WNT2b

肖姝喆¹，成燕玲¹，朱　云²，唐　芮¹，古建标¹，兰　林³，何志华¹，刘丹琼¹，耿岚岚¹，程　旸¹，龚四堂¹

¹广州医科大学附属广州市妇女儿童医疗中心，广东省儿童健康与疾病临床医学研究中心，消化科，广东　广州 510120；²器官衰竭防治国家重点实验室，广东省病毒性肝炎研究重点实验室，南方医科大学南方医院感染内科，广东　广州　510515；³南方医科大学第一临床医学院，广东　广州　510515

摘要：目的　探讨WNT2b高表达成纤维细胞破坏肠黏膜，促进炎症性肠病(IBD)进展的机制。方法　通过体外细胞共培养及条件培养，构建成纤维细胞对Caco-2细胞的作用模型。实验组为加入20%成纤维细胞条件培养基或与WNT2b高表达成纤维细胞共培养，对照组为不含条件培养基或与野生型成纤维细胞共培养，通过跨膜电阻和荧光黄渗透率测定，评估Caco-2细胞屏障通透性的变化。与WNT2b高表达或对照肠道成纤维细胞共培养后，使用免疫荧光法检测Caco-2细胞β-catenin入核情况，使用Western blot法检测ZO-1、E-Cadherin等紧密连接蛋白表达情况。采用DSS(葡聚糖硫酸钠)对C57小鼠进行类IBD肠炎造模，实验组使用Salinomycin(5 mg/kg，腹腔注射)，对照组为对应溶剂生理盐水，分别对小鼠进行处理，评价该抑制剂对肠炎的治疗作用。结果　与对照组相比，过表达WNT2b基因可致成纤维细胞分泌WNT2b蛋白显著增加，并促进Caco-2细胞β-catenin入核(P<0.01)，紧密连接蛋白表达下降；Caco-2细胞中敲低FZD4表达可逆转这一作用。Salinomycin可明显减轻对DSS小鼠肠道炎症并使肠黏膜紧密连接蛋白表达增多。结论　WNT2b高表达成纤维细胞破坏肠黏膜屏障功能，是治疗IBD的潜在靶点。

关键词：炎症性肠病；成纤维细胞；WNT2b；WNT/β-catenin信号通路

Fibroblasts overpressing WNT2b cause impairment of intestinal mucosal barrier

XIAO Shuzhe¹, CHENG Yanling¹, ZHU Yun², TANG Rui¹, GU Jianbiao¹, LAN Lin³, HE Zhihua¹, LIU Danqiong¹, GENG Lanlan¹, CHENG Yang¹, GONG Sitang¹

¹Department of Digestive Diseases, Guangzhou Women and Children's Medical Center,Guangdong Provincial Clinical Research Center for Child Health, Guangzhou Medical University,Guangzhou 510623,China; ²State Key Laboratory of Organ Failure Research, Guangdong Provincial Key Laboratory of Viral Hepatitis Research, Department of Infectious Diseases, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China; ³First School of Clinical Medicine, Southern Medical University, Guangzhou 510515, China

Abstract: Objective To investigate the mechanism by which fibroblasts with high WNT2b expression causes intestinal mucosa barrier disruption and promote the progression of inflammatory bowel disease (IBD). **Methods** Caco-2 cells were treated with 20% fibroblast conditioned medium or co-cultured with fibroblasts highly expressing WNT2b, with the cells without treatment with the conditioned medium and cells co-cultured with wild-type fibroblasts as the control groups. The changes in barrier permeability of Caco-2 cells were assessed by measuring transmembrane resistance and Lucifer Yellow permeability. In Caco-2 cells co-cultured with WNT2b-overexpressing or control intestinal fibroblasts, nuclear entry of β-catenin was detected with immunofluorescence assay, and the expressions of tight junction proteins ZO-1 and E-cadherin were detected with Western blotting. In a C57 mouse model of dextran sulfate sodium (DSS)-induced IBD-like enteritis, the therapeutic effect of intraperitoneal injection of salinomycin (5 mg/kg, an inhibitor of WNT/β-catenin signaling pathway) was evaluated by observing the changes in intestinal inflammation and detecting the expressions of tight junction proteins. **Results** In the co-culture system, WNT2b overexpression in the fibroblasts significantly promoted nuclear entry of β-catenin (P<0.01) and decreased the expressions of tight junction proteins in Caco-2 cells; knockdown of FZD4 expression in Caco-2 cells obviously reversed this effect. In DSS-treated mice, salinomycin treatment significantly reduced intestinal inflammation and increased the expressions of tight junction proteins in the intestinal mucosa. **Conclusion** Intestinal fibroblasts overexpressing WNT2b causes impairment of intestinal mucosal barrier function and can be a potential target for treatment of IBD.

Keywords: inflammatory bowel disease; fibroblasts; WNT2b; WNT/β-catenin signaling pathway

作者简介：肖姝喆，在读硕士研究生，E-mail: xiaoshuzhe8@163.com；成燕玲，在读硕士研究生，E-mail: cyldyx61@163.com。肖姝喆、成燕玲共同为第一作者
通信作者：龚四堂，主任医师，博士生导师，sitangg@126.com；程　旸，博士，副研究员，E-mail: chengyang2872@sina.com

炎症性肠病(IBD)是一种慢性非特异性肠道炎性疾病，其发病机制尚不完全清楚，现有的治疗药物也存在缺陷。对IBD发病机制和治疗手段的探讨，一直是消化系统疾病领域的研究热点[1,2]。

肠黏膜屏障主要由肠上皮细胞及细胞间的紧密连接构成，是维持肠道免疫微环境稳态的重要屏障[3]。目前研究发现，肠黏膜屏障损伤是IBD发病过程中极其重要的一环[4]。肠上皮屏障损伤主要包括肠上皮细胞坏死损伤及肠上皮细胞紧密连接蛋白缺失[5,6]。但是，肠屏

障损伤的诱因并不完全清楚,目前的研究主要围绕免疫细胞展开。

成纤维细胞是肠道的正常组成部分,过去认为主要发挥组织修复的作用。近年来研究发现成纤维细胞具有多种表型,包括炎性成纤维细胞、肌成纤维细胞和抗原提呈成纤维细胞等[7]。有证据显示,成纤维细胞可能在IBD进展过程中起重要作用:肌成纤维细胞与克罗恩病肠纤维化相关;炎性成纤维细胞受细菌抗原激活分泌IL-1β等炎症因子,趋化并激活肠道免疫细胞[8,9]。目前没有研究报道成纤维细胞可直接破坏肠道屏障。我们前期研究报道,IBD患者肠道组织中存在一群特殊类型成纤维细胞,该群成纤维细胞以高表达WNT2b为特征,其表型介于炎性成纤维细胞和肌成纤维细胞之间[10]。有关该群成纤维细胞在IBD中发挥的具体作用目前尚无相关报道。WNT2b是一种外分泌蛋白,属于WNT蛋白家族[11]。WNT2b主要结合卷曲蛋白4(FZD4)受体并激活WNT/β-catenin信号通路[12]。WNT/β-catenin信号通路激活可抑制肠上皮细胞紧密连接蛋白表达[13,14]。据此我们推测,WNT2b高表达的成纤维细胞可能通过分泌WNT2b破坏肠上皮屏障,促进肠道炎症。本研究拟探讨成纤维细胞对肠上皮屏障功能的破坏作用,并进一步探究其相关机制,以期为IBD的治疗提供新的靶点和理论依据。

1 材料和方法

1.1 主要材料与试剂

胎牛血清、DMEM培养液、青霉素链霉素双抗、胰蛋白酶均(Gibco),细胞培养瓶、Transwell小室均(Corning),跨膜电阻测量仪(Bio-Rad),抗人ZO-1(D6L1E)抗体、抗人E-Cadherin(24E10)抗体、抗人β-Catenin(D10A8)抗体(Cell Signaling Technology),稀释比例1∶1000;抗人GAPDH抗体(Proteintech),稀释比例1∶1000;抗人a-Tubulin抗体(锐抗生物科技有限公司),稀释比例1∶5000。

1.2 细胞培养

Caco-2细胞系购自中国科学院典型培养物保藏委员会上海细胞所,培养于DMEM细胞培养液,培养液添加10%胎牛血清和1%青霉素-链霉素;人肠道成纤维细胞株(HIF,上海中乔新舟生物科技有限公司),培养于Sciencell细胞培养液,培养液添加1%促成纤维细胞生长因子、1%双抗、2%血清。以上细胞均在37 ℃恒温、5% CO₂细胞培养箱中培养。

1.3 实验分组

细胞培养实验:选用Caco-2细胞和HIF为实验对象。实验组为加入20%成纤维细胞条件培养基或与WNT2b高表达HIF共培养,对照组为不含条件培养基

或与野生型HIF共培养,采用Western blot、ELISA、4D蛋白组学、IF、Conforcol、HE、细胞跨膜渗透及电阻测定等实验方法分别进行验证。动物实验:使用DSS对10只雄性c57小鼠进行类IBD肠炎造模,并随机分为对照组和实验组,每组5只,实验组使用Salinomycin(5 mg/kg,腹腔注射),对照组为对应溶剂生理盐水。所有动物实验均通过本院伦理委员会审批,(审批号:NFYY-2021-0623)。

1.4 实验方法

1.4.1 Western blot检测 裂解细胞提取蛋白样品后,进行SDS-PAGE胶电泳并转膜。5%脱脂奶粉和2% BSA室温封闭1 h,4 ℃一抗孵育过夜;二抗室温孵育1 h,显影检测。荧光成像系统拍照。

1.4.2 免疫荧光 细胞经过处理后4%多聚甲醛室温固定细胞30 min,0.5% TritonX-100-PBS缓冲液通透20 min,5%脱脂牛奶及2% BSA-PBS缓冲液封闭1 h后4 ℃一抗孵育过夜。荧光二抗避光室温孵育60 min后,滴加DAPI孵育5min染核,封片后在激光共聚焦显微镜下拍照。

1.4.3 4D蛋白质组学 Shotgun法检测成纤维细胞培养基中蛋白。使用蛋白酶将成纤维细胞培养基消化为肽段混合物,在高效液相色谱分离后串联导入高分辨质谱仪中进行分析,得到各肽段的质荷比(m/z),同时对肽段离子进行进一步轰击,获得二级质谱信号。采用检索软件以及相应的蛋白质组数据库对质谱数据进行分析,还原样本中蛋白质组信息。

1.4.4 ELISA 收集细胞培养上清液,按照ELISA试剂盒说明书中的步骤检测上清液蛋白浓度。

1.4.5 上清蛋白浓缩提取 收集成纤维细胞培养上清液,放入已预冷的MilliQ水湿润超滤管中,置离心机中以4 ℃、4000×g,离心40 min。取出的浓缩液50 μL,加入20 μL 5×Loading Buffer, 100 ℃加热10 min,对细胞培养液中蛋白进行浓缩提取。

1.4.6 细胞跨膜电阻测定 Caco-2细胞接种于Transwell小室上层,在温度为37 ℃、5% CO₂的培养箱中培养,细胞每隔2 d换液并使用细胞电阻仪测量跨膜电阻值,待电阻值稳定于某一数值不再改变,表示细胞紧密连接模型构建成功[15]。模型构建成功后开始进行刺激并继续记录跨膜电阻值变化。

1.4.7 细胞跨膜渗透 细胞形成紧密连接后刺激48 h,PBS冲洗3次后,在transwell上室中加入300 μL浓度为10 μg/mL的荧光黄溶液,下室加入800 μL PBS,孵育30 min后取下室溶液200 μL,采用酶标仪检测荧光黄浓度。激发波长500 nm,发射波长500 nm。

1.5 统计方法

采用Graphpad Prism 8.0及EXCEL软件对实验结果进行统计分析及绘图。各项实验均独立重复3次,计

量资料以均数±标准差表示,两个独立样本均数间的比较采用 t 检验;单因数方差分析比较多组间均数差异,P<0.05认为差异具有统计学意义。

2　结果

2.1　成纤维细胞条件培养基抑制肠上皮细胞屏障功能

第14天,实验组与对照组细胞屏障功能无显著差异,而换用不同培养基培养后,实验组 TEER 值迅速下降,而对照组则缓慢下降,在第16天和第18天组间出现显著性差异(P<0.001,图1A),。在第18天进行荧光黄渗透实验,发现实验组细胞荧光黄渗透程度显著高于对照组(P<0.01,图1B)。我们对两组 Caco-2 细胞进行 Western blot 检测,发现 ZO-1 和 E-Cadherin 的表达在成纤维细胞条件培养基组均显著下降(P<0.01,图1C)。
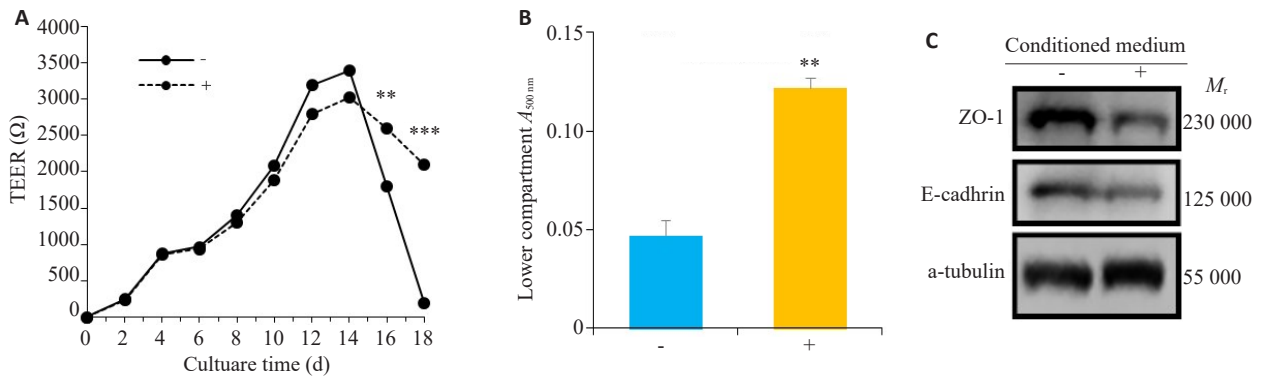

图1　成纤维细胞条件培养对肠上皮细胞屏障功能的影响

Fig.1　Effect of conditioned culture medium of fibroblasts on intestinal epithelial cell barrier function. **A**: Detection of intestinal epithelial cell barrier function by measuring cell transmembrane resistance. **B**: Cell transmembrane permeability assay. **P<0.01,***P<0.001 vs control. **C**: Western blotting of ZO-1 and E-cadherin proteins. +: Cells were treatment with 20% fibroblast conditioned medium. -: Cells without treatment.

2.2　成纤维细胞培养液蛋白鉴定

4D蛋白组学分析发现,成纤维细胞培养液中蛋白组含有 WNT2b 蛋白(表1)。

2.3　过表达 WNT2b 显著增强成纤维细胞对 Caco-2 紧密连接蛋白的抑制作用

Western blot 结果显示,与对照组相比,过表达组 WNT2b-GFP 融合蛋白表达明显,说明过表达成功(图2A)。过表达组培养液中 WNT2b 浓度明显上升(图2B,P<0.001)。检测 Caco-2 细胞紧密连接蛋白表达显示,与对照组相比,WNT2b 过表达 HIF 共培养组 Caco-2 细胞 ZO-1 和 E-Cadherin 表达水平明显更低(图2C)。

2.4　WNT2b 通过激活 WNT/β-catenin 信号通路抑制紧密连接蛋白形成

与对照组相比,β-catenin 入核信号在过表达组 (OV)显著升高(图3A、B,P<0.01)。使用 WNT/β-catenin 信号通路抑制剂 Salinomycin 进行处理,可部分抑制 WNT2b 蛋白对 Caco-2 细胞跨膜电阻和细胞屏障的破坏作用(图3C、D,P<0.05)。由于 FZD4 是主要表达于肠上皮细胞表面的卷曲蛋白家族受体,我们使用 siRNA 敲低 Caco-2 细胞的 FZD4,发现可部分恢复 WNT2b 对

Caco-2 细胞紧密连接蛋白 ZO-1 和 E-Cadherin 抑制作用(图3E)。

2.5　抑制 WNT/β-catenin 信号通路可减轻 DSS 诱导的肠道炎症,同时减轻肠上皮屏障的破坏

Salinomycin 组类 IBD 肠炎小鼠造模期间肠道炎症评分明显更低,第6天和第7天与对照组相比有显著性差异(P<0.001,图4A)。HE 染色炎症浸润,且 Salinomycin 组肠道炎症组织病理学评分显著更低 (P<0.05,图4B、C)。Salinomycin 组 β-catenin 入核信号明显下调,差异具有统计学意义(P<0.05,图4D、E)。Salinomycin 组 ZO-1 和 E-Cadherin 表达水平显著更高,差异具有统计学意义(P<0.05,图4F、G、H)。

3　讨论

肠上皮屏障是维持肠道免疫微环境稳态的核心[16]。肠屏障缺失是 IBD 重要的病理特征[17,18]。目前研究认为,异常的免疫细胞是肠屏障的主要破坏者[19,20]。本文通过成纤维细胞条件培养基和共培养实验,发现成纤维细胞可直接抑制肠上皮细胞紧密连接形成,破坏肠屏障。既往研究报道,成纤维细胞分泌多种炎症因子参与 IBD 进展过程[21]。而本文通过进一步研究发现成纤维细胞主要通过分泌 WNT2b 蛋白而非炎症因子激活

表1　成纤维细胞培养液蛋白鉴定
Tab.1　Identification of proteins in fibroblast culture medium

| Accession	Gene name	Description	Coverage	Peptides	PSMs	Unique peptides
P30086	PEBP1	Phosphatidylethanolamine-binding protein 1 OS=Homo sapiens OX=9606 GN=PEBP1 PE=1 SV=3	21.92513369	3	3	3
P02768	ALB	Albumin OS=Homo sapiens OX=9606 GN=ALB PE=1 SV=2	21.83908046	14	182	14
P05452	CLEC3B	Tetranectin OS=Homo sapiens OX=9606 GN=CLEC3B PE=1 SV=3	21.78217822	3	4	3
Q99969	RARRES2	Retinoic acid receptor responder protein 2 OS=Homo sapiens OX=9606 GN=RARRES2 PE=1 SV=1	21.47239264	3	4	3
P11021	HSPA5	Endoplasmic reticulum chaperone BiP OS=Homo sapiens OX=9606 GN=HSPA5 PE=1 SV=2	21.40672783	12	14	10
Q93097	WNT2B	Protein Wnt-2b OS=Homo sapiens OX=9606 GN=WNT2B PE=1 SV=2	21.2560993	3	3	3
P10599	TXN	Thioredoxin OS=Homo sapiens OX=9606 GN=TXN PE=1 SV=3	20.95238095	2	2	2
P07996	THBS1	Thrombospondin-1 OS=Homo sapiens OX=9606 GN=THBS1 PE=1 SV=2	20.76923077	23	27	22
P05109	S100A8	Protein S100-A8 OS=Homo sapiens OX=9606 GN=S100A8 PE=1 SV=1	20.43010753	2	2	2
O43707	ACTN4	Alpha-actinin-4 OS=Homo sapiens OX=9606 GN=ACTN4 PE=1 SV=2	20.30735456	15	15	6
P62258	YWHAE	14-3-3 protein epsilon OS=Homo sapiens OX=9606 GN=YWHAE PE=1 SV=1	20	5	5	3
Q9Y6C2	EMILIN1	EMILIN-1 OS=Homo sapiens OX=9606 GN=EMILIN1 PE=1 SV=3	19.88188976	16	18	16
P07195	LDHB	L-lactate dehydrogenase B chain OS=Homo sapiens OX=9606 GN=LDHB PE=1 SV=2	19.76047904	6	8	4
P05067	APP	Amyloid-beta precursor protein OS=Homo sapiens OX=9606 GN=APP PE=1 SV=3	19.74025974	12	13	11
P00749	PLAU	Urokinase-type plasminogen activator OS=Homo sapiens OX=9606 GN=PLAU PE=1 SV=2	19.72157773	9	14	9
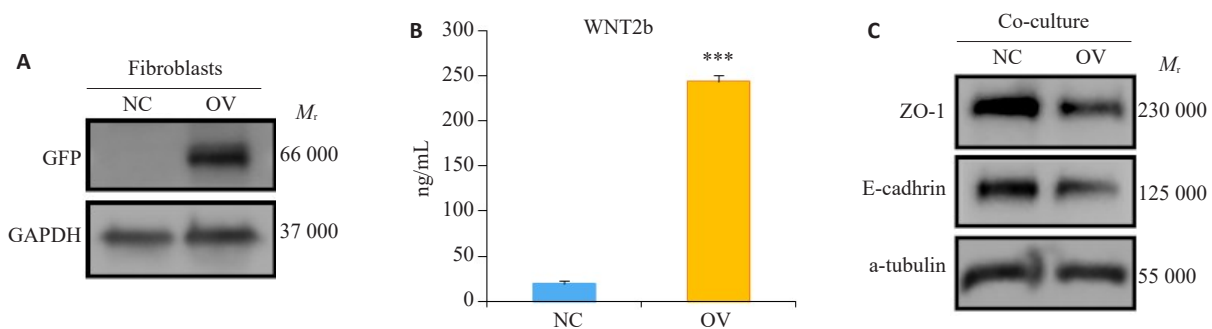
图2　过表达WNT2b显著增强成纤维细胞对Caco2紧密连接蛋白的抑制作用

Fig.2　Effect of WNT2b overexpression in fibroblasts on expressions of tight junction proteins in Caco2 cells in the co-culture system. **A**: Western blotting of WNT2b-GFP fusion protein. **B**: ELISA for detecting the expression level of WNT2b-GFP fusion protein (***$P<0.001$ *vs* NC). **C**: Western blotting for detecting the effect of WNT2b overexpression in fibroblasts on ZO-1 and E-cadherin proteins. NC: Negative control; OV: Overexpression group.

肠上皮细胞WNT/β-catenin信号通路,抑制紧密连接蛋白表达。

　　成纤维细胞主要分化为肌成纤维细胞和炎性成纤维细胞:前者通过分泌细胞外基质促进损伤修复,过度活化可导致肠道狭窄和息肉形成;后者分泌IL-1β等多种炎症因子激活免疫细胞,参与肠道炎症反应[22,23]。我们前期研究发现成纤维细胞存在其他亚群[10],例如本文研究的WNT2b高表达成纤维细胞,该群细胞在肠炎中明显富集,但功能不明。本研究通过肠道成纤维细胞HIF中作过表达WNT2b的方式模拟WNT2b高表达成
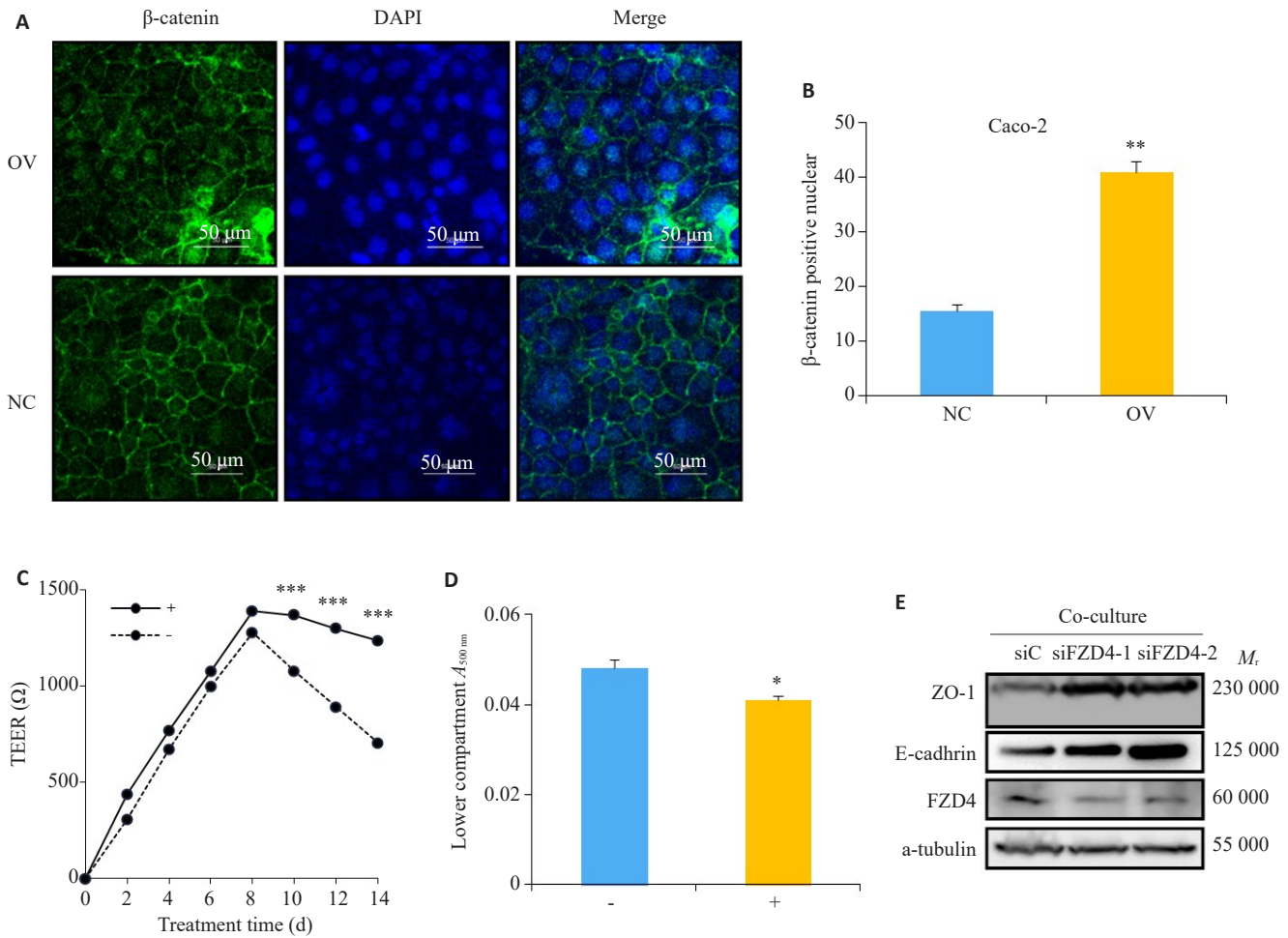
图3 WNT2b通过FZD4激活WNT/β-catenin信号通路抑制紧密连接蛋白形成

Fig.3 WNT2b inhibits tight junction protein formation by FZD4-mediated activation of the WNT/β-catenin signaling pathway. **A**, **B**: β-catenin nuclear entry signaling is significantly increased in the overexpression group (**P<0.01 *vs* NC). **C**, **D**: Effect of salinomycin, an inhibitor of WNT/β-catenin signaling pathway, on cell transmembrane resistance and cell barrier function (*P< 0.05, ***P<0.001 *vs* control). **E**: Western blotting for examining the effect of FZD4 knockdown on ZO-1 and E-Cadherin proteins in Caco2 cells. +: Cells were treatment with 20% fibroblast conditioned medium. -: Cells without treatment.

纤维细胞。我们研究发现肠道成纤维细胞本身可分泌WNT2b蛋白,而高表达WNT2b基因后WNT2b分泌随之增加,说明WNT2b很可能是该群细胞产生作用的关键介质。

    WNT/β-catenin信号通路是在物种进化过程中高度保守的信号通路,成纤维细胞分泌的WNT2b蛋白激活了WNT/β-catenin信号通路,该通路主要通过WNT2b蛋白与细胞膜表面的卷曲蛋白家族受体(FZD4)结合,抑制胞质中的β-catenin降解来诱发上皮细胞-间充质化(EMT)转换,胞内大量增加的β-catenin会转移进入细胞核,作为转录因子亚单位诱导大量基因的表达,这些靶基因的表达产物中有很多都是能诱导EMT转换过程的转录因子,而EMT主要特征有紧密连接蛋白ZO-1及E-Cadherin的表达减少[24, 25]。该通路抑制紧密连接蛋白形成,是破坏IBD肠道屏障的关键信号通路之一[26, 27]。本研究发现WNT/β-catenin通路抑制剂可部分抑制小鼠

DSS肠炎,并上调肠黏膜屏障蛋白表达。我们进一步确认,外源性WNT2b蛋白可激活肠上皮细胞的WNT/β-catenin信号通路,促进β-catenin入核,抑制ZO-1、E-Cadherin蛋白表达。FZD4是肠上皮细胞表达的主要卷曲蛋白受体[28],本文进一步确认,成纤维细胞分泌的WNT2b蛋白作用于FZD4受体产生作用。

    综上所述,本研究阐述了WNT2b高表达肠道成纤维细胞的作用:抑制肠上皮屏障。其机制是通过分泌WNT2b蛋白作用于肠上皮细胞FZD4受体,激活WNT/β-catenin通路,抑制ZO-1、E-Cadherin表达。本研究提示WNT2b高表达肠道成纤维细胞可能在IBD中起重要作用,是治疗IBD的潜在靶点。

参考文献:

[1] Kaplan GG, Windsor JW. The four epidemiological stages in the global evolution of inflammatory bowel disease [J]. Nat Rev Gastroenterol Hepatol, 2021, 18(1): 56-66.
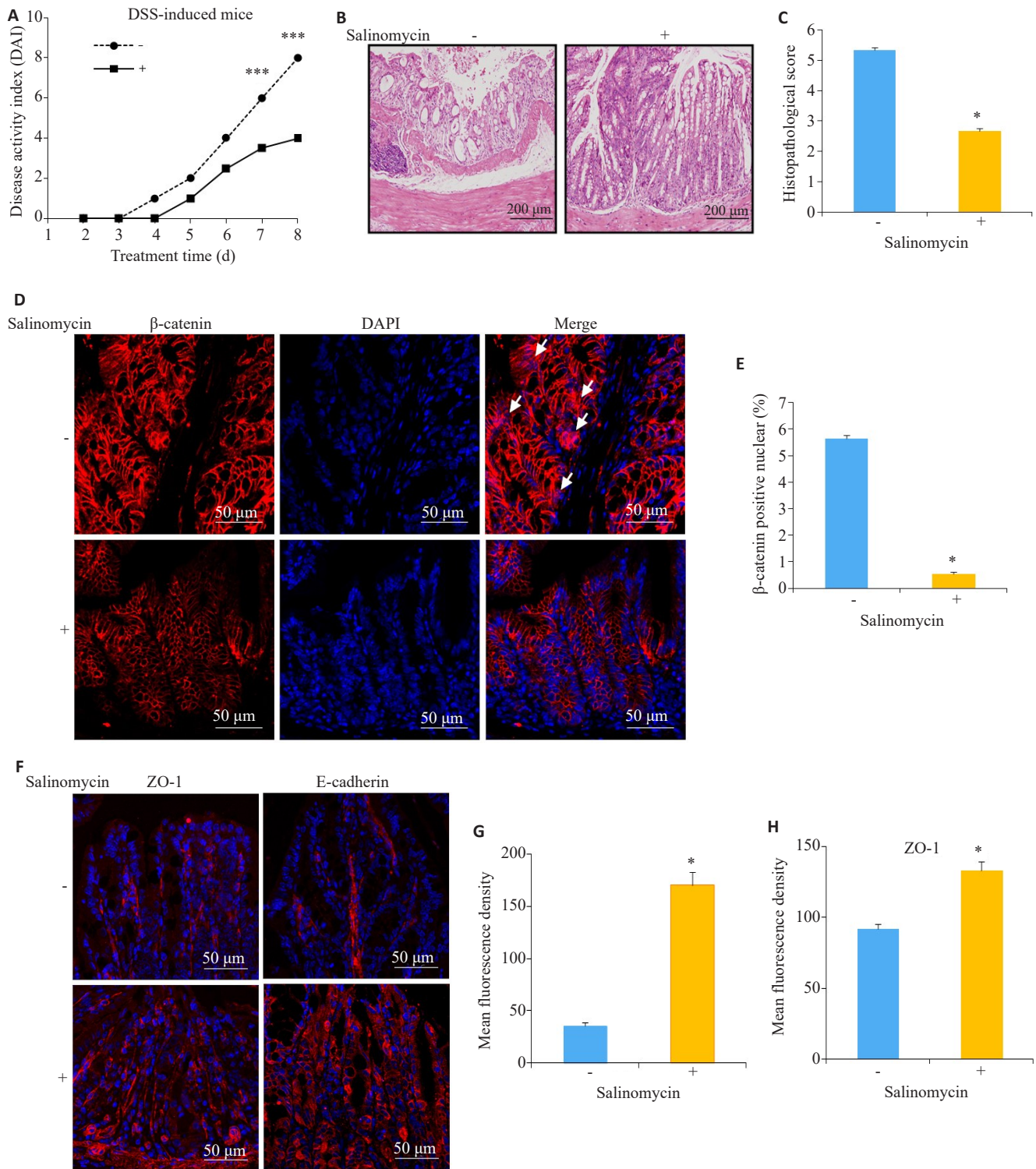
图4 抑制WNT/β-catenin信号通路对DSS诱导的小鼠肠道炎症及肠上皮屏障功能的影响

Fig.4 Effects of inhibiting WNT/β-catenin signaling pathway on intestinal inflammation and intestinal epithelial barrier function in DSS-treated mice. **A**: Intestinal inflammation score in mice with DSS-induced IBD-like enteritis. **B**: HE staining of the intestinal tissue of the mice. **C**: Histopathological score of mice with DSS-induced IBD-like enteritis. **D**, **E**: β-catenin nuclear entry signaling is significantly increased in control group. **F-H**: Immunofluorescence for detecting ZO-1 and E-cadherin proteins expression in the intestinal mucosa of the mice (*$P<0.05$).

[2] Liu S, Zhao WJ, Lan P, et al. The microbiome in inflammatory bowel diseases: from pathogenesis to therapy[J]. Protein Cell, 2021, 12(5): 331-45.

[3] Sommer K, Wiendl M, Müller TM, et al. Intestinal mucosal wound healing and barrier integrity in IBD-crosstalk and trafficking of cellular players[J]. Front Med (Lausanne), 2021, 8: 643973.

[4] Bhat AA, Uppada S, Achkar IW, et al. Tight junction proteins and signaling pathways in cancer and inflammation: a functional

crosstalk[J]. Front Physiol, 2019, 9: 1942.

［5］ Martini E, Krug SM, Siegmund B, et al. The epithelial barrier and its relationship with mucosal immunity in inflammatory bowel disease [J]. Cell Mol Gastroentero Hepatol, 2017, 4(1): 33-46.

［6］ Fabre T, Molina MF, Soucy G, et al. Type 3 cytokines IL-17A and IL-22 drive TGF-β-dependent liver fibrosis[J]. Sci Immunol, 2018, 3 (28): eaar7754.

［7］ Tallquist MD. Cardiac fibroblast diversity[J]. Annu Rev Physiol, 2020, 82: 63-78.

［8］ Neurath MF. Targeting immune cell circuits and trafficking in inflammatory bowel disease[J]. Nat Immunol, 2019, 20(8): 970-9.

［9］ Bettenworth D, Bokemeyer A, Baker M, et al. Assessment of Crohn's disease-associated small bowel strictures and fibrosis on cross-sectional imaging: a systematic review[J]. Gut, 2019, 68(6): 1115-26.

［10］ Huang B, Chen ZH, Geng LL, et al. Mucosal profiling of pediatric-onset colitis and IBD reveals common pathogenics and therapeutic pathways[J]. Cell, 2019, 179(5): 1160-76.e24.

［11］ Wang SZ, Yang TN, He ZX. Investigations on the role of the microRNA-338-5p/wnt family member 2B (WNT2B) axis in regulating the pathogenesis of nasopharyngeal carcinoma (NPC)[J]. Front Oncol, 2021, 11: 684462.

［12］ Schaefer KN, Peifer M. Wnt/beta-catenin signaling regulation and a role for biomolecular condensates[J]. Dev Cell, 2019, 48(4): 429-44.

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

［14］ Lynn KS, Peterson RJ, Koval M. Ruffles and spikes: control of tight junction morphology and permeability by claudins[J]. Biochim Biophys Acta Biomembr, 2020, 1862(9): 183339.

［15］ Felix K, Tobias S, Jan H, et al. Measurements of transepithelial electrical resistance (TEER) are affected by junctional length in immature epithelial monolayers[J]. Histochem Cell Biol, 2021, 156 (6): 609-16.

［16］ 杨靖源, 蒙俊, 杨堃. 肠紧密连接蛋白与肠道屏障功能[J]. 医学综述, 2022, 28(2): 235-9.

［17］ Barbara G, Barbaro MR, Fuschi D, et al. Inflammatory and microbiota-related regulation of the intestinal epithelial barrier[J]. Front Nutr, 2021, 8: 718356.

［18］ di Tommaso N, Gasbarrini A, Ponziani FR. Intestinal barrier in human health and disease[J]. Int J Environ Res Public Heal, 2021, 18 (23): 12836.

［19］ Zhang WJ, Chen SJ, Zhou SC, et al. Inflammasomes and fibrosis[J]. Front Immunol, 2021, 12: 643149.

［20］ Kaminsky LW, Al-Sadi R, Ma TY. IL-1β and the intestinal epithelial tight junction barrier[J]. Front Immunol, 2021, 12: 767456.

［21］ Cho N, Razipour SE, McCain ML. Featured Article: TGF-β1 dominates extracellular matrix rigidity for inducing differentiation of human cardiac fibroblasts to myofibroblasts[J]. Exp Biol Med (Maywood), 2018, 243(7): 601-12.

［22］ Liu SS, Liu C, Lv XX, et al. The chemokine CCL1 triggers an AMFR-SPRY1 pathway that promotes differentiation of lung fibroblasts into myofibroblasts and drives pulmonary fibrosis[J]. Immunity, 2021, 54(9): 2042-56.e8.

［23］ Tai YF, Woods EL, Dally J, et al. Myofibroblasts: function, formation, and scope of molecular therapies for skin fibrosis[J]. Biomolecules, 2021, 11(8): 1095.

［24］ Quandt J, Arnovitz S, Haghi L, et al. Wnt-β-catenin activation epigenetically reprograms Treg cells in inflammatory bowel disease and dysplastic progression[J]. Nat Immunol, 2021, 22(4): 471-84.

［25］ Pai SG, Carneiro BA, Mota JM, et al. Wnt/beta-catenin pathway: modulating anticancer immune response[J]. J Hematol Oncol, 2017, 10(1): 101.

［26］ Nusse R, Clevers H. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities[J]. Cell, 2017, 169(6): 985-99.

［27］ Liu JQ, Xiao Q, Xiao JN, et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities[J]. Signal Transduct Target Ther, 2022, 7: 3.

［28］ Skronska-Wasek W, Mutze K, Baarsma HA, et al. Reduced frizzled receptor 4 expression prevents WNT/β-catenin-driven alveolar lung repair in chronic obstructive pulmonary disease[J]. Am J Respir Crit Care Med, 2017, 196(2): 172-85.

（编辑：吴锦雅）