基于网络药理学的加味佛手散抑制子宫内膜侵袭转移机制研究

张　莹，韦佳慧，张成玲，李攀红，孙文威，徐晓玉，陈　怡*

(西南大学药学院&中医药学院，重庆市高校中药新药筛选重点实验室，
国家中医药管理局中药药理重点建设学科，重庆 400715)

摘要: 加味佛手散是由川芎嗪、阿魏酸加延胡索乙素组成的新中药复方。前期研究发现，加味佛手散能够抑制异位子宫内膜生长，但对异位内膜侵袭转移的作用尚不明确。本研究运用网络药理学方法分析加味佛手散作用靶点，并通过体内模型验证其对异位内膜侵袭转移的作用机制。首先，通过检索 TCMID、TCMSP 和 SEA 数据库获得加味佛手散成分靶点，检索 OMIM、DisGeNET 和 GEO 数据库获得子宫内膜异位症的靶点，采用 Cytoscape 3.5.0 软件构建加味佛手散成分−成分靶点和加味佛手散成分−子宫内膜异位症靶点网络，并将关键靶点的信息上传至 DAVID 数据库进行通路富集分析，发现加味佛手散可能通过调控 MMP2、MMP9、TIMP1、ICAM1 和 VEGFA 等 66 个关键靶点，干预雌激素、HIF-1、TNF 和 GnRH 信号通路等 115 条通路发挥作用，其中 MMP-TIMP 为一类关键靶点。其次，加味佛手散体内能显著抑制异位内膜组织的生长，下调 MMP-2 和 MMP-9，上调 TIMP-1 表达，进而抑制异位内膜侵袭转移。此结果为加味佛手散的研究提供了药效学依据。

关键词: 加味佛手散；子宫内膜异位症；网络药理学；侵袭转移；作用机制

中图分类号: R966　　**文献标识码**: A　　**文章编号**: 0513-4870 (2018) 09-1398-08

Mechanism of *Jiawei Foshou San* on invasion and metastasis in endometriosis based on network pharmacology

ZHANG Ying, WEI Jia-hui, ZHANG Cheng-ling, LI Pan-hong, SUN Wen-wei, XU Xiao-yu, CHEN Yi*

(*College of Pharmaceutical Sciences and Chinese Medicine, Chongqing Key Laboratory of New Drug Screening from Traditional Chinese Medicine, Pharmacology of Chinese Materia Medica-the Key Discipline Constructed by the State Administration of Traditional Chinese Medicine, Southwest University, Chongqing 400715, China*)

Abstract: *Jiawei Foshou San* is a new Chinese medicine compound consisting of ligustrazine, ferulic acid and fumarate. Previously *Jiawei Foshou San* inhibited the growth of endometriosis with unclear mechanism, especially in metastasis and invasion. In this study, network pharmacology analysis was performed to explore potential mechanism of *Jiawei Foshou San* on endometriosis. *Jiawei Foshou San* compound targets were purchase from TCMID, TCMSP and SEA database. Endometriosis targets were collected from OMIM, DisGeNET and GEO database. Networks of *Jiawei Foshou San* compound-compound targets and compound target-endometriosis target were established with Cytoscape 3.5.0 software. Key targets were analyzed for pathway enrichment through DAVID database. It was found that *Jiawei Foshou San* regulated 66 core targets (MMP2, MMP9, TIMP1, ICAM1, VEGFA, et al.) and affected 115 pathways, such as estrogen, HIF-1, TNF and GnRH signaling pathways. MMP-TIMP were uncovered as one cluster of the core targets. Furthermore, *Jiawei Foshou San* significantly suppressed the growth of ectopic endometrium. Meanwhile, invasion and

收稿日期: 2018-07-24; 修回日期: 2018-08-02.

基金项目: 国家自然科学基金资助项目 (81773984, 81402441)；重庆市卫生局中医药科研项目 (zy201602125)；西南大学“国家级大学生创新创业训练计划”项目 (201710635073).

*通讯作者 Tel: 86-23-68251225, E-mail: rachelcy@swu.edu.cn

DOI: 10.16438/j.0513-4870.2018-0674

metastasis were restrained after treating with *Jiawei Foshou San* through decreasing MMP-2 and MMP-9, increasing TIMP-1. In brief, these results provide a pharmacodynamic basis for the study of *Jiawei Foshou San*.

Key words: *Jiawei Foshou San*; endometriosis; network pharmacology; invasion and metastasis; mechanism

具有活性的子宫内膜组织在子宫内膜以外部位生长被称为子宫内膜异位症。尽管子宫内膜异位症被认为是一种良性妇科疾病, 但其具有侵袭、种植及复发率高等恶性生物学行为[1, 2]。子宫内膜异位症的发病机制并未明确, 最广为接受的经血逆流学说认为, 随经血逆流的子宫内膜组织发生侵袭转移是重要的步骤[3, 4]。

中医学认为子宫内膜异位症病机主要为瘀血阻滞胞脉、子宫[5, 6], 治法当以活血化瘀为主[7]。加味佛手散源自妇科经典名方佛手散, 是本课题组研制的一种新的中药单体复方, 由川芎嗪、阿魏酸加延胡索乙素组成。前期研究发现, 加味佛手散对子宫内膜异位症具有较好的疗效, 能抑制异位组织增生, 调节雌激素、孕激素水平, 抗炎和抗血管生成[8], 但对异位内膜侵袭转移的作用机制未见报道。

网络药理学基于生物分子网络构建“药物–靶点–通路”多层次网络分析药效活性成分和可能的分子网络机制, 旨在建立中药及其复方多成分、多途径、多靶点间的协同作用联系[9, 10]。本研究运用网络药理学的思路和方法, 预测加味佛手散抑制子宫内膜侵袭转移作用的靶点, 并利用大鼠模型进行体内靶点验证, 探讨加味佛手散抑制子宫内膜侵袭转移机制。

材料与方法

药品与试剂　加味佛手散由西南大学中药研究所研制提供, 阿魏酸 (批号: ZL2016030815, 纯度 98%)、川芎嗪 (批号: ZL2016061382, 纯度 98%) 和延胡索乙素 (批号: ZL2016051235, 纯度 98.1%) 均购自于南京泽朗公司。BCA 试剂盒、qPCR 引物购自北京鼎国生物技术公司; PrimeScript™ RT 试剂盒购自 Takara 公司, SYBR™ Green Master Mix 购自美国 Thermo Fisher 公司, MMP2 和 MMP9 抗体购自武汉博士德公司, TIMP1 和 β-actin 抗体购自武汉三鹰生物技术公司, 荧光素标记的山羊抗兔二抗购自北京中杉金桥生物技术公司。

动物　SPF 级雌性 SD 大鼠 (180～200 g), 购自重庆市中药研究院, 生产许可证号: SCXK (渝) 2012-0006。本研究严格遵循西南大学动物伦理委员会要求 (伦理批件编号 0002183)。动物自然昼夜节律光照, 适应 1 周后进行实验。

仪器　荧光分光光度计 (美国 Thermo Fisher 公司); 电泳仪 (上海伯乐公司); Tanon5200 全自动化学发光图像分析系统 (上海天能科技公司); CFX 96 Real-Time PCR 仪 (美国 BIO-RAD 公司)。

靶点收集　利用中药综合数据库 TCMID[11] (http://www.megabionet.Org/tcmid/search/)、中药成分系统药理学数据库 TCMSP[12] (http://lsp.nwu.edu.cn) 和相似性集合方法数据库 SEA[13] (http://sea.bkslab.org/) 收集加味佛手散中 3 种成分的作用靶点构建加味佛手散成分–成分靶点网络。利用人类在线孟德尔遗传 OMIM[14] (https://omim.org/)、基因疾病数据库协会 DisGeNET[15] (http://www.disgenet.org/) 和高通量基因表达数据库 GEO[16] (https://www.ncbi.nlm.nih.gov/geo/) 构建加味佛手散成分–子宫内膜异位症靶点网络。使用 STRING[17]数据库 (https://string-db.org/) 进行蛋白相互作用查询, 选择打分值高于 0.4 的置信度数据靶点。上述所有靶点均通过 UniProt[18]数据库中的 UniProtKB 搜索功能 (http://www.uniprot.org/) 获取其官方的 UniProt ID 信息, 并用于后续网络构建。

网络构建与通路分析　基于以上靶点预测结果, 采用 Cytoscape 3.5.0 软件构建加味佛手散成分–成分靶点和加味佛手散成分–子宫内膜异位症靶点网络, 并将关键靶点的信息上传至 DAVID[19]数据库 (https://david.ncifcrf.gov/) 进行 GO (Gene Ontology) 生物学过程富集分析和 KEGG (KEGG pathway analysis) 通路富集分析, 选取具有统计学意义 ($P < 0.05$) 的所有通路。

动物造模与分组　60 只发情期 SD 雌鼠进行子宫内膜自体移植手术。造模 28 天后再次开腹观察, 模型成功标准[20]: ① 移植物体积增大, 表面覆盖有结缔组织或大网膜, 呈隆起透亮小囊状, 表面有血管, 内有积液, 且积液高度≥2 mm; ② 游标卡尺测量移植物的体积大小, 异位组织体积 = 0.52×长×宽×高, 移植物体积≥8 mm^3; ③ 切取移植物送检, 证实移植物中有子宫内膜上皮细胞、腺体及间质的生长。将造模成功的 50 只大鼠随机分为 5 个组: 模型组, 加味佛手散低、中、高浓度组和孕三烯酮组, 各组给药前异位内膜体积无显著性差异, 另设正常雌性大鼠 10 只为空

白组。空白组和模型组灌胃 0.5%羧甲基纤维素钠混悬液，加味佛手散低、中、高浓度组分别给予 45、90、180 mg·kg^{-1}加味佛手散，孕三烯酮组给予 50 mg·kg^{-1}孕三烯酮，每天灌胃给药 1 次，连续给药 4 周。以体积变化率 =(给药前移植内膜体积 − 给药后移植内膜体积)/给药前移植内膜体积×100%作为指标[21]。

qPCR 和 Western blot 法测 MMP2、MMP9、TIMP1 空白组取正常子宫内膜组织，模型组和药物组取异位内膜组织，Trizol 法提取总 RNA，按照 Takara 试剂盒说明书进行逆转录反应合成 cDNA，按照 SYBR 试剂盒说明书检测 MMP-2、MMP-9 和 TIMP-1 的 mRNA 水平，MMP-2 引物序列为：上游 5'-GGCCCTGTCACTCCTGAGAT-3'，下游 5'-GGCATCCAGGTTATCGGGGA-3'；MMP-9 引物序列为：上游 5'-TGGACGATGCCTGCAACGTG-3'，下游 5'-GTCGTGCGTGTCCAAAGGCA-3'；TIMP-1 引物序列为：上游 5'-CAATTCCGACCTCGTCATCAG-3'，下游 5'-CTTGGAACCCTTTATACATCTTGG-3'；GAPDH 引物序列为：上游 5'-AATGGGCAGCCGTTAGGAAA-3'，下游 5'-GCCCAATACGACCAAATCAGAG-3'。用内源对照 GAPDH 标准化的 $2^{-\triangle\triangle Ct}$ 方法计算目的基因相对表达量。

取内膜组织，提取总蛋白上样，经过 10% SDS-PAGE 电泳使蛋白转移至 PVDF 膜上封闭后，孵育一抗 (MMP-2 1∶300, MMP-9 1∶300, TIMP-1 1∶500) 4 ℃过夜，洗膜后室温孵育二抗 (1∶2000) 1 h。使用全自动化学发光图像分析系统进行图像显影定影。*β*-actin 设为内参蛋白。

统计分析 计数资料以 ($\bar{x} \pm s$) 表示。组间数据采用单因素方差分析进行比较，采用 SPSS21 统计软件包进行统计学分析。$P<0.05$ 有显著性差异，$P<0.01$ 有极显著差异。

结果

1 加味佛手散成分−成分靶点网络构建与分析

3 种加味佛手散成分共获得 275 个潜在靶点，其中涉及川芎嗪 10 个，阿魏酸 86 个，延胡索乙素 179 个。通过分析每一种成分的靶点构建网络发现，ADRB2、CA2、F3、LTA4H、PTGS1、PTGS2、SLC6A2 和 SLC6A3 靶点是川芎嗪、阿魏酸和延胡索乙素 3 种成分共有靶点 (图 1)，提示这些共同靶点可能是多成分、多靶点中药协同作用的基础。

2 加味佛手散成分−子宫内膜异位症靶点网络构建与分析

整合数据获得与疾病相关靶点 401 个，将 3 个成分的靶点映射到疾病靶点获得 22 个共同靶点 (表 1)。

Table 1 Common targets of *Jiawei Foshou San* (JFS)-treatment and endometriosis-development

No.	Gene name	Protein name	Uniprot ID	Betweenness	Degree
1	AKR1B1	Aldose reductase	P15121	0.001 9	16
2	AKR1C3	Aldo-keto reductase family 1 member C3	P42330	0.004 5	21
3	BAX	Apoptosis regulator BAX	Q07812	0.003 8	61
4	BCL2	Apoptosis regulator Bcl-2	P10415	0.036 3	117
5	COPS2	COP9 signalosome complex subunit 2	P61201	0.000 4	9
6	CYP1A1	Cytochrome P450 1A1	P04798	0.010 8	39
7	EEF1A1	Elongation factor 1-alpha 1	P68104	0.011 4	28
8	ESR2	Estrogen receptor beta	Q92731	0.005 0	37
9	GANAB	Neutral alpha-glucosidase AB	Q14697	0.006 5	4
10	HSD11B1	Corticosteroid 11-beta-dehydrogenase isozyme 1	P28845	0.003 6	20
11	HSD17B3	Testosterone 17-beta-dehydrogenase 3	P37058	0.000 3	11
12	IL8	Interleukin-8	P10145	0.032 3	113
13	MAOA	Amine oxidase [flavin-containing] A	P21397	0.002 6	19
14	MAOB	Amine oxidase [flavin-containing] B	P27338	0.001 2	14
15	MMP1	Interstitial collagenase	P03956	0.001 1	43
16	MMP2	72 kDa type IV collagenase	P08253	0.012 5	68
17	MMP9	Matrix metalloproteinase-9	P14780	0.010 9	82
18	NCOA1	Nuclear receptor coactivator 1	Q15788	0.013 0	49
19	NOS3	Nitric oxide synthase	P29474	0.018 5	89
20	PTGS2	Prostaglandin G/H synthase 2	P35354	0.022 5	96
21	RAF1	RAF proto-oncogene serine/threonine-protein kinase	P04049	0.004 6	47
22	VEGFA	Vascular endothelial growth factor A	P15692	0.054 2	144

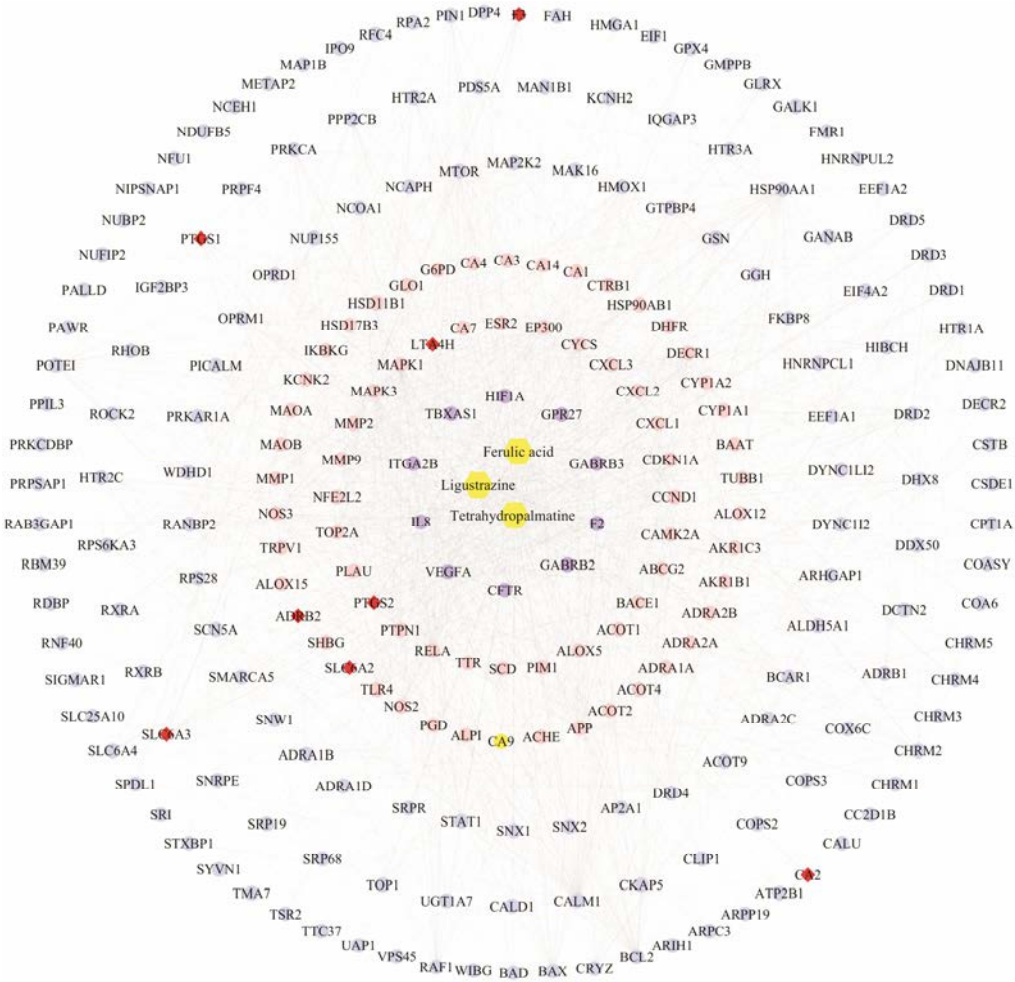

Figure 1　The JFS compound-target network.　Purple circles, pink circles, blue circles and red diamonds represented the targets of ligustrazine, ferulic acid, tetrahydropalmatine and the common targets for all three components respectively.　Yellow hexagons represented the three compounds of JFS

然后提取 22 个共同靶点及其相邻的靶点构建核心蛋白质−蛋白质相互作用网络，再对网络节点的拓扑属性分析进行靶点筛选，节点的“度”和“介数”的平均值分别为 29.860 3 和 0.003 9，选择“度”≥29.860 3 且“介数”≥0.003 9 的 66 个节点作为关键靶点。其中，MMP2、MMP-9、TIMP-1、ICAM1 和 VEGFA 被认为是与活血化瘀功效相关的靶点。

3　关键靶点的通路富集分析

通过 DAVID 数据库对 66 个关键靶点进行 GO 生物学过程、细胞组分和分子功能分析和通路富集分析。图 2 列出了 GO 分析生物学过程、细胞组分和分子功能各类别功能中显著富集排名前 6 的项目，可以看出这些靶点主要涉及凋亡过程的负调节、DNA 模板化、细胞增殖的正调节等生物学过程，涉及核、细胞质、核质等细胞组分，涉及蛋白质结合、酶结合、DNA 结合等分子功能。在 KEGG 数据库中获得与关键靶点相关的通路 115 条，结果表明加味佛手散可能主要通过调节雌激素、转录因子 HIF-1、肿瘤坏死因子和促性腺释放激素等 10 条信号通路过程抑制内膜侵袭转移，其中 MMP/TIMP 在通路中起关键作用（图 3）。

4　加味佛手散作用靶点验证

4.1　加味佛手散能显著缩小异位内膜体积　造模 28 天后，模型组大鼠异位内膜生长良好，体积增大，黏连严重，透亮小囊表面的血管清晰可见且丰富，表面覆盖有结缔组织或大网膜，液体积聚高度≥2 mm，体积≥8 mm^3，送检结果显示为子宫内膜，说明造模成功。分组给药 28 天后，加味佛手散组体积明显缩小，异位内膜黏连减少，表面血管减少，提示加味佛手散对异位内膜生长有较明显的抑制作用。其中加味佛手散 90 和 180 $mg \cdot kg^{-1}$ 异位内膜体积变化率分别为 0.58±0.10、0.82±0.04，与模型组相比显著减小（$P<0.05$）。与模型组相比，孕三烯酮组异位内膜体积变化率为 0.81±0.25（$P<0.05$），且加味佛手散的作用效果呈现剂量依赖性（图 4）。

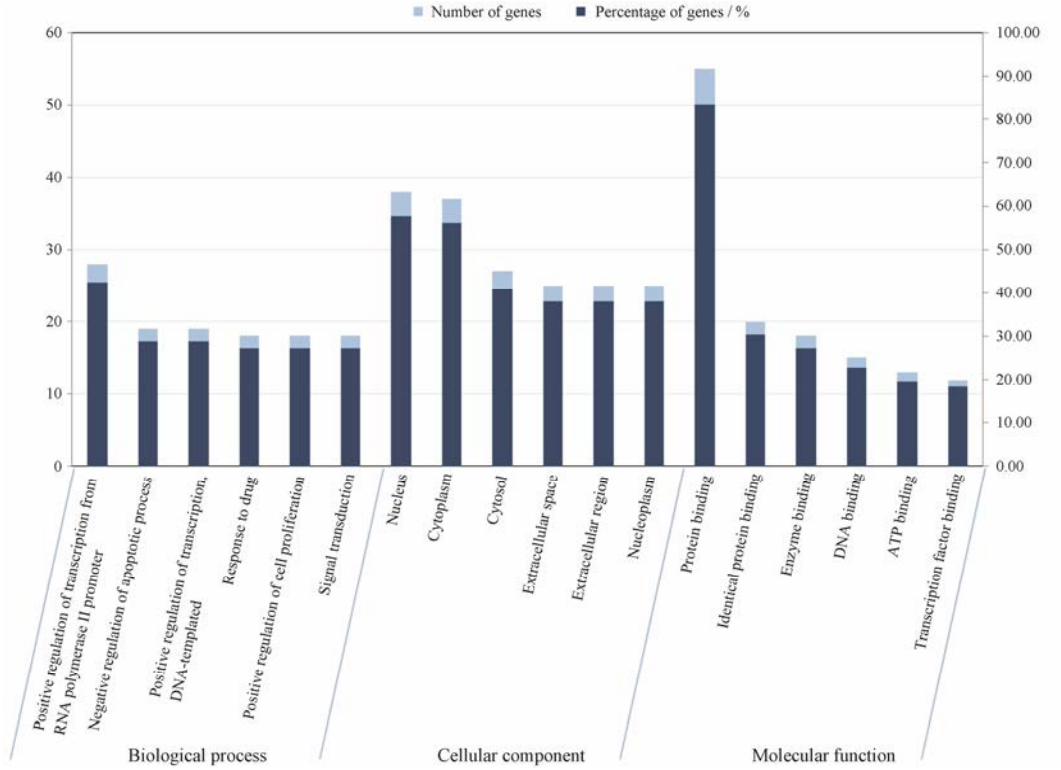

Figure 2　Gene ontology (GO) analysis of candidate targets.　GO enrichment analysis in DAVID database showed the 6 remarkably enriched items in the biological processes, cell component, and molecular function

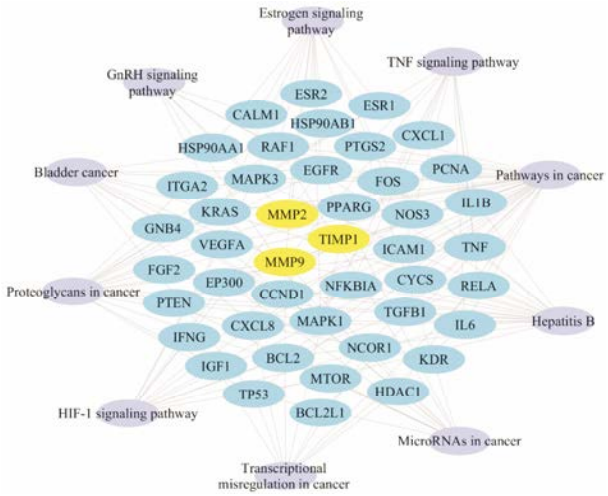

Figure 3　Pathway including MMP2, MMP9 and TIMP1

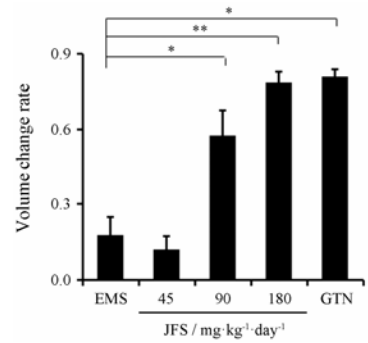

Figure 4　Effect of JFS on the volume change rat of endometrium.　EMS: Endometriosis; GTN: Gestrinone.　$n=6$, $\bar{x}\pm s$. $^{*}P<0.05$, $^{**}P<0.01$

4.2　加味佛手散能调节侵袭转移基因和蛋白的表达

结果表明，与空白组相比，模型组 MMP-2、MMP-9 mRNA 水平显著上调 ($P<0.05$), TIMP-1 mRNA 水平显著下调 ($P<0.05$)。与模型组相比，加味佛手散组显著下调 MMP-2 ($P<0.05$) 和 MMP-9 ($P<0.01$) mRNA 水平，加味佛手散 90 和 180 mg·kg^{-1} 组显著上调 ($P<0.05$) TIMP-1 mRNA 水平 (图 5)。

Western blot 结果表明，与空白组相比，模型组 MMP-2、MMP-9 蛋白表达水平显著上调 ($P<0.05$), TIMP-1 蛋白表达水平显著下调 ($P<0.05$)。与模型组相比，加味佛手散组 MMP-2 和 MMP-9 蛋白表达水平呈剂量依赖性显著下调 ($P<0.05$), TIMP-1 蛋白表达水平均显著上调 ($P<0.05$) (图 6)。综上，加味佛手散对侵袭转移的作用机制与调节 MMP-2、MMP-9 和 TIMP-1 基因和蛋白的表达有关。

讨论

经血逆流学说认为，异位内膜的侵袭转移是发生子宫内膜异位症的重要步骤。MMP-2、MMP-9 和 TIMP-1 是重要的侵袭转移蛋白[22], MMP-2 和 MMP-9

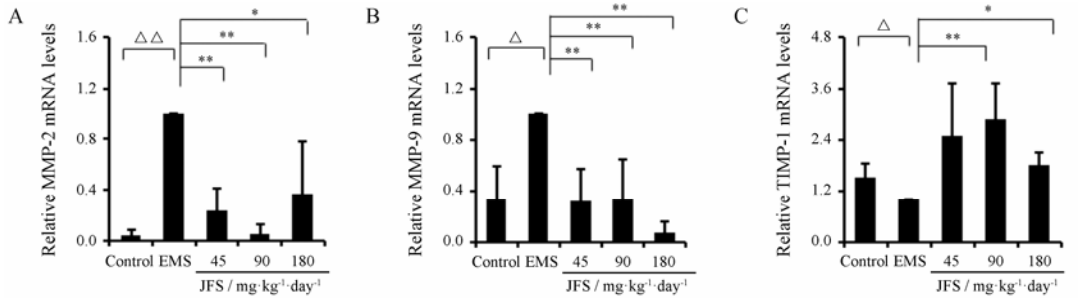

Figure 5　Effect of JFS on invasion and metastasis.　The mRNA levels of MMP-2 (A), MMP-9 (B) and TIMP-1 (C) were detected by qPCR in different groups.　$n=3$, $\bar{x}\pm s$.　$^{\triangle}P<0.05$, $^{\triangle\triangle}P<0.01$; $^{*}P<0.05$, $^{**}P<0.01$

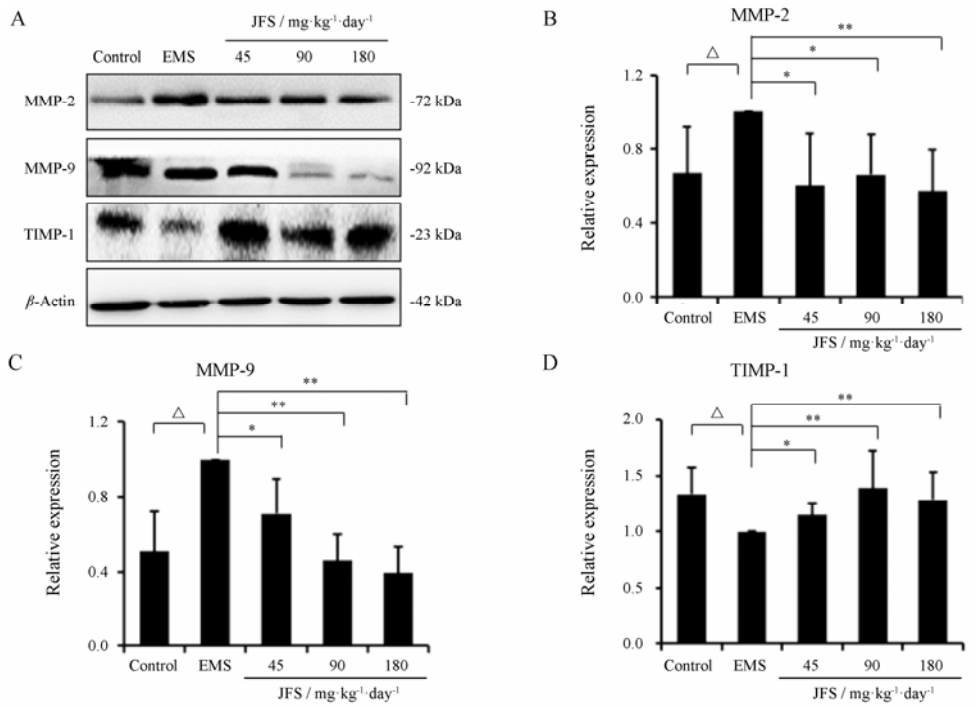

Figure 6　Effect of JFS on invasion and metastasis.　The protein levels of MMP-2, MMP-9 and TIMP-1 were detected by Western blot (A), and the ratio of MMP-2 (B), MMP-9 (C) and TIMP-1 (D) with β-actin were shown.　$n=3$, $\bar{x}\pm s$.　$^{\triangle}P<0.05$; $^{*}P<0.05$, $^{**}P<0.01$

会促进细胞外基质降解，增加异位内膜侵袭力，TIMP-1 能与 MMP-9 形成复合体，进而抑制细胞外基质的降解，它们之间的平衡可调节细胞外基质的降解。通过查阅文献发现，子宫内膜异位内膜的 MMP-2、MMP-9 表达明显增高，而 TIMP-1 表达降低[23−26]。通过抑制 MMP-2、MMP-9，促进 TIMP-1 表达已被报道为抗子宫内膜异位症的有效疗法[24, 27, 28]。本研究发现，在自体移植的子宫内膜异位症 SD 大鼠模型中，异位内膜中 MMP-2 和 MMP-9 基因和蛋白的表达明显增强，而 TIMP-1 基因和蛋白的表达则明显减弱，这与已有研究结果相吻合。同时加味佛手散能显著抑制 MMP-2 和 MMP-9 的表达，显著上调 TIMP-1 的表达，从而抑制异位内膜的侵袭转移。因此，其他如 MMP-1、MMP-7 和 TIMP-2 等在异位内膜侵袭转移中的作用，以及加味佛手散对其的影响都值得进一步探讨研究。

加味佛手散来源于著名活血化瘀名方佛手散，通过分析加味佛手散成分−子宫内膜异位症靶点网络中的 66 个核心靶点，其中 MMP-2、MMP-9、TIMP-1、ICAM1、ACE 和 VEGFA 被认为是与活血化瘀功效相关的靶点。除了子宫内膜异位症，MMP-2、MMP-9 和 TIMP-1 被发现是多种血瘀证的靶点，包括肝癌、颈动脉血栓、毒热血瘀证、缺血再灌注损伤[29−32]。ICAM1、ACE 和 VEGFA 也被认为是痛风、脑缺血、冠心病和子宫内膜异位症的靶点之一[33−36]。通过网络药理学，对 CPPI 网络中的核心靶点进行通路富集分析，发现 GnRH、TNF 和雌激素等信号通路与 MMP-TIMP 相关，且 GnRH、TNF 和雌激素都是维持子宫内膜稳态的关键介质[37−40]。此外，有研究表明 MMP 和 TIMP 蛋白表达受这 3 种途径调节[41−43]。课题组前期研究还发现，加味佛手散可以抑制 GnRH、

雌激素和 TNF-α 的表达[8]，其作用机制可能与调节 MMP/TIMP 平衡有关。因此，预测所得加味佛手散抑制子宫内膜侵袭转移机制可能与作用于 MMP-TIMP 靶点密切相关，表明基于网络药理学的技术手段探讨中药复方药效具有一定的准确性与实用性，为加味佛手散的评估提供合理支持，也为进一步深入探讨药效及发病机制奠定了良好基础。

References

[1] Ma X, Hui Y, Lin L, et al. Possible relevance of tumor-related genes mutation to malignant transformation of endometriosis [J]. Eur J Gynaecol Oncol, 2016, 37: 89−94.

[2] Mihailovici A, Rottenstreich M, Kovel S, et al. Endometriosis-associated malignant transformation in abdominal surgical scar: a PRISMA-compliant systematic review [J]. Medicine, 2017, 96: e9136.

[3] Borrelli GM, Abrao MS, Taube ET, et al. Immunohisto-chemical investigation of metastasis-related chemokines in deep-infiltrating endometriosis and compromised pelvic sentinel lymph nodes [J]. Reprod Sci, 2015, 22: 1632−1642.

[4] Chui MH, Wang TL, Shih IM. Endometriosis: benign, malignant, or something in between? [J]. Oncotarget, 2017, 8: 78263−78264.

[5] Shan J, Cheng W, Zhai DX, et al. Meta-analysis of Chinese traditional medicine Bushen Huoxue prescription for endometriosis treatment [J]. Evid Based Complement Alternat Med, 2017, 2017: 5416423.

[6] Wen Y, Wang Y, Feng TT, et al. Differential proteomics analysis of endometriosis in blood stasis syndrome [J]. Chin J Integr Med, 2017. DOI: 10.1007/s11655-017-2401-4.

[7] Zhao RH. Strategies for activating blood circulation-regulating Gan (liver)-tonifying Shen (kidney) sequential therapy of endometriosis-associated infertility [J]. Chin J Integr Med, 2018. DOI: 10.1007/s11655-018-2931-9.

[8] Tang Q, Shang F, Wang X, et al. Combination use of ferulic acid, ligustrazine and tetrahydropalmatine inhibits the growth of ectopic endometrial tissue: a multi-target therapy for endometriosis rats [J]. J Ethnopharmacol, 2014, 151: 1218−1225.

[9] Ning K, Zhao X, Poetsch A, et al. Computational molecular networks and network pharmacology [J]. Biomed Res Int, 2017. DOI: 10.1155/2017/7573904.

[10] Wang Y, Lin W, Li C, et al. Multipronged therapeutic effects of Chinese herbal medicine Qishenyiqi in the treatment of acute myocardial infarction [J]. Front Pharmacol, 2017, 8: 98−110.

[11] Ru JL. Construction and Utilization of Traditional Chinese Medicine System Pharmacology Database and Analysis Platform (中药系统药理学数据库和分析平台的构建和应用) [D]. Xianyang: North West Agriculture and Forestry University, 2015.

[12] Ru J, Li P, Wang J, et al. TCMSP: a database of systems pharmacology for drug discovery from herbal medicines [J]. J Cheminform, 2014, 6: 13−19.

[13] Keiser MJ, Roth BL, Armbruster BN, et al. Relating protein pharmacology by ligand chemistry [J]. Nat Biotechnol, 2007, 25: 197−206.

[14] Li X, Wu LH, Fan XH, et al. Network pharmacology study on major active compounds of Fufang Danshen formula [J]. China J Chin Mater Med (中国中药杂志), 2011, 36: 2911−2915.

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

[16] Li G, Xu B, Liang XZ, et al. Study on the molecular mechanism of osteoporosis treated by epimedium based on network pharmacology [J]. Chin Pharm Bull (中国药理学通报), 2018, 34: 267−273.

[17] 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.

[18] Chai X, Meng YK, Bai ZF, et al. Mechanism of anti-hepatitis B virus activity of tonkinensis based on biological targets network [J]. Acta Pham Sin (药学学报), 2018, 53: 396−402.

[19] Jiao X, Sherman BT, Huang DW, et al. DAVID-WS: a stateful web service to facilitate gene/protein list analysis [J]. Bioinformatics, 2012, 28: 1805−1806.

[20] Liu W, Wang LX, Guo CX. The effects of lauromacrogol injection into rat endometrial cysts: a preliminary experimental study [J]. Arch Gynecol Obstet, 2016, 294: 556−559.

[21] Duan LJ, Wang JY, Sun XT, et al. The role and significance of endomorphin-1 and μ-opioid receptor in rats with endometriosis [J]. Gynecol Endocrinol, 2016, 32: 912−915.

[22] Li Z, Takino T, Endo Y, et al. Activation of MMP-9 by membrane type-1 MMP/MMP-2 axis stimulates tumor metastasis [J]. Cancer Sci, 2017, 108: 347−353.

[23] Jana S, Chatterjee K, Ray AK, et al. Regulation of matrix metalloproteinase-2 activity by COX-2-PGE2-pAKT axis promotes angiogenesis in endometriosis [J]. PLoS One, 2016, 11: e0163540.

[24] Huang YH, Shen L, Cai A, et al. Effect of warming Yang and removing blood stasis method on matrix metalloproteinases /

tissue inhibitor metalloproteinases levels secreted by cultured endometrial cells from patients with endometriosis [J]. J Tradit Chin Med (中医杂志), 2015, 35: 571−576.

[25] Yi KW, Kim SH, Ihm HJ, et al. Increased expression of p21-activated kinase 4 in adenomyosis and its regulationof matrix metalloproteinase-2 and -9 in endometrial cells [J]. Fertil Steril, 2015, 103: 1089−1097.

[26] Szymanowski K, Mikolajczyk M, Wirstlein P, et al. Matrix metalloproteinase-2 (MMP-2), MMP-9, tissue inhibitor of matrix metalloproteinases (TIMP-1) and transforming growth factor-β2 (TGF-β2) expression in eutopic endometrium of women with peritoneal endometriosis [J]. Ann Agric Environ Med, 2016, 23: 649−653.

[27] Li Z, Liu H, He Z, et al. Effects of cisplatin and letrozole on surgically induced endometriosis and comparison of the two medications in a rat model [J]. Eur J Pharm Sci, 2016, 93: 132−140.

[28] Kim JH, Woo JH, Kim HM, et al. Anti-endometriotic effects of pueraria flower extract in human endometriotic cells and mice [J]. Nutrients, 2017, 9: 212−226.

[29] Gao L, Wang KX, Zhou YZ, et al. Uncovering the anticancer mechanism of compound kushen injection against HCC by integrating quantitative analysis, network analysis and experimental validation [J]. Sci Rep, 2018, 8: 624−639.

[30] Xue M, Zhang L, Yang L, et al. Effect of Chinese herbal medicine for activating blood circulation and detoxifying on expression of inflammatory reaction and tissue damage related factors in experimental carotid artery thrombosis rats [J]. Chin J Integr Med, 2010, 16: 247−251.

[31] Sun J, Zhu Y, Zhang L, et al. Effects of xuelian injection on cerebral TNF-α, IL-1β and MMP-9 in rats experienced focal cerebral ischemia/reperfusion [J]. Int J Clin Exp Med, 2014, 7: 2632−2638.

[32] Salameh A, Dhein S. Strategies for pharmacological organoprotection during extracorporeal circulation targeting ischemia-reperfusion injury [J]. Front Pharmacol, 2015, 6: 296−308.

[33] Zhao FL, Li GC, Yang YH, et al. A network pharmacology approach to determine active ingredients and rationality of herb combinations of modified-Simiaowan for treatment of gout [J]. J Ethnopharmacol, 2015, 168: 1−16.

[34] Zhou W, Wang Y. A network-based analysis of the types of coronary artery disease from traditional Chinese medicine perspective: potential for therapeutics and drug discovery [J]. J Ethnopharmacol, 2014, 151: 66−77.

[35] Fei X, Zhang X, Wang Q, et al. Xijiao Dihuang decoction alleviates ischemic brain injury in MCAO rats by regulating inflammation, neurogenesis, and angiogenesis [J]. Evid Based Complement Alternat Med, 2018. DOI: 10.1155/2018/5945128.

[36] Chen ZZ, Gong X. Effect of Hua Yu Xiao Zheng decoction on the expression levels of vascular endothelial growth factor and angiopoietin-2 in rats with endometriosis [J]. Exp Ther Med, 2017, 14: 5743−5750.

[37] Granese R, Perino A, Calagna G, et al. Gonadotrophin-releasing hormone analogue or dienogest plus estradiol valerate toprevent pain recurrence after laparoscopic surgery for endometriosis: a multi-center randomized trial [J]. Acta Obstet Gynecol Scand, 2015, 94: 637−645.

[38] Leavy O. Reproductive immunology: evading immunosurveillance in endometriosis [J]. Nat Rev Immunol, 2015, 15: 729.

[39] Simmen RC, Kelley AS. Reversal of fortune: estrogen receptor-beta in endometriosis [J]. J Mol Endocrinol, 2016, 57: F23−F27.

[40] Tosti C, Biscione A, Morgante G, et al. Hormonal therapy for endometriosis: from molecular research to bedside [J]. Eur J Obstet Gynecol Reprod Biol, 2017, 209: 61−66.

[41] Voloshenyuk TG, Gardner JD. Estrogen improves TIMP-MMP balance and collagen distribution in volume-overloaded hearts of ovariectomized females [J]. Am J Physiol Regul Integr Comp Physiol, 2010, 299: R683−R693.

[42] Raga F, Casan EM, Wen Y, et al. Independent regulation of matrix metalloproteinase-9, tissue inhibitor of metalloproteinase-1 (TIMP-1), and TIMP-3 in human endometrial stromal cells by gonadotropin-releasing hormone: implications in early human implantation [J]. J Clin Endocrinol Metab, 1999, 84: 636−642.

[43] Yang YN, Wang F, Zhou W, et al. TNF-alpha stimulates MMP-2 and MMP-9 activities in human corneal epithelial cells *via* the activation of FAK/ERK signaling [J]. Ophthalmic Res, 2012, 48: 165−170.