四川大学学报（医学版） 2025，56（6）：1556－1565
J Sichuan Univ（Med Sci）

在线全文

养阴益气活血方对干燥综合征模型小鼠口腔微生态的影响及机制研究*

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【摘要】 目的 探讨养阴益气活血方对干燥综合征模型小鼠口腔微生态的影响，并探究其作用机制。**方法** 将12只8周龄非肥胖型糖尿病（non-obese diabetic，NOD）小鼠随机分为模型组、中药组和羟氯喹组，每组4只；另设4只BALB/c小鼠作为正常对照组。中药组给予养阴益气活血方〔15 g/（kg·d）〕灌胃，羟氯喹组给予羟氯喹〔0.08 g/（kg·d）〕灌胃，正常对照组和模型组正常饲养。治疗8周后，采集唾液样本，通过16S rRNA基因测序分析口腔菌群，并进行Alpha多样性、Beta多样性及功能预测分析。**结果** Alpha多样性分析提示养阴益气活血方能显著提高NOD小鼠口腔微生物群的多样性（$P<0.05$）。物种组成分析显示，给药组通过增加变形菌门，减少厚壁菌门以改善口腔微生态结构（$P<0.01$），而羟氯喹组中厚壁菌门出现异常降低。Beta多样性分析提示，给药组与模型组的微生物分群显著分离，中药组在变形菌门聚集，且其组内离散程度低于羟氯喹组。功能预测分析显示，中药组和羟氯喹组在氨基酸转运与代谢、转录等相关功能方面具有调控潜力。KEGG通路富集分析提示，中药组在细胞过程、环境信息处理及疾病相关通路中的菌群功能丰度高于羟氯喹组（$P<0.05$）。**结论** 养阴益气活血方可以恢复干燥综合征模型小鼠口腔菌群多样性，改善菌落结构，为中药调控口腔微生态的作用提供了实验依据。

【关键词】 非肥胖型糖尿病小鼠 养阴益气活血方 口腔微生物群 序列分析 干燥综合征

Effects and Mechanisms of Yangyin Yiqi Huoxue Formula on the Oral Microecology in Sjögren's Syndrome Model Mice

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[Abstract] Objective To investigate the effect of the Yangyin Yiqi Huoxue formula on the oral microecology in a mouse model of Sjögren's syndrome (SS), and to explore the underlying mechanisms. **Methods** A total of 12 8-week-old non-obese diabetic (NOD) mice were randomly assigned to a model group, a traditional Chinese medicine (TCM) group, and a hydroxychloroquine (HCQ) group, with 4 mice in each group. In addition, 4 BALB/c mice were used as the normal control group. The TCM group was administered Yangyin Yiqi Huoxue formula (15 g/[kg·d]) via gavage and the HCQ group received HCQ (0.08 g/[kg·d]) via gavage. The normal control and model groups were maintained under standard feeding conditions without intervention. After 8 weeks of treatment, saliva samples were collected for 16S rRNA gene sequencing to analyze the oral microbiota. Alpha diversity, beta diversity, and functional prediction analyses were performed. **Results** Alpha diversity analysis showed that the Yangyin Yiqi Huoxue formula significantly increased oral microbiome diversity in NOD mice ($P < 0.05$). Species composition analysis indicated that the formula increased the abundance of the phylum Proteobacteria and decreased the abundance of the phylum Firmicutes ($P < 0.01$), while HCQ led to an abnormal decrease in the abundance of the phylum Firmicutes. Beta diversity analysis revealed distinct microbial clustering in the treatment groups and the model group, with the TCM group showing clustering in the phylum Proteobacteria and exhibiting lower intragroup dispersion than the HCQ group did. According to the functional

* 国家自然科学基金（No. 82574934）、浙江省自然科学基金（No. LTGY24H270001）、国家中医药管理局-浙江省中医药管理局共建重点项目（No. GZY-ZJ-KJ-23075）和宁波市自然科学基金（No. 2021J016）资助

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出版日期：2025-11-20

prediction analysis, both the TCM and HCQ groups demonstrated regulatory potential in terms of amino acid transport and metabolism, transcription, and other related functions. KEGG analysis found greater microbial enrichment in cellular processes, environmental information processing, and disease-related pathways in the TCM group compared to the HCQ group ($P < 0.05$). **Conclusion**　The Yangyin Yiqi Huoxue formula can restore oral microbiome diversity and improve the colony structure in in a mouse model of SS, providing experimental evidence for the advantages of TCM in regulating oral microecological functions.

[Key words]　Non-obese diabetic mice　Yangyin Yiqi Huoxue formula　Oral microbiota　Sequence analysis　Sjögren's syndrome

干燥综合征(Sjögren's syndrome, SS)是一种复杂的慢性自身免疫性疾病,其典型临床表现为口干、眼干等[1],严重影响患者的日常生活、进食与吞咽功能[2-3]。目前,西医的治疗方法主要以激素、羟氯喹(hydroxychloroquine, HCQ)及免疫抑制剂等为主[4],但此类疗法常存在疗效不佳、副作用较大、患者主观症状改善不明显等问题[5-6]。相比之下,中医药在控制疾病进展和改善患者生存质量方面展现出了显著的优势。在临床中,多数医患群体对中医治疗持积极态度[7]。

SS患者存在口干、猖獗龋等口腔问题,这些表现与口腔微生态失衡密切相关[8]。患者唾液分泌量显著减少,导致口腔湿润度下降,为有害微生物的增殖创造了条件,从而引发口腔微生态的失衡[9-10]。研究显示,SS患者口腔中的变形菌门相对丰度较低,可能与疾病的发生和发展有关[11]。此外,患者的口腔微生物群落多样性普遍降低,可能进一步加剧口腔环境失衡与疾病进展[12-13]。近年来,中医药在调节口腔菌群方面显示出独特的优势。例如,黄芪多糖可通过抑制韦荣氏菌属等口腔致病菌的增殖来改善口干症状,黄连素通过调节变形菌门丰度缓解口腔炎症[14-15]。在SS的中医药干预中,有研究发现,使用麦冬、沙参、甘草、枸杞等中药泡水漱口,可以增加患者口腔微生物群落的丰富度[16]。本课题组前期研究也证实,养阴益气活血方能够显著缓解患者的口干症状,并改善腺体分泌与免疫功能[17-18]。

非肥胖型糖尿病(non-obese diabetic, NOD)小鼠是研究SS的经典动物模型。研究表明,随着疾病进展,NOD小鼠的唾液流量可减少约70%,血清和唾液中抗SSA和抗SSB抗体滴度显著升高[19]。同时,NOD小鼠易发生严重的唾液腺淋巴细胞浸润和分泌功能障碍[20]。这与SS患者临床病理表现高度相似,因此本研究拟采用NOD小鼠作为疾病研究的模型。此外,研究发现SS患者和NOD小鼠在菌群结构上具有较高相似性,二者肠道中拟杆菌门比例均显著升高,尤其是脆弱拟杆菌的丰度增加与唾液腺淋巴细胞浸润程度呈正相关;与此同时,粪便中厚壁菌门均呈现减少趋势[21-22]。动物与临床研究均提示,口腔和肠道微生物群落相互关联,共同参与机体免疫调节,影响疾病的发生与发展[23]。本课题组前期工作发现,养阴益气活血方可提升NOD小鼠肠道菌群多样性,并促进有益菌的增殖[24]。基于肠道-口腔菌群的交互作用,本研究将进一步探究该复方对NOD小鼠口腔微生态的直接影响,从而为阐释其治疗SS的多靶点作用机制提供实验依据。

1　材料与方法

1.1　养阴益气活血方冻干粉的制备

养阴益气活血方购自浙江大学医学院附属第一医院,由太子参24 g、白芍15 g、黄精15 g、女贞子15 g、山药30 g、五味子9 g、乌梅15 g、红景天15 g、肿节风12 g等中药组成。将药物称量后放入药煲,加入1 L去离子水浸泡30 min,武火煮40 min,文火熬1 h,高温浓缩。过滤药液,移入50 mL离心管,高速离心10 min(5 000 r/min)。取上清液,置于新的离心管中,—80 ℃冰箱过夜。次日,冻干药物,设置冻干机温度为—60 ℃,压力为1.1 Pa。冻干后称重粉末。

1.2　实验动物准备

选择8周龄SPF级NOD小鼠12只,BALB/c小鼠4只作为正常对照组(空白对照组)。购自上海西普尔-必凯实验动物有限公司,动物许可证号:SCXK(沪)2013-0016。本研究通过浙江大学医学院附属第一医院实验动物伦理委员会批准,批件号:(2022)实动快审第(1359)号。实验过程在保证动物够用的前提下,使用较少的动物。将12只NOD小鼠随机分为模型组、中药组与羟氯喹组,每组4只。中药组和羟氯喹组分别单独给予养阴益气活血方15 g/(kg·d)和羟氯喹 0.08 g/(kg·d)灌胃治疗。模型组和正常对照组每天正常饲养。药物治疗组于实验开始后每天定时给药1次,给药量按动物与人等效剂量换算表计算。给药8周后收集唾液样本。

1.3　口腔微生态检测

唾液样本采集前,使用蒸馏水冲洗小鼠口腔,并禁食禁水。二氧化碳吸入法安乐处死小鼠后立即用无菌拭子

采集口腔样本，每只小鼠采集时间不少于1 min，确保唾液量不低于1 mL。样本收集后分装于1.5 mL无菌离心管中，经低温高速离心(16 200 ×*g*, 4 ℃, 15 min)后，弃上清液，沉淀部分用于后续微生物分析，所有样本均保存于−80 ℃备用。采用试剂盒提取样本总DNA，针对16S rRNA基因V3-V4区进行PCR扩增。扩增产物经纯化后，使用Illumina MiSeq平台进行双端测序，获得相关序列数据进行后续生物信息学分析。

1.4 生物信息分析流程

利用Miseq测序得到的PE reads通过Flash 1.2.11软件根据overlap关系进行拼接，随后对序列进行质量控制和过滤，并区分不同样本。拼接后的序列经Uparse 7.0.1090软件完成操作分类单元(OTU)聚类；采用RDP Classifier 2.11软件对OTU代表序列进行物种分类学注释；通过Usearch 7.0软件统计各样本OTU的相对丰度。基于OTU数据开展多样性分析。使用Mothur 1.30.2软件评估样本内微生物群落的丰富度和多样性，进行Alpha多样性分析。通过Qiime 1.9.1软件计算样本间的Beta多样性距离，分析组间微生物群落结构的相似性或差异性。在上述分析的基础上，通过功能基因数据库FunGene 9.6对序列注释，利用PICRUSt 1.1.0软件对16S rRNA基因序列进行KEGG、COG、Pfam功能预测，统计不同组间在细胞过程、环境信息处理及疾病通路等方面的菌群富集差异。

1.5 统计学方法

运用SPSS 23.0软件开展数据分析。计量数据以$\bar{x} \pm s$表示，组间比较采用独立样本*t*检验、单因素方差分析(ANOVA)和双因素方差分析，组间两两比较采用Dunnet'*t*检验，$P<0.05$为差异有统计学意义。

2 结果

2.1 口腔微生物群落多样性分析

对4组共16个样本进行的多样性数据分析显示，门水平丰度最高的5类物种包括：变形菌门(Proteobacteria)、厚壁菌门(Firmicutes)、放线菌门(Actinobacteriota)、拟杆菌门(Bacteroidota)、绿弯菌门(Chloroflexi)。属水平丰度最高的5类物种包括：变形菌属(*Proteus*)、乳杆菌属(*Lactobacillus*)、链球菌属(*Streptococcus*)、双歧杆菌属(*Gemella*)、链球菌属(*Stenotrophomonas*)。

在多样性指数柱状图中，正常对照组的口腔微生物多样性最高，模型组生物多样性显著降低，给药组可以有效恢复NOD小鼠唾液菌群生物多样性，见图1。

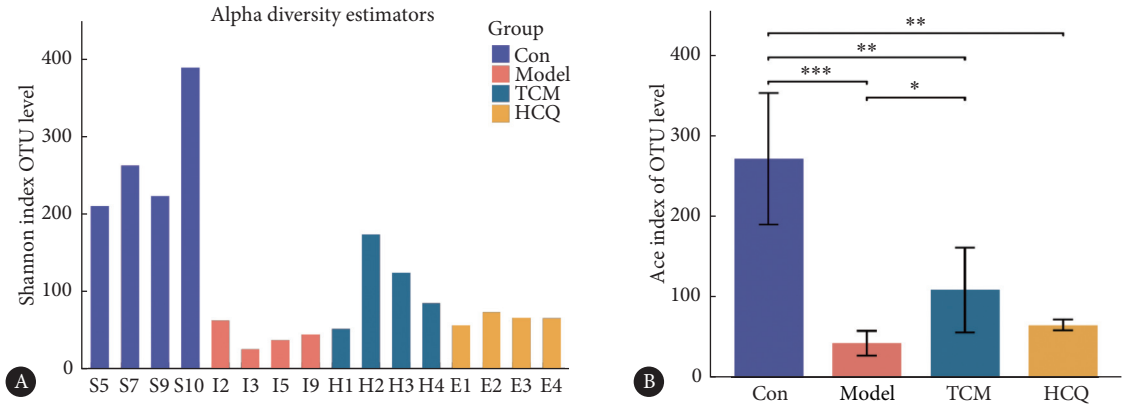

图 1 NOD小鼠口腔微生物群落多样性分析

Fig 1 Analysis of oral microbial community diversity in NOD mice

Con: the control group (4 samples: S5, S7, S9, and S10); Model: the Sjögren's syndrome model group (4 samples: I2, I3, I5, and I9); TCM: traditional Chinese medicine group (4 samples: H1, H2, H3, and H4); HCQ: hydroxychloroquine group (4 samples: E1, E2, E3, and E4). A, the bar graph of alpha diversity analysis index; B, bar graph of intergroup differences in alpha diversity analysis ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.005$).

2.2 口腔微生物物种组成分析

各组样本的物种分析显示，正常对照组有617种，模型组有60种，中药组有294种，羟氯喹组有181种，其中正常对照组物种最丰富，养阴益气活血方对物种恢复作用更好。韦恩图分析显示中药组与正常对照组共同物种多达72种，而羟氯喹组与正常对照组共同物种只有24种，见图2A。聚类分析显示，厚壁菌门在模型组中丰度最高，但是在羟氯喹组中异常减少。另外，给药组能有效恢复变形菌门在NOD小鼠口腔唾液中的丰度，见图2B。对各组别菌群群落分析提示，模型组中厚壁菌门的占比最高可达90% ~ 100%，而变形菌门显著减少。给药组能有效增加变形菌门丰度，中药组变形菌门占比可达60% ~ 80%。

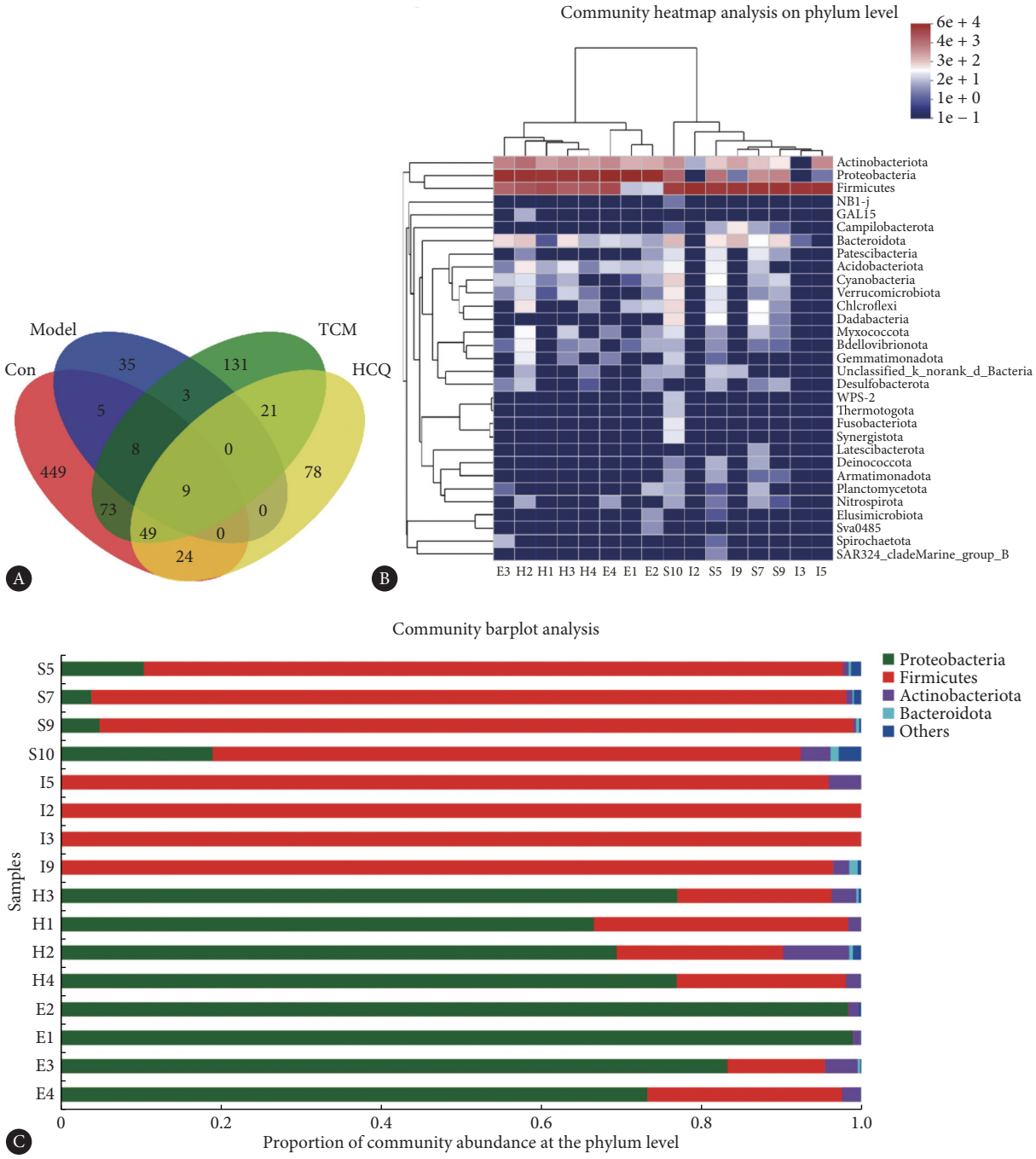

图 2 微生物群落物种组成分析

Fig 2 Analysis of microbial community species composition

The abbreviations are explained in the note to Fig 1. A, Venn diagram analysis of species composition in each group community; B, the heatmap of species in each sample community; C, bar chart of the proportion of different phyla in each sample.

值得注意的是, 在羟氯喹组中厚壁菌门占比异常减少, 提示羟氯喹对厚壁菌门可能存在破坏作用, 见图2C。

2.3　组间群落组成差异性分析

主坐标分析(principal co-ordinates analysis, PCoA)显示, 正常对照组、模型组和给药组群落组成具有明显的差异性, 但是养阴益气活血方和羟氯喹对群落组成的影响没有明显差异, 见图3A。菌群分型分析提示, 在模型组和正常对照组中厚壁菌门占优势, 在给药组中变形菌门占优势, 见图3B。进一步进行组间物种差异分析, 养阴益气活血方有效降低模型组中厚壁菌门丰度(P= 0.004 1), 羟氯喹组变形菌门丰度高于中药组(P= 0.003 6), 见图3C。

2.4　样本相关物种共现性网络分析

通过网络节点分析发现, 正常对照组样本相关物种最丰富, 因此节点数最高, 见附图1(网络资源附件)。给

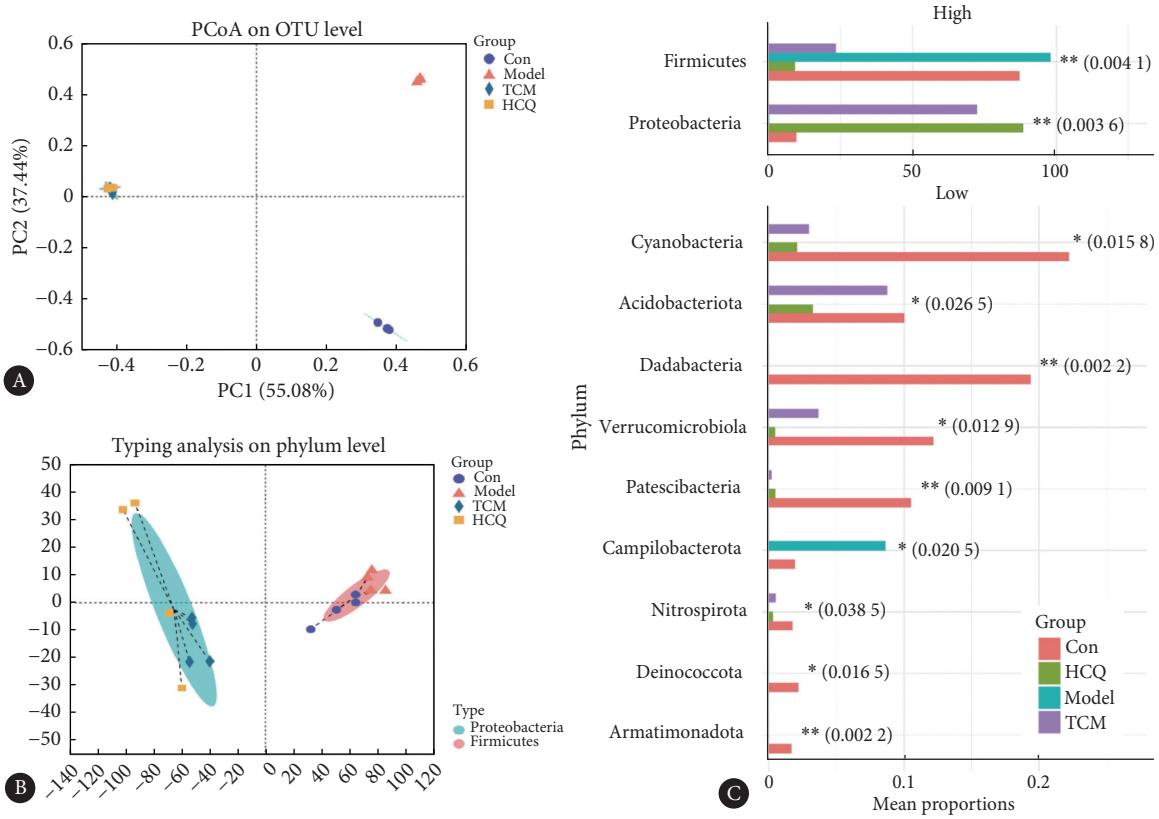

图 3 组间群落组成差异性分析

Fig 3 Analysis of inter-group community composition differences

The abbreviations are explained in the note to Fig 1. A, PCoA analysis chart by sample groups; B, microbiome typing analysis chart for different sample groups; C, multi-species comparison bar chart (the data in the brackets represent the *P* value). $^{*}P<0.05$, $^{**}P<0.01$.

药组相关物种存在重叠现象,模型组样本中相关物种最少。另外,两个给药组均可增加样本相关物种(TCM vs. Model, $P<0.05$),且养阴益气活血方相关的物种更丰富,见图4。

2.5 菌群功能机制聚类分析

2.5.1 COG功能分析

中药组和羟氯喹两组的COG功能聚类结果相近,其中氨基酸运输和代谢(aminoacid transportand metabolism)、转录(transcription)、细胞骨架(cytoskeleton)、脂质运输和代谢(lipid transportand metabolism)、细胞内运输(intracellular tratficking)、分泌作用和囊泡运输(secretion, and vesicular transport)等COG功能的物种丰度均高于模型组,见图5。

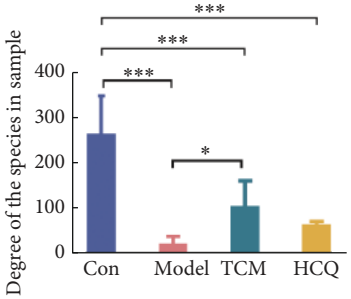

图 4 样本物种相关性

Fig 4 Species correlation in the samples

The abbreviations are explained in the note to Table 1. $^{*}P<0.05$, $^{***}P<0.005$.

2.5.2 FUNGuild功能预测分析

FUNGuild功能预测分析结果揭示中药组和羟氯喹组较模型组的植物病原菌的组成成分明显升高。其中,羟氯喹组的植物病原菌丰度比中药组高,见图6。

2.6 KEGG丰度统计

对中药组和羟氯喹组进行KEGG通路分析的丰度统计显示,菌群富集在代谢(metabolism)、环境信息处理(environmental information processing)、遗传信息处理(genetic information processing)、细胞过程(cellular processes)、人类疾病(human diseases)和生物系统通路上(organismal systems)(图7A)。进一步比较养阴益气活血方和羟氯喹对菌群在各个通路上富集影响的差别,分别对这两组样本在各个通路上的菌群丰度均值进行比较。中药组干预后菌群在细胞过程通路、环境信息处理

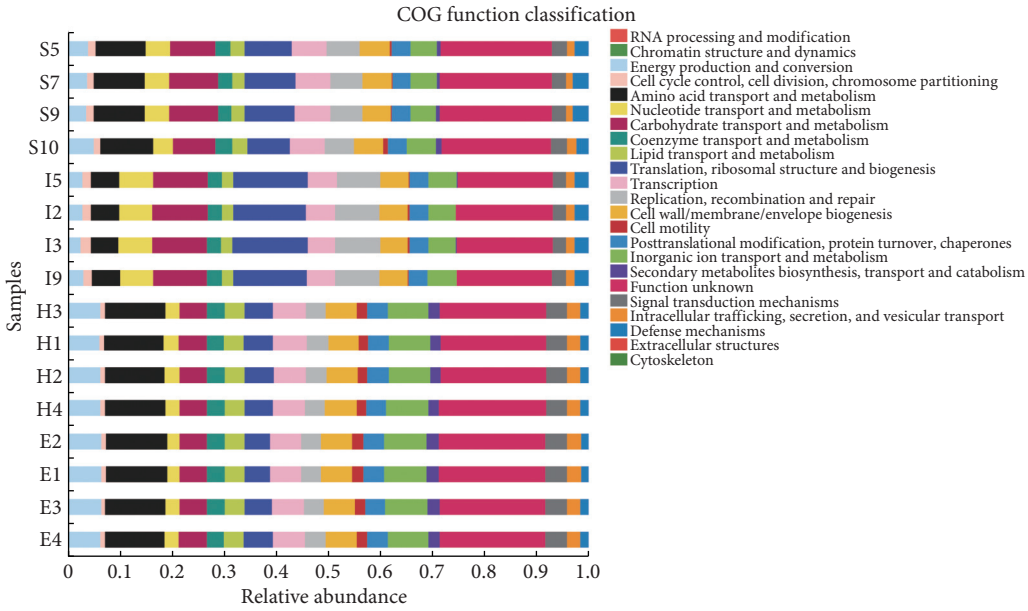

图 5 COG功能分析

Fig 5 COG functional analysis diagram

The sample designations are explained in the note to Table 1.

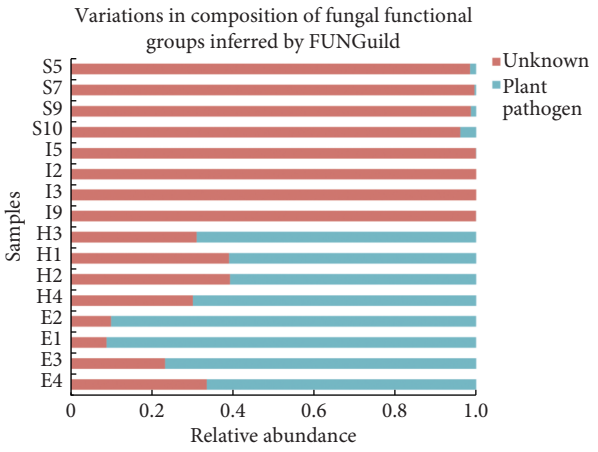

图 6 FUNGuild功能预测分析结果

Fig 6 Analysis plot of FUNGuild function prediction

The sample designations are explained in the note to Table 1.

通路以及人类疾病通路上较羟氯喹组富集明显(图7B)。

3 讨论

口腔菌群的结构和多样性较为稳定, 口腔菌群主要来自6个门: 厚壁菌门, 拟杆菌门, 放线菌门, 变形菌门, 梭杆菌门和疣微菌门[25-26], 发生多样性的改变提示口腔微生态的失衡[27]。本研究发现, 模型组小鼠的口腔微生物多样性明显降低, 而经过养阴益气活血方治疗后, 这一指标显著恢复, 且效果优于羟氯喹。提示养阴益气活血方可以有效提高NOD小鼠口腔微生态的物种多样性。

厚壁菌门是口腔微生态中的主要成分, 其组成结构、定植部位和丰度增加都有可能导致口腔疾病的发生。研究表明SS患者的口腔微生物群落中厚壁菌门的丰度增加与口腔干燥症状的严重程度有关[28]。本研究证实了养阴益气活血方可以有效降低NOD小鼠口腔中的厚壁菌门丰度。但是值得注意的是, 口腔微生物物种组成分析提示羟氯喹对厚壁菌门存在异常的破坏作用, 可能对NOD小鼠口腔微生态造成二次伤害。有研究发现, 长期服用羟氯喹可能会导致口腔黏膜出现疼痛或溃疡, 其疗效及安全性均劣于口服中药, 且不良反应发生率显著高于口服中药组[29]。有动物实验表明, 高剂量羟氯喹(100 mg/kg)灌胃14 d后, 显著降低厚壁菌门的相对丰度, 同时提升拟杆菌门(Bacteroidetes)比例, 这种变化提示羟氯喹可能对厚壁菌门具有选择性抑制作用, 但具体分子机制尚未明确[30]。本研究发现, 羟氯喹组的植物病原菌丰度比中药组高, 提示羟氯喹可能增加SS患者口腔感染的风险。但是, 羟氯喹对口腔菌群的破坏作用目前仅在NOD小鼠口腔菌群中被验证, 后续研究将考虑扩展至人类样本, 以验证这一发现, 并进一步阐明相关机制。相比之下, 养阴益气活血方调控NOD小鼠唾液菌群的结构更加合理, 体现了养阴益气活血方在安全性方面的优势。

变形菌门是唾液中的主要菌门之一, 占总序列数的27.78%[31]。研究发现, 在牙龈炎、牙周炎、龋齿及口腔鳞状细胞癌等多个疾病中, 变形菌门的比例增高, 提示其可能会促进口腔尤其是齿龈的炎症[32]。但是, 尽管SS患者多伴有龋齿等口腔问题, 变形杆菌在SS中的丰度却明显

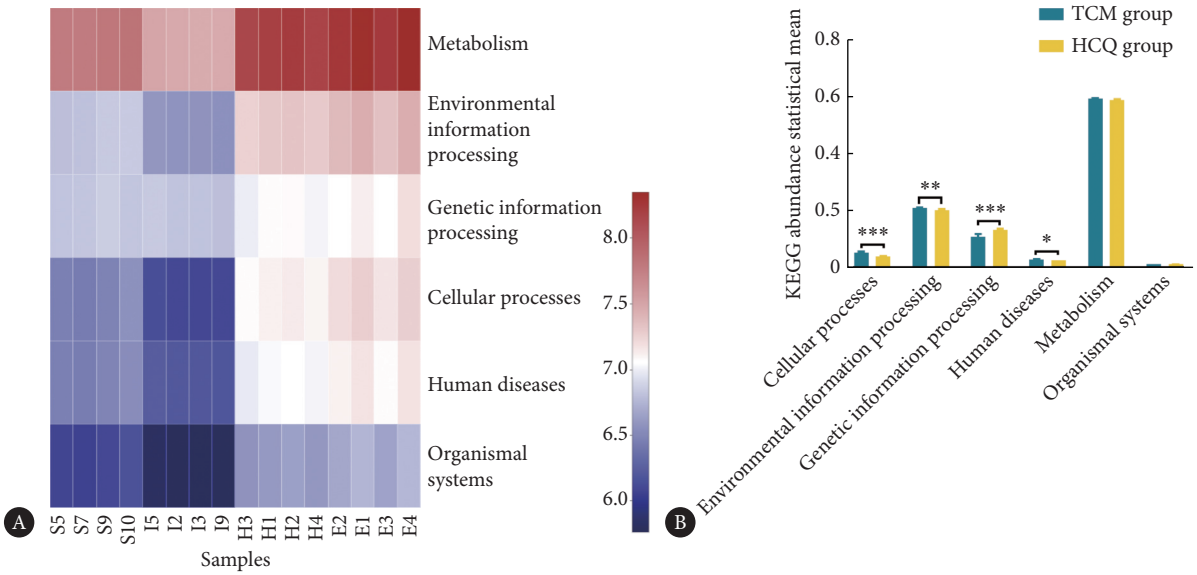

图 7 KEGG通路聚类物种丰度统计

Fig 7 KEGG pathway species abundance statistics

A, KEGG pathway analysis of the 4 different groups (The sample designations are explained in the note to Table 1.); B, KEGG abundance statistical mean of the TCM group and the HCQ group. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.005$.

下降。多个研究证实,与健康口腔菌群相比,SS患者口腔中的变形菌门偏低[33-34]。本研究也证实,模型组小鼠的变形菌门比例较正常对照组显著降低。养阴益气活血方干预后,变形菌门的比例显著增高。因此,基于本次SS模型小鼠的研究结果,深入研究变形菌门在口腔中的定植差异和不同疾病优势菌属类别差异,将揭示变形菌门不同菌属在SS中的作用和机制,可能成为治疗SS口腔微生态的新靶点。此外,如何控制养阴益气活血方增加变形菌门丰度对口腔微生态环境的副作用,需要定量分析和更深入的机制研究。

除了菌群丰度及组成结构发生的变化外,菌群相关代谢产物的改变也通过多途径直接调控宿主免疫稳态与炎症应答水平[35]。变形菌门可以通过其代谢产物,如短链脂肪酸、胆汁酸、氧化三甲胺等,与宿主细胞上的特定受体结合,调节免疫反应[36]。厚壁菌门的代谢产物,如H_2S,可以刺激口腔白细胞,促进炎症因子的产生,从而加剧炎症反应[37]。进一步研究表明,中药可通过调节短链脂肪酸合成等宿主代谢通路和免疫因子的表达,间接影响口腔菌群的稳定性[38-39]。例如,双黄连口服液通过恢复色氨酸代谢通路中N-乙酰血清素等代谢物的水平,减轻口腔炎症反应,进而抑制病原菌的过度生长[40]。有研究证实,养阴益气活血方中芍药的主要成分芍药苷调节肠道微生物产生吲哚-3-乳酸和上皮自噬以减轻小鼠结肠炎,体现了通过菌群代谢产物调控免疫的作用[41]。本研究中的功能分析揭示养阴益气活血方干预后口腔菌群发生了显著的功能转变,尤其是在氨基酸和脂质等成分的代谢和运输途径方面,提示该方在改变NOD小鼠口腔菌群结构和丰度的同时,进一步影响口腔菌群代谢产物及运输,从而调控免疫反应。

根据菌群丰度进行KEGG富集分析,结果显示:与羟氯喹组相比,中药组在细胞过程、环境信息处理的途径中表现出更高的微生物丰度。这表明养阴益气活血方可以通过细胞、口腔黏膜免疫环境和代谢途径等多个方面发挥治疗作用。在课题组前期研究中已证实养阴益气活血方在增加唾液分泌,调节免疫细胞平衡,改善免疫环境等方面具有显著效果[42-43]。有研究显示,方中太子参的成分太子参多糖能恢复臭杆菌的相对丰度,维持免疫抑制小鼠脾淋巴细胞的免疫平衡[44]。本方中芍药配伍炙甘草,可上调NOD小鼠唾液腺中AQP5和M3R的表达,降低血清中抗SSA、SSB、α-Fodrin抗体含量,从而改善腺体分泌能力,缓解口干症状[45]。另外,研究发现芍药总多糖对浮游状态下的变异链球菌、血链球菌、黏性放线菌的生长及变异链球菌、血链球菌产糖、产酸有不同程度抑制作用,初步证实了芍药总多糖的防龋潜能[46]。这些研究为进一步探究养阴益气活血方如何通过调节口腔微生态来缓解SS病情提供了重要实验参考。

中医药在调节口腔菌群方面的独特优势在于其多成分、多靶点的作用机制。例如,中药中的多糖类物质能够通过抑制炎症因子(如 IL-8)的表达,从而间接影响口腔菌群的平衡,进而降低口腔癌的风险[47]。中药中的酚类

化合物能够通过抑制口腔致病菌的生长，抑制其黏附能力和生物膜形成，减少有机酸和挥发性硫化合物的积累，进而维持口腔微生态平衡[48]。本研究中的养阴益气活血方由9味药组成，有研究发现，其中太子参有可能通过调节肠道微生物群的结构和组成，促进丁酸的产生来缓解脾虚症[49]。方中组成药物黄精的主要成分黄精多糖能显著调节微生物群落的丰富度，提高其多样性，特别是窄定义梭状芽孢杆菌和拟杆菌门[50]。体外厌氧发酵实验显示，山药及其活性成分可调节肠道菌群结构和吲哚类代谢产物[51]。另外，有研究发现，方中乌梅的提取物对口腔致病细菌具有抗菌活性，可能是一种潜在的口腔抗菌剂，可以作为控制或预防口腔致病菌相关疾病的候选药物[52]。总之，中药在调节口腔菌群方面可通过多种途径发挥作用，从而改善口腔微生态环境，最终达到治疗疾病的作用。

综上，本研究揭示了养阴益气活血方对NOD小鼠口腔微生物群落组成及功能的影响，特别是在治疗后变形菌门的丰度显著升高，而厚壁菌门丰度显著下降。通过功能分析提示，养阴益气活血方可能是通过调控代谢途径和改善口腔黏膜环境等机制恢复口腔黏膜稳态，发挥治疗作用。当前中药对SS口腔微生物影响的研究仍相对有限，本研究的结果为中医药在SS治疗中的应用提供了新的动物实验证据，并强调了调节口腔微生物群的重要性。今后研究可以进一步验证养阴益气活血方针对NOD小鼠口腔中变形菌门和厚壁菌门的调控机制，深入其对代谢途径和口腔黏膜环境影响的机制研究。

*　　*　　*

作者贡献声明　吴方平负责论文构思、正式分析、研究方法、可视化、初稿写作和审读与编辑写作，卢雯雯负责数据审编、调查研究和研究方法，吴旦斌负责研究方法、提供资源和可视化，周杨青负责数据审编和经费获取，鲁海峰负责研究方法和提供资源，应李雄负责验证，吴国琳负责论文构思、经费获取、研究项目管理和审读与编辑写作。所有作者已经同意将文章提交给本刊，且对将要发表的版本进行最终定稿，并同意对工作的所有方面负责。

Author Contribution　WU Fangping is responsible for conceptualization, formal analysis, methodology, visualization, writing--original draft, and writing--review and editing. LU Wenwen is responsible for data curation, investigation, and methodology. WU Danbin is responsible for methodology, resources, and visualization. ZHOU Yangqing is responsible for data curation and funding acquisition. LU Haifeng is responsible for methodology and resources. YING Lixiong is responsible for validation. WU Guolin is responsible for conceptualization, funding acquisition, project administration, and writing--review and editing. All authors consented to the submission of the article to the Journal. All authors approved the final version to be published and agreed to take responsibility for all aspects of the work.

利益冲突　所有作者均声明不存在利益冲突

Declaration of Conflicting Interests　All authors declare no competing interests.

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（2024 – 10 – 30收稿, 2025 – 08 – 28修回）

编辑　刘　华