPI)网络。最后，将扶正抑瘤方筛选所得活性成分、成分作用靶点、免疫检查点相关核心靶点通路等节点信息与映射关系导入 Cytoscape 软件进行可视化分析并对各项属性进行调整，构建“药物－成分－靶点－通路”网络图。

2.4. GO 富集分析与 KEGG 通路富集分析

于 Rstudio 软件中加载 clusterProfiler 扩展包，对 PPI 网络中的核心靶点进行 Kyoto Encyclopedia of Genes and Genomes (KEGG)通路富集和 Gene Ontology (GO)功能富集分析，进一步利用 ggplot2 扩展包绘图使结果可视化。

3. 结果

3.1. 扶正抑瘤方活性化合物与靶标

经 TCMSP 及 ETCM 数据库筛选汇总得到扶正抑瘤方中各味药物活性成分 195 个(见表 1)，通过 SwissTargetPrediction 数据库及 STITCH 数据库预测，去重后得到对应靶点 676 个。

3.2. 靶点映射与网络构建

将筛选得到的扶正抑瘤方中所含化合物作用靶点经过 Uniprot 数据库归一转换为 Gene Symbol 格式后，与 MalaCards、DisGeNET 等数据库搜集得到的免疫检查点相关靶标进行对应映射，绘制花瓣图(见图 1)，得免疫检查点相关核心靶标 44 个，其中 16 个靶点与扶正抑瘤方作用靶点重合。

通过 STRING 数据库对上步所得的免疫检查点相关核心靶标－扶正抑瘤方作用靶点交集基因进行分析。选择分析模式为 Multiple Proteins，设定物种类别为 Homo Sapiens，互作分数阈值设为高度置信(0.700)，得到 16 个节点，边数 58 条，平均节点度值 7.25，平均局部聚类系数 0.752 的 PPI 网络图(见图 2)。

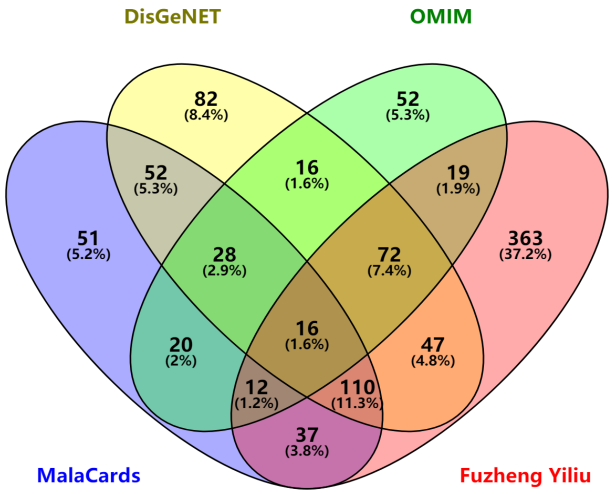

Figure 1. The petals diagram of drug targets associated with immune checkpoint
图 1. 药物靶点与免疫检查点相关靶点花瓣图

Table 1. Active compounds contained in Fuzheng Yiliu decoction
表 1. 扶正抑瘤方所含活性化合物

Mol	Herb	Mol	Herb	Mol	Herb
(−)-ABA	Dioscoreae Rhizoma	DTY	Dioscoreae Rhizoma	lucidenic acid L	Ganoderma
(−)-alpha-Pinene	Ganoderma	EIC	Ganoderma	lucidenolactone	Ganoderma
	Ligustri Lucidi Fructus		Astragali Radix	lucidone A	Ganoderma
()-Borneol	Ligustri Lucidi Fructus	ergosta-4,6,8(14), 22-tetraene-3-one	Ganoderma	lucidone B	Ganoderma
()-Cuparene	Ganoderma	Ergosta-7,22-dien-3-one	Ganoderma	lucidone C	Ganoderma
(−)-Drimenol	Ganoderma	ergosta-7,22E-dien-3beta-ol	Ganoderma	Lucidumoside D	Ligustri Lucidi Fructus
(−)-nopinene	Ligustri Lucidi Fructus	ergosta-7,9(11), 22-trien-3β,5α,6α-triol	Ganoderma	Lucidumoside D_qt	Ligustri Lucidi Fructus
(−)-Olivir	Ligustri Lucidi Fructus	ergosterol	Ganoderma	lupeol	Astragali Radix
(−)-taxifolin	Dioscoreae Rhizoma		Dioscoreae Rhizoma	luteolin	Ligustri Luci-di Fructus
()-Terpinen-4-ol	Ligustri Lucidi Fructus	eriodictyol	Ligustri Lucidi Fructus	luteolin-7-o-glucoside	Ligustri Lucidi Fructus
(+)-alpha-Curcumene	Ganoderma	eugenol	Ligustri Lucidi Fructus	L-Valin	Dioscoreae Rhizoma
(+)-Syringaresinol	Astragali Radix	FA	Astragali Radix	L-Xyl	Dioscoreae Rhizoma

Continued

(R)-linalool	Ganoderma	FERULIC ACID (CIS)	Astragali Radix	Mairin	Astragali Radix
	Ligustri Lucidi Fructus	Flavaxin	Astragali Radix	methly lucidenate L	Ganoderma
(R)-p-Menth-1-en-4-ol	Ganoderma	formononetin	Astragali Radix	Methose	Dioscoreae Rhizoma
(S)-Allantoin	Dioscoreae Rhizoma	Fucopyranose, L-	Astragali Radix	Methylcinnamate	Ligustri Lucidi Fructus
(s)-carvone	Ganoderma	FUM	Ganoderma	Mnk	Ganoderma
1,8-Cineole	Ganoderma	GABA	Ganoderma	MTL	Ganoderma
3,9-di-O-methylnissolin	Astragali Radix		Dioscoreae Rhizoma		Ligustri Lucidi Fructus
3-Hydroxy-2-picoline	Astragali Radix		Astragali Radix	Mucronulatol	Astragali Radix
7-O-methylisomucronulatol	Astragali Radix	ganoderal B	Ganoderma	myristic acid	Ganoderma
ABK	Dioscoreae Rhizoma	ganoderenic acid H	Ganoderma	Nerol	Ligustri Lucidi Fructus
acteoside	Ligustri Lucidi Fructus	ganoderic acid E	Ganoderma	nicotinic acid	Astragali Radix
AIDS180907	Dioscoreae Rhizoma	Ganoderic acid T	Ganoderma	Nonanal	Ganoderma
alexandrin	Astragali Radix	ganoderiol I	Ganoderma	nonane	Ganoderma
alpha-humulene	Ligustri Lucidi Fructus	ganolucidic acid E	Ganoderma	nonanoic acid	Ganoderma
AMG	Ligustri Lucidi Fructus	GCS	Ganoderma	Nonanol	Ganoderma
anethole	Ganoderma	geraniol	Ligustri Lucidi Fructus	Nouracid CS 80	Ganoderma
apigenin	Ligustri Lucidi Fructus	GLB	Dioscoreae Rhizoma	OCTENAL	Ganoderma
Arabinose,d	Astragali Radix	GLY	Dioscoreae Rhizoma	o-Cymol	Ganoderma
ASI	Dioscoreae Rhizoma	Gulutamine	Dioscoreae Rhizoma	oleanolic acid	Ligustri Lucidi Fructus
Astraisoflavanin	Astragali Radix	GUP	Dioscoreae Rhizoma	Oleoside dimethyl ester_qt	Ligustri Lucidi Fructus
beta-Bazzanene	Ganoderma	hancinone C	Dioscoreae Rhizoma	Olitoriside_qt	Ligustri Lucidi Fructus

Continued

betaine	Ganoderma	hederagenin	Astragali Radix	Ononin	Astragali Radix
	Astragali Radix	hexanoic acid	Ganoderma	Ostreasterol	Dioscoreae Rhizoma
beta-Irone	Ganoderma	Hirsutrin	Astragali Radix	palmitic acid	Astragali Radix
beta-sitosterol	Ganoderma	h-Met-h	Dioscoreae Rhizoma	PEL	Ligustri Lucidi Fructus
	Ligustri Lucidi Fructus	Hyacinthin	Ganoderma	Pellitorin	Dioscoreae Rhizoma
BGC	Dioscoreae Rhizoma	hydroxytyrosol	Ligustri Lucidi Fructus	PENTADECYLIC ACID	Ganoderma
Bifendate	Astragali Radix	isoferulic acid	Astragali Radix	PENTYLFURAN	Ganoderma
C09704	Ganoderma	Isofucosterol	Dioscoreae Rhizoma	PHA	Dioscoreae Rhizoma
Caffeate	Astragali Radix	isomucronulatol-7, 2'-di-O-glucosiole	Astragali Radix	piperlonguminine	Dioscoreae Rhizoma
caffeic acid	Ligustri Lucidi Fructus	isorhamnetin	Astragali Radix	Prolinum	Dioscoreae Rhizoma
Calycosin	Astragali Radix	Istidina	Dioscoreae Rhizoma		Astragali Radix
campesta-7,22E-dien-3beta-ol	Ganoderma	Jaranol	Astragali Radix	quercetin	Ligustri Lucidi Fructus
campesterol	Dioscoreae Rhizoma	Kadsurenone	Dioscoreae Rhizoma		Astragali Radix
caprylic acid	Ganoderma	kaempferol	Ligustri Lucidi Fructus	QUOSP	Ligustri Lucidi Fructus
Cedrol	Ganoderma		Astragali Radix	RAM	Ligustri Lucidi Fructus
	Ligustri Lucidi Fructus	ketologanin_qt	Ligustri Lucidi Fructus		Astragali Radix
Cerevisterol	Ganoderma	kingiside_qt	Ligustri Lucidi Fructus	Rhamnocitrin	Astragali Radix
cis-p-Coumarate	Astragali Radix	L-	Dioscoreae Rhizoma	rhamnocitrin-3-O-glucoside	Astragali Radix
cis-Thujopsene	Ligustri Lucidi Fructus		Astragali Radix	rutin	Ligustri Lucidi Fructus
citral	Ganoderma	Lariciresinol	Astragali Radix		Astragali Radix
CLR	Dioscoreae Rhizoma	lauric acid	Ganoderma	salidroside	Ligustri Lucidi Fructus

Continued

Coniferol	Ligustri Lucidi Fructus	L-Bornyl acetate	Ligustri Lucidi Fructus	Sinapyl alcohol	Ligustri Lucidi Fructus
copaene	Ganoderma	l-carvone	Ganoderma	Sitogluside	Ligustri Lucidi Fructus
Cosmetin	Ligustri Lucidi Fructus	Leucinum	Dioscoreae Rhizoma	ST5330609	Ganoderma
coumarin	Astragali Radix	lignoceric acid	Ganoderma	stearic acid	Ganoderma
Crystal VI	Astragali Radix	L-Ile	Dioscoreae Rhizoma	Stigmasterol	Dioscoreae Rhizoma
daidzein	Ligustri Lucidi Fructus	LINALOOL (D)	Ganoderma	sucrose	Ligustri Luci-di Fructus
	Astragali Radix	linolenic acid	Astragali Radix	taxifolin	Ligustri Lucidi Fructus
Damascenone	Ligustri Lucidi Fructus	L-Limonen	Ligustri Lucidi Fructus	Tetracosane	Ganoderma
Daucene	Ganoderma	L-Lysin	Dioscoreae Rhizoma	Threonin	Dioscoreae Rhizoma
DBP	Ligustri Lucidi Fructus	LPG	Dioscoreae Rhizoma	Tormentic acid	Ligustri Lucidi Fructus
dec-2-enal	Ganoderma	L-Serin	Dioscoreae Rhizoma	trans-2,4-decadienal	Ganoderma
Decanal	Ganoderma	Lucialdehyde B	Ganoderma	Tyrosol	Ligustri Lucidi Fructus
delta-amorphene	Ganoderma	lucidenic acid B	Ganoderma	undecanal	Ganoderma
denudatin,a	Dioscoreae Rhizoma	lucidenic acid D1	Ganoderma	ursolic acid	Ligustri Lucidi Fructus
Dioscoreside C_qt	Dioscoreae Rhizoma	lucidenic acid E1	Ganoderma	vanillic acid	Astragali Radix
diosgenin	Dioscoreae Rhizoma	lucidenic acid G	Ganoderma	Vomifoliol	Ligustri Lucidi Fructus
dl-Thujone	Ganoderma	Lucidenic acid K	Ganoderma	XLS	Astragali Radix

将扶正抑瘤方中各味中药、对应活性成分、作用基因靶点等各个节点及映射关系导入 Cytoscape 软件，进行可视化分析并对其各项属性进行设置，得到如图 3 所示“药物－成分－靶点－通路”网络图，图中左侧绿色菱形代表扶正抑瘤方中各味中药，外圈黄色椭圆表示各有效成分，内圈三角形代表相对应的基因靶点，右侧蓝色方形表示关联性较高的通路。

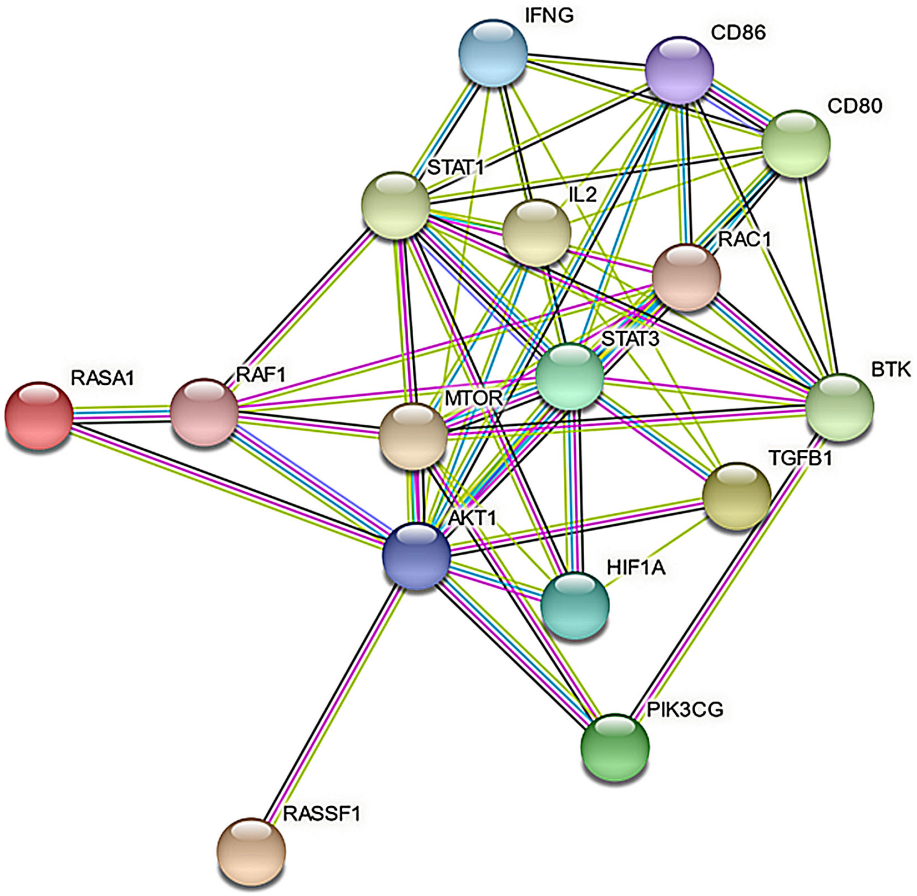

Figure 2. Protein-protein interaction network diagram
图 2. 蛋白－蛋白互作网络图

Figure 3. Drug-component-target-pathway network (Green: single Chinese medicine, yellow: Chinese medicine chemical composition, orange: protein target, blue: acting pathway)
图 3. 药物－成分－靶点－通路网络图(绿色：单味中药，黄色：中药化学成分，橙色：蛋白靶标，兰色：作用途径)

3.3. 靶点生物学功能分析

于 Rstudio 软件中加载 clusterProfiler 扩展包，对 PPI 网络中筛选出的核心靶点进行 KEGG 通路富集和 Gene Ontology (GO)功能富集分析，pvalueCutoff 与 qvalueCutoff 值均设定为 0.05，得到扶正抑瘤方抗肝癌过程中的生物过程(Biological Process, BP)、细胞组分(Cellular Component, CC)、分子功能(Molecular Function, MF)与 KEGG 通路富集情况，进一步利用 ggplot2 扩展包绘图使结果可视化，绘制条形图及气泡图。

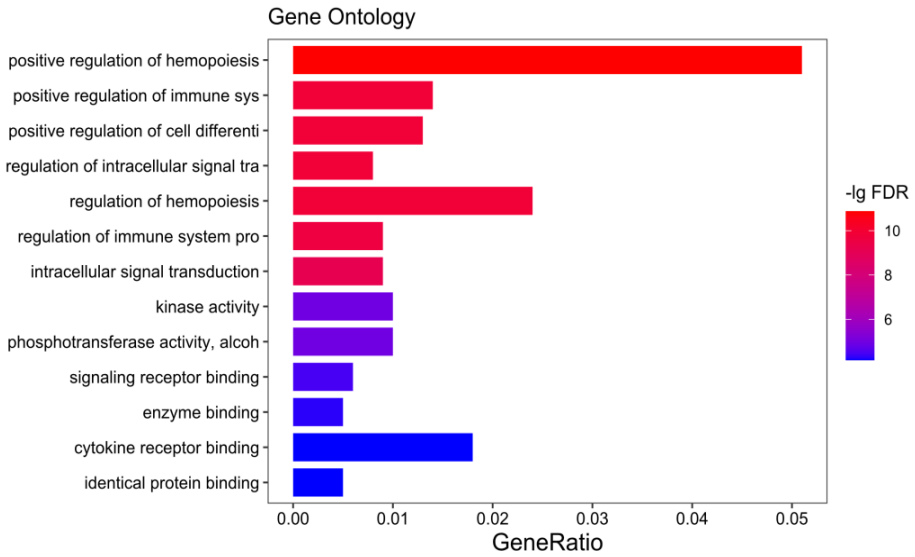

Figure 4. The GO enrichment analysis chart
图 4. GO 富集分析图

由图 4 可知，靶点参与免疫系统过程的正向调节、细胞分化的正调控、细胞内信号转导的调节、免疫系统过程的调节、细胞内信号转导等生物过程，主要分布在质膜有界细胞投影及肥大细胞颗粒，涉及磷酸转移酶活性、信号受体结合、细胞因子受体结合、酶结合等分子功能。

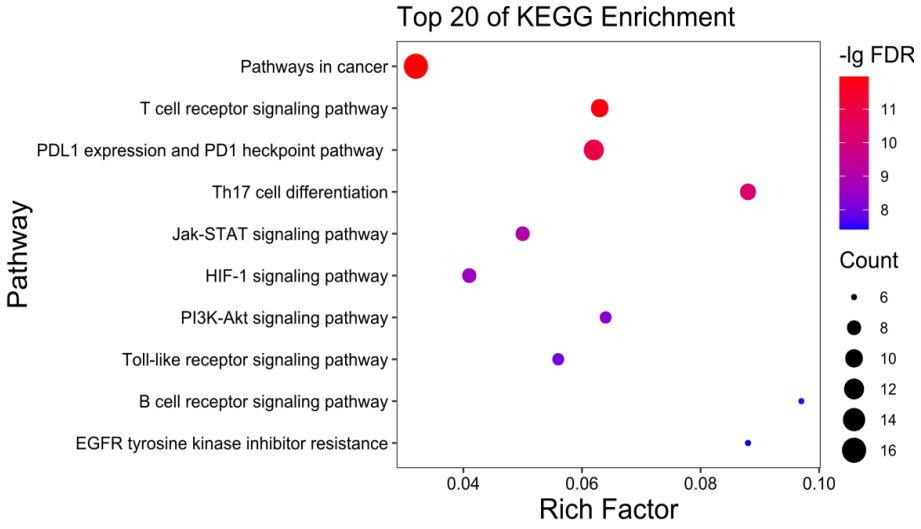

Figure 5. The KEGG enrichment analysis chart
图 5. KEGG 富集分析图

由图 5 结果可以推断，扶正抑瘤方抗肿瘤作用机制可能和 PD-L1 表达与 PD-1 检查点通路(PDL1 expression and PD1 checkpoint pathway in cancer)及 T 细胞受体信号通路(T cell receptor signaling pathway)高度相关。

PD-1 及其配体 PD-L1 是关键的调节性生理免疫检查点，其可调节 T 细胞和 B 细胞在其他免疫细胞类型中的活化程度，维持生物体的自我耐受[5]。在实体瘤中，肿瘤细胞通过增加细胞表面 PD-L1 的表达以沉默免疫系统。PD-L1 的调控受 HIF-1，STAT1，STAT3 影响[6]，阻断 PD-1/PD-L1 途径的治疗性抗体纳武利尤单抗(Opdivo)在肿瘤的临床治疗上已被广泛使用，具有较好的疗效[7]。

受 CTLA4 介导的 T 细胞受体以及共刺激分子(例如 CD28)通过与 CD28 反受体 B7-1/B7-2 分别相关的外源抗原(CD80, CD86)的相互作用导致一系列信号级联反应[8]，包括蛋白质－酪氨酸激酶，磷酸酶，GTP 结合蛋白和衔接子蛋白等[9]，从而对 T 细胞增殖，细胞因子产生和分化成效应细胞产生影响。

4. 讨论

本研究通过 ETCM、TCMSP 数据库筛选扶正抑瘤方中各味药物活性成分 195 个，共涉及相关作用靶标 676 个。从 MalaCards、DisGeNET 等数据库中筛选得到免疫检查点相关核心靶标 44 个，其中 16 个靶点与扶正抑瘤方作用靶点重合。通过构建“药物－成分－靶点－通路”网络图，体现中医药治疗疾病中多成分多靶点联合调控的作用特点。

研究提示，扶正抑瘤方主要通过 PD-L1 表达于 PD-1 检查点通路及 CTLA4 介导的 T 细胞受体信号通路发挥抗肿瘤作用，涉及 HIF1A、STAT1、STAT3、CD80、CD86 等关键靶标。在临床上，PD1 抑制剂纳武利尤单抗(Opdivo)与 CTLA4 抑制剂伊匹木单抗(Yervoy)联用是美国 FDA 批准的首个也是唯一一个双重免疫疗法，“OY 组合”已获 FDA 批准运用于涵盖肝细胞癌，结直肠癌，肾细胞癌、黑色素瘤、非小细胞肺癌在内的五种类型癌症[10]。二者同属免疫检查点抑制剂，分别作用于抗肿瘤免疫的不同阶段。CTLA4 抑制剂主要作用于 T 细胞发育早期，PD1 抑制剂则主要在 T 细胞的效应阶段起作用。二者联用具有协同增效作用，III 期临床试验证明，与传统化疗相比，双重免疫疗法显著改善了患者总生存期(OS)，($p < 0.05$)及总体缓解率(ORR)，($p < 0.05$) [11]。

5. 结论

本研究通过网络药理学分析构建了扶正抑瘤方“药物－成分－靶点－通路”复杂网络图，探讨扶正抑瘤方抗肿瘤的可能作用机制。结果表明扶正抑瘤方可通过多成分、多靶点、多通路发挥抗肿瘤作用，主要涉及 PD-L1 表达与 PD-1 检查点通路及 CTLA4 介导的 T 细胞受体信号通路，为后续深入研究扶正抑瘤方治疗癌症的作用机制奠定基础。

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