网络药理学−分子对接联合体内外实验验证解析创新中药YCNHF I/II的炎症性肠病线粒体保护机制

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摘要: 本研究旨在探讨创新中药复方YCNHF I (栀子等6味药食同源药材) /II (黄芩等6味非药食同源药材) 治疗炎症性肠病 (inflammatory bowel disease, IBD) 的多靶点作用机制。通过多数据库挖掘筛选复方活性成分及IBD相关靶点, 构建“化合物−靶点−通路”网络并进行分子对接验证; 采用超高效液相色谱−质谱联用鉴定入血成分, 检测抗氧化能力, 在脂多糖 (lipopolysaccharide, LPS) 诱导的人结直肠癌上皮细胞Caco-2炎症模型中检测白细胞介素-6 (interleukin-6, IL-6)、白细胞介素-1β (interleukin-1β, IL-1β) 表达及线粒体膜电位; 建立葡聚糖硫酸钠盐 (dextran sulfate sodium, DSS) 诱导的溃疡性结肠炎 (ulcerative colitis, UC) 大鼠模型, 灌胃给药后通过苏木素−伊红 (hematoxylin and eosin, HE) 染色观察结肠病理, Western blot检测经过磷酸化修饰的蛋白激酶B (phospho-protein kinase B, p-Akt)表达。结果显示, YCNHF I和YCNHF II分别与IBD靶点形成191、186个交集靶点, 富集于磷脂酰肌醇3激酶 (phosphatidylinositol 3 kinase, PI3K)-Akt等信号通路及线粒体功能调控网络; 槲皮素等成分与IL-6等靶点结合能力较强; 两复方分别有53、72个入血成分具抗氧化能力, 可抑制促炎因子表达、改善线粒体膜电位, 且能改善UC大鼠病理损伤、调控PI3K-Akt通路。本实验获得延安大学医学伦理委员会批准 (批准号: IIT-G-2025064)。

关键词: 炎症性肠病; 中药复方; 网络药理学; 分子对接; 抗氧化; 线粒体保护

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Network pharmacology-internal and external experiments of molecular docking consortium to verify and analyze the mitochondrial protection mechanism of innovative traditional Chinese medicine YCNHF I/II in inflammatory bowel disease

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Abstract: This study aimed to explore the multi-target mechanism of action of the innovative Chinese herbal compounds YCNHF I (comprising 6 herbs with both edible and medicinal values, including Gardenia jasminoides and others)/II (comprising 6 herbs without edible value, including Scutellaria baicalensis and others) in treating

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inflammatory bowel disease (IBD). We screened the active components of the compounds and IBD-related targets by mining multiple databases, then constructed a "compound-target-pathway" network and validated it using molecular docking. Ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) was employed to identify components that entered the bloodstream, and their antioxidant capacity was assessed. In lipopolysaccharide (LPS)-induced inflammatory Caco-2 cell models, we measured the expression of interleukin-6 (IL-6) and interleukin-1β (IL-1β), as well as mitochondrial membrane potential. Additionally, we established a rat model of ulcerative colitis (UC) induced by dextran sulfate sodium (DSS), after administering the compounds *via* intragastric gavage, colonic pathology was observed *via* hematoxylin and eosin (HE) staining, and p-Akt expression in colon tissues was detected by Western blot. Results showed that YCNHF I and YCNHF II shared 191 and 186 common targets with IBD-related targets, respectively. These targets were mainly enriched in signaling pathways such as PI3K-Akt and in mitochondrial function regulatory networks. Components like quercetin showed strong binding affinity to targets including IL-6. The two compounds had 53 and 72 blood-entering components, respectively, exhibited antioxidant activity, inhibited pro-inflammatory factor expression, and improved mitochondrial membrane potential. They also alleviated pathological damage in UC rats and regulated the PI3K-Akt pathway. This experiment was approved by the Medical Ethics Committee of the Yan'an University (approval number: IIT-G-2025064).

Key words: inflammatory bowel disease; Chinese medicine compound; network pharmacology; molecular docking; anti-oxidation; mitochondrial protection

炎症性肠病 (inflammatory bowel disease, IBD) 是一类以肠道慢性非特异性炎症为特征的自身免疫性疾病, 主要包括克罗恩病 (Crohn's disease, CD) 和溃疡性结肠炎 (ulcerative colitis, UC)[1], 全球发病率逐年升高, 亚洲新兴工业化国家尤为显著[2]。其发病机制涉及肠道菌群失调、免疫异常及氧化应激等多因素[3], 其中线粒体功能紊乱被证实是介导肠上皮细胞损伤的核心环节[4]。

在IBD患者肠上皮细胞中, 线粒体融合蛋白2 (mitofusin 2, MFN2) 和视神经萎缩因子1 (optic atrophy 1, OPA1) 表达下调, 导致线粒体网络断裂、碎片化及超微结构异常[5]。患者样本及基因缺陷小鼠模型均证实, 线粒体体积缩小、膜电位丢失及呼吸功能障碍, 线粒体功能紊乱加剧活性氧蓄积并激活NOD样受体热蛋白结构域相关蛋白3 (NOD-like receptor thermal protein domain associated protein-3, NLRP3) 炎症小体, 释放白细胞介素1β (interleukin-1β, IL-1β)、白细胞介素18 (interleukin-18, IL-18) 等促炎因子, 最终导致肠上皮细胞损伤及免疫细胞浸润[6,7]。

临床一线药物如5-氨基水杨酸 (5-amino salicylic acid, 5-ASA) 虽能抑制炎症, 但缺乏线粒体调控作用, 长期使用存在线粒体毒性风险[8]。生物制剂如抗肿瘤坏死因子α (tumor necrosis factor-α, TNF-α) 单抗虽能缓解症状, 但存在价格昂贵及免疫原性强等局限性[9]。因此开发靶向线粒体的新型治疗策略成为IBD研究领域的重要方向。中药复方在调控线粒体功能方面独具优势。中药活性成分黄芩苷通过抑制线粒体通透性转换孔 (mitochondrial permeability transition pore, mPTP) 开放维持膜电位稳定[10]; 厚朴酚可通过单磷酸腺苷激活的蛋白激酶−过氧化物酶体增殖物激活受体γ辅激活子1α [adenosine 5'-monophosphate (AMP)-activated protein kinase-peroxisome proliferator-activated receptor gamma coactivator 1α pathway, AMPK-PGC-1α] 通路信号上调沉默信息调节因子3 (silent information regulators 3, SIRT3) 表达增强线粒体抗氧化能力[11]; 槲皮素则通过AMPK/SIRT1/PGC-1α轴促进线粒体生物合成相关蛋白 (PGC-1α、核呼吸因子1、线粒体转录因子A) 表达[12], 三者协同改善线粒体功能。

本研究筛选并挖掘的创新药物组方YCNHF I (含栀子及砂仁等) 和YCNHF II (含黄芩及厚朴等) 中富含黄芩苷、厚朴酚和槲皮素等活性成分, 通过网络药理学构建“成分−线粒体靶点−通路”网络, 结合分子对接验证关键成分与线粒体蛋白互作; 采用中药入血成分分析、抗氧化实验、JC-1膜电位检测评估线粒体抗氧化活性, 通过实时荧光定量聚合酶链式反应 (quantitative real-time polymerase chain reaction, qRT-PCR) 检测线粒体相关炎症因子白细胞介素-6 (interleukin-6, IL-6)、IL-1β表达, 在3%葡聚糖硫酸钠盐 (dextran sulfate sodium, DSS) 诱导的急性UC大鼠模型中, 灌胃不同剂量YCNHF I/II后, 采用苏木素−伊红 (hematoxylin and eosin, HE) 染色观察结肠组织病理变化, Western blot技术检测结肠组织经过磷酸化修饰的蛋白激酶B (phospho-protein kinase B, p-Akt) 表达水平。

材料与方法

细胞、动物与试剂　本实验中的中药材购自金方千草药业有限公司, 原产地位于安徽亳州。美沙拉嗪肠溶片 (成分: 5-ASA) 购自葵花药业集团; 人结直肠癌上皮细胞Caco-2细胞株购自中国典型培养物保藏中心细胞库; 180 ± 20 g雄性SD大鼠购自江苏华创信诺医药科技有限公司, 许可证号为SCXK (苏) 2020-0009; 细胞生化试剂、细胞生物学试剂和分子生物学试剂等均来自北京索莱宝科技有限公司; qRT-PCR引物均合成于生工生物工程 (上海) 股份有限公司 (引物序列 *IL-6* For: GTGTTGCCTGCTGCCTTCC, *IL-6* Rev: TCTGAAGAGGTGAGTGGCTGTC; *IL-1β* For: GCACCTGTACGATCACTGAACTG, *IL-1β* Rev: CACTTGTTGCTCCATATCCTGTCC), *GAPDH* 基因货号为B661104-0001; 植物乳杆菌、嗜酸乳杆菌、鼠李糖乳杆菌、肠膜明串珠菌肠膜亚种、戊糖片球菌混合益生菌菌包 (mixed probiotics, MP) 购自江苏新申奥生物科技有限公司; DSS购自上海麦克林生化科技股份有限公司; Western blot试剂购自武汉三鹰生物技术有限公司。本实验获得延安大学医学伦理委员会批准 (批准号: IIT-G-2025064)。

基于网络药理学进行IBD相关复方挖掘及分析　基于TCMSP (https: //tcmsp-e.com/)、ETCM2.0 (http://www.tcmip.cn/ETCM2/front/#/) 和TCMIO (http://tcmio.xielab.net/) 数据库, 结合IBD中医证型“湿热内蕴”[13]、“脾虚湿困”[14]、“肝郁脾虚”[15]等挖掘古籍复方, 经《中华人民共和国药典2020版》[16]和《中华本草 第5册》[17]规范药名后进行频数分析。筛选频数前50的药物, 采用SPSS Modeler 18的Apriori算法 (支持度 ⩾ 10%、置信度 ⩾ 40%) 提取高频药物组合, 构建核心复方YCNHF I: 栀子、砂仁、陈皮、甘草、当归、干姜 (药食同源) 与YCNHF II: 黄芩、厚朴、陈皮、木香、甘草、当归 (非药食同源), 并未按照中药配伍原则进行组方。其次, 通过TCMSP筛选OB ⩾ 30%且DL ⩾ 0.18的活性成分, 结合CNKI (https://kns.cnki.net/kns8s/)、Swiss Target Prediction (http://www.swisstargetprediction.ch/) 补充高类药性与生物利用度吸收成分, 经UniProt (https://www.uniprot.org/) 标准化人源靶点。同时, 从Genecards (https://www.genecards.org/)、TTD (https://db.idrblab.net/ttd/)、OMIM (https://omim.org/) 数据库获取IBD核心靶点, 通过韦恩图筛选复方与疾病的共同靶点, 最终选取核心成分与靶点进行AutoDock Vina分子对接验证。

YCNHF I/II的制备　将YCNHF I/II按药材与水1∶10比例浸泡1 h, 武火煮沸后转文火煎煮40 min, 过滤取汁; 药渣再加8倍量水煎煮, 合并两次滤液, 冷冻干燥成粉末, 冻干粉得率为10%。

YCNHF I/II入血成分活性鉴定　将SD大鼠随机分为空白组、YCNHF I组 (0.531 25 $g \cdot kg^{-1} \cdot d^{-1}$) 和YCNHF II组 (0.5 $g \cdot kg^{-1} \cdot d^{-1}$), 每组5只, 连续灌胃7天后眼眶取血, 分离含药血浆。采用UPLC-MS/MS技术, 通过Waters ACQUITY UPLC HSS T3色谱柱 (1.8 μm, 2.1 mm × 100 μm) 进行梯度洗脱 (流动相A为0.1%甲酸/水, B为0.1%甲酸/乙腈), 结合Q Exactive HF-X质谱仪在ESI+/-模式下检测。血浆样本经70%甲醇超声提取后, 通过XCMS程序进行峰提取, 保留时间校正和代谢物鉴定。

YCNHF I/II抗氧化能力分析　根据实验步骤[18]分别在593和536 nm检测吸光度值, 计算总抗氧化能力和羟自由基清除力。

CCK-8法检测细胞毒性　取对数生长期的Caco-2细胞以每毫升 2.5×10^4 个细胞重悬后, 按每孔5 000个细胞接种于96孔板, 空白组为基础培养基, 常规培养24 h后, 分别给予0～320 $\mu g \cdot mL^{-1}$ 复方药物或0～320 $\mu mol \cdot mL^{-1}$ 5-ASA (n = 3), 继续孵育24 h, 采用CCK-8法于450 nm波长检测细胞活力, 细胞存活率计算公式为: 存活率 (%) = (给药组吸光度值−空白组吸光度值) / (对照组吸光度值−空白组吸光度值) × 100%。

qRT-PCR检测YCNHF I/II对炎症因子*IL-6*、*IL-1β* mRNA表达的影响　取对数生长期Caco-2细胞以每毫升 2.5×10^4 个细胞重悬后, 按每孔 3×10^5 个细胞接种于6孔板常规培养24 h, 实验分组如下: LPS组 (1 $\mu g \cdot mL^{-1}$ LPS)、给药组 (40 $\mu g \cdot mL^{-1}$ 复方 + 1 $\mu g \cdot mL^{-1}$ LPS或20 $\mu mol \cdot mL^{-1}$ 5-ASA + 1 $\mu g \cdot mL^{-1}$ LPS), 继续孵育24 h后提取总RNA, 采用qRT-PCR技术检测*IL-6*、*IL-1β*及*GAPDH*基因表达。反应体系含10 μL 2× SYBR green supermix、2 μL引物 (10 $\mu mol \cdot L^{-1}$) 及100 ng cDNA, 通过CFX Connect实时PCR仪进行扩增 (95 ℃预变性5 min, 40个循环: 95 ℃ 30 s, 58 ℃ 30 s)。通过 $2^{-\Delta\Delta Ct}$ 法计算目的基因相对mRNA水平。

JC-1染色检测YCNHF I/II对LPS诱导Caco-2细胞线粒体膜电位 (MMP) 的影响　取对数生长期Caco-2细胞以每毫升 2.5×10^4 个细胞重悬后, 按每孔 3×10^5 个细胞接种于6孔板常规培养24 h, 实验分组如下: LPS组 (1 $\mu g \cdot mL^{-1}$ LPS)、给药组 (1 $\mu g \cdot mL^{-1}$ LPS+40 $\mu g \cdot mL^{-1}$ 复方), 使用JC-1线粒体膜电位检测试剂盒评估MMP。共聚焦荧光显微镜检测荧光从绿色到红色的变化情况, Image J分析各组荧光含量以评估线粒体膜电位。

YCNHF I/II治疗UC作用

造模及给药方法　SD大鼠适应性喂养7天后随机分为正常对照组 (control)、模型组 (DSS)、YCNHF I

低剂量组 (YCNHF I-L, 0.229 5 g·kg^{-1}·d^{-1})、YCNHF I 中剂量组 (YCNHF I-M, 0.459 g·kg^{-1}·d^{-1})、YCNHF I 高剂量组 (YCNHF I-H, 0.918 g·kg^{-1}·d^{-1})、YCNHF II 低剂量组 (YCNHF II-L, 0.238 5 g·kg^{-1}·d^{-1})、YCNHF II 中剂量组 (YCNHF II-M, 0.477 g·kg^{-1}·d^{-1})、YCNHF II 高剂量组 (YCNHF II-H, 0.954 g·kg^{-1}·d^{-1})、混合益生菌组 (MP, 0.5 g·kg^{-1}·d^{-1})、5-ASA 组 (5-ASA, 0.36 g·kg^{-1}·d^{-1}), 每组 5 只。1 周后将正常对照组之外的其他组饮用水都换为 3% DSS, 在第 9 天 90% 的动物出现便血或肛门处粘连血渍, 则判断模型成功。随后造模组换饮去离子水, 正常对照组每日灌胃去离子水, 阳性对照组及各治疗组均给予为期 10 天的灌胃给药。

体重变化及疾病活动指数 (disease activity index, DAI) 评分　每天在固定时间对大鼠实施干预, 并对大鼠的状态进行观察, 对大鼠体重进行记录, 依照 DAI 评分标准 (表 1) 记录腹泻和便血程度, DAI = (腹泻分数 + 便血分数 + 体重分数)/3。

样本的收集和预处理　最后一次给药后, 所有大鼠禁食不禁水 12 h, 将所有大鼠均实施 1% 戊巴比妥钠安乐死并解剖, 收集脾及结肠组织。取出结肠后立即测量盲肠前端至直肠末端的长度。分离脾脏进行称重, 小鼠脾脏系数的计算公式为: 脾脏质量 (g) /体重 (g) × 10。测量结束后, 将大鼠的远端结肠段用无菌 PBS 清洗, 再于 4% 多聚甲醛中过夜固定, 剩余结肠与其他样本于−80 ℃保存。

HE 染色　取各大鼠相同部位结肠组织, 用 4% 多聚甲醛固定后, 常规石蜡包埋切片。切片处理后, HE 染色, 于光学显微镜下观察结肠组织病理学变化。

结肠组织 p-Akt 蛋白表达测定　采用 Western blot 法检测结肠组织中 p-Akt 蛋白, 冰上裂解组织获取蛋白, 二喹啉甲酸 (bicinchoninic acid, BCA) 蛋白定量法测定蛋白浓度并调整上样量。收集样品上清液, 加入十二烷基硫酸钠−聚丙烯酰胺凝胶电泳上样缓冲液, 经煮沸变性后进行电泳分离。使用湿法转膜将蛋白转移至聚偏二氟乙烯膜。室温封闭 2 h 后, 一抗 4 ℃孵育过夜, 次日加入二抗震荡孵育 2 h 后显影, Image J 软件计算条带灰度值并绘制统计图。

统计学分析　采用 SPSS 27.0 对数据进行统计学分析, 计量资料以平均值 ± 标准差 ($\bar{x} \pm s$) 表示, 多组间比较采用单因素方差分析, 两组间比较采用独立样本 t 检验。$P < 0.05$ 为差异有统计学意义。

结果

1　IBD 中药复方挖掘

1.1　中药复方筛选　基于 TCMM、ETCM2.0 和 TCMIO 数据库挖掘 IBD 相关古籍复方共 1 457 种。对中药名称进行转置规范, 筛选 750 味中药 (累计用药 9 752 频次), 统计前 50 高频药物 (表 2)。

1.2　获得 IBD 中药复方　基于 SPSS Modeler 18.0 构建前 50 味中药的复杂网络 (强链接 ⩾ 40, 弱链接 ⩽ 15), Cytoscape 3.10.0 生成网络贡献图 (图 1)。采用 Apriori 算法进行关联规则分析 (支持度 ⩾ 10%、置信度 ⩾ 40%), 获得 26 组有效组合 (提升度 > 1), 包含 23 个 2 项组合和 3 个 3 项组合, 值得注意的是当归和甘草支持度可达到 23.59%。结合《实用中医药膳学》[19]构建 6 个复方 (YCNHF 1～6), 其中药食同源中药复方为 YCNHF 1 (栀子∶砂仁∶陈皮∶甘草∶当归∶干姜 = 10∶8∶9∶9∶10∶5)、YCNHF 2 (砂仁∶陈皮∶山楂∶甘草∶黄芪∶干姜 = 8∶9∶10∶9∶10∶5)、YCNHF 3 (栀子∶陈皮∶山楂∶甘草∶当归∶肉豆蔻 = 10∶9∶10∶9∶10∶9); 非药食同源 YCNHF 4 (黄连∶陈皮∶木香∶甘草∶白芍∶干姜 = 6∶9∶5∶9∶15∶5)、YCNHF 5 (黄芩∶厚朴∶陈皮∶木香∶甘草∶当归 = 10∶10∶9∶5∶9∶10)、YCNHF 6 (黄连∶厚朴∶陈皮∶木香∶肉豆蔻∶甘草 = 6∶10∶9∶5∶9∶9)。

2　网络药理学分析

2.1　中药复方靶点获取　于 TCMSP 筛选 OB ⩾ 30% 且 DL ⩾ 0.18 的活性成分, 结合 CNKI 和 Swiss Target Prediction 补充高类药性与生物利用度吸收成分, 通过 UniProt 映射至人源靶点并标准化, 剔除重复无效靶点, 获 YCNHF 1～6 靶点分别为 256、260、259、260、247、257 个。

2.2　IBD 靶点获取　在 Genecard 数据库用“inflammatory bowel disease”检索 IBD 相关疾病基因, 按 Score 值排序, 选 Score 值 ⩾ 中位数的基因, 结合 TTD 和 OMIM 补充并去重, 得 4 509 个综合 IBD 相关靶点。将 YCNHF 1～6 潜在靶点与 IBD 相关靶点上传至 Venny 2.1.0 在线网站进行 Venn 分析, 获 YCNHF 1～6 交集靶点分别为 193、193、192、188、186、183 个。

Table 1　Scoring criteria for the disease activity index (DAI)

Weight	Bloody stool	Hardness of stool	Score
Not reduced or increased	No bloody stool	Good and the texture is hard	0
Weight loss of 1%−5%	Mild hematochezia	Soft forming	1
Weight loss of 6%−10%	Moderate hematochezia	Soft and unshaped	2
Weight loss of 10%−15%	Severe hematochezia	Watery stool	3
Weight loss of >15%	Severe hematochezia	Watery stool	4

Table 2 The frequency statistics of oral traditional Chinese medicine compound medication for inflammatory bowel disease (IBD) included in the study. The frequency refers to the quantity that appears in 1 457 traditional Chinese medicine prescriptions for IBD. The frequency is the percentage it occupies in the total frequency

Medicine	Frequency	Percent/%	Class of action
Licorice	484	4.96	Replenishing Qi
Chinese angelica	345	3.54	Replenish blood
Coptis chinensis	338	3.47	Clearing heat
Costus	279	2.86	Promoting Qi to relieve pain
Tangerine peel	258	2.65	Regulating Qi and strengthening spleen
Citrus aurantii	246	2.52	Promoting Qi to eliminate distension
Radix angelicae	229	2.35	Dry dampness stop belt
Poria cocos	216	2.21	Tonifying spleen
Radix paeoniae alba	211	2.16	Restrain Yin and prevent perspiration
Magnolia officinalis	181	1.86	Eliminating dampness
Scutellaria baicalensis	174	1.78	Clearing heat
Rhizoma zingiberis	167	1.71	Winry
Ginseng	159	1.63	Tonify deficiency
Rheum officinale	156	1.60	Purgation
Areca nut	138	1.42	Promoting circulation of Qi
Garden burnet	123	1.26	Clearing heat
Astragalus membranaceus	114	1.17	Tonify deficiency
Fructus amomi	107	1.10	Regulating the flow of Qi
Ligusticum chuanxiong	105	1.08	Promoting blood circulation and removing blood stasis
Rhizoma atractylodis	99	1.02	Resolving dampness
Monkshood	99	1.02	Winry
Fructus chebulae	98	1.00	Arresting astringency
Myristica fragrans	97	0.99	Arresting astringency
Rehmannia	94	0.96	Clearing heat
Radices sileris	89	0.91	Relieving superficies
Sophora flower	84	0.86	Cooling blood to stop bleeding
Pericarpium citri reticulatae viride	83	0.85	Regulating the flow of Qi
Cinnamon	83	0.85	Winry
Rattletop	75	0.77	Promoting Qi to relieve pain
Chinese peony	73	0.75	Replenish blood
Hawthorn	72	0.74	Digestion and accumulation
Dark plum	71	0.73	Astringent
Golden cypress	70	0.72	Clearing heat
Rhizoma alismatis	70	0.72	Relieve water and permeate dampness
Medicated leaven	69	0.71	Help digestion
Cyperus rotundus	64	0.66	Regulating the flow of Qi
Pericarpium papaveris	62	0.64	Arresting astringency
Alumen	60	0.62	Arresting astringency
Radix paeoniae rubra	60	0.62	Clearing heat
Sophora fruit	59	0.61	Clearing heat
Colla corii asini	55	0.56	Tonify deficiency
Pinellia ternata	55	0.56	Warming cold phlegm
Halloysitum rubrum	54	0.55	Arresting astringency
Chinese honey locust	52	0.53	Warming cold phlegm
Fresh ginger	51	0.52	Relieving superficies
Syzygium aromaticum	50	0.51	Winry
Fructus evodiae	49	0.50	Winry
Radix angelicae	48	0.49	Relieving superficies
Radix bupleuri	48	0.49	Relieving superficies
Fructus Gardeniae	47	0.48	Clearing heat

2.3　确定IBD中药复方　通过Metascape数据库对YCNHF 1～6与IBD交集靶点进行KEGG富集分析，预测YCNHF 1和YCNHF 5可能通过抑制丝裂原活化蛋白激酶 (mitogen-activated protein kinase, MAPK)、高级糖基化终产物–受体 (advanced glycation end products-receptor for advanced glycation end products, AGE-RAGE) 及磷脂酰肌醇3激酶 (phosphatidylinositol 3-kinase, PI3K)-蛋白激酶B (protein kinase B, 又称Akt) 等通路 (表3) 调控IBD。将YCNHF 1和YCNHF 5重命名为YCNHF I和YCNHF II用于后续实验。

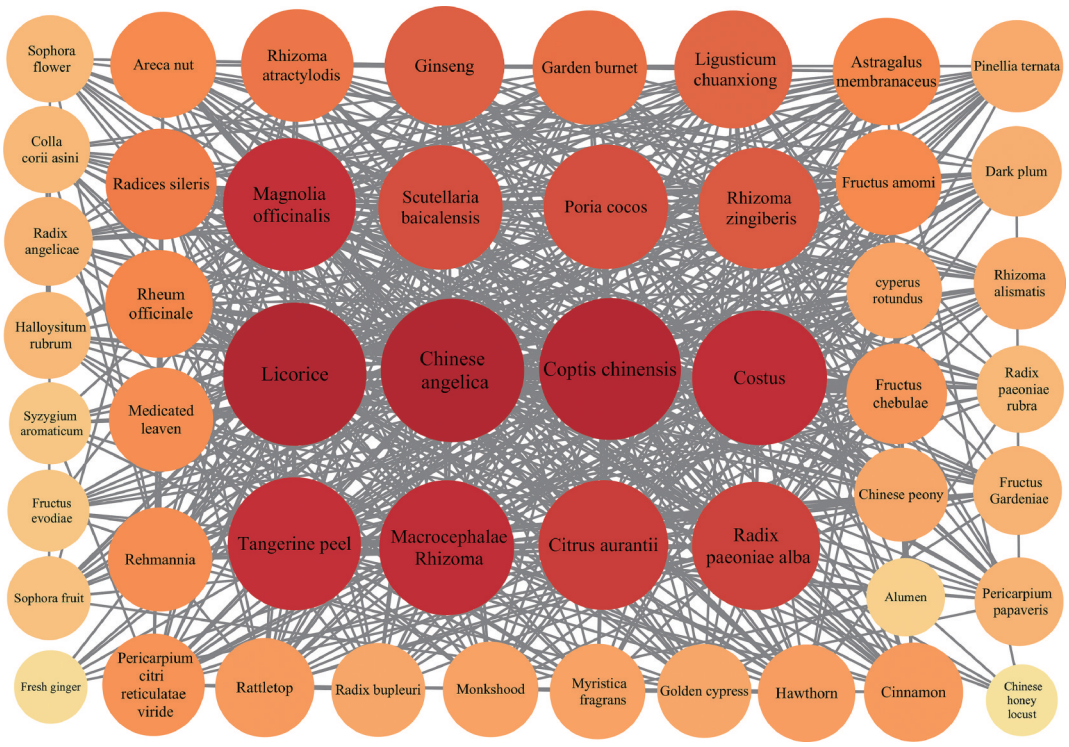


Figure 1 Network contribution diagram of IBD traditional Chinese medicine compounds. The higher the potency value, the larger the circle, the darker the color, the more connections it has with other traditional Chinese medicines, and the more important it is in the network

Table 3 Correlation analysis between YCNHF 1−6 and IBD

Name	Target	Channel
YCNHF 1	*AKT1*, *STAT3*, *HSP90AA1*, *MAPK3*	MAPK, AGE-RAGE, PI3K-Akt
YCNHF 2	*ESR1*, *MAPK1*, *IL1B*, *CXCL8*	Cancer, lipids and atherosclerosis, chemical carcinogenesis-receptor activation
YCNHF 3	*ESR1*, *MAPK10*, *TNF*, *IL6*	Cancer, lipids and atherosclerosis, PI3K-Akt
YCNHF 4	*ESR1*, *HSP90AA1*, *TNF*, *IL6*	Cancer, lipids and atherosclerosis, chemical carcinogenesis-receptor activation
YCNHF 5	*AKT1*, *IL6*, *STAT3*, *HSP90AA1*	MAPK, PI3K-Akt, AGE-RAGE
YCNHF 6	*ESR1*, *TNF*, *MAPK1*, *IL1B*	Cancer, lipids and atherosclerosis, chemical carcinogenesis-receptor activation

2.4 分子对接 选取YCNHF I/II中排名前10位的靶点基因：*JUN*、*AKT1*、*IL6*、*STAT3*、*TNF*、*HSP90AA1*、*ESR1*、*MAPK1*、*MAPK3*、*IL1B*与前5位的活性成分：槲皮素、豆甾醇、山柰酚、*β*-谷甾醇、柚皮素分别用AutoDock Vina软件进行分子对接验证。发现结合能均小于−5 kcal·mol^{-1} (表4)，具有良好的结合能力。

3 YCNHF I/II入血成分活性分析

YCNHF I中共有53个成分入血。其中12个以原型成分入血，剩余41个以代谢物成分方式入血；YCNHF II中共有72个成分入血。其中12个以原型成分入血，剩余60个以代谢物成分方式入血 (图2A～E)。将YCNHF I/II中入血成分经PubChem (https://pubchem.ncbi.nlm.nih.gov/) 网站筛选分别得到585和774个靶点，其中与IBD交集靶点分别为365和464个 (图2F、G)。进一步筛选出前10个高degree靶点并构建核心PPI网络 (图2H、I)。选取高degree靶点经过DAVID数据库进行KEGG富集分析，发现YCNHF I/II可能通过PI3K-Akt、MAPK、AGE-RAGE等通路调节IBD。上述结果与前期网络药理学研究预测结论高度吻合，提示相关信号通路可作为后续药效物质基础及

Table 4 Binding energy (kcal·mol^{-1}) of active components and key target molecules of YCNHF I/II for IBD treatment

Component	Target									
	JUN	*AKT1*	*IL6*	*STAT3*	*TNF*	*HSP90AA1*	*ESR1*	*MAPK1*	*MAPK3*	*IL1B*
Quercetin	−6.4	−6.0	−7.1	−8.1	−6.9	−6.5	−5.9	−7.6	−6.9	−7.0
Stigmasterol	−6.4	−7.3	−5.7	−6.9	−6.1	−6.6	−6.7	−6.8	−6.7	−6.0
Kaempferol	−6.3	−6.0	−6.5	−7.8	−8.1	−8.8	−6.9	−6.4	−8.9	−6.8
β-Sitosterol	−6.4	−6.9	−5.6	−5.3	−6.3	−6.9	−6.7	−6.5	−6.8	−6.0
Naringenin	−6.2	−6.2	−6.6	−6.8	−6.7	−6.6	−5.9	−6.2	−9.0	−6.8

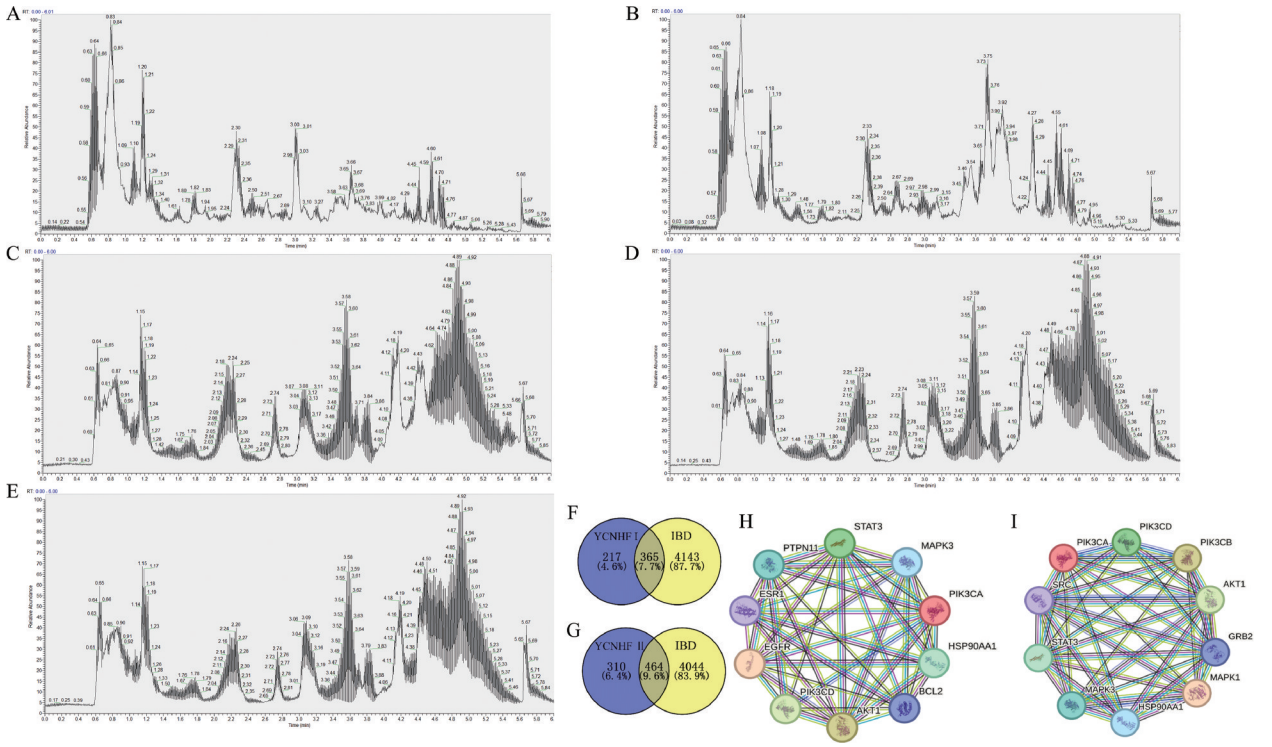


Figure 2　Analysis of YCNHF components entering the bloodstream and network pharmacological analysis. A: Total ion current diagram of YCNHF I; B: Total ion current diagram of YCNHF II; C: Blank serum total ion current diagram; D: YCNHF I serum total ion current diagram; E: YCNHF II serum total ion current diagram; F: Venn diagram of the intersection target of YCNHF I and IBD; G: Venn diagram of the intersection target of YCNHF II and IBD; H: PPI diagram of the core target of YCNHF I and IBD; I: PPI diagram of the core target of YCNHF II and IBD

作用机制研究的关键。

4　中药复方体外抗氧化能力

在YCNHF I/II的体外羟自由基清除能力实验和总抗氧化活性实验中, 结果显示在所测浓度范围内, YCNHF I/II的羟自由基清除率和体外总抗氧化能力均呈浓度依赖性升高 (图3A、B), 且YCNHF II的效果明显优于YCNHF I。

5　YCNHF I/II在高浓度下对Caco-2细胞无毒性

CCK-8实验显示, YCNHF I和YCNHF II在0～320 μg·mL^{-1}浓度下处理Caco-2细胞24 h后, 细胞存活率分别为100.5%和101.3%, 均对Caco-2细胞无显著毒性作用, 差异有统计学意义 (P < 0.05, 图3C～E)。提示其具有良好的生物相容性, 为后续实验提供了安全浓度依据。

6　YCNHF I/II均下调炎症因子*IL-6*、*IL-1β* mRNA表达

qRT-PCR实验结果显示, 与正常对照组相比, 1 μg·mL^{-1} LPS处理组*IL-6*和*IL-1β* mRNA表达显著增高, 而与1 μg·mL^{-1} LPS处理组相比, 40 μg·mL^{-1} YCNHF I/II联合LPS共处理24 h后下调Caco-2细胞中*IL-6*和*IL-1β* mRNA表达 (图3F), 差异有统计学意义 (P < 0.000 1), YCNHF II表现出更加显著的优势。该结果表明, YCNHF I/II通过下调促炎细胞因子表达发挥抗炎作用。

7　YCNHF I/II改善LPS诱导Caco-2细胞膜电位水平

与正常组相比, 各组膜电位水平均有所下降, 1 μg·mL^{-1} LPS处理组下降最为显著。40 μg·mL^{-1} YCNHF I/II联合LPS药物共处理24 h后, 各组线粒体膜电位水平与LPS诱导组相比均有提高, YCNHF II效果更为明显 (图3G、H), 差异有统计学意义 (P < 0.000 1)。

8　YCNHF I/II改善3% DSS诱导大鼠的UC病理症状

实验结果显示, 与正常对照组相比, 造模期间各个组大鼠体重下降且DAI评分增高 (图4A、B); 给药期间, 与模型组相比, YCNHF I/II各给药组大鼠体重缓解更为明显, DAI评分也明显降低且YCNHF II高剂量组作用最明显 (图4C、D)。表明YCNHF I/II显著改善了DSS诱导的UC大鼠体重减轻及降低其DAI评分。此外, 正常对照组大鼠结肠细长无水肿充血等症状, 肠道内容物饱满呈现颗粒状; DSS诱导后各组大鼠不同程度地出现了结肠变短的现象, 表明大鼠急性结肠炎模型成功建立, 其中模型组大鼠结肠缩短最为严重。给予不同剂量的YCNHF I/II后UC大鼠结肠长度显著变长 (图4E、F), YCNHF II-H与5-ASA组差异有统计学意义 (P < 0.05), 同时对DSS诱导的脾指数增大具有明显改善作用 (图4G)。上述结果表明, YCNHF I/II有效

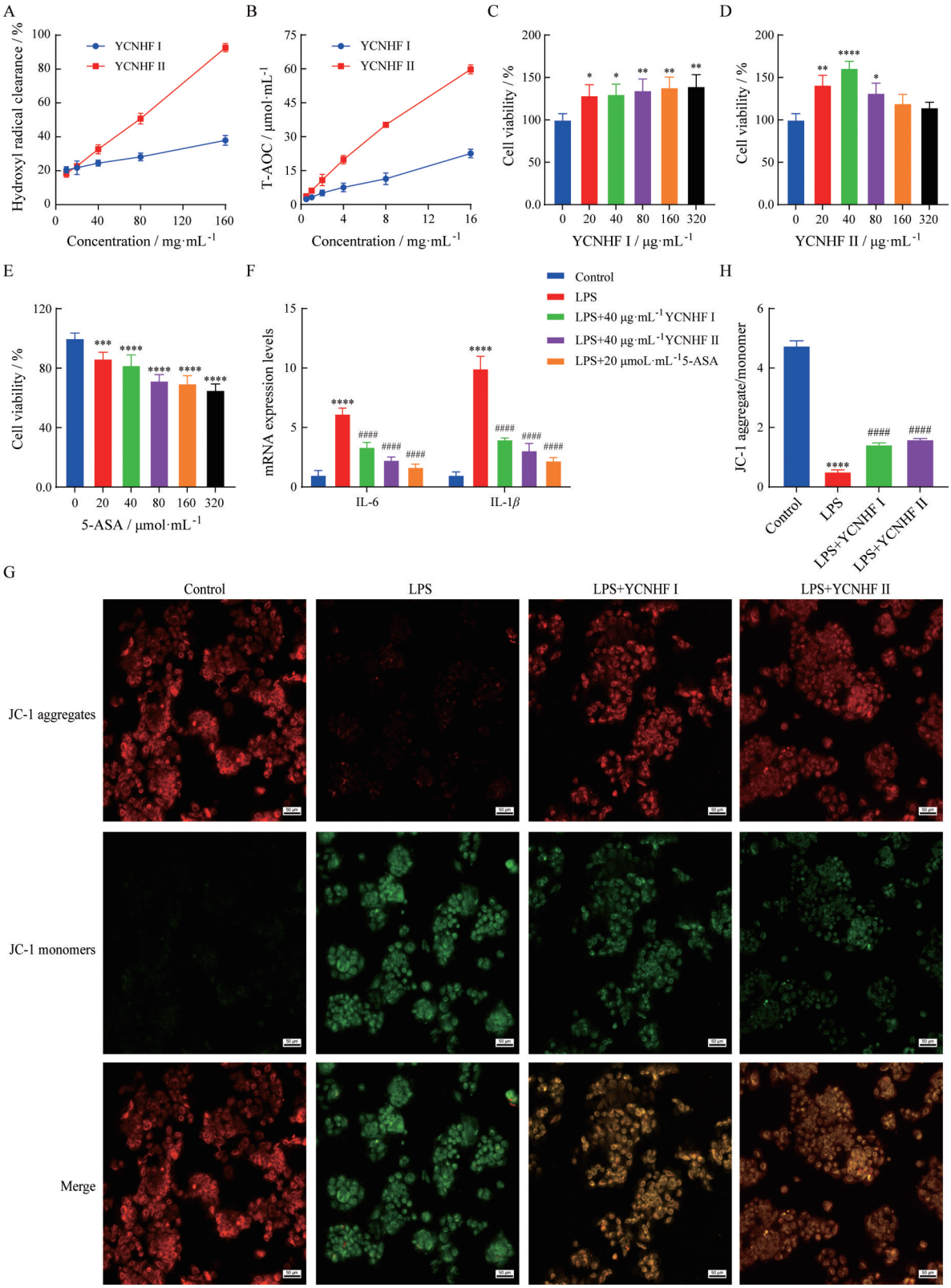


Figure 3 *In vitro* activity analysis of YCNHF. A: Hydroxyl radical clearance rate of YCNHF I/II; B: Total antioxidant activity of YCNHF I/II; C−E: Effects of drug treatment alone (C: YCNHF I; D: YCNHF II; E: 5-ASA) for 24 h on cell survival rate; F: The effect of drugs on LPS-induced cellular inflammatory factors; G, H: The effect of drugs on the membrane potential level induced by LPS. The former is the fluorescence image of membrane potential in each group, and the latter is the fluorescence content image analyzed by Image J (scale bars: 50 μm, 100×). $n = 3$, $\bar{x} \pm s$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.000\ 1$ *vs* control group; $^{\#\#\#\#}P < 0.000\ 1$ *vs* LPS group. T-AOC: Total antioxidant capacity; 5-ASA: 5-Amino salicylic acid; LPS: Lipopolysaccharide

改善了DSS诱导的UC症状。

正常对照组大鼠结肠黏膜结构完整, 肠腺排列紧密, 上皮细胞形态正常, 无溃疡形成; 杯状细胞数量较多, 隐窝呈规律性指状分布, 形态清晰, 未见破坏或缺失。模型组大鼠结肠则呈现严重急性炎症反应, 具体表现为上皮细胞糜烂或脱落; 杯状细胞数量显著减少, 隐窝出现不同程度破坏甚至消失, 可见密集的蓝紫色炎症细胞浸润; 黏膜层组织结构紊乱, 局部可见溃疡形成及糜烂加重, 黏膜损伤程度严重。各给药组大鼠结肠组织病理学损伤均得到不同程度改善, 其中

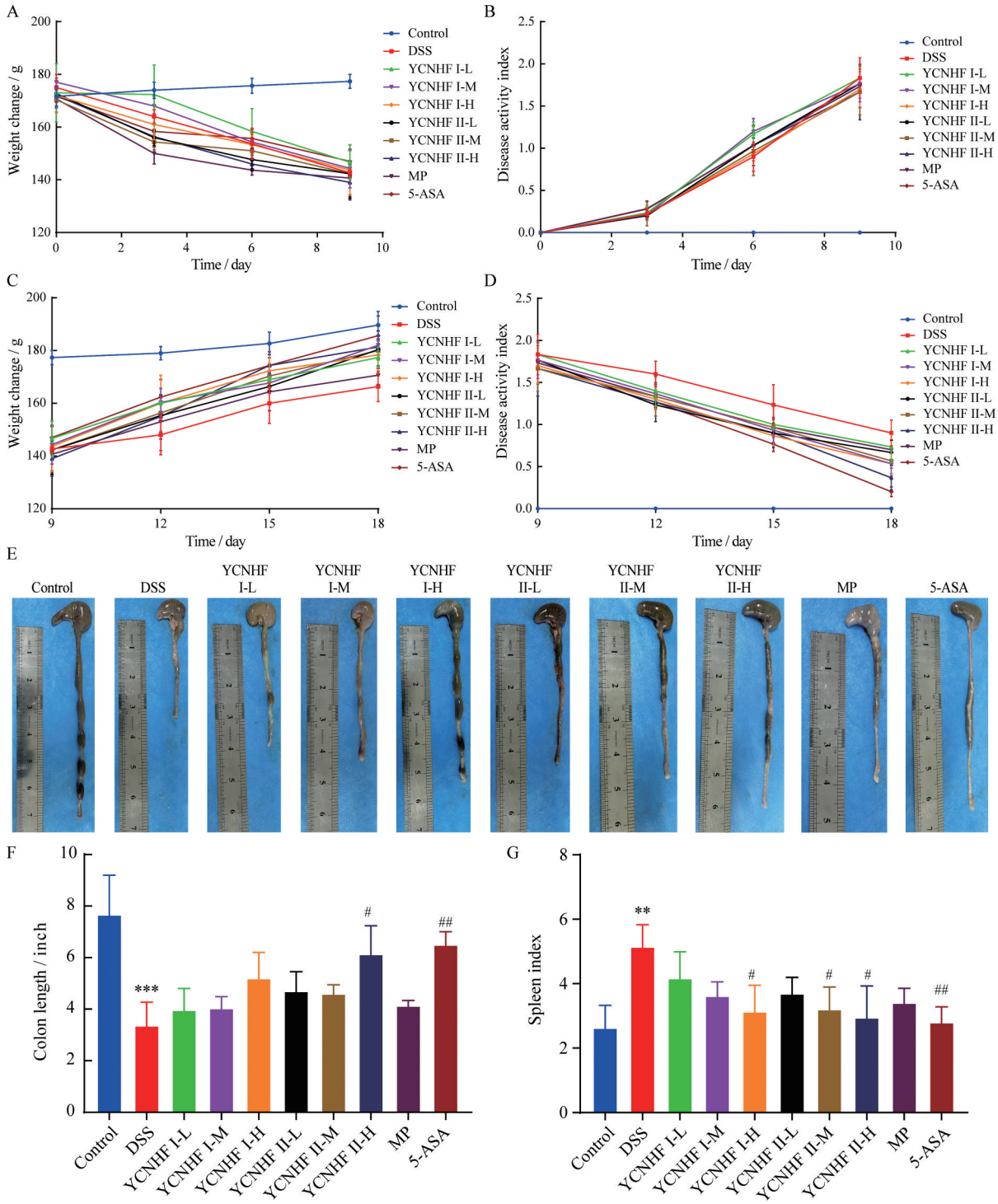


Figure 4　Detection of various indicators of YCNHF *in vivo* activity. A: Weight records during the modeling period; B: DAI scores during the modeling period; C: Weight records during administration; D: DAI scores during administration; E: Colon lengths of rats; F: The colon lengths of rats were statistically analyzed; G: Spleen index of each group; $n = 5$, $\bar{x} \pm s$. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ *vs* DSS group. MP: Mixed probiotics; DSS: Dextran sulfate sodium

YCNHF II高剂量组对结肠病理损伤的治疗作用最显著, 杯状细胞数量较模型组显著增多, 局部隐窝脓肿明显减轻 (图5)。

9 YCNHF I/II改善3% DSS诱导大鼠的p-Akt蛋白含量表达

Western blot实验结果显示, DSS处理组较正常对照组结肠组织中p-Akt的表达水平呈现显著上调趋势, 差异有统计学意义 ($P < 0.001$), 该结果提示DSS诱导的病理状态可能通过激活PI3K-Akt信号通路参与结肠组织的损伤或炎症反应过程。而在给予不同治疗方案后, 与DSS模型组相比, YCNHF I/II大鼠结肠组织中的p-Akt蛋白表达均呈现不同程度的下降趋势 (图6), 差异有统计学意义 ($P < 0.05$)。表明YCNHF I/II可能通过调控Akt信号通路的激活水平, 对DSS诱导的结肠病理改变发挥潜在调节作用。进一步对比可见, YCNHF II高剂量组的p-Akt蛋白下调幅度更为明显 ($P < 0.001$)。提示在本实验条件下, 高剂量YCNHF II对DSS诱导的结肠组织p-Akt异常激活具有更优的调控作用。

讨论

本研究基于网络药理学挖掘IBD高频药物组成中药复方YCNHF I/II, 药食同源复方YCNHF I中栀子苦寒清热, 陈皮理气, 砂仁和干姜温化湿寒, 当归和甘草补气养血, 体现“清热不伤阳, 理气兼顾虚”。而非药食同源复方YCNHF II中黄芩和厚朴清热燥湿[20], 木香和陈皮行气导滞, 当归和甘草补气养血, 体现“急则治标”的抗炎侧重, 这一点与YCNHF II表现出更强的羟自由基清除能力、总抗氧化能力、显著下调IL-6和IL-1β炎症因子, 以及明显改善UC大鼠的病理病症表达相符合。

经过UPLC-MS/MS对YCNHF I/II复方进行入血成分分析, 发现YCNHF I有53个成分入血, YCNHF II中则有72个成分入血。进一步将得到的入血活性成分与IBD进行网络药理学分析, 结果显示其共同指向核心靶点PIK3CD、STAT3、PIK3CA、MAPK3、AKT1、HSP90AA1, 这些靶点可能通过PI3K-Akt、MAPK、AGE-RAGE等通路调控IBD, 与前期网络药理学预测结果高度吻合。

在结肠组织p-Akt蛋白含量表达实验结果中, 发现YCNHF I/II大鼠结肠组织中的p-Akt蛋白表达与DSS组比较均呈现不同程度的下降趋势, 表明YCNHF I/II通过调控PI3K-Akt信号通路的激活水平, 对DSS诱导的结肠病理改变发挥潜在调节作用。线粒体功能紊乱是IBD肠上皮损伤的核心环节, 受肠道

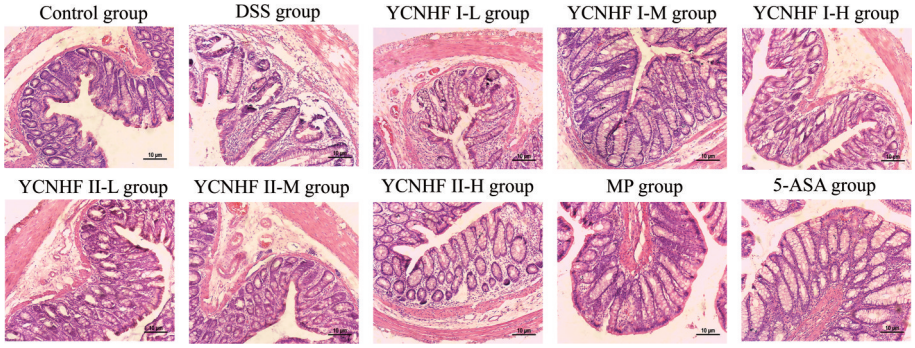


Figure 5 YCNHF improves the pathological damage of colonic tissue in UC rats induced by DSS. Scale bars: 10 μm, 200×

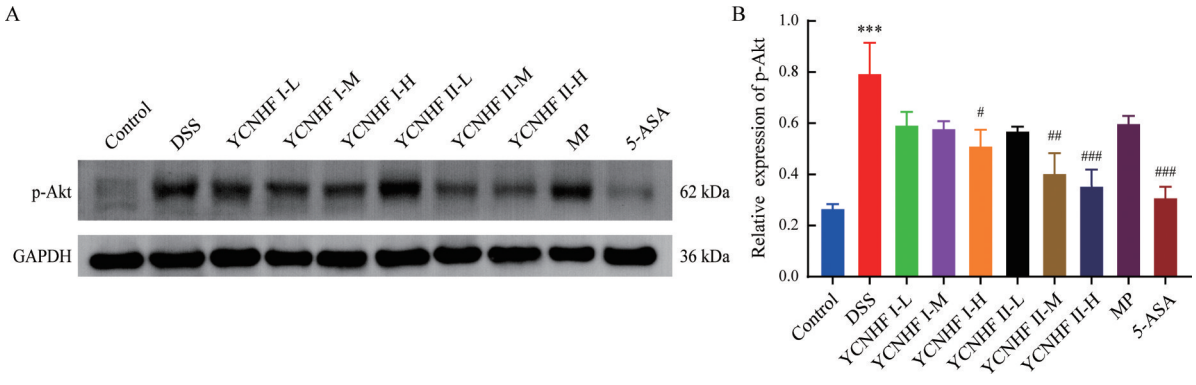


Figure 6 YCNHF improves the expression of p-Akt protein content in rats induced by DSS. A: Regulation of p-Akt protein by YCNHF I/II; B: Analysis of the gray values of Western blot strips by Image J. $n = 3$, $\bar{x} \pm s$. $^{***}P < 0.001$ *vs* control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ *vs* DSS group

吸收特性影响, 临床用药5-ASA难以有效作用于结肠细胞线粒体, 且本身缺乏线粒体调控活性[21], 而中药复方YCNHF I/II表现出高羟自由基清除力和总抗氧化能力, JC-1线粒体膜电位实验显示, YCNHF I/II可以改善LPS诱导的线粒体膜电位去极化, 稳定膜电位, 改善肠上皮细胞能量代谢障碍。

YCNHF I/II的多靶点优势弥补了传统单靶点药物的局限性, 其线粒体保护特性为临床用药策略升级提供新思路。然而, 本研究还需结合代谢组学技术解析活性成分与肠道菌群的互作机制, 并探索与生物制剂的联合用药方案, 或基于中医辨证分型指导个性化治疗, 以提升临床精准性。

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利益冲突: 无利益冲突。

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