DOI:10.13210/j.cnki.jhmu.20240429.003
网络出版地址:https://link.cnki.net/urlid/46.1049.R.20240429.1534.006

基于RNA-Seq探讨清解化攻方干预重症急性胰腺炎大鼠肠屏障损伤的潜在作用机制

朱晓东[1],陈国忠[2],卢 洁[2],冯敏超[1],唐曦平[3],刘锟荣[2,4] ✉
(1.广西中医药大学,广西 南宁 530200;2.广西中医药大学第一附属医院,广西 南宁 530023;3.广西医科大学附属肿瘤医院,广西 南宁 530021;4.广西中医药防治医学分子生物重点实验室,广西 南宁 530023)

[摘要] **目的**:采用高通量RNA-Seq探讨清解化攻方干预重症急性胰腺炎大鼠肠屏障损伤的潜在作用机制。**方法**:采用逆行胰胆管注射牛黄胆酸钠方法建立SAP模型,HE观察空白组、模型组、中药组大鼠胰腺和回肠组织病理改变情况,ELISA法检测大鼠血清血淀粉酶、脂肪酶、DAO、内毒素水平;RNA-seq测序检测各组大鼠回肠组织差异性表达基因,筛选清解化攻方干预重症急性胰腺炎大鼠肠屏障损伤的核心基因、生物学功能及信号通路。**结果**:与空白组比较,模型组大鼠胰腺和回肠组织均有明显的病理改变,经清解化攻方干预得到显著改善;ELISA结果提示清解化攻方能够显著改善SAP大鼠血清淀粉酶、脂肪酶、DAO、内毒素含量($P<0.05$)。RNA-Seq结果显示SAP组与BG组差异基因有2 623个,经QJHGD处理后表达明显下调的基因有175个,主要有*Tnip3*、*Lpo*等,而上调的基因有320个,主要有*aicda*、*Tnn*等,参与调节氧化应激等生物过程,并干预TJ、细胞凋亡、Toll样受体、NOD样受体、NF-κB等多条信号通路而发挥作用。**结论**:清解化攻方可能通过调节*Tnip3*、*aicda*等关键基因及调控TJ、细胞凋亡、Toll样受体等多条信号通路起到抑制重症急性胰腺炎肠屏障损伤作用。

[关键词] RNA-Seq;重症急性胰腺炎;肠屏障损伤;清解化攻方
[中图分类号] R285.5 [文献标志码] A [文章编号] 1007-1237(2024)16-1220-10

RNA-Seq-based exploration of the potential mechanism of action of Qingjie Huagong decoction in intervening intestinal barrier injury in rats with severe acute pancreatitis

ZHU Xiaodong[1], CHEN Guozhong[2], LU Jie[2], FENG Minchao[1], TANG Xiping[3], LIU Kunrong[2,4] ✉
(1. *Guangxi University of Chinese Medicine*, *Nanning* 530200, *China*; 2. *The First Affiliated Hospital of Guangxi University of Traditional Chinese Medicine*, *Nanning* 530023, *China*; 3. *Guangxi Medical University Affiliated Cancer Hospital*, *Nanning* 530021, *China*; 4. *Guangxi Key Laboratory of Molecular Biology for the Prevention and Treatment of Chinese Medicine*, *Nanning* 530023, *China*)
[*Foundation Project*]: *This study was supported by National Natural Science Foundation of China* (82160890); 2022 *Guangxi University of Traditional Chinese Medicine Doctoral Research Start-up Fund* (2022*BS*029)
[*Author*]: *ZHU Xiaodong*, *M.M.*, *E-mail*: 2082553933@qq.com.
[*Correspondence to*]: *LIU Kunrong*, *Attending Physician*, *M.D.*, *E-mail*: *glory38@yeah.net.*
Received: 2024-01-15 *Revised*: 2024-04-17 *JHMU*, 2024;30(16):1220-1229

View from specialist: It is creative, and of certain scientific and educational value.

[Abstract] **Objective**: To explore the potential mechanism of action of Qingjie Huagong decoction (QJHGD) in intervening the intestinal barrier damage in rats with severe acute pancreatitis by using high-throughput RNA-Seq. **Methods**: A SAP model was established by retrograde pancreaticobiliary injection of sodium oxybate, HE was used to observe the histopathological changes in the pancreas and ileum of rats in the blank group, model group and traditional Chinese medicine group, and the levels of serum amylase, lipase, DAO and endotoxin in rats were detected by ELISA method; the differentially expressed genes were detected in ileal tissues of rats in each group by RNA-seq sequencing, so as to screen the core genes, biological functions and signaling

[基金项目] 国家自然科学基金资助项目(82160890);2022年广西中医药大学引进博士科研启动基金项目(2022BS029)
[作者简介] 朱晓东,硕士,E-mail:2082553933@qq.com。
[通讯作者] 刘锟荣,主治医师,博士,E-mail:glory38@yeah.net。
[收稿日期] 2024-01-15 [修回日期] 2024-04-17 网络出版时间:2024-04-30 09:31:00

pathways of the QJHGD for intervening in the intestinal barrier damage in rats with severe acute pancreatitis. **Results**: Compared with the blank group, the pancreas and ileum tissues of rats in the model group showed obvious pathological changes, which were significantly improved by the intervention of QJHGD; the ELISA results suggested that QJHGD could significantly improve the serum amylase, lipase, DAO and endotoxin levels in rats with SAP ($P<0.05$). The RNA-Seq results showed that there were 2 623 genes differentiated in the SAP group from the BG group, and 175 genes were significantly down-regulated after QJHGD treatment, mainly *Tnip3* and *Lpo*, while 320 genes were up-regulated, mainly *aicda* and *Tnn*, and so on. There were 175 genes whose expression was significantly down-regulated after QJHGD treatment, mainly *Tnip3*, *Lpo*, *etc.*, while 320 genes were up-regulated, mainly *aicda*, *Tnn*, *etc.*, which were involved in the regulation of biological processes such as oxidative stress and intervened in multiple signaling pathways such as TJ, apoptosis, Toll-like receptor, NOD-like receptor, NF-κB and so on, and played a role in the regulation of oxidative stress. **Conclusion**: QJHGD may inhibit the intestinal barrier injury of severe acute pancreatitis by regulating key genes such as *Tnip3* and *aicda*, as well as multiple signaling pathways such as TJ, apoptosis, and Toll-like receptor.

[**Key words**] RNA-Seq; Severe acute pancreatitis; Intestinal barrier injury; Qingjie Huagong decoction

急性胰腺炎(acute pancreatitis,AP)是由胰腺外分泌功能异常引起的有害性炎症性疾病,主要由胆道结石、暴饮暴食、代谢和免疫功能障碍等引起,是常见的急性危重症之一[1,2]。大约20%～35%的AP患者会进展为严重急性胰腺炎(severe acute pancreatitis,SAP)。SAP的特征是持续性多器官衰竭和微循环障碍(尤其是肠道),死亡率高达30%[3,4],是一种与肠道屏障功能障碍密切相关的胰腺急性炎症性疾病[5,6],SAP的发生和发展,及全身炎症反应、菌群紊乱和易位、感染性坏死等都与肠道屏障功能障碍有密切关系。清解化攻方(Qingjie Huagong decoction,QJHGD)是陈国忠教授根据SAP的关键病机创立的院内制剂,并已获国家发明专利(No. ZL201811021893.2),临床上联合西药治疗SAP能显著改善患者的临床症状,降低淀粉酶、脂肪酶和炎症指标[7,8],课题组前期基础实验证明QJHGD可减轻肠道炎症,抑制肠黏膜屏障损伤的功能[9,10],但具体分子机制有待进一步研究。

转录组是在细胞或组织中表达的所有RNA转录物的集合[11]。运用转录组测序(RNA-Seq)是对细胞中所有RNA转录本的cDNA文库进行测序,以监测组织或细胞在单核苷酸水平上的整体转录活性,可分析转录本的结构和表达水平,在疾病诊断、治疗和药物作用机制的阐明等方面显示出巨大的潜力。因此,运用转录组学能够从整体水平上探索中药干预后组织或细胞中基因的转录变化情况,筛选出关键基因和靶点[12]。鉴于SAP与肠道屏障功能障碍的密切关系,积极研究SAP肠道屏障功能障碍的分子机制,对于后续深入的基础研究和改善SAP肠道损伤至关重要,因此,本研究对SAP大鼠回肠组织进行转录组测序,生物信息分析筛选QJHGD干预SAP肠屏障损伤的相关基因和信号通路,阐明QJHGD抑制SAP肠屏障损伤的分子机制。

1 材料和方法

1.1 动物

于湖南斯莱克景达实验动物有限公司[生产许可证号:SCXK(湘)2019-0004]购买清洁剂级雄性SD大鼠12只,8周龄,体质量(180±20) g,在广西中医药大学动物实验室喂养,使用许可证号:SYXK(桂)2019-0001。实验室温度在(24±2) ℃、昼日光照12 h,适应性喂养1周;本研究已通过实验动物伦理(批号:DW20220310-020)。

1.2 药物及试剂

QJHGD由大黄、枳实、厚朴、柴胡、黄芩、桃仁、莱菔子、木香、延胡索、丹参等组成,药材经广西中医药大学中药真伪鉴定技术中心认定为正品,药液熬制后过滤,用旋转蒸发仪浓缩成1 g/mL比例的生药;牛磺胆酸钠(美国Sigma公司,S0900000);苏木精和伊红(H&E)染色试剂盒(solarbio公司,G1120);ELISA试剂盒包括血清脂肪酶、淀粉酶DAO、内毒素(贝茵莱生物科技,RA21218、RA21767、RA20028、RA20354)

1.3 分组及给药

按随机数字表法,将大鼠分为空白组(BG组)、模型组(SAP组)、清解化攻方组(QJHGD组),每组4只。参考文献[13]采用逆行胰胆管注射牛黄胆酸钠方法建立SAP模型,在大鼠腹正中剑突下约1.5 cm切口开腹,显露、提取十二指肠,辨认胰胆管,用显微血管夹将肝门端胰胆管在距胰腺约0.5 cm处夹闭,左手持血管夹,右手持4号半针头从胰胆管远端行逆行穿刺,0.2 mL/min缓慢匀速向胰胆管注入5%牛磺胆酸钠(1 mL/kg),注药完毕后,加压阻闭针口1 min,去除血管夹,见胰腺快速充血、水肿,提示造模成功。根据汤家铭/陈民利主编的《医学实验动物学》中实验动物与人的体表面积比例进行等比例换算中药给药剂量,并根据课题组前期实验已

发现中剂量效果更佳，故本研究QJHGD组予9 g/kg体质量灌胃，BG组、SAP组予相同容积0.9%氯化钠溶液灌胃，干预3 d，早晚给药各1次。干预结束后，予腹腔注射戊巴比妥钠麻醉后取材。

1.4　苏木精-伊红染色法（hematoxylin-eosin staining，HE）观察胰腺和回肠组织病理

各组大鼠胰腺和回肠石蜡包埋经切片、烤片、脱蜡等步骤，按照HE试剂盒说明书步骤染色，然后光学显微镜观察病理变化。

1.5　ELISA法检测大鼠血清血淀粉酶、脂肪酶、DAO、内毒素水平

腹主动脉采血收集各组大鼠的血液，室温静置3 h沉淀，3 500 r/min离心15 min，抽取上清液。按相应ELISA试剂盒说明书操作检测血淀粉酶、脂肪酶、DAO、内毒素的浓度。

1.6　转录组测序

1.6.1　测序流程　测序流程经对BG组、SAP组、QJHGD组共12个回肠组织进行样本检测，采用Agilent 2100 bioanalyzer，精确检测RNA完整性和总量；然后文库构建与质检；库检合格后，进行Illumina测序（委托诺禾致源生物科技有限公司完成）。

1.6.2　生物信息分析　首选测序片段被高通量测序仪测得的图像数据经CASAVA碱基识别转化为序列数据（reads），为了保证数据分析的质量及可靠性，需要对原始数据进行过滤所有分析均是基于clean data进行的高质量分析；然后直接从基因组网站下载参考基因组和基因模型注释文件，使用HISAT2 v2.0.5构建参考基因组的索引，并使用HISAT2 v2.0.5将配对末端clean reads与参照基因组比对；采用StringTie（1.3.3b）（Mihaela Pertea.et al. 2015）进行新基因预测；然后featureCounts（1.5.0-p3）用于计算映射到每个基因的读数，根据基因的长度计算每个基因的FPKM，并计算映射到该基因的读数，对基因表达水平定量；然后对于有生物学重复的样本，使用DESeq2软件（1.20.0）进行两个比较组合之间的差异表达分析，筛选差异表达基因；通过clusterProfiler（3.8.1）软件实现差异表达基因的GO和KEGG通路富集分析，筛选潜在信号通路；基于已知和预测蛋白质-蛋白质相互作用的STRING数据库进行差异表达基因的蛋白互作网络（protein-protein interaction，PPI）分析，筛选核心靶点。

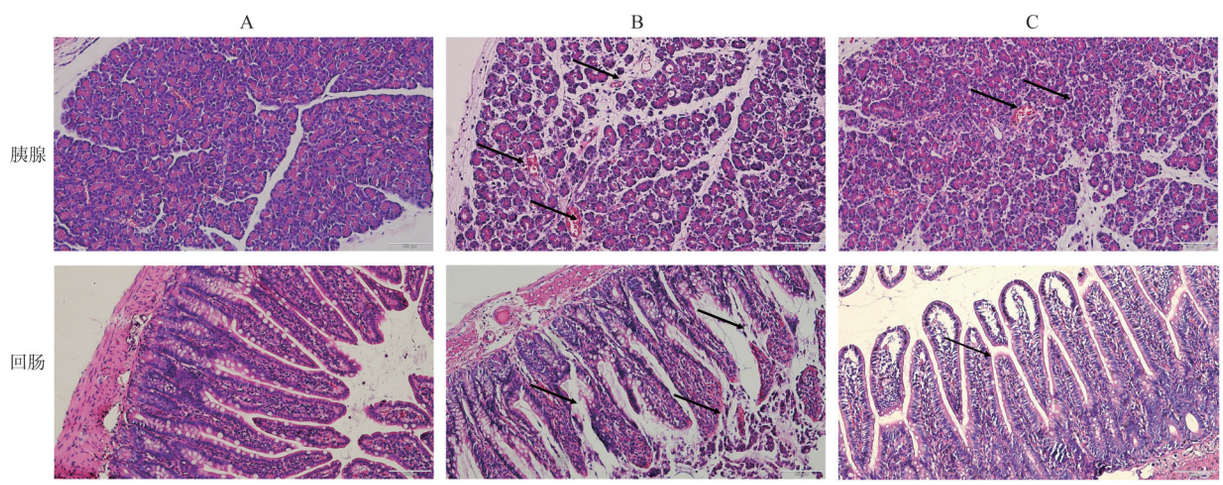
1.7　统计学处理

运用SPSS23.0软件进行统计学分析。计量资料表示为（$\bar{x}\pm s$），不同组比较用One-way ANOVA分析，进行两两比较。$P<0.05$表示差异有统计学意义。

2　结果

2.1　各组大鼠胰腺及回肠组织病理变化的影响

HE染色观察胰腺和回肠组织的病理改变如图1所示。BG组大鼠胰腺组织排列紧密、形态完整，小叶间隙正常，未见出血灶，回肠组织肠上皮细胞形态正常，肠黏膜未见脱落；SAP组大鼠胰腺组织肿大、细胞间隙隙增宽、可见出血灶，回肠组织肠上皮细胞结构紊乱，间隙增宽稀疏，肠黏膜上皮脱落。QJHGD治疗组大鼠的胰腺组织形态较好、水肿减轻、出血灶减少，肠上皮细胞结构、肠黏膜上皮脱落情况较SAP组明显改善。



A：BG组；B：SAP组；C：QJHGD组

图1　各组大鼠胰腺和回肠组织病理性变化情况（HE染色×200倍）

Fig 1　Histopathological changes in the pancreas and ileum of rats in various groups（HE，×200）

2.2 各组大鼠血清淀粉酶、脂肪酶、DAO、内毒素水平变化

与BG组比较，SAP组大鼠血清中淀粉酶、脂肪酶、DAO、内毒素的含量均有显著提升（$P<0.05$）；QJHGD量组均可降低淀粉酶、脂肪酶、DAO、内毒素含量，且与SAP组相比有显著性差异（$P<0.05$）。见表1。

表1 各组大鼠血清淀粉酶、脂肪酶、DAO、内毒素指标检测结果（n=3，$\bar{x}\pm s$）

Tab 1 Results of serum amylase, lipase, DAO, and endotoxin indexes in rats of each group（n=3，$\bar{x}\pm s$）

组别	淀粉酶(U/L)	脂肪酶(U/L)	DAO(mg/mL)	内毒素(ng/mL)
BG组	824.51±49.24	582.23±45.34	0.29±0.13	0.79±0.23
SAP组	2 571.24±134.56*	3 535.34±57.41*	3.28±0.51*	2.54±0.35*
QJHGD组	1 520.46±56.64#	1 328.78±68.49#	1.39±0.45#	1.58±0.23#

注：与BG组比较，*$P<0.05$；与SAP组比较，#$P<0.05$。

2.3 测序结果和分析

2.3.1 数据质量汇总 基于Illumina测序平台完成BG组、SAP组和QJHGD组12个回肠组织的转录组测序，各样品获得raw reads平均为4.29×10^7，raw bases平均为6.44G，clean reads平均为4.16×10^7，clean bases平均为6.24G，数据整体测序错误率在0.2或0.3，质控Q20碱基百分比平均为97.71%，Q30碱基百分比平均为93.91%，见表2，表明此次测序获得数据质量较好，可用于后续研究。

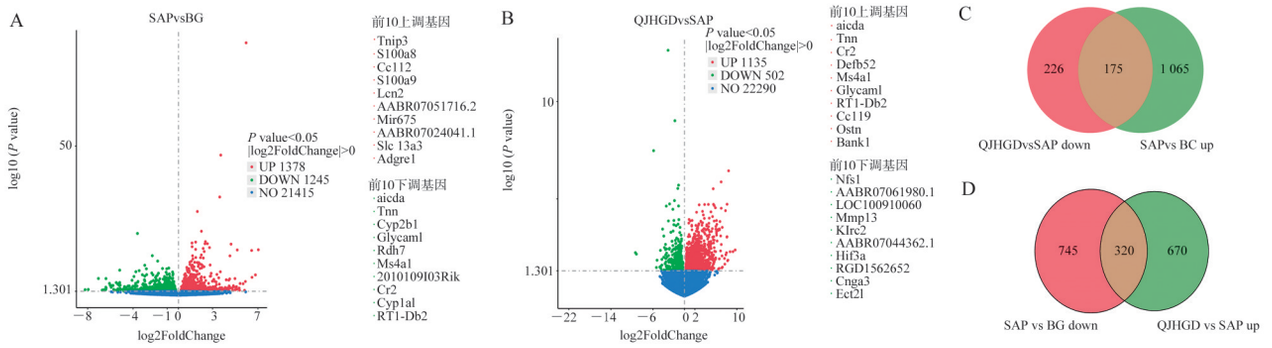
表2 测序数据汇总

Tab 2 Summary of sequencing data

sample	raw reads	raw bases	clean reads	clean bases	error rate	Q20	Q30	GC pct
BG1	40793994	6.12G	39497062	5.92G	0.03	97.69	93.89	49.54
BG2	44064246	6.61G	42445414	6.37G	0.03	97.70	94.04	48.51
BG3	43158704	6.47G	41560992	6.23G	0.03	97.21	92.84	47.67
BG4	45576204	6.84G	44359542	6.65G	0.02	97.96	94.42	49.23
SAP1	44123666	6.62G	43063074	6.46G	0.03	97.69	93.86	48.48
SAP2	43093206	6.46G	41979142	6.30G	0.03	97.78	94.01	49.81
SAP3	42703636	6.41G	41843654	6.28G	0.03	97.85	94.26	48.44
SAP4	44925128	6.74G	43883730	6.58G	0.03	97.76	93.93	49.88
QJHGD1	39972422	6.00G	38901046	5.84G	0.03	97.72	93.90	48.95
QJHGD2	43832448	6.57G	42177748	6.33G	0.02	97.96	94.45	49.34
QJHGD3	41569020	6.24G	39993850	6.00G	0.03	97.44	93.38	50.09
QJHGD4	41171636	6.18G	39652474	5.95G	0.03	97.71	93.99	48.54

2.3.2 差异表达基因（differentially expressed genes，DEGs）分析 测序数据完成质控、比对、定量后，对其表达数据进行统计学分析，筛选样本在不同状态下表达水平显著差异的基因，DEGs的筛选标准为$|\log2(\text{FoldChange})|\geq1$& padj$\leq0.05$。结果显示：SAP组与BG组差异基因有2 623个（图2A），其中上调1 378个，下调1 245个。QJHGD组与SAP组差异基因1 342个（图2B），其中上调611个，下调731个。去除无法注释DEGs，在不同组两两比较，与BG组相比，SAP组中表达上调的基因，QJHGD处理后表达明显下调的基因有175个（图2C），主要有*Tnip3*、*Adgre1*、*Rtn4rl2*、*Lpo*、*Mmp13*等；与BG组相比，SAP组中表达下调基因，QJHGD干预后表达上调的基因有320个（图2D），主要有*aicda*、*Tnn*、*Glycam1*、*Rdh7*、*Ms4a1*等。由此可见，QJHGD可调控SAP大鼠回肠组织内众多基因表达。

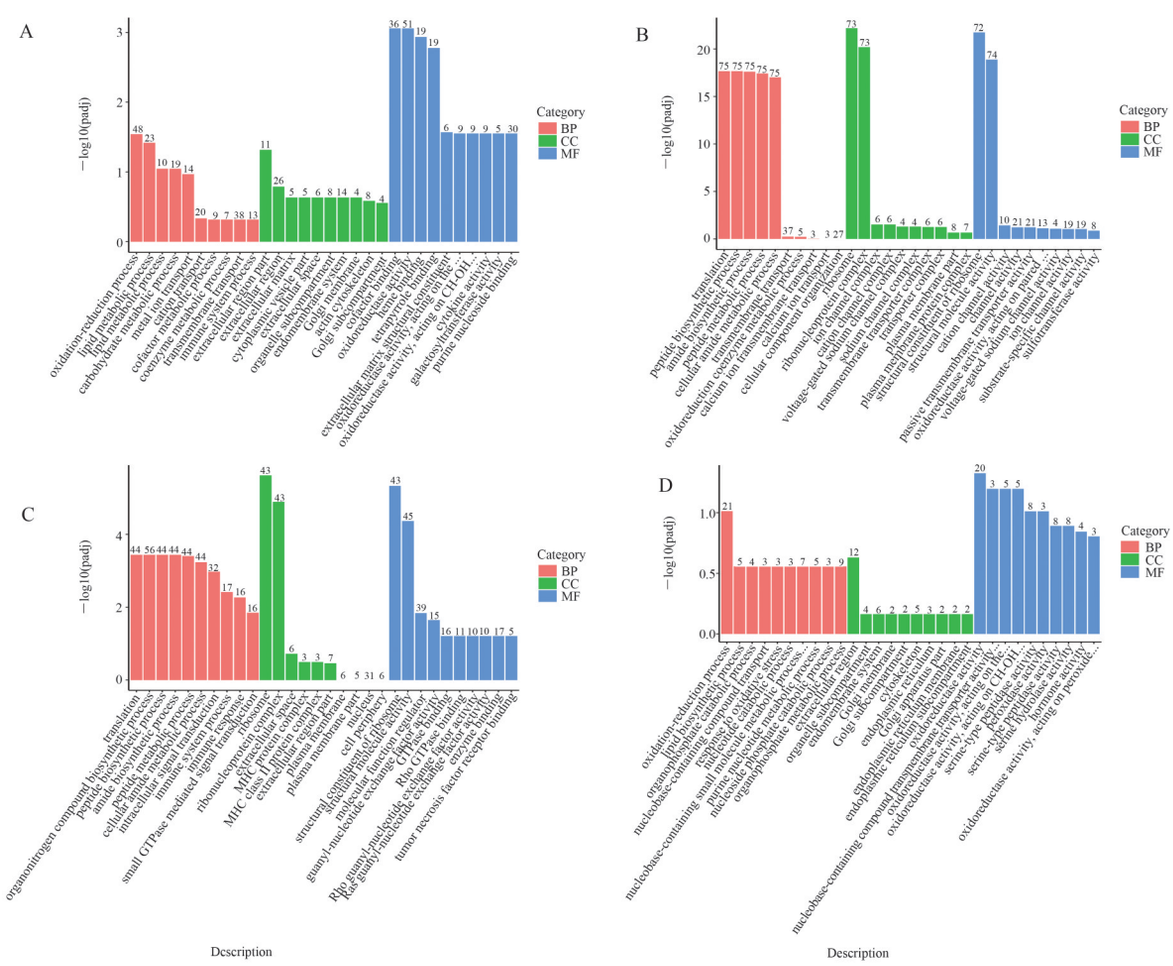
2.3.3 差异基因的GO富集分类分析 采用clusterProfiler软件对差异基因集进行GO功能富集分析，分为生物过程（biological process，BP）和细胞组成（cellular component，CC）分子功能（molecular function，MF）3个部分，筛选出BG组和SAP组、QJHGD组和SAP组比较的显著性上下调差异基因的前十进行绘图，见图3。结果发现，SAP组与BG组比较，上调差异基因中（图3A），BP主要富集于氧化还原（oxidation-reduction process）、脂质代谢（lipid



A：SAP组*vs.* BG组；B：QJHGD组*vs.* SAP组；C：SAP组与BG组上调基因*vs.* QJHGD组与SAP组下调基因；D：SAP组与BG组下调基因*vs.* QJHGD组与SAP组上调基因

图2　不同组别测序的差异表达基因的火山图和韦恩图

Fig 2　Volcano and Venn plots of differentially expressed genes sequenced in different groups



A：SAP组*vs.* BG组上调；B：SAP组*vs.* BG组下调；C：SAP组*vs.* QJHGD组上调；D：SAP组*vs.* QJHGD组下调。

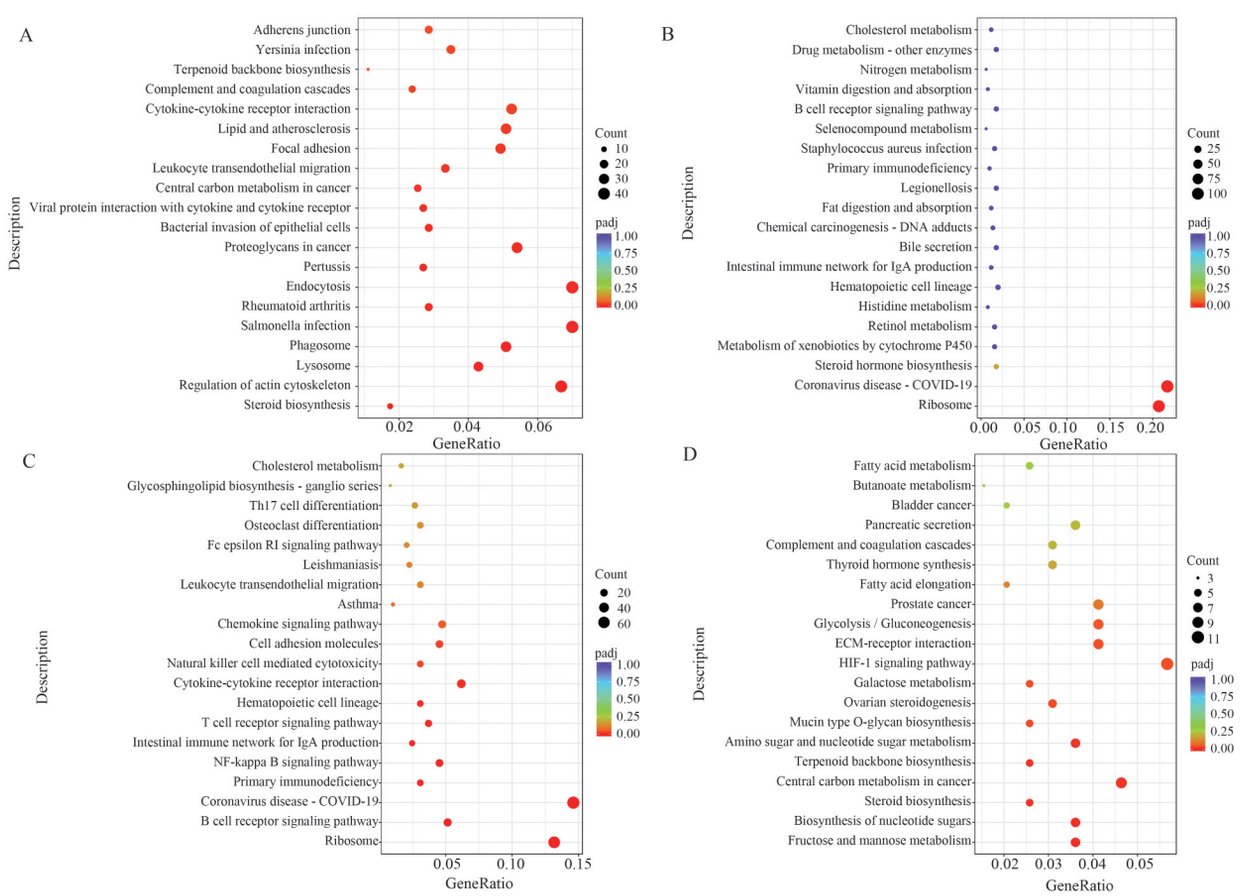
图3　差异基因GO富集分析

Fig 3　Differential gene GO enrichment analysis

metabolic process)等，CC主要富集于细胞外区域部分(extracellular region part)、细胞外基质(extracellular matrix)等，MF主要富集于辅因子结合(cofactor binding)、氧化还原酶活性(oxidoreductase activity)等；下调差异基因中(图3B)，BP主要富集于翻译(translation)、肽的生物合成(peptide biosynthetic process)，CC主要富集于核糖体(ribosome)、核糖核蛋白复合体(ribonucleoprotein complex)，MF主要富集于核糖体的结构成分(structural constituent of ribosome)、结构分子活性(structural molecule activi-

ty)。SAP组与QJHGD组比较,上调差异基因中(图3C),BP主要富集于氧化还原过程(oxidation-reduction process)、应激反应(response to stress)等,CC主要富集于细胞外区域(extracellular region)等,MF主要富集于血红素结合(heme binding)、四吡咯结合(tetrapyrrole binding)等;下调差异基因中(图3D),BP主要富集于离子传输(ion transport)、跨膜运输(transmembrane transport),CC主要富集于转运复合体(transporter complex)、离子通道复合物(ion channel complex),MF主要富集于离子通道活性(ion channel activity)、底物特异性通道活性(substrate-specific channel activity)。结果表明,QJHGD可能通过氧化还原等生物过程发挥缓解SAP肠屏障损伤的作用。

2.3.4 差异表达基因KEGG通路富集分析 对每组的差异表达基因做KEGG通路富集,以padj小于0.05作为显著性富集的阈值,从KEGG富集结果中,选取最显著的20个KEGG通路绘制散点图进行展示,见图4。SAP组*vs.* BG组的上调差异基因(图4A)主要富集于细菌入侵上皮细胞、IL-17信号通路、紧密连接、细胞凋亡、ECM与受体的相互作用、Toll样受体信号通路、NOD样受体信号通路等;SAP组*vs.* BG组的下调差异基因(图4B)主要富集于产生IgA的肠道免疫网络、B细胞受体信号通路、Th17细胞分化、还有跟多种物质代谢相关;SAP组*vs.* QJHGD组,上调差异基因(图4C)中主要富集于B细胞受体信号通路、NF-kappa B信号通路、产生IgA的肠道免疫网络、T细胞受体信号通路、FoxO信号通路、FcγR介导的吞噬作用、Th1和Th2细胞分化、JAK-STAT信号通路、PPAR信号通路等,下调差异基因(图4D)中主要富集于果糖和甘露糖、丁酸盐代谢等物质代谢、HIF-1信号通路、Rap1信号通路、PI3K-Akt信号通路、PPAR信号通路等。由此可见,QJHGD可通过多条信号通路干预SAP肠道损伤。

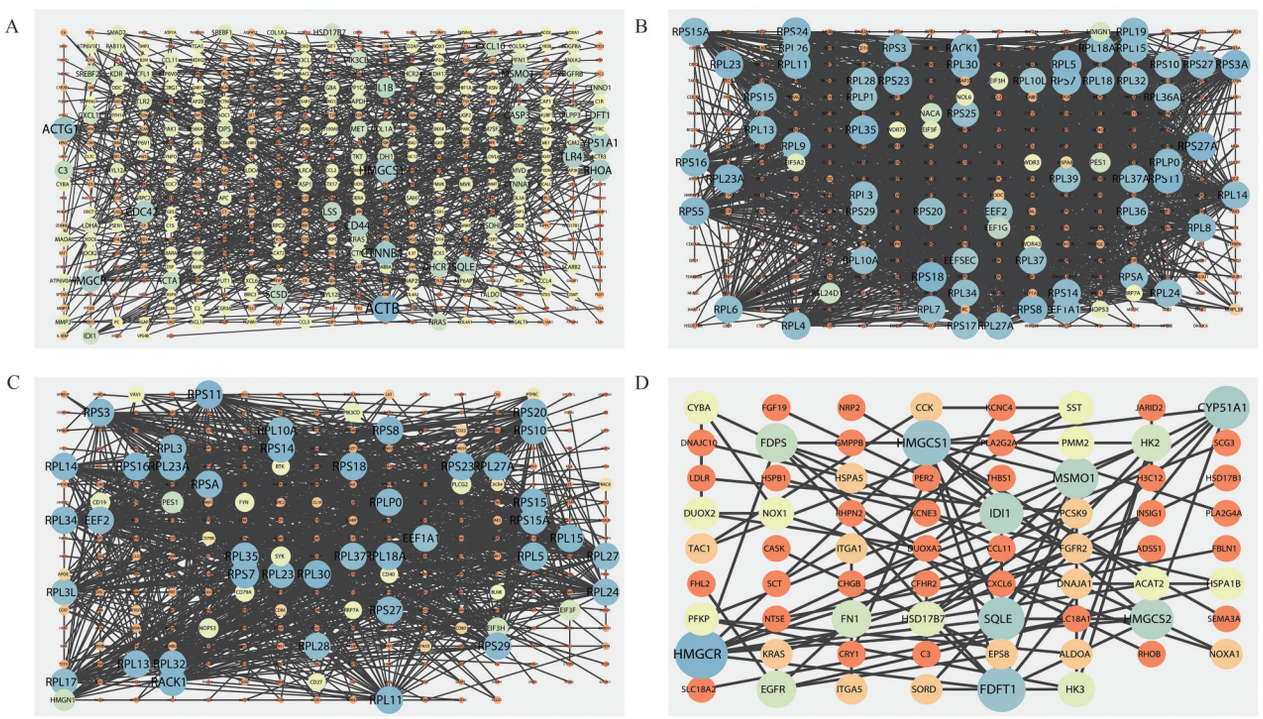


A:SAP组*vs.* BG组上调;B:SAP组*vs.* BG组下调;C:SAP组*vs.* QJHGD组上调;D:SAP组*vs.* QJHGD组下调

图4 差异表达基因的KEGG通路富集分析

Fig 4 KEGG pathway enrichment analysis of differentially expressed genes

2.3.5　差异基因的PPI网络构建　将SAP组与BG组差异基因中上调的1 378个，下调的1 245个，QJHGD组与SAP组差异基因中上调的611个，下调的731个分别输入STRING网站构建PPI网络，运用Cytoscape软件筛选出节点度值前3位的DEGs，见图5。结果显示SAP组与BG组上调DEGs中ACTB、ACTG1、HMGCS1，下调DEGs中RPS11、RPS18、RPS27A；QJHGD组与SAP组上调DEGs中RPS11、RPS7、RPS16，下调DEGs中HMGCR、HMGCS1、FDFT1。由此可见，HMGCS1和RPS11是QJHGD治疗SAP肠屏障损伤的核心靶点。



A：SAP组*vs.* BG组上调；B：SAP组*vs.* BG组下调；C：SAP组*vs.* QJHGD组上调；D：SAP组*vs.* QJHGD组下调

图5　PPI网络核心靶点分析

Fig 5　PPI network core targeting analysis

3　讨论

中医药在临床治疗重症急性胰腺炎中发挥着越来越重要的作用，众多学者投入大量的中医药治疗SAP的研究[14]，并且在SAP肠道损伤也有广泛研究[15-17]，但一直缺乏明确的分子机制研究，这是中医药治疗SAP肠道损伤发展与应用的困扰，然而RNA-Seq分析给这样挑战带来新的突破点。高通量RNA-Seq可以快速筛选出药物干预后RNAs变化，从整体水平上研究细胞和组织中基因转录变化情况，用于研究疾病基因表达量改变、基因功能富集情况及药物干预后差异表达基因的筛选，以明确药物的主要作用靶标和信号通路，有助于更快探究和阐释中药治疗疾病的具体的分子作用机制。

本研究通过建立SAP大鼠模型，从病理结果显示，SAP可导致肠黏膜损伤，肠上皮细胞结构会紊乱，组织间隙增宽稀疏，细胞水肿，肠黏膜上皮脱落，经QJHGD治疗后，上述问题得到明显改善，并且QJHGD能显著降低SAP大鼠血清淀粉酶、脂肪酶、DAO、内毒素含量，说明QJHGD可有效改善SAP大鼠肠黏膜损伤。本研究通过高通量RNA-Seq获得BG组、SAP组和QJHGD组的12个结肠组织的转录组数据，共检测到表达基因38 073个，SAP组与BG组差异基因有2 623个，其中上调1 378个，下调1 245个；QJHGD组与SAP组差异基因有1 342个，其中上调611个，下调731个。通过分析SAP显著差异基因而经QJHGD干预后逆转基因结果，并查阅相关文献发现关键基因，如*TNIP3*（Tumor Necrosis Factor Alpha -Induced Protein 3），是维持体内平衡NF-κB活化和抑制肠道环境中的炎症的关键mRNA，与NFκB的激活呈负相关，TNIP3缺乏会引起严重肠道炎症[18]，并且TNIP3能够维持肠道屏障功能并支持上皮细胞紧密连接[19]；LPO是一种过氧化物酶，可增强屏障抗菌防御，从而减轻肠道炎症[20]；Mmp13为基质金属蛋白酶，是

炎症过程中的重要介质,可通过激活TNF来调节炎症性疾病中的肠上皮屏障完整性[21];而Aicda缺乏显着增加了回肠中炎性细胞因子的基因表达[22];Rdh7是调节肠道淋巴细胞的视黄酸信号传导和IL-22产生所必需,可驱动肠道中的保护性或致病性免疫反应[23]。这些差异基因参与氧化还原、脂质代谢、应激反应、防御反应等生物学过程。由此可见,SAP致肠屏障损伤有多个显著差异基因,而QJHGD干预后可逆转这些基因,起到治疗SAP肠屏障损伤的作用。

本研究对这些差异基因进行了KEGG富集通路分析,根据分析结果并查阅相关文献,发现富集通路中与SAP肠屏障损伤密切相关的通路有:(1)紧密连接(tight junction,TJ):TJ在肠黏膜上皮细胞之间形成物理屏障,对调节肠黏膜屏障功能至关重要[24],SAP时,TJ屏障被破坏,使肠黏膜通透性增加,从而促进细菌移位,引发或促进全身炎症反应[25];(2)细胞凋亡:SAP中非胰腺器官的损伤主要是由细胞凋亡诱导的[26],SAP引起的炎性细胞因子的过量产生是胰腺和肠道屏障功能障碍的发病机制,进一步增加通透性并促进肠黏膜细胞凋亡[27,28];(3) Toll样受体信号通路:Toll样受体(Toll-like receptors, TLRs)与炎症反应密切相关系[29],在SAP的炎症反应、肠道炎症和肠屏障中起着关键作用[30,31];(4)NOD样受体信号通路:NOD样受体(Nod-like receptor, NLR)家族是机体最大的一类模式识别受体,可通过自身寡聚化组装形成大型信号分子机器,如NLRP3,NLRC4等形成的炎症小体,从而激活NF-κB、MAPK通路、细胞焦亡等,释放炎性细胞因子,介导下游一系列免疫炎症级联反应[32,33],密切参与大鼠SAP肠屏障损伤[34,35];(5)NF-kappa B信号通路:NF-κB作为负责调节参与免疫和炎症的多种基因的转录因子,是AP炎症进展的早期和核心事件[36],可诱导激酶调节肠道免疫和体内平衡[37]。同时,PI3K-Akt信号通路[38],肠道菌群代谢产物[39]、Th1和Th2细胞[40]等信号通路也得到研究。此外,B细胞受体、FoxO、JAK-STAT、PPAR等信号通路有待研究。由此可见,QJHGD可通过调节上述信号通路关键基因发挥抑制SAP肠屏障损伤的作用。

综上所述,本研究采用高通量RNA-Seq验证了QJHGD抑制SAP大鼠肠道损伤具体分子机制,发现QJHGD能够调控*TNIP3*、*LPO*、*Mmp13*、*Aicda*、*Rdh7*等基因表达,在TJ、细胞凋亡、Toll样受体、NOD样受体、NF-κB等信号通路发挥作用。本研究对QJHGD干预SAP肠屏障损伤的分子机制进行了深入探究,为后续研究提供了新思路和新方向。但本研究未通过Western-Blot、RT-qPCR等技术验证转录组学数据,这也是课题组接下来对发现的关键基因及信号通路进行更全面、更深层次的探究。

作者贡献度说明:

朱晓东、刘锟荣:构思并设计了该研究和实验;朱晓东、卢洁:负责数据收集处理及文章撰写;冯敏超、唐曦平、陈国忠、刘锟荣:负责实验思路及最终文章的修改。

所有作者声明不存在利益冲突关系。

参考文献

1 Boxhoorn L, Voermans RP, Bouwense SA, et al. Acute pancreatitis [J]. Lancet, 2020, 396 (10252): 726-734.

2 Wang Z, Liu J, Wang Y, et al. Identification of key biomarkers associated with immunogenic cell death and their regulatory mechanisms in severe acute pancreatitis based on WGCNA and machine learning [J]. Int J Mol Sci, 2023,24(3):3033.

3 Duan F, Wang X, Wang H, et al. GDF11 ameliorates severe acute pancreatitis through modulating macrophage M1 and M2 polarization by targeting the TGFbetaR1/SMAD-2 pathway [J]. Int Immunopharmacol, 2022, 108:108777.

4 Li W, Ou L, Fu Y, et al. Risk factors for concomitant infectious pancreatic necrosis in patients with severe acute pancreatitis: A systematic review and meta-analysis [J]. Clin Res Hepatol Gastroenterol, 2022, 46 (5): 101901.

5 Tu XH, Huang SX, Li WS, et al. Mesenchymal stem cells improve intestinal integrity during severe acute pancreatitis[J]. Mol Med Rep, 2014,10(4):1813-1820.

6 Zhang JW, Zhang GX, Chen HL, et al. Therapeutic effect of Qingyi decoction in severe acute pancreatitis-induced intestinal barrier injury [J]. World J Gastroenterol, 2015,21(12):3537-3546.

7 彭鸿,陈国忠,方丽娇,等.清解化攻法合并高强度综合治疗在重症急性胰腺炎早期的作用[J].辽宁中医杂志,2019,46(5):991-994.
Peng H, Chen GZ, Fang LJ, et al. Clearing and resolving and purging method combined high strength training in early stage for severe acute pancreatitis [J]. Liaoning J TraditChin Med, 2019,46(5):991-994.

8 彭鸿.清解化攻方治疗湿热毒瘀型中重症急性胰腺炎早期的临床观察[D].广西:广西中医药大学,2019.
Peng H. Clinical observation on early stage of moderate and severe acute pancreatitis of damp-heat toxin-stasis

type treated by Qingjie Huagong Prescription [D]. Guangxi: Guangxi University of Traditional Chinese Medicine, 2019.

9 杨昕，秦百君，唐曦平，等.清解化攻方调控HMGB1/TLR9/NF-κB信号通路对重症急性胰腺炎大鼠肠黏膜屏障保护作用的研究[J].时珍国医国药，2022，33(10)：2346-2349.
Yang X, Qin BJ, Tang XP, et al. Study on the protective effect of HMGB1/TLR9/NF-κB signaling pathway on the intestinal mucosal barrier of rats with severe acute pancreatitis by regulating HMGB1/TLR9/NF-κB signaling pathway with Qingxie Huaxing Formula [J]. Lishizhen Med Materia Med Res, 2022, 33(10): 2346-2349.

10 秦百君，唐曦平，杨昕，等.清解化攻方调节重症急性胰腺炎模型大鼠肠道菌群及对肠黏膜屏障的影响[J].中国药房，2022，33(15)：1825-1832.
Qin BJ, Tang XP, Yang X, et al. Study on the effects of Qingjie huagong decoction on the regulation of intestinal flora and intestinal mucosal barrier in severe acute pancreatitis model rats[J]. Chin Pharm, 2022, 33(15): 1825-1832.

11 Nassar SF, Raddassi K, Wu T. Single-cell multiomics analysis for drug discovery [J]. Metabolites, 2021, 11(11): 729.

12 张改君，苗静，郭丽颖，等.多组学联用在中药作用机制研究中的应用[J].中草药，2021，52(10)：3112-3120.
Zhang CJ, Miao J, Guo LY, et al. Application of multi-omics combination in mechanism studies of traditional Chinese medicine [J]. Chin Tradit Herbal Drugs, 2021, 52(10): 3112-3120.

13 凤振宁，金世柱.急性胰腺炎动物模型构建方法的研究[J].胃肠病学和肝病学杂志，2020，29(04)：388-391.
Feng ZN, Jin SZ. Study on the establishment of animal model of acute pancreatitis [J]. Chin J Gastroenterol Hepatol, 2020, 29(04): 388-391.

14 金涛，李兰，张潇颖，等.《急性胰腺炎中西医结合诊疗指南》解读[J/OL].中国普外基础与临床杂志，1-7[2023-12-07].
Jin T, Li L, Zhang XY, et al. Interpretation of integrated traditional Chinese and western medicine practice guidelines for diagnosis and treatment of acute pancreatitis[J/OL]. Chin J Bases Clin General Surg, 1-7[2023-12-07].

15 刘家军，熊明，杜磊超，等.通腑化瘀汤联合微生态制剂对重症急性胰腺炎患者肠道黏膜屏障及胃肠动力的影响[J].中国中西医结合消化杂志，2023，31(9)：696-700.
Liu JJ, Xiong M, Du LC, et al. Effect of Tongfu Huayu Decoction combined with microecological preparation on intestinal mucosal barrier and gastrointestinal motility in patients with severe acute[J]. Chin J Int Tradit Western Med Digest, 2023, 31(9): 696-700.

16 王惠雨，聂宁，周荣.重症急性胰腺炎肠黏膜屏障功能障碍的研究进展[J].临床急诊杂志，2022，23(10)：738-742.
Wang HY, Nie N, Zhou R. Research progress of intestinal mucosal barrier dysfunction in severe acute pancreatitis[J]. J Clin Emerg, 2022, 23(10): 738-742.

17 艾飞艳，王文豪，邓敏子，等.黄连提取物对重症急性胰腺炎大鼠肠道菌群和黏膜屏障功能的影响研究[J].中国临床药理学杂志，2022，38(18)：2192-2196.
Ai FY, Wang WH, Deng MZ, et al. Effect of coptidis extract on the intestinal microflora and mucosal barrier function of rats with severe acute pancreatitis [J]. The Chin J Clin Pharmacol, 2022, 38(18): 2192-2196.

18 Majumdar I, Ahuja V, Paul J. Altered expression of tumor necrosis factor alpha -induced protein 3 correlates with disease severity in ulcerative colitis [J]. Sci Rep, 2017, 7(1): 9420.

19 Kolodziej LE, Lodolce JP, Chang JE, et al. TNFAIP3 maintains intestinal barrier function and supports epithelial cell tight junctions [J]. PLoS One, 2011, 6(10): e26352.

20 Lin Y, Cai Q, Luo Y, et al. Epithelial chemerin-CMKLR1 signaling restricts microbiota-driven colonic neutrophilia and tumorigenesis by up-regulating lactoperoxidase [J]. Proc Natl Acad Sci U S A, 2022, 119(29): e2205574119.

21 Vandenbroucke RE, Dejonckheere E, Van Hauwermeiren F, et al. Matrix metalloproteinase 13 modulates intestinal epithelial barrier integrity in inflammatory diseases by activating TNF[J]. EMBO Mol Med, 2013, 5(7): 1000-1016.

22 Tsuruta T, Muhomah TA, Sonoyama K, et al. Aicda deficiency exacerbates high-fat diet-induced hyperinsulinemia but not gut dysbiosis in mice [J]. Nutr Res, 2021, 93: 15-26.

23 Grizotte-Lake M, Zhong G, Duncan K, et al. Commensals suppress intestinal epithelial cell retinoic acid synthesis to regulate interleukin-22 activity and prevent microbial dysbiosis[J]. Immunity, 2018, 49(6): 1103-1115.e6.

24 Buckley A, Turner JR. Cell biology of tight junction barrier regulation and mucosal disease[J]. Cold Spring Harb Perspect Biol, 2018, 10(1): a029314.

25 Wang J, Li C, Jiang Y, et al. Effect of ceramide-1-phosphate transfer protein on intestinal bacterial translocation in severe acute pancreatitis[J]. Clin Res Hepatol Gastro-

enterol, 2017,41(1):86-92.

26 Zhou Q, Xiang H, Liu H, et al. Emodin alleviates intestinal barrier dysfunction by inhibiting apoptosis and regulating the immune response in severe acute pancreatitis [J]. Pancreas, 2021,50(8):1202-1211.

27 Rychter JW, van Minnen LP, Verheem A, et al. Pretreatment but not treatment with probiotics abolishes mouse intestinal barrier dysfunction in acute pancreatitis [J]. Surgery, 2009,145(2):157-167.

28 Bai X, Song Z, Zhou Y, et al. The apoptosis of peripheral blood lymphocytes promoted by hyperbaric oxygen treatment contributes to attenuate the severity of early stage acute pancreatitis in rats[J]. Apoptosis, 2014,19(1):58-75.

29 Giakoustidis A, Mudan SS, Giakoustidis D. Dissecting the stress activating signaling pathways in acute pancreatitis[J]. Hepatogastroenterology, 2010,57(99-100):653-656.

30 Ni W, Ma YF, Chen T, et al. Toll-Like receptor 9 signaling pathway contributes to intestinal mucosal barrier injury in mice with severe acute pancreatitis[J]. Pancreas, 2022,51(9):1194-1200.

31 Su S, Liang T, Zhou X, et al. Qingyi decoction attenuates severe acute pancreatitis in rats via inhibition of inflammation and protection of the intestinal barrier[J]. J Int Med Res, 2019,47(5):2215-2227.

32 Nozaki K, Li L, Miao EA. Innate sensors trigger regulated cell death to combat intracellular infection[J]. Annu Rev Immunol, 2022,40:469-498.

33 Cao X. Self-regulation and cross-regulation of pattern-recognition receptor signalling in health and disease[J]. Nat Rev Immunol, 2016,16(1):35-50.

34 Xu S, Wei S, Guo Y, et al. Involvement of nucleotide-binding and oligomerization domain-like receptors in the intestinal injury of severe acute pancreatitis in rats[J]. Pancreas, 2018,47(2):245-251.

35 Yan Y, Lu B, Li P, et al. NOD receptor and TLR9 modulation in severe acute pancreatitis-induced intestinal injury[J]. Mol Med Rep, 2017,16(6):8471-8476.

36 Jakkampudi A, Jangala R, Reddy BR, et al. NF-kappaB in acute pancreatitis: Mechanisms and therapeutic potential [J]. Pancreatology, 2016,16(4):477-488.

37 Wang B, Shen J. NF-kappaB inducing kinase regulates intestinal immunity and homeostasis[J]. Front Immunol, 2022,13:895636.

38 Piao X, Liu B, Sui X, et al. Picroside II improves severe acute pancreatitis-induced intestinal barrier injury by inactivating oxidative and inflammatory TLR4-dependent PI3K/AKT/NF-kappaB signaling and improving gut microbiota[J]. Oxid Med Cell Longev, 2020,2020:3589497.

39 Wang Z, Li F, Liu J, et al. Intestinal microbiota - an unmissable bridge to severe acute pancreatitis-associated acute lung injury[J]. Front Immunol, 2022,13:913178.

40 Zhuang Q, Huang L, Zeng Y, et al. Dynamic monitoring of immunoinflammatory response identifies immunoswitching characteristics of severe acute pancreatitis in rats[J]. Front Immunol, 2022,13:876168.

[**本文编辑**] 邹 洲 宋睿璞 朱金月