论著·基础医学

限时进食通过影响再生胰岛衍生蛋白3γ与肠道菌群缓解小鼠结肠炎

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［摘要］ **目的**　初步探究限时进食(time-restricted feeding，TRF)通过影响再生胰岛衍生蛋白3γ(regenerating islet-derived protein 3 gamma，Reg3γ)与肠道菌群缓解小鼠结肠炎的机制。**方法**　将6周龄SPF级C57BL/6小鼠按体质量分层后随机分为(n=7)：自由饮食(ad libitum feeding，AL)组与TRF组，分别给予全时段自由摄食和每天仅在00:00-08:00进食，持续4周；于第4周通过喂养2.5%葡聚糖硫酸钠(dextran sodium sulfate，DSS)溶液6 d诱导结肠炎。通过监测疾病活动指数(disease activity index，DAI)评分、结肠长度、HE染色及病理评分并结合结肠组织中胰岛再生基因3γ(regenerating islet-derived 3 gamma，*Reg3g*)和炎症因子mRNA表达等指标，综合评估结肠炎严重程度及TRF的干预效果。另将6周龄C57BL/6小鼠按体质量分层后随机分为(n=7)：AL+抗生素鸡尾酒(antibiotic cocktail，ABX)组与TRF+ABX组。ABX混合液用于耗竭肠道菌群，以评估TRF缓解结肠炎的菌群依赖性。收集AL与TRF组小鼠粪便样本进行16S rRNA测序(16S ribosomal RNA sequencing，16S rRNA-seq)，检测血清中脂多糖(lipopolysaccharide，LPS)水平；并采集其结肠上皮细胞进行RNA测序(RNA sequencing，RNA-seq)。**结果**　DSS造模后，AL组小鼠表现出体质量下降、腹泻、血便等典型结肠炎症状。TRF干预可显著缓解上述表型，自造模第4天起DAI评分低于AL组(P<0.001)，结肠缩短程度减轻(P<0.01)，组织结构保持相对完整，炎症明显减轻。RT-qPCR分析显示，TRF组结肠组织中*Reg3g*及炎症因子(*IL-1β*、*IL-6*、*TNF-α*)较AL组mRNA水平下调(P<0.05)，抗炎因子*IL-10*上调(P<0.000 1)。ABX处理后，AL+ABX组与TRF+ABX组在DAI评分、结肠长度及病理评分等指标上均无显著差异；TRF+ABX组较AL+ABX组仅*Reg3g*下调(P<0.05)，而*IL-1β*、*IL-6*、*TNF-α*及*IL-10*差异无统计学意义。16S rRNA-seq分析结果表明，TRF显著重塑肠道菌群结构，革兰氏阳性菌丰度升高，革兰氏阴性菌显著减少，伴随血清LPS水平降低(P<0.001)。RNA-seq进一步提示，TRF干预下活化B细胞核因子κ轻链增强子(nuclear factor kappa-light-chain-enhancer of activated B cells，NF-κB)等炎症相关信号通路富集显著受抑制。**结论**　TRF通过下调Reg3γ表达、重塑肠道菌群结构并降低血清LPS水平，从而抑制NF-κB炎症信号通路，减轻DSS诱导的小鼠结肠炎。

［关键词］ 限时进食；结肠炎；再生胰岛衍生蛋白3γ；肠道菌群；NF-κB信号通路

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Time-restricted feeding attenuates DSS-induced colitis in mice by modulating regenerating islet-derived protein 3 gamma and gut microbiota

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[**Abstract**] **Objective** To explore the underlying mechanisms by which time-restricted feeding (TRF) attenuates dextran sodium sulfate (DSS)-induced colitis in mice *via* modulation of regenerating islet-derived protein 3 gamma (Reg3γ) expression and gut microbiota. **Methods** Six-week-old C57BL/6 mice were stratified by body weight and randomized into ad libitum feeding (AL) and TRF groups (n=7). The AL mice were given unrestricted food access, whereas the TRF mice were allowed feeding only during 00:00 and 08:00 daily, for totally 4 weeks. Mouse colitis model was induced at the fourth week by adding 2.5% dextran sodium sulfate (DSS) in drinking water for 6 d. Disease severity and the effects of TRF were assessed with disease activity index (DAI) scoring, colon length measurement, HE staining and histopathological scoring, and mRNA expression levels of regenerating islet-derived 3 gamma (*Reg3g*) and inflammatory cytokines in colonic tissues. Another 14 mice were randomized into AL plus antibiotic cocktail (AL+ABX) and TRF plus antibiotic cocktail (TRF+ABX) groups, with 7 animals in each group. ABX was administered to deplete gut microbiota and evaluate the microbiota dependence of TRF in attenuating colitis. Fecal samples from AL and TRF mice were analyzed by 16S ribosomal RNA sequencing (16S rRNA-seq), and serum lipopolysaccharide (LPS) level was measured. The colonic epithelial cells were collected for RNA-seq. **Results** After modeling, the AL mice exhibited typical colitis symptoms, such as weight loss, diarrhea, and hematochezia. TRF intervention significantly attenuated these above symptoms, with lower DAI scores from day 4 post-modeling (P<0.001), reduced colon shortening (P<0.01), preserved tissue architecture, and decreased inflammation. RT-qPCR analysis showed that TRF down-regulated colonic mRNA expression levels of *Reg3g* and pro-inflammatory cytokines (*IL-1β*, *IL-6*, *TNF-α*) (P<0.05) while up-regulated that of anti-inflammatory factor *IL-10* (P<0.000 1) when compared with the corresponding levels in AL mice. ABX treatment led no significant differences between the AL+ABX and TRF+ABX groups in term of DAI score, colon length, or histopathology. Obviously down-regulated *Reg3g* was observed in the TRF+ABX group than the AL+ABX group (P<0.05), whereas *L-1β*, *IL-6*, *TNF-α* and *IL-10* showed no notable changes. 16S rRNA-seq revealed that TRF markedly reshaped gut microbiota composition, with increased Gram-positive bacterial abundance, reduced Gram-negative bacteria, with concomitant lower serum LPS level (P<0.001). RNA-seq also indicated significant suppression of NF-κB and other inflammation-related signaling pathways in the TRF group. **Conclusion** TRF attenuates DSS-induced colitis in mice by downr-egulating Reg3γ expression, reshaping gut microbiota, and reducing serum LPS level, and thereby suppressing NF-κB-mediated inflammatory signaling pathways.

[**Key words**] time-restricted feeding; colitis; regenerating islet-derived protein 3; gut microbiota; NF-κB signaling pathway

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炎症性肠病(inflammatory bowel disease, IBD)是一种以肠道慢性、复发性炎症为特征的免疫性疾病[1]。全球疾病负担研究(Global Burden of Disease Study, GBD)显示,近年来IBD的发病率及患病率在全球范围内持续上升[2]。1990–2021年,我国IBD发病率年均增长2.09%[3-4]。该病以腹痛、腹泻、血便为主要临床

特征,常伴有多种肠外表现。病程迁延不愈,往往需要终身治疗,严重影响患者生活质量并造成较大的心理和经济负担[2]。IBD病因复杂,目前普遍认为是在遗传易感基础上,由肠道菌群失衡异常激活免疫系统而引起的持续炎症反应[5]。

已有研究证实,饮食会影响IBD患者的肠道菌群组成及肠道炎症的发生发展[6]。部分患者因担心进食诱发症状而主动减少进食,而现有药物疗效有限,难以实现长期缓解。因此,寻找安全、有效、易于长期坚持的饮食干预策略具有重要临床意义。限时进食(time-restricted feeding, TRF)作为一种新型间歇性禁食(intermittent fasting, IF)方式,通过限制每日进食时间窗,在不减少总营养摄入的前提下,可促进代谢节律同步、降低炎症水平、改善肠道环境,具有潜在的抗炎作用[7-9]。研究表明,TRF不仅可通过重塑昼夜节律及肠道菌群周期性波动改善代谢、减轻炎症、延缓疾病进展[10-13],还可能通过优化营养摄入时段激活细胞修复机制[14],从而缓解结肠炎。

肠道菌群失调被认为是IBD的重要发病机制之一[15-16]。菌群-宿主互作网络的失衡可触发级联反应,包括条件致病菌过度增殖损伤肠屏障功能、微生物代谢功能紊乱致使代谢产物异常蓄积,最终引起固有免疫和适应性免疫应答失衡[17-18]。饮食作为核心环境调节因子,可通过直接或间接途径影响肠道菌群的生态网络[19-21]。然而,TRF是否通过调节肠道菌群发挥对IBD的保护作用,其具体机制尚不明确。

本研究以葡聚糖硫酸钠(dextran sodium sulfate, DSS)诱导的小鼠结肠炎作为IBD动物模型,初步探讨TRF对结肠炎的干预效果,并结合肠道菌群及相关信号通路变化,揭示其缓解结肠炎的潜在机制,为临床探索饮食干预IBD提供实验依据和新的研究方向。

1 材料与方法

1.1 主要试剂

DSS购自美国MP Biomedicals公司;尿粪隐血检测试剂盒(联苯胺法)购自翌圣生物科技(上海)股份有限公司;万古霉素盐酸盐、氨苄青霉素钠、硫酸新霉素、甲硝唑购自生工生物工程(上海)股份有限公司;TRIzol™ 试剂购自美国赛默飞世尔科技有限公司,PrimeScriPt RT reagent Kit with gDNA Eraser (Perfect Real Time)、TB Green Premix *Ex Taq* Ⅱ (Tli RNaseH Plus)购自日本TaKaRa公司,引物由生工生物有限公司(上海)股份有限公司合成;小鼠脂多糖(lipopolysaccharide, LPS)定量检测试剂盒购自江苏晶美生物科技有限公司。

1.2 动物实验

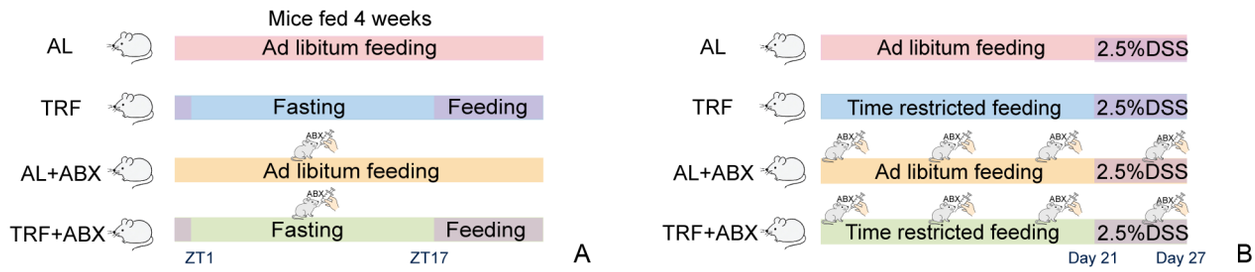
5周龄、体质量18~21 g的SPF级C57BL/6雄性小鼠购自成都药康生物科技有限公司,实验前适应性饲养1周。本研究所涉及的全部动物实验方案经陆军军医大学动物实验伦理审查委员会批准(AMUWEC20255354),实验严格遵守《实验动物管理条例》以及《实验动物伦理审查办法》等相关规定。动物饲养与实验均在新桥医院实验动物中心的SPF级动物实验室进行,环境温度控制在20~25 ℃、相对湿度为50%~60%,实行12 h昼夜交替光照。实验过程中采用异氟烷吸入麻醉以减少动物疼痛;所有动物在实验终点前每日监测健康状态和体质量,一旦出现严重不适即实施安乐死处理,执行人道终点原则。实验期间未观察到严重不良事件。

1.3 实验方法

1.3.1 结肠炎小鼠模型建立和分组 实验前将小鼠按体质量从小到大排序后实施分层随机化,确保分组间体质量均衡。将小鼠随机分为(n=7):自由饮食(ad libitum feeding,AL)组和TRF组。饮食干预持续4周,其中TRF组每天仅在00:00-08:00[同期节律时间(zeitgeber time,ZT)17~1,即从黑暗开始后5 h至光照开始后1 h]期间进食,符合小鼠"昼伏夜出"的活动规律;AL组则全天自由摄食(图1A)。DSS是一种硫酸化多糖,通过破坏肠道上皮屏障,引发免疫细胞浸润和炎症反应,模拟IBD的病理特征,主要表现为体质量减轻、腹泻和血便[22]。第4周(Day 21)开始,通过在饮水中连续添加2.5%DSS溶液6 d,构建小鼠结肠炎模型(图1B)。

1.3.2 抗生素鸡尾酒(antibiotic cocktail, ABX)处理小鼠模型的建立和分组 小鼠适应1周后,按体质量从小到大排序并采用分层随机化方法,将14只小鼠随机分为(n=7)AL+ABX组和TRF+ABX组,分别接受全天自由饮食或每日仅在ZT17~1期间供食的TRF干预(图1A),饮食处理持续4周。期间小鼠隔日灌胃给予ABX溶液,溶液由万古霉素盐酸盐(100 mg/kg)、氨苄青霉素钠(200 mg/kg)、硫酸新霉素(200 mg/kg)和甲硝唑(200 mg/kg)组成,现配现用,每只按0.1 mL/10 g体质量剂量灌胃1次,持续4周以清除肠道菌群。第4周起,小鼠在继续饮食干预与ABX灌胃的基础上,给予2.5%DSS溶液诱导结肠炎,持续6 d构建ABX处理下的急性结肠炎模型(图1B)。

1.3.3 疾病活动指数(disease activity index, DAI)评分 在使用DSS造模期间,连续6 d每天固定时间记录小鼠的体质量,观察粪便性状及血便情况,出血



A: Diagram of dietary intervention protocol; B: Flowchart of DSS-induced colitis modeling procedure

图1　不同饮食干预条件下DSS诱导结肠炎小鼠模型的建立方法

Figure 1　Establishment of mouse model of DSS-induced colitis under different dietary interventions

情况依据尿粪隐血检测试剂盒结果判定。根据体质量下降程度、粪便稠度和出血情况评估DAI,评分方法参考文献[23-24],总分由3项指标评分累加得出,分值越高表示炎症程度越严重。

1.3.4　结肠长度测量、HE染色及组织病理学分析

实验结束时,所有小鼠在异氟烷吸入麻醉后采用颈椎脱臼法处死,开腹收集结肠并测量其长度。切取部分新鲜结肠组织,经无菌生理盐水清洗、吸干水分后展平肠腔,卷成"瑞士卷"形状,置于4%多聚甲醛溶液中固定12 h。随后进行石蜡包埋、切片、HE染色,并使用组织切片扫描仪进行扫描与观察。染色实验由上海瑞雨生物科技有限公司完成。其余结肠组织转移至2 mL离心管中,置于超低温冰箱保存。

1.3.5　RNA提取及RT-qPCR　　取出1.3.4剩余结肠组织(1 cm),加入经过4 ℃预冷的TRIzol™试剂,用匀浆机充分研磨结肠组织,采用氯仿-异丙醇方法提取结肠组织总RNA。根据逆转录试剂盒说明书将RNA逆转录为cDNA,采用RT-qPCR技术对cDNA进行定量检测。以GAPDH作为内参,采用$2^{-\triangle\triangle Ct}$计算目标基因的相对表达量,所用引物序列见表1。

表1　RT-qPCR引物序列

Table 1　RT-qPCR primer sequences

Primer Name	Primer Sequence(5′→3′)
IL-1β	Forward primer:TTCAGGCAGGCAGTATCACTC
	Reverse primer:GAAGGTCCACGGGAAAGACAC
IL-6	Forward primer:CTTCCATCCAGTTGCCTTCT
	Reverse primer:CTCCGACTTGTGAAGTGGTATAG
TNF-α	Forward primer:GAAAGCATGATCCGGGACGTG
	Reverse primer:GATGGCAGAGAGGAGGTTGAC
IL-10	Forward primer:GCTCTTACTGACTGGCATGAG
	Reverse primer:CGCAGCTCTAGGAGCATGTG
Reg3g	Forward primer:TTCCTGTCCTCCATGATCAAAA
	Reverse primer:CATCCACCTCTGTTGGGTTCA

1.3.6　16S rRNA测序(16S ribosomal RNA sequencing,16S rRNA-seq)　　小鼠粪便样本经DNA提取、PCR扩增、纯化后,使用NEBNext® Ultra™ Ⅱ FS DNA PCR-free Library Prep Kit(New England Biolabs, Ipswich, MA, USA)进行文库构建。构建好的文库经过Qubit和qPCR定量,文库合格后,使用NovaSeq 6 000进行PE 250上机测序。利用QIIME2软件进行物种注释。针对门、纲、目、科、属和种等不同分类层级,筛选各组中丰度最高的前10个物种,利用Perl的SVG函数绘制相对丰度分布柱状图。革兰氏阴性菌(Gram-negative bacteria,GNB)为循环系统LPS的主要来源[25],其相对丰度由16S rRNA-seq数据计算得到。测序由北京诺禾致源科技股份有限公司完成。

1.3.7　酶联免疫吸附测定法(enzyme-linked immunosorbent assay, ELISA)检测小鼠血清LPS分泌水平　　小鼠麻醉后取外周血,室温静置1 h,使其充分凝固后,于室温以3 000 r/min离心10 min,收集上清液作为血清样本。血清按照ELISA试剂盒说明书进行检测,绘制标准曲线,计算小鼠血清中LPS水平。

1.3.8　结肠组织RNA测序(RNA sequencing,RNA-seq)及转录组分析　　从超低温冰箱取出适量结肠组织样本,提取总RNA后进行纯化和质量检测,确保RNA完整性(RIN≥7.0)。对高质量RNA进行片段化,逆转录合成cDNA并构建测序文库。采用基于Illumina平台的第二代高通量测序技术(next-generation sequencing,NGS)对文库进行双末端(paired-end, PE)测序,测序读长为150 bp。对原始下机数据进行质量控制,过滤低质量序列,得到高质量数据。将高质量数据比对至参考基因组,并拼接转录本以重构序列,计算基因表达量。进行差异表达基因分析、基因功能富集和聚类分析,探讨TRF对DSS造模小鼠结肠组织基因表达的调控作用及潜在机制。所有提取纯化、文库构建、测序及数据分析由上海拜谱生物科技有限公司完成。

1.4 统计学分析

采用GraphPad Prism 8.0.2软件进行统计学分析,计量资料以$\bar{x}\pm s$表示,2组间比较采用Shapiro-Wilk检验评估数据正态性,正态数据采用独立样本t检验,非正态数据采用Mann-Whitney U检验,多组间比较采用单因素方差分析,P<0.05认为差异具有统计学意义。

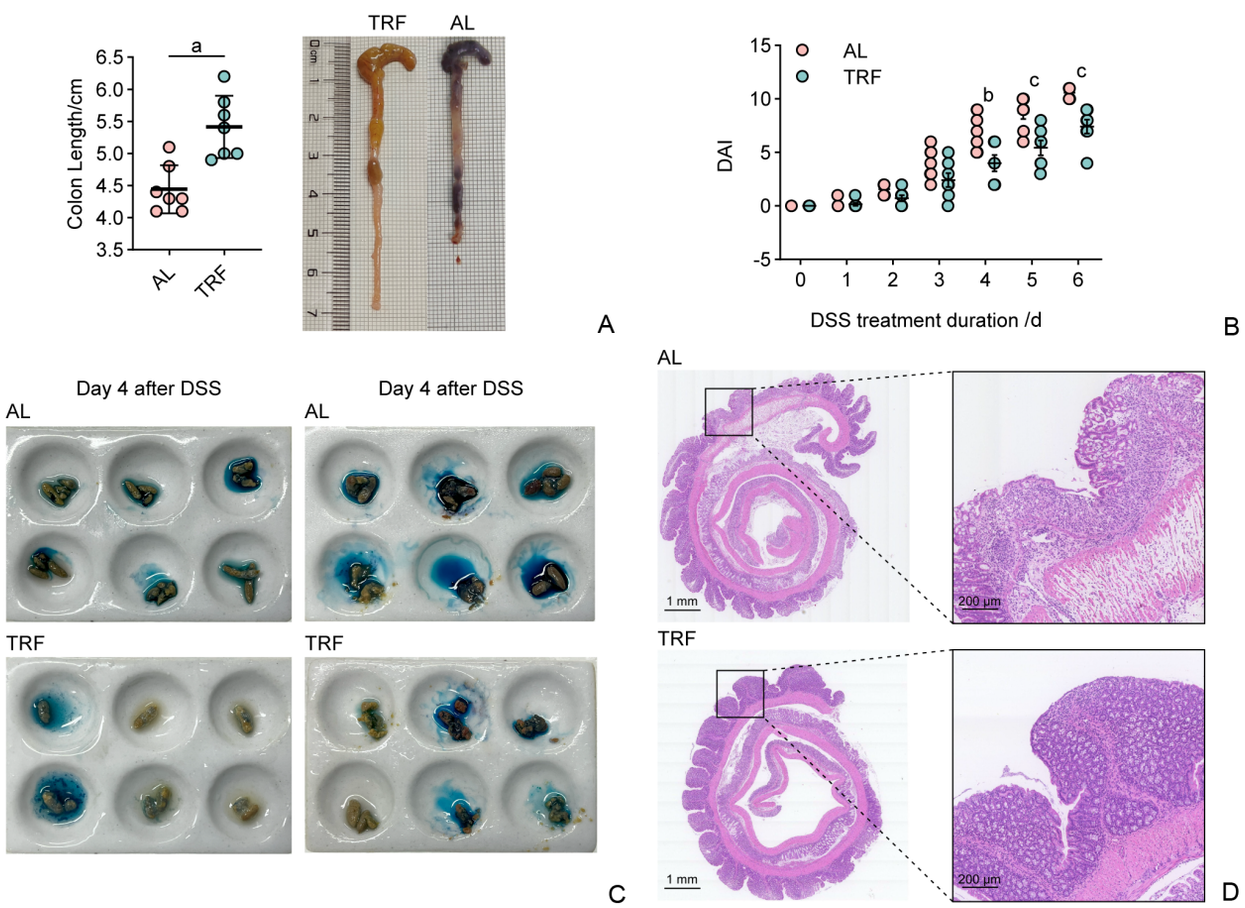
2 结果

2.1 TRF显著缓解DSS诱导的小鼠结肠炎

为探索TRF对IBD的潜在影响,本研究基于DSS诱导的急性结肠炎小鼠模型,进行不同饮食方式的干预实验,以评估TRF对结肠炎相关表型及肠道组织损伤的影响。实验结果显示,AL组小鼠结肠长度明显缩短(图2A),DAI评分自造模第4天起显著升高(图2B),开始出现活动减少、腹泻及血便(图2C),提示结肠炎症状呈进行性加重;而经TRF饮食干预后,小鼠结肠长度缩短程度减轻(P<0.01,图2A),DAI评分自第4天起较AL组显著降低(P<0.001,图2B),血便情况也明显改善(图2C)。HE染色结果显示,AL组小鼠结肠壁呈现整体增厚,黏膜下固有层可见大量炎性细胞浸润,黏膜上皮受损,腺体及隐窝结构紊乱,同时伴有大面积溃疡形成;而TRF干预后的小鼠结肠黏膜相对完整,结肠壁增厚不明显,腺体基本规则,充血与水肿程度降低,炎性浸润减少(图2D)。结果表明,TRF干预有助于保护肠道组织结构完整性,并有效缓解DSS诱导的小鼠结肠炎症状。

2.2 TRF通过调节肠道菌群影响结肠炎进程

为验证TRF缓解结肠炎是否依赖于肠道菌群,本研究采用ABX清除小鼠肠道菌群。结果显示,TRF+



A: Intergroup comparison of colon length with representative images; B: Intergroup comparison of DAI score during DSS modeling; C: Results of fecal occult blood detection; D: Histological observation of mouse colon tissues by HE staining. a: P<0.01; b: P<0.001; c: P<0.000 1

图2 不同饮食方式干预下DSS诱导小鼠结肠炎的表型特征及组织学改变(n=7,$\bar{x}\pm s$)

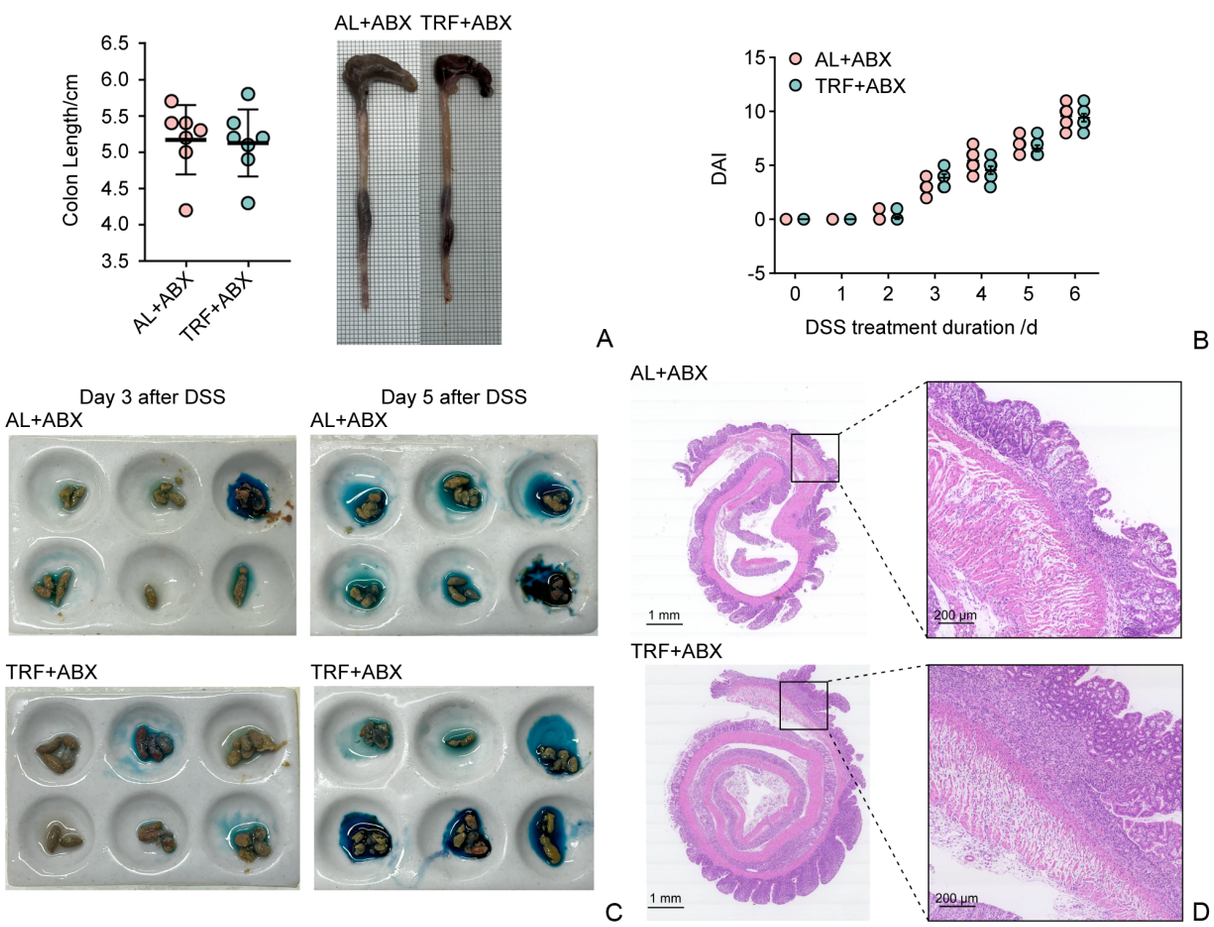
Figure 2 Phenotypic characteristics and histological changes of DSS-induced colitis in mice under different dietary interventions (n=7,$\bar{x}\pm s$)

ABX组与AL+ABX组在DSS诱导下均出现明显的结肠缩短(图3A)、体质量下降、腹泻和粪便带血等疾病表型(图3B、C),2组间差异无统计学意义。HE染色结果也显示,TRF+ABX组的结肠组织炎症程度与AL+ABX组相当,表现为黏膜结构破坏、隐窝紊乱和显著的炎性细胞浸润,TRF原本对上皮损伤和炎症反应的缓解作用不再显著(图3D)。上述结果提示,ABX处理可消除TRF所带来的保护效应,进一步表明TRF缓解结肠炎依赖于肠道菌群的存在及其调节功能。

2.3　TRF下调再生胰岛衍生蛋白3γ(regenerating islet-derived protein 3 gamma,Reg3γ)表达并显著减少GNB丰度

为深入挖掘TRF缓解结肠炎的潜在机制,本研究对AL组和TRF组DSS造模小鼠的结肠组织进行了RNA-seq分析,比较其基因表达差异及相关通路变化(图4A)。基因本体论(Gene Ontology,GO)分析结果显示,TRF干预显著下调多个与炎症及免疫密切相关的生物过程,主要包括炎性反应、细胞因子反应、细菌应答和先天性免疫应答(图4B)。差异表达分析进一步揭示,在上述通路中,与炎症调控密切相关的关键基因如胰岛再生基因3γ(regenerating islet-derived 3 gamma,*Reg3g*)、分化抗原簇14(cluster of differentiation 14,*Cd14*),肿瘤坏死因子(tumor necrosis factor,*Tnf*)在TRF组中表达水平均显著下调(图4C),提示其可能是TRF干预炎症反应调节的潜在靶点。

为进一步探究TRF是否通过调控肠道菌群进而影响*Reg3g*的表达水平,本研究检测了4组小鼠(AL、TRF、AL+ABX、TRF+ABX)结肠上皮细胞中*Reg3g*的



A: Intergroup comparison of colon length and representative images in mice under ABX treatment; B: Intergroup comparison of DAI score during DSS modeling under ABX treatment; C: Results of fecal occult blood detection after ABX intervention; D: Histological observation of mouse colonic tissues by HE staining under ABX treatment

图3　ABX处理下不同饮食方式对DSS诱导小鼠结肠炎的影响(n=7,$\bar{x}\pm s$)

Figure 3　Effects of different dietary interventions on DSS-induced colitis in mice under ABX treatment (n=7,$\bar{x}\pm s$)

mRNA表达。结果显示,TRF组与AL组(P<0.01)、TRF+ABX组与AL+ABX组(P<0.05)之间,*Reg3g*表达水平均存在显著差异(图4D)。这一结果表明,即使在ABX消除菌群的条件下,TRF仍可显著下调*Reg3g*的表达,提示*Reg3g*的转录下调可能并非由肠道菌群变化引发,而是TRF直接作用的结果,进而可能通过调控*Reg3g*影响肠道菌群组成,参与结肠炎的缓解过程。

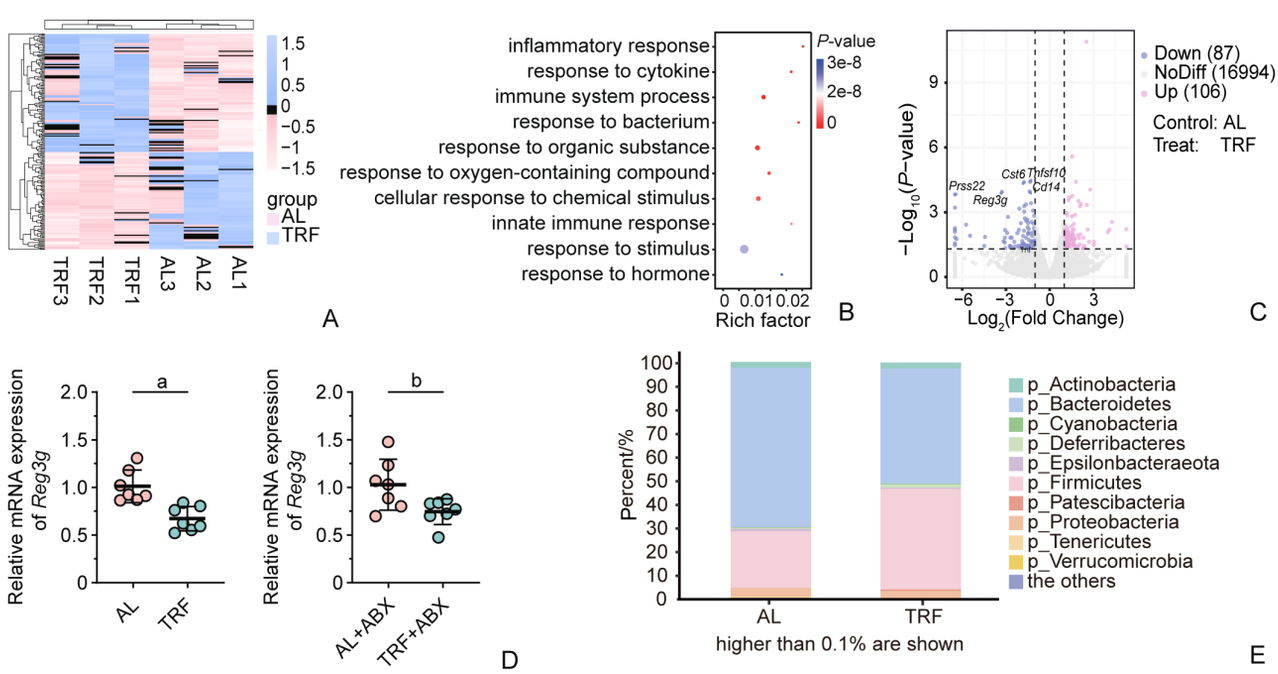
为评估TRF可能对肠道菌群产生的影响,本研究对AL组与TRF组小鼠的粪便样本进行了16S rRNA-seq分析。菌群丰度分析显示,TRF干预可显著改变小鼠肠道菌群组成。在门水平上,AL组以GNB为主,主要包括拟杆菌门和变形菌门;而TRF组中,厚壁菌门和铁还原菌门等革兰氏阳性菌(Gram-positive bacteria,GPB)的相对丰度明显升高,GNB比例相应减少,但总体比例较GPB仍略高(图4E)。这种GPB占比显著上升、GNB下降的菌群变化趋势与Reg3γ表达水平的下调相对应。作为一种主要靶向GPB的抗菌肽,Reg3γ表达减少可能减弱了对这类菌群的抑制,从而改变肠道菌群结构。这一结果提示,TRF通过调控Reg3γ表达影响肠道菌群组成,可能是其缓解结肠炎的重要机制之一。

2.4 RNA-seq提示TRF可能通过抑制LPS/NF-κB通路发挥抗炎作用

为进一步探讨GNB变化对循环系统的影响,本研究测定了血清LPS水平。结果显示,TRF组小鼠血清LPS显著低于AL组(P<0.001,图5A),表明TRF可能通过减少肠道GNB丰度降低循环系统中LPS含量。

转录组分析结果显示,TRF组小鼠结肠组织中与炎症相关的活化B细胞核因子κ轻链增强子(nuclear factor kappa-light-chain-enhancer of activated B cells,NF-κB)信号通路、TNF信号通路及细胞因子-细胞因子受体互作通路表达显著下调(图5B、C)。基因集富集分析(Gene Set Enrichment Analysis,GSEA)进一步表明,炎症性肠病相关基因集和NF-κB信号通路基因集主要富集于表达下调区域,显示通路相关基因表达受到抑制(图5D)。

在经典炎症信号通路中,LPS通过激活Toll样受体,引发NF-κB信号级联反应。本研究结果显示,TRF干预降低血清LPS水平的同时,NF-κB通路活性明显下调,表明该信号轴整体受到抑制。为验证该通路功能变化的实际影响,检测其下游关键炎症因子的

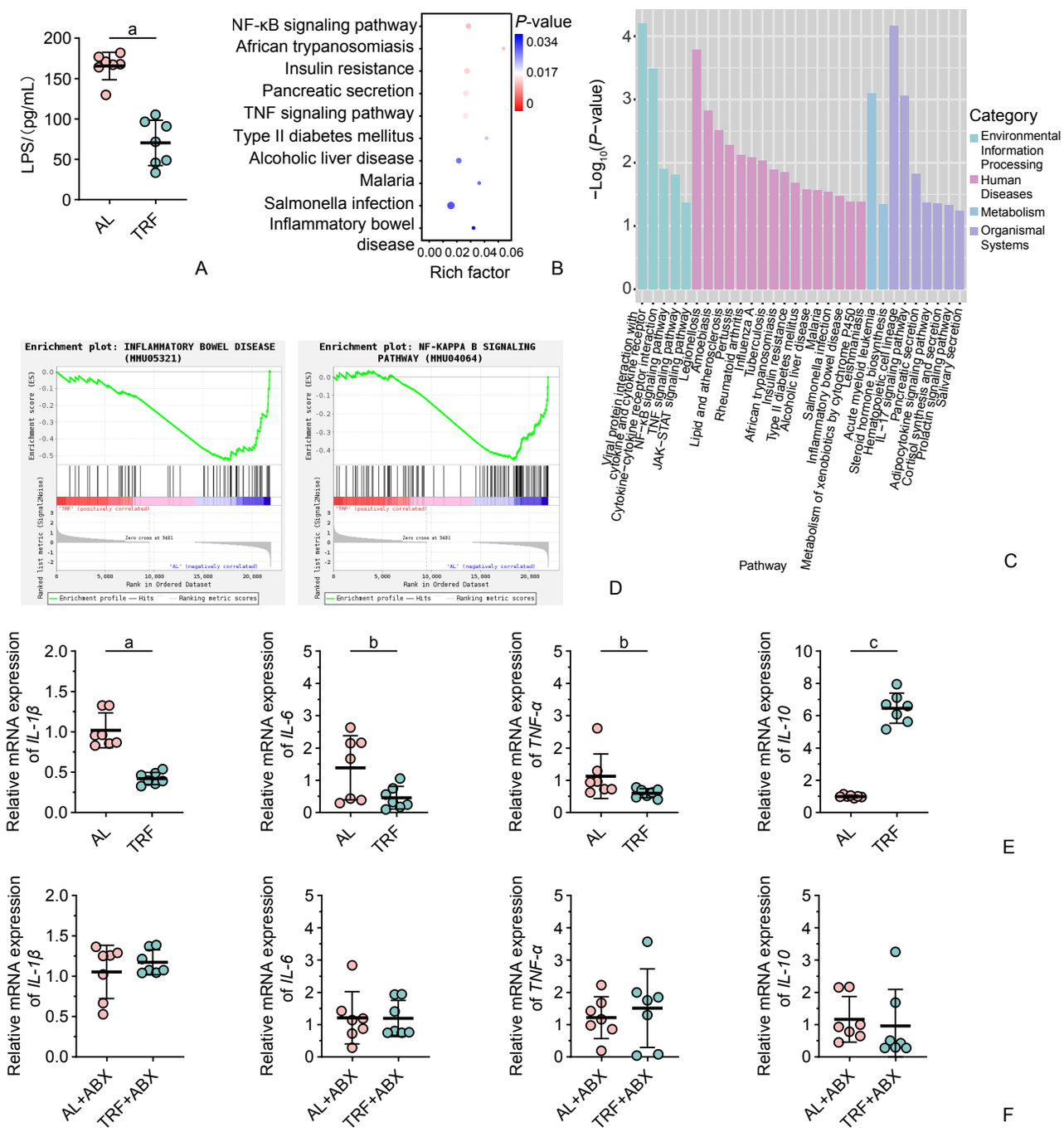


A: Heatmap of differentially expressed genes; B: GO enrichment bubble plot of significantly down-regulated biological processes in the TRF group; C: Volcano plot of differentially expressed genes; D: Relative mRNA level of *Reg3g* in colonic tissue from DSS-treated mice between paired groups, AL and TRF, AL+ABX and TRF+ABX (n=7, $\bar{x}\pm s$). a: P<0.01, b: P<0.05; E: Phylum-level composition of gut microbiota based on 16S rRNA-seq in the AL and TRF groups

图4 TRF干预引起的*Reg3g*表达变化及相关菌群特征分析

Figure 4 *Reg3g* expression changes and associated microbial characteristics induced by TRF intervention

mRNA表达水平。结果显示,TRF组*IL-1β*、*IL-6*及*TNF-α*表达水平均显著低于AL组(P<0.05),同时*IL-10*表达水平显著升高(P<0.000 1,图5E),结肠炎症水平下降与血清LPS水平及NF-κB通路活性下降趋势一致。在ABX处理后,上述因子表达差异消失(图5F),提示TRF抗炎效应依赖于肠道菌群介导的LPS水平调节。



A: Serum LPS level in AL and TRF mice; B: KEGG bubble plot of down-regulated genes between AL and TRF groups; C: KEGG bar chart of down-regulated genes between AL and TRF groups; D: KEGG gene set enrichment analysis (GSEA) of differentially expressed genes between AL and TRF groups; E: mRNA expression levels of colonic inflammatory cytokines in AL and TRF mice; F: mRNA expression levels of colonic inflammatory cytokines after antibiotic (ABX) treatment in TRF+ABX and AL+ABX mice. a: P<0.001, b: P<0.05, c: P<0.000 1

图5　TRF干预对LPS水平及NF-κB通路相关炎症因子与*IL-10*表达的影响(n=7,$\bar{x}\pm s$)

Figure 5　Effects of TRF intervention on LPS level and NF-κB pathway-related inflammatory factors and *IL-10* (n=7,$\bar{x}\pm s$)

3 讨论

3.1 基于NF-κB通路的TRF抗炎机制及多疾病应用前景

当前TRF研究多聚焦于体质量控制和代谢改善,重点强调其对昼夜节律重建的作用[26]。仅有少数研究初步评估TRF对IBD患者的潜在益处,其内在作用机制尚未明确[27]。本研究通过多组学整合分析与实验验证发现,TRF可显著下调小鼠结肠组织中NF-κB信号通路的活性,并抑制其下游炎症因子的释放,提示其在小鼠结肠炎中表现出的抗炎作用,可能为IBD提供潜在干预策略。

NF-κB作为经典的炎症信号通路,其持续激活可导致肠黏膜屏障受损及促炎因子过度释放,是驱动IBD慢性炎症的重要机制之一[28-29]。此外,NF-κB也是多种慢性疾病的核心炎性通路,其异常激活与非酒精性脂肪性肝炎、动脉粥样硬化、糖尿病肾病及神经退行性疾病的发生密切相关[30-33]。本研究结果不仅为TRF在结肠炎中的应用提供了分子机制支持,也为其在其他NF-κB驱动性疾病中的应用奠定理论基础,提示TRF有望成为多种疾病的共同干预策略之一。尽管既往研究已证实TRF具备抗炎与抗氧化应激效应[11, 34],但不同疾病背景下NF-κB的激活机制及组织微环境存在差异[35-37],TRF干预的靶向性与适应性仍需在更广泛模型中进一步验证。

3.2 基于GNB功能视角,TRF通过LPS轴调控局部及系统性炎症

IBD被认为是一种菌群相关性疾病,肠道菌群失调会增强机体对炎症的易感性[38-39]。肠道菌群由数百种细菌组成,菌属之间存在复杂的协同与拮抗关系,仅依赖单一菌属的变化难以全面反映其整体生态功能[40],因此,本研究并未聚焦于特定菌属,而是将关注范围扩展至GNB,从其作为LPS主要来源的角度切入,探讨其在炎症诱导中的作用及TRF的调控效应。

LPS作为典型的病原体相关分子模式(pathogen-associated molecular pattern,PAMP),可通过激活Toll样受体并募集衔接蛋白,触发下游NF-κB级联反应,诱导多种促炎因子的转录表达[41-42]。因此,LPS被视为推动肠道慢性炎症的重要因子[43]。本研究发现,TRF可通过调节肠道菌群组成,有效降低血清中LPS水平,从而抑制炎症反应。此外,有研究发现TRF还可维持结肠组织完整性,减少肠源性LPS向循环系统的渗漏,缓解LPS诱导的神经炎症和氧化应激,从而改善IBD相关的行为障碍[44]。上述结果提示,TRF的抗炎效应可能不局限于肠道,还可通过调节肠-脑轴发挥更广泛的系统性保护作用。

3.3 TRF通过调控Reg3γ表达参与肠道菌群调节与炎症缓解

Reg3γ是一种由潘氏细胞和肠上皮细胞分泌的抗菌肽(antimicrobial peptide, AMP),主要针对GPB发挥抑菌作用,在维持菌群-宿主空间分离、增强黏膜屏障中发挥重要作用[45-47]。Reg3γ的表达一般是由黏膜表面微生物-上皮直接接触诱导,与结肠炎的发生发展密切相关[48]。以往研究表明,ABX处理或无菌环境会抑制Reg3γ表达,而补充益生菌可增强其转录水平[46,49]。

然而,本研究在有菌与无菌状态下均观察到TRF组Reg3γ mRNA表达低于AL组,提示*Reg3g*可能受到TRF直接调控,而非完全依赖菌群刺激。尽管该结果与*Reg3g*作为"保护性抗菌肽"在炎症状态中通常上调的传统认知不一致[48],但考虑到*Reg3g*具有昼夜节律性表达特征[45],TRF可能通过恢复其节律模式实现功能优化,而非简单提高表达水平。

此外,TRF干预显著降低GNB比例和外周LPS水平。尽管Reg3γ主要针对GPB,其表达减少可能导致GPB数量增加,进而通过生态位竞争间接抑制GNB定植,影响LPS水平,但这一机制尚缺乏实验证据。后续拟通过*Reg3g*基因敲除及肠道GNB定植实验,进一步验证其在TRF介导炎症调节中的作用,以增强因果链条的系统性与严谨性。

3.4 TRF作为IBD辅助干预手段的临床潜力

TRF通过调控Reg3γ表达,减少GNB及其产生的LPS,抑制NF-κB信号通路激活,最终有效缓解了小鼠结肠炎。本研究初步揭示了TRF对结肠炎模型的抗炎机制,为其作为IBD辅助干预手段提供了理论依据。

作为一种非药物、低成本且依从性高的饮食干预策略,TRF在减少体质量、改善代谢、调节免疫等方面的综合效应[9, 50-52],使其在IBD辅助治疗中具有良好应用前景。鉴于目前IBD药物治疗存在副作用和耐药等问题,TRF为IBD患者提供了一种安全有效的干预方案。但未来仍需进一步开展临床试验,评估其疗效与安全性,同时深化机制研究,推动TRF的临床转化应用。

利益冲突 本文所有作者声明不存在利益冲突

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