基于肠道代谢组的中药药效物质基础和作用机制研究

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摘要: 中药药效物质是中药作用机制和质量控制研究的基础,也是中医药传承和创新发展的关键科学问题。然而中药多组分、多靶点、整体性的复杂特点, 以及现代科学研究技术方法的限制, 为中药药效物质研究带来了极大挑战。中药与肠道菌群的相互作用提供了一种新思路。基于肠道代谢组的中药药效物质基础与作用机制研究, 多以中药的有效作用以及肠道菌群与疾病的相关性假设为基础, 探究微生物群和宿主表型之间的关系, 逐步深入, 最终聚焦肠道菌株和肠道代谢物在分子水平的联系。本文对该模式的研究策略及关键技术进行了归纳总结, 以期为本模式的应用提供参考。

关键词: 肠道菌群; 肠道代谢物; 中药; 药效物质基础; 研究策略

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Research on material basis and mechanism of traditional Chinese medicine based on intestinal metabolomics

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Abstract: The pharmacodynamic substance of traditional Chinese medicine (TCM) is an important basis for its mechanism and quality control, and also a key scientific issue for the inheritance and development of TCM. However, the complex characteristics of multi-component, multi-target and integrity of TCM, as well as the limitations of modern scientific research technical methods, have brought great challenges to the research. The interactions between Chinese medicine and intestinal flora provide us with a new idea. Based on the effective role of TCM and the hypothesis of correlation between intestinal flora and disease, the research on the material basis and mechanism of action of TCM based on intestinal metabolomics mostly explored the relationship between microflora and host phenotype, gradually deepening, and finally focused on the relationship between intestinal strains and molecular levels. This paper summarized the research ideas and key technologies of this model, in order to provide reference for the application of this model.

Key words: Intestinal flora; intestinal metabolite; traditional Chinese medicine; material basis; research ideas

中药药效物质是指中药单方或复方发挥药效的主要化学成分, 是中药作用机制和质量控制研究的基础, 也是中药传承和创新发展的关键科学问题。然而中药多组分、多靶点、整体性的复杂特点, 为中药药效物质基础研究带来了极大挑战。目前国内外学者做了很多中药药效物质基础的研究方法的探索性研究, 并建立了系统性的研究方法, 如血清药物化学[1]、目标成分敲除/敲入技术[2]、中药谱效学[3]、中药整体药代动力学方

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法[4]、网络药理学技术[1]、结构中药学[5]等。这些方法对于中药活性成分和物质基础研究各有优势, 多种方法综合运用使中药的药效物质基础研究更加全面。

近年来大量的研究[6-8]表明, 中药药效的发挥和中药与肠道菌群的相互作用有关, 中药可通过调节肠道菌群平衡及肠道代谢物发挥作用[9]。肠道代谢物是沟通菌群与宿主相互作用的桥梁, 其变化是菌群与宿主功能的直接反映, 具有参与能量代谢、细胞间通讯、宿主免疫、维持黏膜完整性等多种生理功能[10,11]。肠道代谢物鉴定和功能研究有助于更加深入地理解其对宿主生理病理过程的影响和调控。基于粪便或肠道内容物的代谢组学分析可通过检测肠道代谢物的变化, 清晰展示肠道菌群和宿主的代谢状态。因此, 肠道代谢组也是肠道菌群功能研究的重要手段。

基于肠道代谢组的中药药效物质基础与作用机制研究需要考虑宿主本身、肠道菌群、宿主代谢物、菌群代谢产物、中药活性成分等多种因素, 为肠道菌群相关研究带来了更大的挑战。目前在基于肠道菌群的中药药效及机制研究中, 多以肠道菌群与药效的相关性假设为基础, 探究微生物群和宿主表型之间的关系, 逐步深入, 最终阐明其分子机制[12]。然而, 明确肠道菌群与中药药效作用的因果关系还充满了挑战, 需要有充分的证据来确证。因此, 本文将对基于肠道菌群和肠道代谢组的中药药效物质基础和作用机制研究策略及应用方法进行综述, 希望对本模式的应用提供参考。

1 研究策略

1.1 中药化学成分制备、表征及药效评价

首先制备中草药提取物, 或分离得到不同的提取部位, 并进行化学成分表征或定量。通过临床试验或动物模型评价中药提取物、提取部位或制剂的药效。通过行为学、病理指标、生化指标等分析疾病的发生发展, 评价中药的治疗作用, 此过程是整个基于肠道菌群的中药药效物质基础和作用机制研究的基础。

Zeng 等[13]首先优化了柑橘中多甲氧基黄酮的提取纯化过程, 并对其中的多甲氧基黄酮类成分进行定量测定, 并通过生理生化指标来评价其对代谢综合征的改善作用, 在此基础上才进行肠道微生物相关的深入研究。Sun 等[14]研究了杜仲多糖对肥胖饮食诱导的认知和社交功能障碍的调节作用, 首先对多糖进行分离纯化及表征, 通过动物行为学等指标来评估其药效。

1.2 肠道菌群和中药药效间的关联分析

目前微生物组学和代谢组学联合分析是阐明肠道菌群和中药药效之间相互关系的有效手段。16S rRNA 测序、宏基因组、16S PicBio SMRT 等测序技术可用于分析肠道菌群组成及其相对丰度, 以发现与疾病或药效相关的菌群差异。小分子代谢物是协调微生物与宿主相互作用的关键中间体, 因此可以采用非靶向或靶向代谢组学分析表征代谢物, 发现差异代谢表型, 有助于研究受到疾病或中药影响的与肠道微生物相关的代谢途径, 寻找相关生物标记物以及中药治疗靶点。进一步通过 Pearson、Spearman、GRaMM[15]等方法对微生物组和代谢组数据或疾病表型进行关联分析, 阐明微生物及其代谢物与药效之间的关系。另外, 宏基因组还可以表征代谢功能, 可以进一步结合靶向代谢组学方法对代谢物进行更精准地分析。

例如, 为了探究小檗碱 (berberine, BBR) 对葡聚糖硫酸钠盐 (dextran sulfate sodium, DSS) 诱导的结肠炎大鼠肠道微生物的调节作用, Jing 等[16]采用 16S rDNA 测序技术发现, BBR 可以通过降低结肠炎大鼠厚壁菌门丰度而降低厚壁菌门/拟杆菌门比率。通过血清代谢组学分析发现, BBR 可以回调 DSS 所致的代谢物水平变化[16]。另外 Zeng 等[13]也通过小鼠粪便中细菌 16S rDNA 的测序分析发现柑橘多甲氧基黄酮 (polymethoxyflavone-rich extract, PMFE) 可以缓解高脂饮食 (high-fat diet, HFD) 诱导的肠道菌群失调。采用粪便非靶向代谢组学分析发现 PMFE 可以改变 HFD 小鼠的支链氨基酸 (branched-chain amino acids, BCAAs) 水平, 并且进一步采用血清靶向代谢组学验证此结果, Spearman 相关分析表明这些氨基酸的水平与体重增加、血脂、胰岛素抵抗等代谢特征呈正相关[13]。Huang 等[17]通过 16S rRNA 测序发现, 普洱茶可以降低人粪便中 *Lactobacillus*, *Bacillus*, *Streptococcus* 和 *Lactococcus* 的丰度, 这些菌属的共同功能是产生胆盐水解酶 (bile-salt hydrolase, BSH)。为了识别小肠中存在的微生物基因的相关功能通路, 采用宏基因组学表征肠道菌群功能, 结果显示 BSH 相关蛋白在普洱茶给药组中均有所减少, 因此研究者在此基础上进一步对聚焦的 BSH 活性以及机制进行后续分析[17]。本课题组也采用 16S rRNA 结合非靶向代谢组学技术探究了黄芪-党参-枣水提取物的抗疲劳功效[7], 以及黄芪抗顺铂诱导的肝损伤作用[8]。

1.3 无菌或伪无菌小鼠验证中药发挥药效依赖于肠道菌群

无菌 (germ-free, GF) 动物和伪无菌动物 (抗生素处理) 是用来探究中药发挥药效是否依赖于肠道菌群的两种模型。采用这两种模型使肠道菌群耗竭, 然后分析药效指标, 初步明确肠道菌群与中药药效的因果关系, 但无法确定哪些微生物发挥作用。这两种模型各有优缺点, 虽然无菌小鼠模型通常被认为是肠道菌群研究的黄金标准, 但抗生素处理模型是一种更为快速、

廉价和容易实施的替代方法[18]。进一步的分子机制需要采用传统的动物模型或生物学研究方法来探究。

为了研究PMFE的代谢保护作用是否依赖于肠道微生物群, Zeng等[13]在PMFE给药前使用混合抗生素处理HFD小鼠, 当肠道菌群被抗生素抑制时, PMFE的代谢保护作用被消除。除了使用混合抗生素验证肠道菌群对药效的影响, 还可以采用单一抗生素筛选敏感菌株。在冬虫夏草 (*Hirsutella sinensis* mycelium, HSM) 多糖H1部位给药之前, 分别给予HFD小鼠单一抗生素包括克林霉素、甲硝唑、青霉素、万古霉素和新霉素, 结果显示仅有新霉素可以消除H1的抗肥胖作用, 因此, 新霉素敏感菌可能是H1抗肥胖作用的关键菌[19]。

1.4 粪菌移植验证肠道菌群和中药药效的因果关系

粪菌移植实验 (fecal microbiome transplantation, FMT) 涉及将供体的粪便物质转移到受体动物或人身上, 以确定是否转移供体微生物, 是否转移疾病或给药供体相关的表型。和清除肠道菌群方法一样, 该方法能够反映肠道菌群和疾病表型的因果关系, 但是不能反映分子机制。

Wu等[19]分别将高脂饮食、正常饮食、给予生理盐水、冬虫夏草多糖小鼠的粪便微生物群移植入HFD受体小鼠体内, 与其他两组相比, 经正常饮食和冬虫夏草多糖小鼠粪菌移植的受体体重减轻, 肥胖特征减弱, 表现出明显的抗肥胖作用, 说明肠道菌群可以介导HSM的抗肥胖作用。

另外还有研究将患者粪便物质转移给动物, 再给予动物药物治疗以验证药物对菌群的影响。例如, 对于非小细胞肺癌免疫治疗, 有一部分患者对免疫治疗不响应, Huang等[20]将*α*PD-1非敏感性患者的粪便移植给无菌小鼠, 造模后, 采用人参多糖结合*α*PD-1联合治疗, 结果表明联合治疗可以显著延缓肿瘤生长, 改善免疫反应, 并且和临床治疗一样, 可以调节相同的菌群相关代谢。

1.5 验证微生物菌株能否产生相应药效

通过上述研究筛选出与中药药效相关的微生物菌株后, 进一步探究微生物菌株是否能表现出疾病表型, 产生相应的生理生化特征, 有利于研究中药作用的分子机制。可以选择动物模型或细胞模型测定微生物菌株作用后的疾病指标或分子。如Hu等[21]在明确BBR可以激活AhR (aryl hydrocarbon receptor), 恢复结肠炎大鼠肠道屏障后, 进一步采用Caco-2模型验证BBR作用的分子机制, 将结肠炎大鼠及BBR干预后大鼠的肠道菌群与Caco-2共同培养, 结果表明, BBR调节后的肠道菌群可以激活AhR受体。Wu等[19]筛选出敏感菌株*P. goldsteinii*, 是H1抗肥胖作用的关键菌, 进一步将*P. goldsteinii*灌胃给予HFD小鼠8周, 验证其抗肥胖作用, 结果表明可以显著降低HFD小鼠体重增加、降低内脏脂肪、抗胰岛素性稳态模式评估法指数、血清白介素-1*β* (IL-1*β*)、内毒素水平和肠道通透性。

也可以对菌株进行体外培养, 明确菌株是否可以产生相应的关键代谢物, 阐释微生物和宿主之间的相互作用机制。Zeng等[13]采用相关分析确定*Bacteroides*为PMFE治疗代谢综合征的关键菌属后, 分别将PMFE与*Bacteroides*属的单个菌株进行体外培养, 根据菌株丰度及关键代谢物变化 (BCAAs) 筛选出*B. ovatus*为关键菌株。进一步将*B. ovatus*给予HFD小鼠验证其对疾病指标和关键代谢物的影响, 结果显示*B. ovatus*显示出明显的作用, 而灭活的*B. ovatus*无治疗作用[13]。

1.6 验证肠道代谢物能否产生相应的药效

通过上述前四个层次聚焦到关键的代谢物后, 进一步对宿主、微生物及代谢物之间的关系进行验证, 如采用体外培养肠道菌群测定相关代谢物变化, 或者分析抗生素处理或粪菌移植对肠道代谢物的影响。最后探究关键代谢物对药效指标的影响, 有利于对中药作用机制有更深入的理解。

如Li等[22]通过代谢组学分析发现大麦叶可以升高结肠炎小鼠粪便中肌苷、鸟苷的含量, 并通过抗生素处理、体外培养等多重验证确定肌苷、鸟苷与肠道菌群、疾病 (结肠炎) 的关系。最终对代谢物肌苷、鸟苷的作用机制进行体内外实验验证, 体外细胞实验证明肌苷而非鸟苷能激活人结肠上皮细胞中的过氧化物酶体增殖物激活受体 (peroxisome proliferator-activated receptor, PPAR) *γ*信号及功能, 同样动物实验也证明肌苷通过腺苷受体 (adenosine 2A receptor, A2AR)/PPAR*γ*改善肠道功能及结肠炎, 因此肌苷是大麦叶改善结肠炎的关键药效物质。

中药与肠道菌群相关研究, 可以巧妙应用上述方法, 研究思路环环相扣, 最终阐明中药的作用机制, 明确其药效物质 (图1), 具体案例如表1[13,16,17,19-21,23-28]。首先从药效指标及初步的作用机制出发, 明确中药的药效作用。通过16S rRNA及宏基因组等测序方法筛选药物作用的关键菌群, 通过代谢组学筛选出药物作用的肠道代谢物, 从肠道菌群“结构”与“功能”方面共同揭示肠道菌群结构改变对功能代谢物的影响。这个过程可以先筛选关键菌, 再根据功能和相关分析聚焦相关代谢物, 如案例3[17]; 也可以先筛选代谢物, 再聚焦分析相关的肠道菌, 如案例9[25]、11[27]; 或者同时对肠道菌群和代谢物分别进行非靶标分析, 进一步对差异菌和差异代谢物进行相关分析, 聚焦关键代谢通路或代谢物类型, 如案例1[13] (表1)。

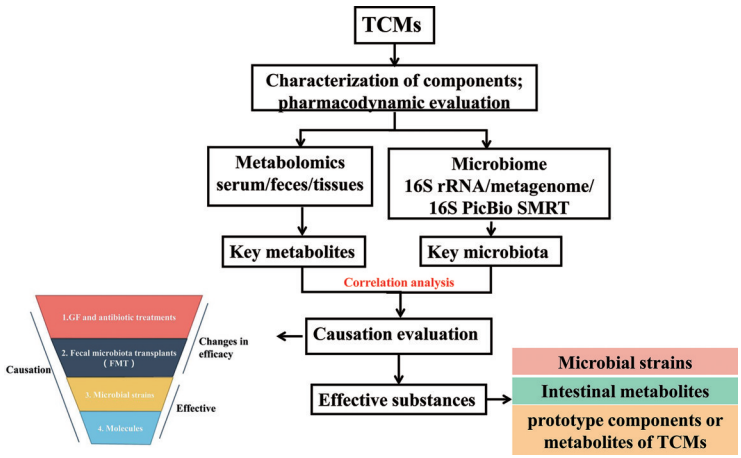

Figure 1 The research idea of traditional Chinese medicine (TCM) material basis and mechanism based on intestinal flora. GF: Germ-free; FMT: Fecal microbiome transplantation

Table 1 Case studies on pharmacodynamic substances and mechanism of TCMs based on intestinal microbiota. PMFE: Polymethoxyflavone-rich extract; BSH: Bile salt hydrolase; BA: Bile acid; FXR: Farnesoid X receptor; FGF15: Fibroblast growth factor 15; GE: Ginseng; MA: Myristoleic acid; LCFA: Long chain fatty acids; BAT: Brown adipose tissue; GPs: Ginseng polysaccharides; NSCLCs: Non-small cell lung cancers; AOS: Alginate oligosaccharide; BL: Barley leaf; PBM: Gegen Qinlian decoction; M-LCFAs: Medium and long chain fatty acids; AAs: Amino acids; APS: Astragalus polysaccharides; NAFLD: Nonalcoholic fatty liver disease; BBR: Berberine; Prob: Probiotic; PL: Postprandial lipidemia

No.	Object of study (TCM)	Desease model	Research design	Effective substances and molecular mechanisms	Ref.
1	Citrus PMFE	Metabolic syndrome	1. metabolic protective effects; 2. 16S rRNA-focous on Bacteroides; 3. Fecal untargeted metabolomics + serum targeted metabolomics-key metabolites; 4. Cocktail of antibiotics; 5. FMT; 6. Fecal batch-culture fermentation *in vitro*-key bacterial strain-efficacy evaluation.	PMFE improves metabolic dysfunction involving the enrichment of *B. ovatus*, a potentially beneficial intestinal bacterium.	[13]
2	BBR	Colitis	1. Pharmacodynamic indexes; 2. 16S rRNA; 3. Cocktail of antibiotics; 4. Metabolomics analysis; 5. *In vitro* mechanism: Caco-2 cell monolayer model + gut microbiota.	BBR treated DSS-induced colitis in rats through the regulation of gut microbiota associated tryptophan metabolite to activate AhR, which can greatly improve the disrupted gut barrier function.	[16]
3	Pu-erh tea	Hypercholesterolemia	1. Pharmacodynamic indexes and preliminary mechanism (human + mice); 2. 16S rRNA + metagenome-BSH; 3. Determination of bile acids; 4. Correlation analysis of components of Pu-erh and BSH bacteria; 5. Theabrownin reduced BSH bacteria abundance and BSH activity; 6. Ileal conjugated BAs inhibited FXR-FGF15 to promote BA synthesis.	Theabrownin increases the levels of ileal conjugated BAs which, in turn, inhibit the intestinal FXR-FGF15 signaling pathway, resulting in increased hepatic production and fecal excretion of BAs, reduced hepatic cholesterol, and decreased lipogenesis.	[17]
4	*H. sinensis* mycelium polysaccharides	Obesity	1. Pharmacodynamic indexes; 2. FMT verification; 3. Cocktail of antibiotics + single antibiotic-neomycin sensitive gut bacteria; 4. FMT: faecal microbiota with single antibiotics *ex vivo* prior to FMT; 5. Single strain culture and transplant.	HSM polysaccharides enrichs the gut bacterium *Parabacteroides goldsteinii*, prevents body weight gain, improves intestinal integrity and reduces inflammation and insulin resistance.	[19]

Continued

No.	Object of study (TCM)	Desease model	Research design	Effective substances and molecular mechanisms	Ref.
5	GPs	NSCLCs	1. Pharmacodynamic indexes and preliminary mechanism: GPs increased the antitumour response to *α*PD-1; 2. 16S PicBio SMRT- Muribaculaceae increased; 3. Metabolomic profling- short-chain fatty acids and tryptophan; 4. Clinical analysis: Intestinal flora difference of NSCLC responders and non-responders; 5. FMT: LLC bearing mice transplanted with feces from non-responders, GPs reinstate the response to *α*PD-1 mAb treatment.	GPs potentiate the antitumour effect of *α*PD-1 mAb by enhancing CD^{8+} T cell function and reducing the suppressive effect of Tregs, which might be addressed by reshaping the *P. distasonis* and *B. vulgatus* and tryptophan metabolism.	[20]
6	BL	Ulcerative colitis	1. Pharmacodynamic indexes and mucosal barrier function; 2. Transcriptomics: BL alters gut gene expression profile and activates the PPAR*γ* signaling pathway; 3. Metabolomics: derived purine metabolites; 4. BL fermentation: enrichment of inosine and guanosine; 5. Function of metabolites *in vitro*: inosine activates PPAR*γ* signaling in human colon epithelial cells; 6. Function of metabolites *in vivo*: inosine improves intestinal functions and protects against colitis *via* A2AR/PPAR*γ*.	BL enriches microbiota- derived purine metabolite inosine, which could activate PPAR*γ* signaling to protect against colitis.	[21]
7	GE	Obesity	1. Pharmacodynamic indexes and preliminary mechanism; 2. 16S rRNA-GE treatment enriches *Enterococcus faecalis*; 3. *E. faecalis* reduces obesity: *E. faecalis* treatment; 4. Mechanism of *E. faecalis*: serum metabolomics-MA; 5. The efficacy of MA-increasing BAT activity; 6. Genes functional verification.	GE-*E. faecalis*- LCFA (specifically MA) axis reduces obesity by increasing BAT activity and beige fat formation.	[23]
8	AOS	Small intestinal mucositis	1. FMT-16S rRNA (FMT-AOS/FMT-CON); 2. FMT- Pharmacodynamic indexes; 3. Serum metabolomics; 4. Correlation analysis of microbiome and metabolomics.	Gut microbiota from AOS-treated donor improves small intestine function and blood metabolome.	[24]
9	Rhein	Ulcerative colitis	1. Pharmacodynamic indexes; 2. Non-targeted metabolomics-rhein altered purine metabolism and decreased uric acid level; 3. Function of metabolites: uric acid led to intestinal barrier damage; 4. 16S rRNA+PCR-*Lactobacillus* level increased; 5. Cultured *Lactobacillus* sp. *in vitro*-uric acid decreased; 6. FMT determine whether gut microbiota altered by rhein had therapeutic benefits.	Rhein increases *Lactobacillus*, which indirectly changes purine metabolism and subsequently alleviated colitis.	[25]
10	PBM	Ulcerative colitis	1. Pharmacodynamic indexes and mucosal barrier function; 2. Cocktail of antibiotics; 3. FMT; 4. 16S rRNA; 5. Targeted metabolomics (SCFAs + M-LCFAs + AAs + BAs); 6. Correlation analysis of microbiome and metabolomics.	PBM could improve colonic inflammation through intestinal mucosal barrier, increase the production of propionate and total SCFAs regulate M-LCFAs, maintain BAs, and regulate AAs metabolism.	[26]

Continued

No.	Object of study (TCM)	Desease model	Research design	Effective substances and molecular mechanisms	Ref.
11	APS	Nonalcoholic fatty NAFLD	1. Pharmacodynamic indexes; 2. 16S rRNA; 3. Co-housing experiment assess the microbiota dependent anti-NAFLD effect of APS; 4. SCFAs determination-acetic acid were elevated in APS-supplemented mice; 5. Acetic acid producing bacterium-metagenomics-*Desulfovibrio vulgaris*; 6. *D. vulgaris* anti-NAFLD efficacy.	APS enriched *D. vulgaris* is effective on attenuating hepatic steatosis possibly through producing acetic acid, and modulation on hepatic lipids metabolism in mice.	[27]
12	Prob+BBR	Type 2 diabetes	1. Clinical study: Prob+BBR improves PL; 2. Lipidomic profle: MCFA and phospholipids decreased; 3. Recovering fecal enrichment of *Bifdobacterium breve* could be responsible for Prob+BBR induced PL improvement; 4. *In vitro* culture: BBR induces the expression of *fadD* genes regulating FFA simulation in *B. breve.*	The activation of *fadD* by BBR could enhance MCFA import and mobilization in *B. breve* and diliminish the intraluminal lipids for absorption to mediate the effect of Prob+BBR on PL.	[28]

聚焦关键的肠道菌或代谢物后, 采用多种技术方法进行正反验证, 通过抗生素干预及FMT证明中药作用依赖于肠道菌群, 通过体外培养或体内移植验证菌株的生物学功能, 通过细胞或动物模型、分子生物学手段验证代谢物的生物学功能, 最终阐明中药的作用机制。整个肠道菌群相关研究经历了从菌群到菌株、从关联到因果、从表型到机制的过程。

2 关键技术

2.1 微生物组学与代谢组学关联分析

由于肠道内容物物质组成十分复杂, 除了成千上万的小分子或代谢物, 还包含脱落的结肠上皮细胞, 未消化的食物残渣和大分子 (纤维、蛋白质、DNA、粘多糖等)[29]。因此, 如何对活性成分或药效物质进行有效的初步筛选是重要的第一步。由于测序和质谱技术的不断发展和完善, 微生物组学、代谢组学等技术能够在不同维度上反映生物体形貌, 从多角度提供有效信息。

2.1.1 微生物组学 分别通过16S rRNA基因测序或宏基因组学的“霰弹枪”测序分析肠道菌群的组成或进行肠道菌群功能预测, 然后对操作分类单元 (OTUs) 和其他功能DNA片段进行生物信息学分析[6]。基于三代测序技术的全长Marker基因测序方法也日益成熟, 可以获得基因的全长序列, 物种区分较二代测序的基因局部序列分析更加精细[30]。通过对肠道微生物群落进行深入分析, 以及对宿主肠道和微生物群落之间的相互作用分析, 能够更好地发现微生物之间的相互作用, 以及微生物和宿主表型的相互作用关系。

2.1.2 代谢组学 除了肠道微生物, 还存在多种肠道代谢物: 宿主产生的内源性代谢物, 经肠道菌群消化代谢后的外源性成分, 肠道菌群发酵膳食成分产生的代谢物, 如短链脂肪酸 (short-chain fatty acids, SCFAs), 以及肠道菌群修饰后的宿主代谢物, 如吲哚及其衍生物等; 由微生物从头合成的代谢物等, 如脂多糖、肽聚糖[31]。基于各种体液, 如尿液、血浆, 尤其是粪便或肠道内容物的代谢组学分析可通过检测代谢物的变化, 清晰展示肠道菌群和宿主的代谢状态, 有助于研究受到疾病或中药影响的与肠道微生物相关的代谢途径, 寻找相关生物标记物以及药物治疗靶点。其中, 基于质谱的代谢组学技术是检测和识别肠道代谢物, 探索其功能作用的关键技术之一, Bauermeister等[32]对微生物组研究中基于质谱的代谢组学技术进行了综述, 目前用于代谢组学分析的质谱联用技术有液相色谱-质谱联用 (LC-MS)、气相色谱-质谱联用 (GC-MS)、离子淌度质谱 (ion mobility mass spectrometry, IMS) 等。对于有潜在目标代谢物的研究, 如SCFAs、胆汁酸等, 可以采用靶向代谢组学分析, 针对特定分子调整工作流程和仪器以提高检测灵敏度和特异性。而非靶向代谢组学无目标分析物, 可以无偏向地获得生物样本中尽可能多的代谢物信息, 但是数据分析和代谢物注释仍具有很大挑战。

微生物分析也可以用不同的质谱技术以空间方式 (成像) 进行, 从简单的微生物培养到组织切片, 质谱成像可用于了解两个或两个以上微生物的代谢交换[33]。结合其他数据类型, 如宏基因组、16S RNA或转录组数据, 可用于建立分子空间模式与微生物群落的关系。

2.1.3 转录组学和蛋白组学 除了微生物组学和代谢组学, 转录组学和蛋白质组学也可以对多维信息进行

一定的补充。宿主转录组学能够从整体上揭示细胞中基因的表达情况及调控规律; 蛋白质组学针对某一生物、组织或细胞在特定生理或病理条件, 系统性研究所有蛋白质的特征、数量和功能, 能够获得包括功能多样性肠道菌群在内的环境样本, 揭示正在进行的代谢过程以及受环境变化如疾病状况的影响, 更有可能反映宿主与微生物生态系统的实际情况[34]。

2.1.4 相关分析方法 相关分析是一种统计方法, 用于评估和预测两个或多个变量之间可能的联系, 这些变量可以是定量的, 也可以是分类的。Pearson, Spearman相关分析用−1到1的相关系数来评价微生物−代谢物的相关关系。如Yu等[35]为了阐明茱萸丸对结肠炎的干预作用机制, 采用Spearman相关分析分析粪便代谢物与粪便微生物之间的相关性。除了单变量分析, 还有很多多变量分析方法可用来研究微生物和代谢物之间的相互关系, 如偏最小二乘分析 (partial least square, PLS)、规范相关分析 (canonical correlation analysis, CCA)、协惯性分析 (coinertia analysis, CIA) 等, Ling等[30]对多变量分析方法在肠道微生物相关分析中的应用适用性和局限性进行了综述。

Procrustes分析可以通过集成不同的数据类型 (包括微生物组、代谢组、转录组等), 并根据其负载之间的相关性将成对组学数据可视化, 塑造了同一系统的两个或多个数据集或矩阵的分布[32]。相较于单组学技术手段, 多组学联合分析具有系统性与整体性的特点且组学间交叉验证, 与传统的免疫、生理和病理分析协同工作, 能够获得整体分析结果[34]。

2.2 微生物功能评价模型

为了进一步验证确定肠道微生物和代谢物之间的因果关系, 研究者结合多种体外体内模型 (化学定义的细菌培养介质, 类器官和动物) 来进行评价。其中, 细菌培养介质和动物模型应用较多[9,15]。小鼠或人体类器官模型, 也称为肠类或结肠管, 用来研究体外研究微生物代谢物对肠上皮的影响, 以确定肠上皮产生神经递质的肠内分泌细胞如何对细菌产物做出反应。与传统的癌细胞或永生细胞系相比, 具有以下几个优点: ① 保留片段特异性; ② 表达各种肠道细胞; ③ 肠黏液层的产生; ④ 干细胞的维持; ⑤ 激素的分泌[36]。Horvath等[36]已经建立了基于LC-MS靶向代谢组学、体外细菌和类器官培养模型以及体内小鼠模型的整合评价体系, 用于肠-脑轴的相关研究。

2.3 中药化学成分肠道菌群生物转化分析技术

与内源性的宿主代谢物相比, 肠道代谢物中中药原型成分及其经肠道菌群代谢的产物的分析更为复杂。生物转化研究主要通过体外方法进行: ① 肠液转化, 肠道液生物转化可实现转化产物的大规模制备; ② 用宿主菌群样本孵育。该方法可检测原型药物和代谢物的种类和数量, 它的优点是可以准确地反映个体的整个肠道微生物群; ③ 代表性菌株的培养[37,38]。将无菌/抗生素处理的动物与常规动物进行比较也是一种常用手段, 以证明肠道微生物在天然产物代谢中的关键作用[39]。另外, 对临床试验受试者粪便进行菌群测序和代谢物分析, 可以全面反映天然产物在体内的代谢过程, 并用于解释个体差异[38]。通过体内或体外肠道菌群的转化后, 再采用LC-MS对中药原型成分及代谢物进行定性定量分析, 或进一步验证其药效。

3 总结与展望

随着对肠道菌群功能的研究不断深入, 中药对肠道菌群的作用研究也日渐深入, 但是要明确中药、宿主和微生物之间的关系需要反复的确证。本文对目前中药通过作用于肠道菌群发挥治疗作用的研究方法及策略进行了总结。采用层层递进的“漏斗”法进行研究将更有利于对中药治疗疾病的机制进行深入挖掘, 为中药作用机制的阐明及药效物质的发现提供了一种新的研究思路与方法。

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