牛磺鹅去氧胆酸抗炎作用机制的网络药理学研究

李　磊[1], 图门巴雅尔[2], 李培锋[3], 刘　畅[1*], 李升和[1*], 宁康健[1], 姜锦鹏[1]

(1. 安徽科技学院动物科学学院, 农业农村部猪肉质量安全控制重点实验室, 安徽 凤阳 233100;
2. 山西省动物疫病预防控制中心, 山西 太原 030027; 3. 内蒙古农业大学兽医学院, 农业农村部动物疾病临床诊疗技术重点实验室, 内蒙古 呼和浩特 010018)

摘要: 为了探讨牛磺鹅去氧胆酸 (taurochenodeoxycholic acid, TCDCA) 的抗炎作用机制, 通过 PubChem 查找 TCDCA 的分子结构并下载 SDF 格式文件, 经 PharmMapper 服务器、GeneCards 数据库预测并筛选 TCDCA 抗炎靶点。将靶点导入 STRING 数据库得到蛋白互作关系并通过 Cytoscape 进行可视化处理, 然后将靶点导入 STRING 数据库进行 GO 及 KEGG 通路分析, 通过分子对接对 TCDCA 与主要靶点结合活性进行验证, 并通过 DisGeNET 数据库获取靶点类型信息。筛选得到 TCDCA 涉及抗炎作用的靶点 89 个, 网络分析结果表明, TCDCA 主要涉及刺激反应 (response to stimulus)、多细胞生物过程 (multicellular organismal process)、单细胞生物过程 (single-multicellular organism process)、对化学刺激反应 (response to chemical) 及有机物反应 (response to organic substance) 等 68 个生物过程, 通过调节癌症相关通路 (pathways in cancer)、孕酮介导的卵母细胞成熟 (progesterone-mediated oocyte maturation)、MAPK 信号通路 (MAPK signaling pathway)、蛋白多糖在癌症中的作用 (proteoglycans in cancer) 等 51 条信号通路来发挥抗炎作用。本研究反映出 TCDCA 经多靶点、多途径发挥抗炎作用的特点, 为后续开展其抗炎作用机制的研究指明了方向。

关键词: 牛磺鹅去氧胆酸; 炎症; 网络药理学; 药理机制; 分子对接

中图分类号: R967　　**文献标识码**: A　　**文章编号**: 0513-4870 (2018) 12-2064-12

Mechanisms of anti-inflammation of taurochenodeoxycholic acid based on network pharmacology

LI Lei[1], TUMEN Ba-ya-er[2], LI Pei-feng[3], LIU Chang[1*], LI Sheng-he[1*], NING Kang-jian[1], JIANG Jin-peng[1]

(*1. Key Laboratory of Quality and Safety Control for Pork, Ministry of Agriculture and Rural, College of Animal Science, Anhui Science and Technology University, Fengyang 233100, China; 2. Shanxi Animal Disease Control Center, Taiyuan 030027, China; 3. Key Laboratory of Clinical Diagnosis and Treatment Techniques for Animal Disease, Ministry of Agriculture and Rual, College of Veterinary Medicine, Inner Mongolia Agricultural University, Hohhot 010018, China*)

Abstract: To investigate the anti-inflammatory mechanisms of taurochenodeoxycholic acid (TCDCA), the molecule structure file of TCDCA was downloaded from PubChem database, PharmMapper and GeneCards were used to predict and screen the targets of TCDCA. STRING database and Cytoscape software were used to construct protein interactions network. GO and KEGG analysis was preformed through STRING database. The key targets were validated by molecular docking and the targets type was attributed by DisGeNET database.

收稿日期: 2018-07-01; 修回日期: 2018-07-31.

基金项目: 国家自然科学基金资助项目 (31802242); 安徽省高校自然科学研究重点项目 (KJ2017A503, KJ2017A504); 安徽科技学院人才引进项目 (ZRC2014447); 安徽科技学院重点学科建设项目 (AKZDXK2015A04).

*通讯作者 Tel: 86-550-6732040, E-mail: liuchang052@126.com; lish@ahstu.edu.cn

DOI: 10.16438/j.0513-4870.2018-0600

The network showed that 89 targets were involved in 68 biological processes including response to stimulus, multicellular organismal process, single-multicellular organism process, response to chemical, response to organic substance, by adjusting 51 signaling pathways, such as pathways in cancer, progesterone-mediated oocyte maturation, MAPK signaling pathway, proteoglycans in cancer. These findings provide an overview of anti-inflammation of TCDCA, which reflects the characteristic of multi-targets and multi-pathways of TCDCA. It pointed out the direction for further research on anti-inflammatory mechanism of TCDCA.

Key words: taurochenodeoxycholic acid; inflammation; network pharmacology; pharmacological mechanism; molecular docking

胆汁, 性味苦寒, 具有清热解毒、清肝明目、清肺止咳、滋阴润肺之功效, 为我国传统动物来源中药, 应用历史悠久, 因其来源广泛, 疗效确切, 备受关注[1]。早在《神农本草经》中就有关于牛胆的记载, 后来的《唐本草》中还记载了熊胆汁的应用, 《本草纲目》与《中华本草》中更是分别收录了 31 和 44 种动物胆汁[2], 上千年的用药历史证明了胆汁的确切疗效。

现代药理学研究发现, 动物胆汁在治疗呼吸系统疾病 (如急慢性气管炎、咳嗽等)、肝胆疾病 (如胆固醇性结石)、消化系统疾病 (如小儿单纯性消化不良) 等方面具有显著作用[3, 4], 此外, 动物胆汁还具有解热镇痛[5]、抑菌、抗炎[6, 7]、免疫调节[8]等多方面的药理作用。不同动物胆汁组成成分略有差异, 但均主要包含胆汁酸、胆色素、脂类、蛋白、微量元素等成分, 其中胆汁酸被认为是胆汁发挥功效的物质基础。按照来源可将胆汁酸分为初级和次级胆汁酸, 前者由胆固醇经多次酶促反应在肝脏合成, 后者主要由前者在肠道细菌水解后经 7-位脱羟基作用而产生; 按照化学结构可将胆汁酸分为游离型和结合型胆汁酸。

在各类型胆汁酸中, 牛磺鹅去氧胆酸 (taurochenodeoxycholic acid, TCDCA, 图 1) 作为结合型胆汁酸主要存在于多种动物 (如鸡、鸭、鹅、蛇等) 胆汁中, 不同动物胆汁中 TCDCA 含量有一定差异, 其中以鸡、鸭、鹅等家禽胆汁含量较高, 均超过 38%[9]。课题组前期研究中发现, TCDCA 对多种原因引起的急慢性炎症反应均具有良好的抑制作用[10], 但其确切作用机制尚未阐明, 本研究拟通过网络药理学分析 TCDCA 抗炎作用机制, 为深入揭示 TCDCA 的药理作用提供支持。

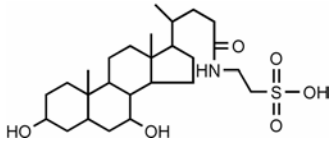

Figure 1　The chemical structure of taurochenodeoxycholic acid (TCDCA)

材料与方法

TCDCA 作用靶点的获取　登录 PubChem 服务器, 查询并下载 TCDCA 的 3D 分子结构式, 储存为 SDFile (*.sdf) 格式。然后将 TCDCA.sdf 文件输入 PharmMapper 服务器[11], 获得 TCDCA 作用靶点。利用 UniProt 数据库的 Retrieve/ID Mapping 功能[12], 将靶点的 UniProt ID 转换为 Gene Symbol。

炎症相关靶点的筛选　在 GeneCards 服务器输入 inflammation 和 anti-inflammation 搜索已报道的炎症相关基因[13], 去除重复基因, 与 PharmMapper 返回的 TCDCA 靶点进行匹配, 得到 TCDCA 抗炎作用靶点。

蛋白相互作用网络构建分析　将 TCDCA 抗炎作用靶点信息导入 STRING 数据库 (https://string-db.org/, Version 10.5)[14], 设定物种为人, 可获得靶点蛋白相互作用关系, 从结果中提取 node1、node2 及 combine score 等相关信息导入 Cytoscape 程序构建靶点蛋白网络[15], 并对网络进行分析, 保存分析结果, 设置结点颜色和大小以反映 degree 的大小, 设置边的粗细反映 combine score 的大小, 建立靶点蛋白相互作用网络。

生物功能与通路分析　将 TCDCA 作用靶点信息输入 STRING 数据库, 进行 GO 富集分析和 KEGG 通路注释分析, 保存结果, 设定阈值为 $P<0.05$, 筛选生物过程或通路。

分子对接验证　选择 KEGG 通路中富集的靶点, 查找其 PDB ID, 输入 Systems Dock Web Site (http://systemsdock.unit.oist.jp, Version 2.0) 与 TCDCA 进行分子对接[16], 保存对接结果, 对其对接打分进行分析, 用以评价 TCDCA 与各靶点间的结合活性。

靶点类型归属　将能够与 TCDCA 发生对接的靶点信息输入 DisGeNET 数据库[17], 获取靶点类型相关信息。

结果

1　靶点预测

将 TCDCA 在 PharmMapper 服务器返回的前 100

个潜在作用靶点依据 fit score 进行排序，通过 UniProt 数据库将 UniProt ID 转换为 Gene Symbol，然后与 GeneCards 中炎症相关基因进行比对，筛选出 89 个 TCDCA 抗炎的潜在作用靶点，详见表 1。

2 蛋白相互作用网络构建与分析

将上述靶点蛋白信息导入 STRING 数据库，获得靶点蛋白相互作用关系，利用 Cytoscape 程序对靶点蛋白相互作用网络进行可视化处理 (图 2)。图中的节点表示靶点蛋白分子，边表示各靶点间的相互关系，共包含 85 个节点，356 条边 (其中 HRSP12、RORA、CRAT 和 ISG20 与其他靶点蛋白不存在相互作用，故不在网络中体现)。图中节点颜色和大小反映了靶点蛋白 degree 的值，颜色由绿变红、节点越大表示该靶点蛋白的 degree 值越大，反之表示 degree 值越小。边的粗细程度表示靶点蛋白间的 combine score 值，线条越粗，表示该值越大。

3 GO 分析结果

通过 STRING 数据库分析了 TCDCA 抗炎靶点的

Table 1 Information of potential targets from TCDCA

No.	UniProt ID	Fit score	Gene symbol	Protein name
1	P22830	6.231	FECH	Ferrochelatase
2	P06702	5.847	S100A9	S100 calcium binding protein A9
3	P28845	5.427	HSD11B1	Hydroxysteroid 11-beta dehydrogenase 1
4	P51161	5.344	FABP6	Fatty acid binding protein 6
5	Q04828	3.81	AKR1C1	Aldo-keto reductase family 1 member C1
6	Q96AZ6	3.786	ISG20	Interferon stimulated exonuclease gene 20
7	P24941	3.775	CDK2	Cyclin dependent kinase 2
8	P50579	3.623	METAP2	Methionine aminopeptidase 2
9	P08235	3.61	NR3C2	Nuclear receptor subfamily 3 group C member 2
10	P02768	3.595	ALB	Albumin
11	Q13370	3.584	PDE3B	Phosphodiesterase 3B
12	Q06520	3.571	SULT2A1	Sulfotransferase family 2A member 1
13	P22894	3.553	MMP8	Matrix metallopeptidase 8
14	Q01469	3.513	FABP5	Fatty acid binding protein 5
15	P49638	3.453	TTPA	Alpha tocopherol transfer protein
16	Q16539	3.433	MAPK14	Mitogen-activated protein kinase 14
17	P11142	3.369	HSPA8	Heat shock protein family A (Hsp70) member 8
18	P02774	3.341	GC	GC, vitamin D binding protein
19	Q07343	3.119	PDE4B	Phosphodiesterase 4B
20	Q13231	3.11	CHIT1	Chitinase 1
21	P27487	2.997	DPP4	Dipeptidyl peptidase 4
22	P52895	2.996	AKR1C2	Aldo-keto reductase family 1 member C2
23	P00325	2.981	ADH1B	Alcohol dehydrogenase 1B (class I), beta polypeptide
24	P04818	2.978	TYMS	Thymidylate synthetase
25	P35398	2.975	RORA	RAR related orphan receptor A
26	P10275	2.974	AR	Androgen receptor
27	P18031	2.961	PTPN1	Protein tyrosine phosphatase, non-receptor type 1
28	P00533	2.958	EGFR	Epidermal growth factor receptor
29	P45452	2.956	MMP13	Matrix metallopeptidase 13
30	P11309	2.954	PIM1	Pim-1 proto-oncogene, serine/threonine kinase
31	P47929	2.947	LGALS7	Galectin 7
32	P12931	2.945	SRC	SRC proto-oncogene, non-receptor tyrosine kinase
33	P49354	2.942	FNTA	Farnesyltransferase, CAAX box, alpha
34	P43155	2.939	CRAT	Carnitine *O*-acetyltransferase
35	P09211	2.936	GSTP1	Glutathione *S*-transferase pi 1
36	P80188	2.936	LCN2	Lipocalin 2
37	P00374	2.925	DHFR	Dihydrofolate reductase
38	P00734	2.925	F2	Coagulation factor II, thrombin
39	P00491	2.903	PNP	Purine nucleoside phosphorylase

Continued

No.	UniProt ID	Fit score	Gene symbol	Protein name
40	P02766	2.899	TTR	Transthyretin
41	P78536	2.896	ADAM17	ADAM metallopeptidase domain 17
42	P11586	2.893	MTHFD1	Methylenetetrahydrofolate dehydrogenase, cyclohydrolase and formyltetrahydrofolate synthetase 1
43	P25311	2.893	AZGP1	Alpha-2-glycoprotein 1, zinc-binding
44	P03372	2.888	ESR1	Estrogen receptor 1
45	P15056	2.885	BRAF	Serine/threonine-protein kinase B-raf
46	P53779	2.885	MAPK10	Mitogen-activated protein kinase 10
47	P00742	2.881	F10	Coagulation factor X
48	P00918	2.877	CA2	Carbonic anhydrase 2
49	P26196	2.873	DDX6	DEAD-box helicase 6
50	P00747	2.872	PLG	Plasminogen
51	P06401	2.87	PGR	Progesterone receptor
52	P43235	2.867	CTSK	Cathepsin K
53	P08758	2.864	ANXA5	Annexin A5
54	Q13126	2.853	MTAP	Methylthioadenosine phosphorylase
55	P29474	2.851	NOS3	Nitric oxide synthase 3
56	O14965	2.848	AURKA	Aurora kinase A
57	P04062	2.846	GBA	Glucosylceramidase beta
58	P07900	2.836	HSP90AA1	Heat shock protein 90 alpha family class A member 1
59	P68400	2.836	CSNK2A1	Casein kinase 2 alpha 1
60	P04278	2.829	SHBG	Sex hormone binding globulin
61	P03950	2.809	ANG	Angiogenin
62	P15090	2.806	FABP4	Fatty acid binding protein 4
63	O15530	2.806	PDPK1	3-Phosphoinositide dependent protein kinase 1
64	P02743	2.792	APCS	Amyloid P component, serum
65	P18075	2.785	BMP7	Bone morphogenetic protein 7
66	Q15075	2.78	EEA1	Early endosome antigen 1
67	P20248	2.759	CCNA2	Cyclin A2
68	P23141	2.755	CES1	Carboxylesterase 1
69	P42330	2.732	AKR1C3	Aldo-keto reductase family 1 member C3
70	P14174	2.721	MIF	Macrophage migration inhibitory factor (glycosylation-inhibiting factor)
71	P00915	2.706	CA1	Carbonic anhydrase 1
72	P12643	2.652	BMP2	Bone morphogenetic protein 2
73	P28482	2.646	MAPK1	Mitogen-activated protein kinase 1
74	P62937	2.643	PPIA	Peptidylprolyl isomerase A
75	O14757	2.628	CHEK1	Checkpoint kinase 1
76	P15121	2.622	AKR1B1	Aldo-keto reductase family 1 member B
77	P08842	2.616	STS	Steroid sulfatase (microsomal), isozyme S
78	P02652	2.586	APOA2	Apolipoprotein A2
79	P00751	2.549	CFB	Complement factor B
80	P30044	2.546	PRDX5	Peroxiredoxin 5
81	P07339	2.521	CTSD	Cathepsin D
82	P45983	2.47	MAPK8	Mitogen-activated protein kinase 8
83	P37231	2.377	PPARG	Peroxisome proliferator activated receptor gamma
84	P56817	2.373	BACE1	Beta-secretase 1
85	P42574	2.342	CASP3	Caspase 3
86	P20701	2.256	ITGAL	Integrin subunit alpha L
87	P52758	2.195	RIDA	Reactive intermediate imine deaminase A homolog
88	P49137	2.192	MAPKAPK2	Mitogen-activated protein kinase-activated protein kinase 2
89	P23946	2.055	CMA1	Chymase 1

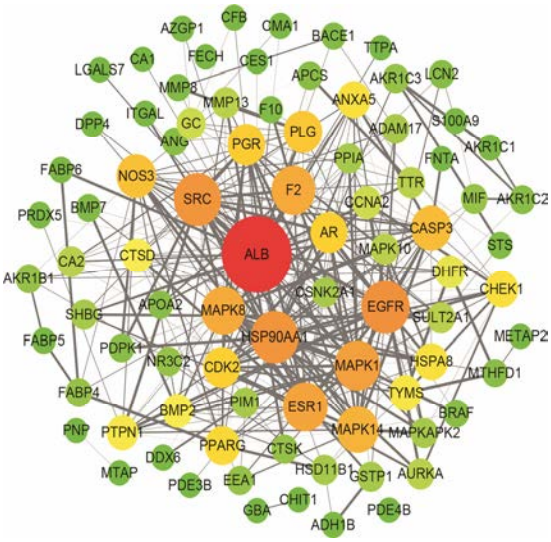

Figure 2 Target protein interaction network of TCDCA

生物学过程、分子功能和细胞成分，结果表明，这些靶点被富集到 68 个生物学过程中 (表 2)，其中排名前 5 的生物学过程主要包括：对刺激的反应 (response to stimulus)、多细胞生物过程 (multicellular organismal process)、单细胞生物过程 (single-multicellular organism process)、对化学刺激的反应 (response to chemical) 及对有机物的反应 (response to organic substance) 等。分子功能 GO 分析表明 (表 3)，TCDCA 的靶点主要富集于蛋白结合 (protein binding)、脂质结合 (lipid binding)、受体结合 (receptor binding)、脂肪酸结合 (fatty acid binding)、受体信号蛋白丝氨酸/苏氨酸激酶活性 (receptor signaling protein serine/threonine kinase activity) 及 MAPK 蛋白激酶活性 (MAP kinase activity) 等 6 方面。在细胞组分方面 (表 4)，这些靶点主要为

Table 2 Gene ontology terms for biological process of TCDCA

GO-ID	GO term	Degree	*P*-value
GO.0050896	Response to stimulus	58	2.06E-02
GO.0032501	Multicellular organismal process	51	1.61E-02
GO.0044707	Single-multicellular organism process	50	1.02E-02
GO.0042221	Response to chemical	47	7.79E-03
GO.0010033	Response to organic substance	39	3.56E-03
GO.0065008	Regulation of biological quality	37	1.54E-02
GO.0009628	Response to abiotic stimulus	31	3.89E-07
GO.0050790	Regulation of catalytic activity	31	2.58E-02
GO.0051246	Regulation of protein metabolic process	31	2.58E-02
GO.0009719	Response to endogenous stimulus	30	3.47E-04
GO.0042127	Regulation of cell proliferation	30	5.75E-03
GO.0002376	Immune system process	29	1.61E-02
GO.0033993	Response to lipid	28	3.51E-06
GO.0009605	Response to external stimulus	28	3.99E-03
GO.0080134	Regulation of response to stress	27	2.02E-03
GO.1901700	Response to oxygen-containing compound	27	3.89E-03
GO.0009725	Response to hormone	26	5.28E-05
GO.0014070	Response to organic cyclic compound	25	2.95E-05
GO.0031399	Regulation of protein modification process	24	2.35E-02
GO.0006952	Defense response	23	1.61E-02
GO.0042592	Homeostatic process	23	1.61E-02
GO.0006955	Immune response	23	2.85E-02
GO.0048545	Response to steroid hormone	21	2.42E-06
GO.0051240	Positive regulation of multicellular organismal process	21	4.73E-02
GO.0051338	Regulation of transferase activity	19	1.61E-02
GO.0010243	Response to organonitrogen compound	18	9.65E-03
GO.0031347	Regulation of defense response	18	1.24E-02
GO.0032101	Regulation of response to external stimulus	18	2.58E-02
GO.0045859	Regulation of protein kinase activity	17	1.92E-02
GO.0009611	Response to wounding	17	2.58E-02
GO.1901698	Response to nitrogen compound	17	2.58E-02
GO.0050878	Regulation of body fluid levels	17	3.69E-02
GO.0042060	Wound healing	16	3.01E-02

Continued

GO-ID	GO term	Degree	*P*-value
GO.0043627	Response to estrogen	15	6.63E-06
GO.0071396	Cellular response to lipid	15	5.47E-03
GO.0007596	Blood coagulation	15	2.48E-02
GO.0032870	Cellular response to hormone stimulus	15	4.84E-02
GO.0097305	Response to alcohol	14	3.04E-03
GO.0071900	Regulation of protein serine/threonine kinase activity	14	1.24E-02
GO.1903034	Regulation of response to wounding	14	1.61E-02
GO.0001775	Cell activation	14	2.06E-02
GO.1901652	Response to peptide	13	1.61E-02
GO.0071407	Cellular response to organic cyclic compound	13	1.70E-02
GO.1901615	Organic hydroxy compound metabolic process	13	4.77E-02
GO.0030198	Extracellular matrix organization	12	1.70E-04
GO.0009314	Response to radiation	11	2.84E-02
GO.0022411	Cellular component disassembly	10	7.79E-03
GO.0071383	Cellular response to steroid hormone stimulus	10	7.79E-03
GO.0044772	Mitotic cell cycle phase transition	10	9.65E-03
GO.0009416	Response to light stimulus	10	1.70E-02
GO.0008202	Steroid metabolic process	10	1.91E-02
GO.0050727	Regulation of inflammatory response	10	2.58E-02
GO.0010038	Response to metal ion	10	3.16E-02
GO.0030168	Platelet activation	9	3.31E-02
GO.0070482	Response to oxygen levels	9	3.78E-02
GO.0022617	Extracellular matrix disassembly	8	1.70E-04
GO.0032355	Response to estradiol	8	1.61E-02
GO.0043401	Steroid hormone mediated signaling pathway	7	3.78E-02
GO.0000302	Response to reactive oxygen species	7	4.38E-02
GO.0009636	Response to toxic substance	7	4.66E-02
GO.2000379	Positive regulation of reactive oxygen species metabolic process	6	4.64E-02
GO.2001057	Reactive nitrogen species metabolic process	5	1.08E-02
GO.0030574	Collagen catabolic process	5	1.26E-02
GO.0031100	Organ regeneration	5	3.78E-02
GO.0046464	Acylglycerol catabolic process	4	1.92E-02
GO.0006730	One-carbon metabolic process	4	3.86E-02
GO.0031659	Positive regulation of cyclin-dependent protein serine/threonine kinase activity involved in G_1/S transition of mitotic cell cycle	3	7.79E-03
GO.0060694	Regulation of cholesterol transporter activity	2	2.58E-02

Table 3　Gene ontology terms for molecular functions of TCDCA

GO-ID	GO term	Degree	*P*-value
GO.0005515	Protein binding	50	3.05E-03
GO.0008289	Lipid binding	19	1.49E-03
GO.0005102	Receptor binding	19	3.99E-02
GO.0005504	Fatty acid binding	6	3.05E-03
GO.0004702	Receptor signaling protein serine/threonine kinase activity	6	2.19E-02
GO.0004707	MAP kinase activity	4	5.87E-03

细胞质 (cytoplasmic part、cytosol)、细胞外成分 (extracellular region)、膜结合囊泡 (membrane-bounded vesicle)、囊泡 (vesicle)、外泌体 (extracellular exosome) 与内涵体 (endosome) 等。

Table 4　Gene ontology terms for cellular components of TCDCA

GO-ID	GO term	Degree	*P*-value
GO.0044444	Cytoplasmic part	56	3.88E-02
GO.0005829	Cytosol	47	7.09E-06
GO.0005576	Extracellular region	46	3.22E-04
GO.0044421	Extracellular region part	44	2.49E-04
GO.0031988	Membrane-bounded vesicle	42	9.87E-03
GO.0031982	Vesicle	42	1.45E-02
GO.0043233	Organelle lumen	40	2.06E-02
GO.0070062	Extracellular exosome	38	3.98E-03
GO.0005615	Extracellular space	31	1.93E-09
GO.0005768	Endosome	11	4.51E-02
GO.0072562	Blood microparticle	9	3.22E-04

4 KEGG 分析结果

TCDCA 所有潜在抗炎靶点中共有 44 个靶点富集到 51 条信号通路 (表 5), 排名靠前的通路包括癌症相关通路 (pathways in cancer)、孕酮介导的卵母细胞成熟 (progesterone-mediated oocyte maturation)、MAPK 信号通路 (MAPK signaling pathway)、蛋白多糖在癌症中的作用 (proteoglycans in cancer) 等。此外, 如胰岛素信号通路 (insulin signaling pathway)、FoxO 信号通路 (FoxO signaling pathway)、雌激素信号通路 (estrogen signaling pathway)、PPAR 信号通路 (PPAR signaling pathway)、NOD 样信号通路 (NOD-like receptor signaling pathway)、Toll 样受体信号通路 (Toll-like receptor signaling pathway)、肿瘤坏死因子信号通路 (TNF signaling pathway) 及血管内皮生长因子受体信号通路 (VEGF signaling pathway) 等均与炎症反应密切相关, 体现了 TCDCA 多途径的作用特点。

5 分子对接分析

将上述富集到 51 条通路中的 44 个靶点的 PDB ID 输入到 System Dock Web Site 服务器中, 采用分子对接的方式进一步确认这些靶点与 TCDCA 的结合活性, 研究发现只有 24 个分子可以与 TCDCA 发生结合, 其中 docking score 大于 7.0 的有 18 个靶点, TCDCA 与其中 5 个靶点的打分甚至超过 8.0 (表 6, 图 3), 介于 5.0～7.0 之间的有 6 个靶点。该对接程序认为 docking score 大于 4.25 表示药物分子与靶蛋白间存在结合能力, 大于 5.0 时表示结合活性较强, 大于 7.0 时表示结合能力极强[18]。分子对接结果表明, TCDCA 与预测靶点的结合能力均较强, 体现了 TCDCA 多靶点的作用特点。

6 作用靶点归属

将上述可与 TCDCA 结合的 24 个靶点信息输入 DisGeNET 数据库查找各靶点作用类型 (protein class, 表 7), 发现这些靶点多数为蛋白激酶、氧化还原酶、转录因子、受体等, 表明 TCDCA 可作为信号分子与这些靶点发生结合, 调控相关靶点的生物学功能, 这与近年来对于胆汁酸生理功能的研究结果相吻合[19, 20]。

Table 5 Enriched KEGG pathways of potential targets of TCDCA

No.	Pathway	Count	*P*-value	No.	Pathway	Count	*P*-value
1	Pathways in cancer	11	3.33E-03	26	NOD-like receptor signaling pathway	5	1.69E-03
2	Progesterone-mediated oocyte maturation	9	3.18E-06	27	Shigellosis	5	1.69E-03
3	MAPK signaling pathway	9	4.48E-03	28	Pertussis	5	2.28E-03
4	Proteoglycans in cancer	8	2.56E-02	29	Metabolism of xenobiotics by cytochrome P450	5	3.23E-03
5	Epithelial cell signaling in *Helicobacter pylori* infection	7	7.95E-05	30	Colorectal cancer	5	3.23E-03
				31	Pancreatic cancer	5	4.31E-03
6	Toxoplasmosis	7	3.23E-03	32	Toll-like receptor signaling pathway	5	6.87E-03
7	Tuberculosis	7	3.23E-03	33	Adherens junction	5	1.02E-02
8	Hepatitis C	7	3.23E-03	34	TNF signaling pathway	5	1.11E-02
9	Neurotrophin signaling pathway	7	4.77E-03	35	VEGF signaling pathway	5	1.30E-02
10	Insulin signaling pathway	7	5.83E-03	36	Fc epsilon RI signaling pathway	5	1.78E-02
11	FoxO signaling pathway	7	7.07E-03	37	Steroid hormone biosynthesis	4	7.32E-03
12	Estrogen signaling pathway	7	7.32E-03	38	Salmonella infection	4	9.12E-03
13	Prostate cancer	7	7.32E-03	39	Complement and coagulation cascades	4	1.20E-02
14	Focal adhesion	7	1.11E-02	40	Chemical carcinogenesis	4	1.23E-02
15	Epstein-Barr virus infection	7	2.08E-02	41	Natural killer cell mediated cytotoxicity	4	2.83E-02
16	ErbB signaling pathway	6	2.28E-03	42	Endometrial cancer	4	3.36E-02
17	GnRH signaling pathway	6	3.23E-03	43	Chagas disease (American trypanosomiasis)	4	3.49E-02
18	Prolactin signaling pathway	6	4.10E-03	44	Retrograde endocannabinoid signaling	4	3.79E-02
19	PPAR signaling pathway	6	4.20E-03	45	One carbon pool by folate	3	5.83E-03
20	Osteoclast differentiation	6	7.32E-03	46	Thyroid cancer	3	9.76E-03
21	Hepatitis B	6	7.32E-03	47	Staphylococcus aureus infection	3	1.75E-02
22	Influenza A	6	8.94E-03	48	Bladder cancer	3	2.15E-02
23	Viral carcinogenesis	6	1.20E-02	49	Type II diabetes mellitus	3	2.75E-02
24	Regulation of actin cytoskeleton	6	1.88E-02	50	RIG-I-like receptor signaling pathway	3	2.89E-02
25	Rap1 signaling pathway	6	2.08E-02	51	Dorso-ventral axis formation	2	4.63E-02

Table 6　Molecular docking of TCDCA

No.	Gene symbol	PDB ID	Docking score (p*K*d/p*K*i)	Count	No.	Gene symbol	PDB ID	Docking score (p*K*d/p*K*i)	Count
1	HSPA8	4H5W	9.115	5	13	MAPK10	2EXC	7.423	31
2	CASP3	2XZD	8.404	12	14	MAPK8	3PZE	7.358	31
3	ADH1B	1U3U	8.234	2	15	GSTP1	2A2R	7.255	4
4	PPARG	1ZGY	8.068	4	16	MAPK14	1A9U	7.051	26
5	HSP90AA1	4BQG	8.035	5	17	EGFR	2GS7	7.026	18
6	MAPK1	3W55	7.865	41	18	ESR1	3CBP	7.025	3
7	HSD11B1	2IRW	7.819	3	19	AR	2PIV	6.291	2
8	CSNK2A1	3OWJ	7.798	2	20	BRAF	3NY5	5.835	19
9	ITGAL	3BQM	7.667	5	21	PLG	1A7C	5.824	3
10	AKR1C1	4YVP	7.653	2	22	SRC	1O4R	5.817	15
11	CCNA2	2CCH	7.595	4	23	PDPK1	1W1H	5.573	11
12	MAPKAPK2	3FYJ	7.499	4	24	CFB	2OK5	5.229	2

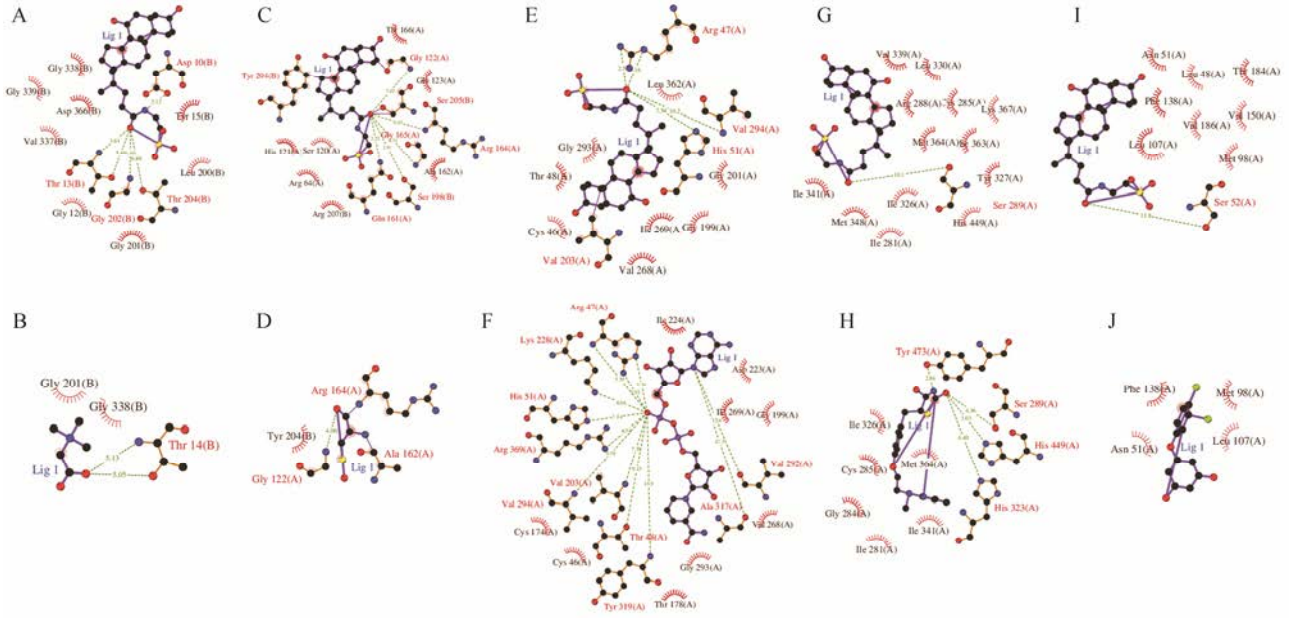

Figure 3　Results of molecular docking.　A, C, E, G, I: Molecular docking results for TCDCA with HSPA8, CASP3, ADH1B, PPARG and HSP90AA1 respectively; B, D, F, H, J: Positive control

Table 7　The protein class of potential anti-inflammation targets of TCDCA

No.	Gene symbol	Protein class	No.	Gene symbol	Protein class
1	HSPA8	Null	14	MAPK8	Transferase; kinase
2	CASP3	Protease; enzyme modulator; hydrolase	15	GSTP1	Null
3	ADH1B	Oxidoreductase	16	MAPK14	Transferase; kinase
4	PPARG	Transcription factor; receptor; nucleic acid binding	17	EGFR	Null
			18	ESR1	Transcription factor; receptor; nucleic acid binding
5	HSP90AA1	Chaperone			
6	MAPK1	Transferase; kinase	19	AR	Transcription factor; receptor; nucleic acid binding
7	HSD11B1	Oxidoreductase			
8	CSNK2A1	Null	20	BRAF	Transferase; kinase
9	ITGAL	Null	21	PLG	Protease; hydrolase
10	AKR1C1	Oxidoreductase	22	SRC	Transferase; kinase
11	CCNA2	Enzyme modulator	23	PDPK1	Transfer/carrier protein; transferase; calcium-binding protein; kinase
12	MAPKAPK2	Cytoskeletal protein; transferase; kinase			
13	MAPK10	Transferase; kinase	24	CFB	Null

讨论

胆汁酸具有乳化脂肪、增强脂肪酶活性，促进脂肪及脂溶性维生素的消化与吸收，促进胆汁分泌防止胆石生成等作用[21, 22]。近年来发现，胆汁酸可作为信号分子，激活细胞内信号转导通路发挥抗炎、免疫调节等药理作用[19, 20]。研究发现，TCDCA 具有显著的抗炎、免疫调节作用，对物理、化学、细菌等原因引起的炎症反应均具有显著的抑制作用[10]，随着研究深入，本课题组以佐剂性关节炎 (adjuvant arthritis, AA) 模型为研究对象探讨了 TCDCA 对糖皮质激素受体 (glucocorticoid receptor, GR) 介导的信号通路的影响，结果表明 TCDCA 可激活 GR，显著抑制 AA 大鼠足跖肿胀，改善 AA 大鼠骨骼关节变形等情况，表现出明显的抗炎作用[23]，TCDCA 对 AA 的治疗作用与其抑制 AA 大鼠滑膜组织与细胞中核因子-κB (nuclear factor-κB, NF-κB) 转录活性，进而抑制肿瘤坏死因子-α (tumor necrosis factor-α, TNF-α)、白细胞介素 (interleukin, IL)-1β、IL-6 等细胞因子及细胞间黏附分子-1 的表达[23, 24]，以及诱导 AA 大鼠成纤维样滑膜细胞凋亡有关[25]，但同时研究也发现，阻断 GR 信号通路并不能完全拮抗 TCDCA 的抗炎作用，这表明还存在其他通路参与了 TCDCA 抗炎作用。因此，通过网络药理学的方式深入挖掘 TCDCA 的作用靶点对于揭示其作用机制具有重要意义。

本研究通过 PharmMapper 服务器与 GeneCards 数据库预测到 TCDCA 的潜在抗炎作用靶点共 89 个。接着通过 STRING 数据库分析发现，在这些靶点中，共有 85 个蛋白存在相互作用关系，这表明 TCDCA 抗炎作用并非依靠作用于单一靶点起效，而是多靶点彼此协同作用。随后利用 STRING 数据库对这些靶点进行了 GO 分析，结果表明，这些靶点主要分布于细胞外、细胞质、膜结合囊泡、外泌体、内涵体等部位，具有与蛋白、脂质、受体、脂肪酸结合的功能，还具有蛋白激酶的活性，可参与机体对多种物质刺激的反应过程，参与蛋白、脂质代谢，调控细胞增殖、免疫应答，调控多种蛋白激酶活性等作用，这表明 TCDCA 的抗炎作用可通过多层次、多途径实现。

对上述靶点进行 KEGG 通路分析发现，共有 44 个靶点可富集到 51 条信号通路中，其中 24 个靶点可与 TCDCA 发生结合，它们主要为酶类 (包括蛋白酶、蛋白激酶、氧化还原酶、转移酶、水解酶等)、受体、转录因子、转运蛋白、钙结合蛋白、伴侣分子、细胞骨架蛋白等。进一步分析发现，这些靶点中 MAPK1 (41)、MAPK8 (31)、MAPK10 (31)、MAPK14 (26)、BRAF (19)、EGFR (18)、SRC (15)、CASP3 (12)、PDPK1 (11) 等均参与了 10 条以上信号通路 (表 6)，以上靶点可能对 TCDCA 的抗炎作用具有重要的影响。

上述靶点中，MAPK1、MAPK8、MAPK10、MAPK14 均为丝裂原活化蛋白激酶 (mitogen-activated protein kinase, MAPK) 家族成员，它们是一组在真核生物中高度保守的蛋白激酶，其介导的信号转导通路在调节细胞增殖、分化、凋亡过程中发挥重要作用，此外，MAPK 通路还参与了机体对外界环境应激的适应、炎症反应等多种生理、病理过程，是多种抗炎药物作用的共同通路[26]。Dong 等[27]发现低浓度胆汁酸能够促进大鼠肝细胞 JNK 蛋白表达，Alpini 等[28]研究发现，TCDCA 的差向异构体牛磺熊去氧胆酸可通过 MAPK 通路抑制胆管上皮癌细胞系 Mz-ChA-1 细胞的增殖，Ko 等[29]报道证实，熊去氧胆酸 (ursodeoxycholic acid, UDCA) 能够调控 MAPK 和 NF-κB 通路抑制脂多糖 (lipopolysaccharide, LPS) 诱导 RAW 264.7 巨噬细胞中 IL-1β、TNF-α、IL-6 等细胞因子的表达，同时还能促进抗炎细胞因子 IL-10 的表达，以上研究表明胆汁酸可以调控不同细胞中的 MAPK 通路。Wang 等[30]报道了 TCDCA 可以通过 PKC/JNK 通路诱导 NR8383 细胞凋亡，这与本研究网络药理学的预测基本一致，表明 MAPK 通路可能是 TCDCA 发挥抗炎作用的重要途径。

BRAF 作为一种蛋白激酶参与了促有丝分裂信号从细胞膜到细胞核的传递，能够磷酸化 MAP2K1 激活 MAPK/ERK 信号通路。在类风湿性关节炎等炎症疾病中，BRAF-MAPK/ERK 信号通路被激活，促进成纤维样滑膜细胞增殖，可加重关节炎症反应[31]，Zhang[32]发现白芍药苷可抑制该通路显著缓解类风湿性关节炎。课题组前期研究发现，TCDCA 可促进细胞凋亡，抑制成纤维样滑膜细胞过度增殖[25]，在这一过程中 TCDCA 对 BRAF-MAPK/ERK 通路的影响尚不清楚。

表皮生长因子受体 (epidermal growth factor receptor, EGFR) 是一种糖蛋白，位于细胞膜表面，属于酪氨酸激酶受体，与其配体结合后形成二聚体，并发生磷酸化激活，活化的 EGFR 可进一步激活下游的 RAS-RAF-MEK-ERK、PI3K-AKT、PLC-PKC、STATs 与 NF-κB 等信号通路，而这些信号通路都与炎症反应密切相关。Im 等[33]发现，UDCA 可以通过调控

EGFR/Raf-1/ERK 通路抑制脱氧胆酸诱导的结肠癌细胞凋亡, 表明胆汁酸可通过调控 EGFR 相关通路发挥生物学效应[34]。Rust 等[35]报道, TCDCA 可以激活 EGFR 下游 PI3K/PKC 通路, 但对于 PI3K 下游另一重要蛋白 AKT 无影响; TCDCA 还可以抑制 NF-κB 的转录活性[36], 但由于 NF-κB 与多条信号通路均存在密切联系, TCDCA 是否能够抑制 EGFR 进而抑制 NF-κB 还需进行实验验证。

蛋白激酶 SRC 广泛分布于组织细胞内, 可与多条信号通路中的关键分子发生相互作用而参与调控细胞代谢、生长、发育和分化等过程。SRC 可直接激活 MAPK 通路上游活化蛋白 RAS, 活化的 RAS 可依次激活 RAF、MEK、MAPK/ERK 引发一系列生物学效应[37]; SRC 还与 EGFR 通路密切相关, EGFR 的活性常随着 SRC 活性升高而升高[38]; 在 LPS 诱导的巨噬细胞炎症反应中, 抑制 SRC 活性可阻断诱导型一氧化氮合酶 (inducible nitric oxide synthase, iNOS)、环氧合酶 (cyclooxygenase, COX)-2 及 TNF-α 的表达产生抗炎作用[39, 40]。TCDCA 对 LPS 诱导的大鼠腹腔巨噬细胞中 iNOS、COX-2 及 TNF-α 的表达均有抑制作用[23, 36], 提示 TCDCA 也可能通过抑制 SRC 活性而产生抗炎作用。

PDPK1 在磷脂酰肌醇-3,4,5-三磷酸存在的条件下可快速磷酸化 AKT 激活 PI3K/AKT 信号通路及下游 NF-κB 通路[41, 42], PDPK1 的高表达间接促进了 NF-κB 通路的活化[43], 然而也有研究发现, 在巨噬细胞中 PDPK1 可以负反馈抑制 Toll 样受体介导的 NF-κB 通路[44], 因此, PDPK1 对于炎症的影响可能是双重的。尽管 PDPK1 在 PI3K/AKT 及 NF-κB 信号通路中发挥重要作用, 胆汁酸也可以通过调控这两条通路产生相关生物学效应, 但目前胆汁酸对 PDPK1 有何影响尚不明确。

除了上述参与多条通路的靶点外, HSPA8、CASP3、ADH1B、PPARG、HSP90AA1 等靶点在与 TCDCA 的分子对接中得分较高, 可能对 TCDCA 的抗炎作用也具有重要作用。这些靶点中, HSPA8、HSP90AA 均与 LPS 诱导细胞分泌 TNF-α 有关, TCDCA 已被证实在多种情况下均可抑制 TNF-α 表达[45, 46]; 前期研究证实 TCDCA 可通过活化 CASP3 诱导佐剂性关节炎模型大鼠成纤维样滑膜细胞凋亡[25]; ADH1B 与 PPARG 可调控体内物质代谢, 前者参与体内乙醇的代谢, 而 PPARG 主要与脂质代谢有关, 同时, PPARG 在调控机体炎症反应方面也显示出重要作用, 但目前对于 TCDCA 与这两个靶点的研究尚未见相关报道。

综合以上信息不难发现, TCDCA 的上述靶点主要集中于调控 RAS-RAF-MAPK、PI3K-AKT、NF-κB 等信号通路, 这些通路均与炎症反应密切相关, 其中部分靶点在 TCDCA 抗炎效果中的作用已有相关文献报道, 在一定程度上确证了本研究有关网络药理学预测的准确性, 其余靶点的作用尚需进一步确证。本研究证明了 TCDCA 的抗炎作用是通过多靶点、多途径、多通路实现的, 为今后更加深入地阐明 TCDCA 的抗炎机制提供了新方向, 也为以胆汁酸为基础的创新抗炎药物研制奠定了基础。

References

[1] Yuan S, Zhao W, Wang J. Research progress on pharmacological activities and clinical application of Sus scrofa domestica Brisson bile [J]. Acta Chin Med Pharmacol (中医药学报), 2014, 42: 166−168.

[2] Zhao W, Wang Y, Lu Y, et al. Medicinal research on animal bile [J]. Inform Tradit Chin Med (中医药信息), 1999, 16: 14−15.

[3] Jiang J, Yang P, Shi X, et al. Research progress on components and pharmacological activities of animal bile [J]. Food Drug (食品与药品), 2017, 19: 227−231.

[4] Hu X, Shi C. Comparison of tauro conjugated bile acids with the corresponding free bile acids in antitussive, expectorant and anti-inflammatory effects [J]. Chin J Clin Pharm (中国临床药学杂志), 2001, 10: 85−88.

[5] Li P, Guan H, Baiyin J. Studies on antipyretic and analgesic effects of taurine and cholic acid in mice and rats [J]. J Tradit Chin Veter Med (中兽医医药杂志), 2003, 22: 3−5.

[6] Hasi S, Li P, Cao J, et al. Studies on antibacterial effects of taurine and cholic [J]. J Tradit Chin Veter Med (中兽医医药杂志), 2001, 20: 3−6.

[7] Li P, Guan H, Hasi S, et al. Studies on anti-inflammatory effects of taurine and cholic [J]. J Tradit Chin Veter Med (中兽医医药杂志), 2002, 21: 7−10.

[8] He X, Li P, Guan H, et al. Effect of taurochenodeoxycholic acid on immune function in mice [J]. J Chin Med Mater (中药材), 2005, 28: 1089−1092.

[9] Li F. Study on the Matrix Selection for Cultivating Bear Bile Powder Analogue *in vitro* Based on Compositions Similarity (基于物质成分相似性的体外培育拟天然熊胆粉的基质选择研究) [D]. Chongqing: Chongqing University (重庆大学), 2015.

[10] Li PF, He XL, Guan H, et al. Taurochenodeoxycholic acid (TCDCA) anti-inflammatory mechanism [J]. Chin J Veter Sci (中国兽医学报), 2008, 28: 1317−1320.

[11] Wang X, Shen Y, Wang S, et al. PharmMapper 2017 update: a web server for potential drug target identification with a comprehensive target pharmacophore database [J]. Nucleic Acids Res, 2017, 45: W356−W360.

[12] Chen C, Huang H, Wu CH. Protein bioinformatics databases and resources [J]. Methods Mol Biol, 2017, 1558: 3 −39.

[13] Rebhan M, Chalifa-Caspi V, Prilusky J, et al. GeneCards: integrating information about genes, proteins and diseases [J]. Trends Genet, 1997, 13: 163.

[14] 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: D362−D368.

[15] Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks [J]. Genome Res, 2003, 13: 2498−2504.

[16] Hsin KY, Matsuoka Y, Asai Y, et al. SystemsDock: a web server for network pharmacology-based prediction and analysis [J]. Nucleic Acids Res, 2016, 44: W507−W513.

[17] Pinero J, Bravo A, Queralt-Rosinach N, et al. DisGeNET: a comprehensive platform integrating information on human disease-associated genes and variants [J]. Nucleic Acids Res, 2017, 45: D833−D839.

[18] Wu D, Gao Y, Xiang H, et al. Exploration into mechanism of antidepressant of Bupleuri radix based on network pharmacology [J]. Acta Pharma Sin (药学学报), 2018, 53: 210−219.

[19] Zhou H, Hylemon PB. Bile acids are nutrient signaling hormones [J]. Steroids, 2014, 86: 62−68.

[20] Guo C, Xie S, Chi Z, et al. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome [J]. Immunity, 2016, 45: 802−816.

[21] de Aguiar VT, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism [J]. Cell Metab, 2013, 17: 657 −669.

[22] Fiorucci S, Distrutti E. Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders [J]. Trends Mol Med, 2015, 21: 702−714.

[23] Liu M, Mao W, Guan H, et al. Effects of taurochenodeoxycholic acid on adjuvant arthritis in rats [J]. Int Immunopharmacol, 2011, 11: 2150−2158.

[24] Li L, Liu C, Li P. Effect of taurochenodeoxycholic acid on the expression of ICAM-1 in fibroblast-like synoviocytes of adjuvant arthritis in rats [J]. Chin J Veter Sci (中国兽医学报), 2017, 37: 1144−1148.

[25] Li L, Liu C, Liu M, et al. Taurochenodeoxycholic acid induces apoptosis of fibroblast-like synoviocytes [J]. Eur J Pharmacol, 2013, 706: 36−40.

[26] Plotnikov A, Zehorai E, Procaccia S, et al. The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation [J]. Biochim Biophys Acta, 2011, 1813: 1619−1633.

[27] Dong X, Zhao H, Ma X. Low concentration of cholic acid up-regulates JNK protein expression: can it promote synthesis of liver cell DNA? [J]. J Clin Rehab Tiss Engin Res (中国组织工程研究与临床康复), 2010, 14: 5818−5822.

[28] Alpini G, Kanno N, Phinizy JL, et al. Tauroursodeoxycholate inhibits human cholangiocarcinoma growth *via* Ca^{2+}-, PKC-, and MAPK-dependent pathways [J]. Am J Physiol Gastrointest Liver Physiol, 2004, 286: G973−G982.

[29] Ko WK, Lee SH, Kim SJ, et al. Anti-inflammatory effects of ursodeoxycholic acid by lipopolysaccharide-stimulated inflammatory responses in RAW 264.7 macrophages [J]. PLoS One, 2017, 12: e180673.

[30] Wang X, Zhang Z, He X, et al. Taurochenodeoxycholic acid induces NR8383 cells apoptosis *via* PKC/JNK-dependent pathway [J]. Eur J Pharmacol, 2016, 786: 109.

[31] Zhang LL, Wei W, Wang QT, et al. Cross-talk between MEK1/2-ERK1/2 signaling and G protein-couple signaling in synoviocytes of collagen-induced arthritis rats [J]. Chin Med J, 2008, 121: 2278−2283.

[32] Zhang L. The Cross Talk between G Protein-AC-cAMP Signaling and Ras-Raf-MEK-ERK Signaling in Fibroblasts Like Synoviocytes of Rat with Collagen-induced Arthritis and Regulation of Paeoniflorin (胶原性关节炎大鼠成纤维样滑膜细胞 G 蛋白-AC-cAMP 与 Ras-Raf-MEK-ERK 信号转导的交叉对话及芍药苷的调节作用) [D]. Hefei: Anhui Medical University (安徽医科大学), 2008.

[33] Im E, Martinez JD. Ursodeoxycholic acid (UDCA) can inhibit deoxycholic acid (DCA)-induced apoptosis *via* modulation of EGFR/Raf-1/ERK signaling in human colon cancer cells [J]. J Nutr, 2004, 134: 483−486.

[34] Feldman R, Martinez JD. Growth suppression by ursodeoxycholic acid involves caveolin-1 enhanced degradation of EGFR [J]. Biochim Biophys Acta, 2009, 1793: 1387−1394.

[35] Rust C, Karnitz LM, Paya CV, et al. The bile acid taurochenodeoxycholate activates a phosphatidylinositol 3-kinase-dependent survival signaling cascade [J]. J Biol Chem, 2000, 275: 20210−20216.

[36] Liu MQ. Studies on The Therapeutic Action and Mechanism of TCDCA in Adjuvant-induced Arthritis Rats (TCDCA 对佐剂性关节炎模型大鼠的治疗作用及其作用机制研究) [D]. Hohhot: Inner Mongolia Agricultural University (内蒙古农业大学), 2012.

[37] Kline CL, Olson TL, Irby RB. Src activity alters alpha3

integrin expression in colon tumor cells [J]. Clin Exp Metastasis, 2009, 26: 77−87.

[38] Hiscox S, Morgan L, Green TP, et al. Elevated Src activity promotes cellular invasion and motility in tamoxifen resistant breast cancer cells [J]. Breast Cancer Res Treat, 2006, 97: 263−274.

[39] Lee YG, Lee WM, Kim JY, et al. Src kinase-targeted anti-inflammatory activity of davallialactone from *Inonotus xeranticus* in lipopolysaccharide-activated RAW264.7 cells [J]. Br J Pharmacol, 2008, 154: 852−863.

[40] Yu T, Lee S, Yang WS, et al. The ability of an ethanol extract of *Cinnamomum cassia* to inhibit Src and spleen tyrosine kinase activity contributes to its anti-inflammatory action [J]. J Ethnopharmacol, 2012, 139: 566−573.

[41] Dieterle AM, Bohler P, Keppeler H, et al. PDK1 controls upstream PI3K expression and PIP3 generation [J]. Oncogene, 2014, 33: 3043−3053.

[42] Wu N, He C, Zhu B, et al. 3-Phosphoinositide dependent protein kinase-1 (PDK-1) promotes migration and invasion in gastric cancer cells through activating the NF-κB pathway [J]. Oncol Res, 2017, 25: 1153−1159.

[43] Du L, Xie Y, Liu M. Expression and significance of PDK1 and NF-κB in the middle ear cholesteatoma [J]. J Clin Otorhinolaryngol Head Neck Surg (临床耳鼻咽喉头颈外科杂志), 2016, 33: 770−773.

[44] Chaurasia B, Mauer J, Koch L, et al. Phosphoinositide-dependent kinase 1 provides negative feedback inhibition to Toll-like receptor-mediated NF-κB activation in macrophages [J]. Mol Cell Biol, 2010, 30: 4354−4366.

[45] He X, Li P, Guan H, et al. Effect of TCDCA on the contents of IL-1β, IL-6, TNF-α and IgG in AA rat serum [J]. Prog Veter Med (动物医学进展), 2010, 31: 49−51.

[46] Wang X, Zhang Z, Li P. Effects of taurochenodeoxycholic acid on the production of tumor necrosis factor-α induced by TGR5 in NR8383 [J]. Chin J Veter Sci (中国兽医学报), 2016, 36: 108−111.