

鞣花酸改善博来霉素诱导肺损伤的机制

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摘要:【目的】探讨鞣花酸(EA)对博来霉素(BLM)诱导的小鼠肺纤维化及相关肺功能障碍的保护作用,并阐明其与白细胞介素(IL)-17/核因子(NF)- κ B/基质金属蛋白酶(MMP)9信号轴的调控关系。【方法】以雄性C57BL/6J小鼠为实验动物,采用气管内注射BLM进行肺纤维化动物造模。分别使用IL-17中和抗体、Ixekizumab、SB-3CT、PDTC或EA干预,以调控IL-17/NF- κ B/MMP9信号轴。通过HE、Masson及天狼星红染色观察肺组织病理变化;采用肺功能测试(PFT)评估肺功能;Western blot和qRT-PCR检测相关蛋白及基因表达。结合网络药理学预测EA作用靶点,通过STRING数据库和Cytoscape构建蛋白互作网络,并采用分子对接验证EA与核心靶点的结合能力。【结果】博来霉素可诱导小鼠出现明显肺纤维化及肺功能障碍,显著升高促炎因子IL-17水平,并通过激活转录因子NF- κ B p65上调促纤维化因子MMP9的表达。抑制IL-17/NF- κ B/MMP9信号轴可明显减轻肺纤维化程度。EA干预可显著抑制博来霉素诱导的IL-17升高,阻断NF- κ B/MMP9通路激活,进而减轻肺纤维化病变并改善肺功能。【结论】EA可通过抑制IL-17/NF- κ B/MMP9信号通路介导的炎症与纤维化反应,有效改善博来霉素诱导的小鼠肺纤维化及肺功能障碍。

关键词:肺纤维化;鞣花酸;博来霉素;IL-17;信号通路

中图分类号:R364.5 文献标志码:A 文章编号:1672-3554(XXXX)XX-0001-14

DOI: 10.11714/jssysu.med.YX20260027

Mechanism of Ellagic Acid in Ameliorating Bleomycin-Induced Lung Injury

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Abstract:【Objective】To investigate the protective effect of ellagic acid (EA) against bleomycin (BLM)-induced pulmonary fibrosis and associated pulmonary function impairment in mice, and to elucidate its relationship with the regulation of the interleukin (IL)-17/ nuclear factor (NF)- κ B/ matrix metalloproteinase (MMP)9 signaling axis.【Methods】A pulmonary fibrosis model was established in male C57BL/6J mice via intratracheal BLM injection.

收稿日期:2026-02-12

录用日期:2026-03-23

基金项目:西藏自治区科技计划项目(XZ202301ZY);西藏民族大学校内项目(26MDY27)

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Interventions included IL-17 neutralizing antibody, Ixekizumab, SB-3CT, PDTC, or EA were used to modulate the IL-17/NF- κ B/MMP9 signaling axis. Pathological changes in lung tissue were observed via HE, Masson, and Sirius red staining. Pulmonary function was assessed using a pulmonary function test (PFT). Western blot and qRT-PCR were employed to detect related protein and gene expression. Network pharmacology was utilized to predict the potential targets of EA. A protein-protein interaction (PPI) network was constructed using the STRING database and Cytoscape. Molecular docking was performed to validate the binding capability of EA to core targets.【Results】BLM successfully induced obvious pulmonary fibrosis and lung dysfunction in mice, significantly elevating the level of the pro-inflammatory cytokine IL-17. This was associated with the activation of the transcription factor NF- κ B p65, leading to the upregulation of the pro-fibrotic factor MMP9. Inhibition of the IL-17/NF- κ B/MMP9 signaling axis markedly alleviated the degree of pulmonary fibrosis. EA intervention significantly suppressed the BLM-induced increase in IL-17, blocked the activation of the NF- κ B/MMP9 pathway, and consequently reduced lung fibrotic lesions and improved pulmonary function.【Conclusion】EA may effectively ameliorate BLM-induced pulmonary fibrosis and lung dysfunction in mice, likely by inhibiting the inflammatory and fibrotic responses mediated through the IL-17/NF- κ B/MMP9 signaling pathway.

Key words: pulmonary fibrosis; ellagic acid; bleomycin; IL-17; signaling pathway

[J SUN Yat-sen Univ (Med Sci)]

肺纤维化(pulmonary fibrosis, PF)是一种致死性、进行性发展的纤维化疾病,临床上表现为肺功能进行性下降,主要症状包括呼吸困难和干咳^[1]。该病的病理特征为肺部持续性炎症和结缔组织的过度沉积^[2]。研究显示,活性氧(reactive oxygen species, ROS)可以显著促进关键炎症细胞因子(如转化生长因子 β (transforming growth factor β , TGF- β)和肿瘤坏死因子 α (tumor necrosis factor α , TNF- α)的表达^[3]。这些细胞因子在驱动成纤维细胞活化、增殖以及胶原蛋白的过度合成与沉积中起着核心作用,最终导致不可逆的纤维化组织重塑^[4-5]。然而,单纯针对清除 ROS 或中和 TNF- α 的治疗策略在阻止或逆转纤维化进程方面效果有限,提示调控肺纤维化进展的完整分子机制仍有待进一步阐明。新近研究表明,炎症失调是影响组织损伤结局的关键调控因素,决定着正常修复与病理性纤维化之间的微妙平衡^[6-7]。白细胞介素-17(interleukin-17, IL-17)作为一种强效促炎细胞因子,与多种自身炎症性疾病密切相关,并广泛参与哮喘、肺炎等炎症性病理过程。此外,IL-17 信号通路与多种器官系统的纤维化发病机制密切相关^[8]。研究进一步表明,IL-17 或其特异性受体 IL-17R 的上调可促进炎症细胞的募集^[9],增强促纤维化生长因子的产生,并加速肝纤维化的进展^[10-11]。值得注意的是,近期研究经由 JAK/STAT3 通路上调的 IL-17 也参

与了肺纤维化的发生^[12]。然而,IL-17 在博来霉素(bleomycin, BLM)诱导的肺纤维化中的具体下游信号机制,目前仍未完全阐明。核因子 κ B(nuclear factor, NF- κ B)作为一种可诱导的转录因子,在炎症和免疫相关基因的表达调控中处于核心地位^[13-14]。研究显示,IL-17 可以通过激活 NF- κ B 和 CCAAT/增强子结合蛋白 β (CCAAT/enhancer-binding protein β , C/EBP β)等多种转录因子发挥生物学功能,从而诱导一系列下游基因的表达。这些 IL-17 下游基因产物,包括多种细胞因子(如 IL-16、GM-CSF、TNF- α)、趋化因子(如 CXCL1、CXCL2、CCL20),以及急性期蛋白和补体成分等炎症介质,参与不同器官的纤维化进程^[7,15]。此外,IL-17 还能上调基质金属蛋白酶(matrix metalloproteinase, MMP)的表达,如 MMP1、MMP3 和 MMP9 等,这些蛋白酶已被证实可通过降解细胞外基质促进组织损伤^[16-17]。然而,IL-17 是否通过 NF- κ B 介导的 MMP9 表达上调而特异性加剧肺纤维化,目前尚不完全明确。鞣花酸(ellagic acid, EA)是一种水解自鞣花单宁的天然多酚,常见于石榴、葡萄、草莓、诃子等果蔬坚果中^[18]。研究表明,EA 在缓解胰腺、心脏等器官纤维化方面显示出治疗潜力^[19-21]。此外,EA 具有显著的抗炎活性,能够调控关键细胞过程。其神经保护作用即与抗炎、抗氧化机制相关,而这些通路同样参与纤维化发

病^[22]。临床研究也表明,EA可通过降低促炎细胞因子、提高抗炎介质水平,改善多发性硬化症患者的身体状况^[23]。然而,EA是否通过调控IL-17/NF- κ B/MMP9通路来特异性缓解肺纤维化及相关功能障碍,目前尚不清楚。

1 材料与方法

1.1 动物

SPF级雄性C57BL/6J小鼠(7-8周龄,体重20-30 g)购自北京维通利华实验动物技术有限公司,生产许可证号为SCXK(京)2021-0011。动物饲养于温度恒定(22±1 °C)、12 h明暗循环的环境中,自由摄食饮水。本研究的动物实验均经东南大学实验动物伦理委员会批准(批准号:SEU-IACUC-2025401004)。实验采用随机分组设计,并遵循“减少、替代、优化”原则,以最大限度减轻动物痛苦并控制使用数量。

1.2 肺纤维化模型的建立

采用气管内注射博来霉素(Bleomycin, MedChem Express, 美国)的方法构建肺纤维化模型,操作参考先前文献并稍作调整^[24]。简述如下:将博来霉素溶于生理盐水,配制成1 g/L母液,分装后于-20 °C保存备用。小鼠在异氟烷麻醉下,于第0天经气管注射0.5 mg/kg博来霉素,第4天再次注射1 mg/kg。

1.3 药物干预

IL-17中和抗体(信诺生物,中国)和IL-17受体抑制剂Ixekizumab(TargetMol,中国)分别配制为1 g/L与0.5 g/L,每只小鼠气管内给予15 μ L。MMP9抑制剂SB-3CT(TargetMol,中国)和PDTC(江苏凯杰生物技术,中国)通过腹腔注射给药。鞣花酸(EA, Felix, 中国上海)以2 g/L的剂量经灌胃给予^[25]。

1.4 组织学染色

取肺组织经4%多聚甲醛灌注固定,继续于40 g/L多聚甲醛中室温固定过夜,随后石蜡包埋。使用旋转切片器(Leica, 德国)切取4 μ m厚度切片,脱蜡后分别进行马松三色染色(Masson's trichrome staining, MT)、天狼星红染色(Picro-Sirius red staining, PSR)和苏木精-伊红染色(Hematoxylin

and eosin staining, HE),操作按试剂盒(Servicebio, 中国武汉)说明书进行。染色切片使用正置显微镜(Nikon Ci-L, 日本)拍摄,采用ImageJ软件对染色阳性区域进行定量分析,评估纤维化程度。

1.5 蛋白免疫印迹(Western blot)

小鼠经戊巴比妥钠深度麻醉后,迅速取出肺组织。组织置于冰上,加入含蛋白酶与磷酸酶抑制剂的RIPA裂解液进行匀浆,于4 °C、12 000 \times g离心10 min,收集上清。采用BCA法测定蛋白浓度。蛋白样品经10% SDS-PAGE电泳分离,转至PVDF膜(Servicebio, 中国武汉),室温封闭1 h后,于4 °C与以下一抗孵育过夜:NF- κ B p65(1:1 000, Abcam, 英国)、p-NF- κ B p65(Ser311)(1:1 000, CST, 美国)、 α -平滑肌肌动蛋白(α -SMA, 1:1 000, CST, 美国)、I型胶原(Collagen-I, 1:1 000, Millipore, 美国)及MMP9(1:500, Abcam, 英国)。随后孵育相应二抗,使用ECL化学发光试剂盒(Servicebio, 中国武汉)显影,并通过ImageJ软件对条带进行灰度值定量分析。

1.6 实时荧光定量PCR(qRT-PCR)

采用RNA提取试剂盒(Servicebio, 武汉)提取小鼠肺组织总RNA。使用PrimeScript® RT试剂盒(Servicebio, 武汉)将RNA反转录为cDNA。PCR反应使用SYBR Green qPCR SuperMix(Invitrogen, 美国)在ABI Prism 7500系统上进行。反应程序如下:95 °C预变性3 min;95 °C 10 s、58 °C 20 s、72 °C 10 s,共40个循环。以 β -actin作为内参基因,采用 $2^{-\Delta\Delta C_t}$ 法计算目标基因mRNA的相对表达量。所用引物序列见表1。

1.7 肺功能检测

采用动物肺功能测试系统(PFT, 上海塔旺智能科技)进行检测。小鼠置于系统内适应后,在未束缚状态下连续记录30 min,测定以下参数:吸气量(inspiratory capacity, IC)、用力肺活量(forced vital capacity, FVC)、吸气峰流速(peak inspiratory flow, PIF)、呼气峰流速(peak expiratory flow, PEF)、100 ms用力呼气容积(forced expiratory volume in 100 ms, FEV₁₀₀)、200 ms用力呼气容积(forced expiratory volume in 200 ms, FEV₂₀₀)及肺动态顺应性(dynamic lung compliance, C_{dyn})。

表1 扩增基因引物序列
Table 1 Amplified gene primers

Gene name	Gene primers
<i>IL17</i>	F: 5'-TGACCCCTAAGAAACCCCA-3' R: 5'-TCATTGTGGAGGGCAGACAA-3'
<i>TNF</i>	F: 5'-CGGGCAGGTCTACTTTGGAG-3' R: 5'-ACCCTGAGCCATAATCCCCT-3'
<i>IL6</i>	F: 5'-AGACAAAGCCAGAGTCTTCAG-3' R: 5'-TGTGACTCCAGCTTATCTCTTGG-3'
<i>ESR1</i>	F: 5'-CCAGGCTTTGGGACTTGAAT-3' R: 5'-GCAAGTTAGGAGCAAACAGGAG-3'
<i>RELA</i>	F: 5'-GATCGCCACCGATTGAAGA-3' R: 5'-TCGGGTAGGCACAGCAATAC-3'
<i>MMP9</i>	F: 5'-GCTAAACCACCTCTCCCGAG-3' R: 5'-GAGTGGATAGCTCGGTGGTG-3'

1.8 网络药理学分析

通过中药系统药理学数据库(TCMSP ;<https://www.91tcmsp.com>)检索藏药诃子的生物活性成分及其靶点,筛选条件为口服生物利用度(oral bioavailability, OB) $\geq 30\%$,且类药性(drug-likeness, DL) ≥ 0.18 。将成分靶点经 UniProt 数据库(<https://www.uniprot.org/>)统一为官方基因符号。从 DisGeNET (<https://disgenet.com>)、GeneCards (<https://www.genecards.org>)、OMIM (<https://omim.org>)及 TTD (<https://omim.org>)数据库获取肺纤维化相关基因,并使用 R 语言绘制维恩图,筛选诃子成分与肺纤维化共有靶点。

利用 STRING 数据库(版本 12.0; <https://cn.string-db.org/>)构建蛋白互作(PPI)网络(置信度阈值 0.4),导入 Cytoscape(版本 3.8.0)进行可视化与拓扑分析,并采用 CytoNCA、CytoHubba 及 MCODE 插件识别核心靶点。最后通过分子对接预测诃子关键成分与核心靶点之间的结合亲和力与作用模式,评估其对肺纤维化相关蛋白的潜在调控作用。

1.9 统计分析

数据以均数 \pm 标准误表示。采用 SPSS 29.0 和 GraphPad Prism 8.0.1 进行统计分析。符合正态分布的两组间比较采用 *t* 检验,多组比较采用单因素方差分析(one-way ANOVA),染色与免疫印迹数

据采用双因素方差分析(two-way ANOVA)并结合 Tukey 法进行多重比较。非正态数据采用非参数检验(Mann-Whitney U 检验或 Wilcoxon 配对符号秩检验)。以 $P < 0.05$ 为差异具有统计学意义。

2 结果

2.1 BLM 诱导小鼠肺纤维化及肺功能障碍

通过 MT 染色与 SPR 染色进行组织病理学评估,结果显示 BLM 处理组小鼠肺泡结构破坏明显,胶原沉积显著增加(图 1A、B)。与这些形态学改变相符的是,Western blot 检测发现 BLM 处理 14 天后,肺组织中纤维化标志物 α -SMA 及 Collagen-I 的蛋白表达均明显上调(图 1C、D)。

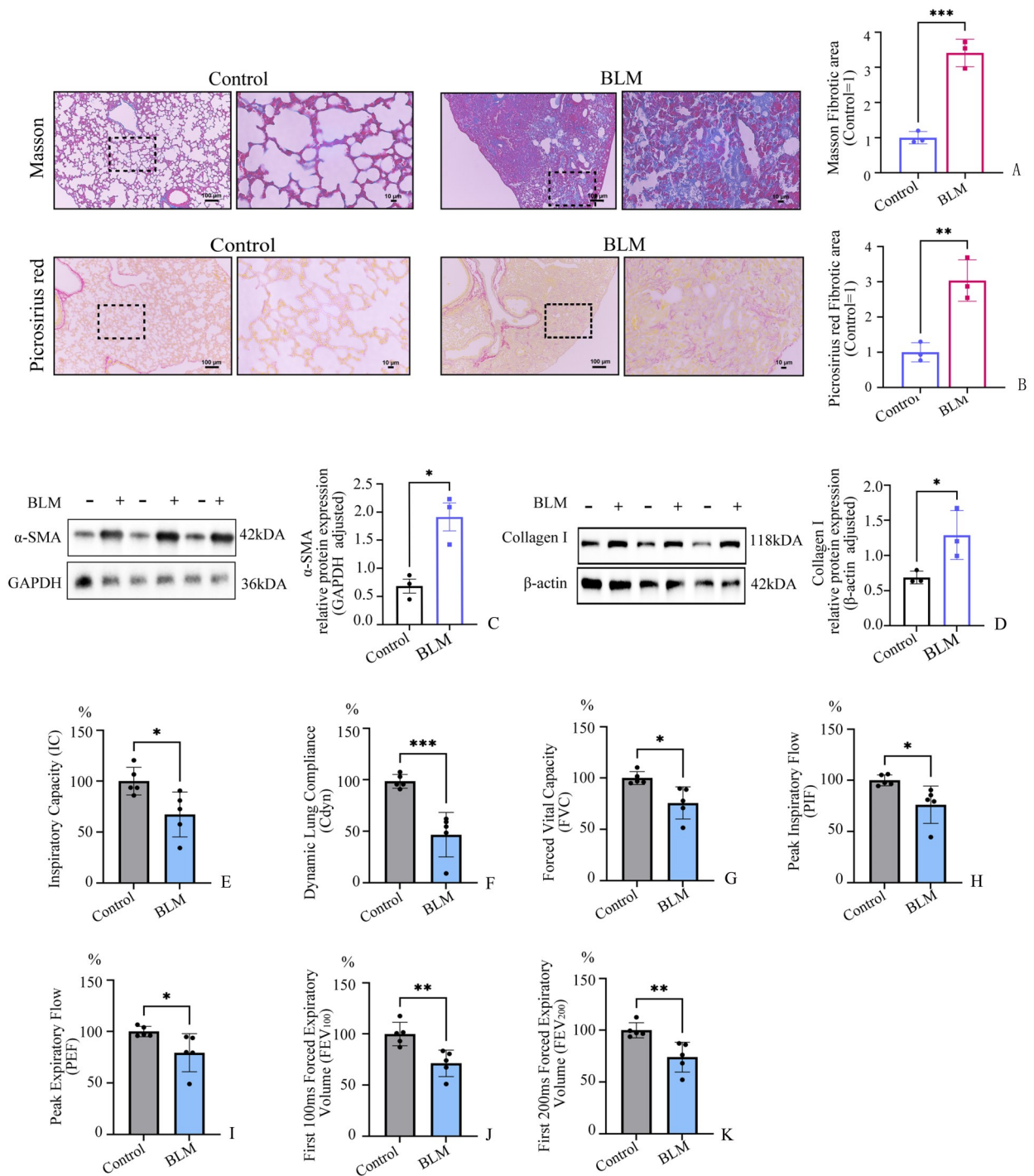
肺功能检测显示,BLM 组小鼠在第 14 天时多项通气参数显著降低,包括 IC、FVC、PIF、PEF、FEV₁₀₀、FEV₂₀₀ 及 Cdyn(图 1E - K)。值得注意的是,采用双因素 ANOVA 分析两组小鼠体重,显示在同一时间点无显著差异(图 S1)。以上结果提示,BLM 成功诱导了肺纤维化模型,并导致明显的肺功能障碍。以上结果提示,BLM 成功诱导了肺纤维化模型,并导致明显的肺功能障碍。

2.2 IL-17 信号通路介导 BLM 诱导的肺纤维化

BLM 处理第 14 天,肺组织出现明显炎症细胞浸润,包括胞质丰富的巨噬细胞及多叶核中性粒细胞,并聚集于肺泡腔与肺间质(图 2A)。进一步,我们检测了多个与器官纤维化相关的细胞因子。qPCR 结果显示,与对照组相比,BLM 组肺组织中 *IL17* mRNA 水平显著升高,*IL6* mRNA 虽有上调趋势但无统计学差异,*TNF* 表达未见明显变化(图 2B - D)。为明确 IL-17 在纤维化中的作用,进一步采用 IL-17 中和抗体及 IL-17 受体抑制剂 Ixekizumab 进行干预。结果显示,两者均能显著抑制 BLM 诱导的 Collagen-I 沉积(图 2E、F)。以上结果表明,IL-17 信号通路在 BLM 诱导的肺纤维化过程中发挥关键作用。

2.3 基于网络药理学的诃子活性成分筛选及其抗肺纤维化靶点预测

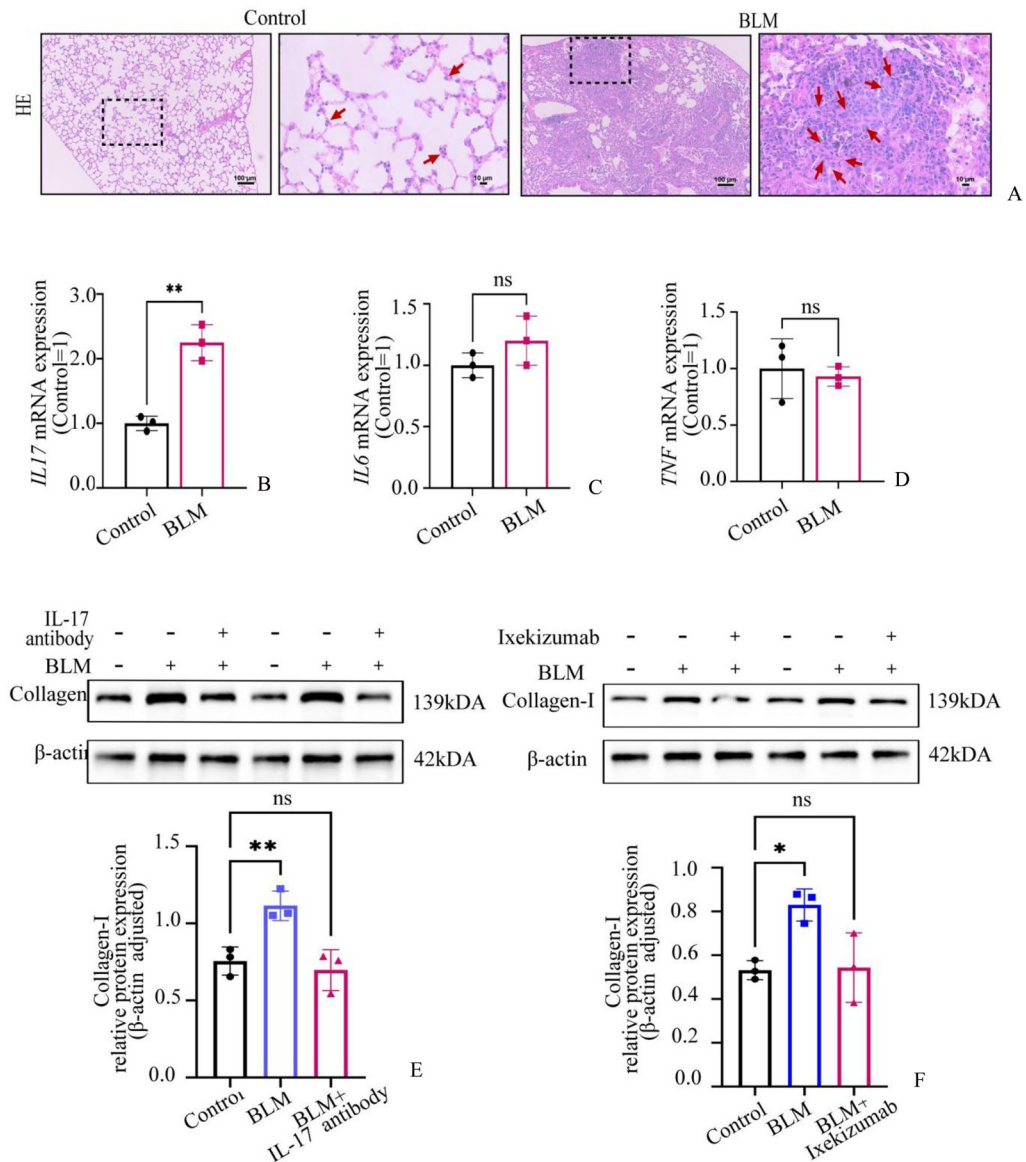
通过中药系统药理学数据库(TCMSP),从诃子中筛选出包括鞣花酸(EA)在内的 7 种生物活性成



A-B: Representative images of lung tissue sections stained with Masson's trichrome and Sirius red 14 days after BLM treatment (A: $t=9.739$, $***P=0.0006$ vs. Control; B: $t=5.446$, $P=0.0055$ vs. Control; $n=3$ per group). C-D: Protein expression levels of fibrosis markers α -SMA and Collagen I detected by Western blot (C: $t=4.403$, $*P=0.0117$ vs. Control; D: $t=2.899$, $*P=0.0442$ vs. Control; $n=3$ per group). E-K: Pulmonary function parameters measured using a whole-body PFT system (including E: $t=2.831$, $*P=0.0221$ vs. Control; F: $t=5.123$, $***P=0.0009$ vs. Control; G: $t=3.209$, $*P=0.0124$ vs. Control; H: $t=2.799$, $*P=0.0232$ vs. Control; I: $t=2.405$, $*P=0.0428$ vs. Control; J: $t=3.709$, $**P=0.0060$ vs. Control; K: $t=3.584$, $**P=0.0071$ vs. Control; $n=5$ per group). Data are presented as the mean \pm SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

图 1 BLM 诱导的小鼠肺纤维化与肺功能损伤

Fig. 1 BLM-induced pulmonary fibrosis and lung function impairment in mice



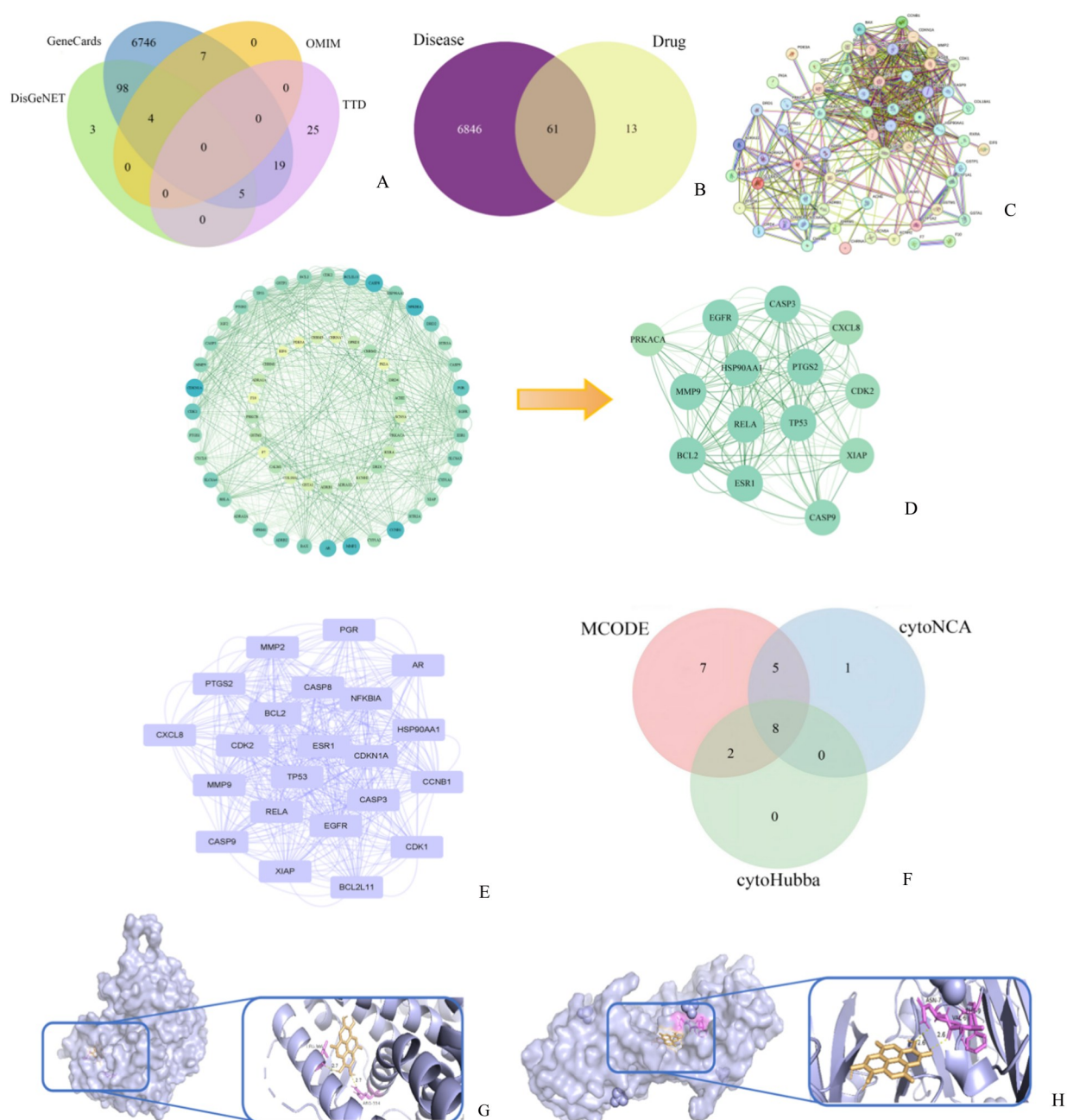
A: HE staining of lung tissues 14 days after BLM treatment ($n = 3$ per group). B-D: mRNA expression levels of *IL17*, *IL6* and *TNF* in lung tissue measured by qPCR on day 14 after BLM treatment (B: $t=7.185$, $P=0.0020$ vs. Control; C: $t=1.549$, $P=0.1963$ vs. Control; D: $t=0.4361$, $P=0.6853$ vs. Control; $n = 3$ per group). E: IL-17 neutralization attenuated BLM-induced Collagen I expression ($F=13.17$, $P=0.0064$, $**P=0.0117$ vs. Control, $n = 3$ per group). F: Ixekizumab, an IL-17 receptor inhibitor, reduced BLM-induced Collagen I upregulation ($F=7.895$, $P=0.0209$, $*P=0.0224$ vs. Control, $n = 3$ per group). Data are presented as the mean \pm SEM. $*P < 0.05$, $**P < 0.01$.

图2 IL-17信号通路介导博来霉素诱导的小鼠肺纤维化

Fig. 2 IL-17 signaling mediates to bleomycin-induced pulmonary fibrosis in mice

分,这7种成份对应124个潜在靶点基因(表S1)。进一步经标准化整理后得到74个有效靶点基因(表S2)。同时,自DisGeNET、GeneCards、OMIM及TTD数据库共收集6,907个肺纤维化相关基因(图3A)。将其与成分靶点取交集,获得61个共同基因(图3B),提示其可能是诃子干预肺纤维化的潜在作用靶点。进一步利用STRING

数据库构建上述61个靶点的蛋白互作(PPI)网络(图3C),并借助CytoNCA、MCODE与CytoHubba综合识别出8个核心靶点(图3D-F,表S3)。分子对接分析显示,EA与ESR1、MMP9、RELA等多个核心靶点具有较强结合亲和力(图3G、H,表2),提示EA可能通过多靶点协同作用发挥抗肺纤维化效应。



A: Potential targets associated with pulmonary fibrosis retrieved from DisGeNET, GeneCards, OMIM, and TTD databases. B: Venn diagram showing overlapping targets between bioactive compounds of *Terminalia chebula* and pulmonary fibrosis. C: Protein-protein interaction (PPI) network of the 61 common targets constructed using STRING. D-E: Identification of core targets based on topological analysis using CytoNCA and MCODE. F: Eight core genes screened from the intersection targets. G: Predicted binding conformation of EA with ESR1. H: Predicted binding conformation of EA with MMP9.

图3 基于网络药理学的诃子干预肺纤维化作用分析

Fig. 3 Network pharmacology-based analysis of *Terminalia chebula* (Hezi) against pulmonary fibrosis

2.4 MMP9上调促进 BLM 诱导的肺纤维化

为探究 ESR1、MMP9 和 RELA 在肺纤维化中的潜在作用,我们通过 qPCR 定量检测了它们的表达水平。结果显示,BLM 处理第 14 天,肺组织中

MMP9 的 mRNA 水平显著升高,而 ESR1 与 RELA 的表达虽有上调趋势但未达到统计学意义(图 4A-C)。Western blot 进一步证实,BLM 组 MMP9 蛋白表达明显增加(图 4D)。为明确 MMP9 在纤维化中

表 2 诃子活性成分与靶基因分子对接后的结合能
Table 2 Molecular docking revealed the *Terminalia chebula* binding energy with target genes

NO.	Component	Target	Binding energy(kal/mol) PyMOL
1	ellipticine	TP53	-8.8
2	ellagic acid	ESR1	-8.7
3	ellagic acid	MMP9	-8.7
4	ellagic acid	RELA	-8.3

的作用,使用 MMP9 抑制剂 SB-3CT($25 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$,腹腔注射,连续 14 d)进行干预。MT 染色和 PSR 染色结果显示,抑制 MMP9 能显著减轻 BLM 诱导的肺纤维化(图 4E、F)。相比之下,单独使用 SB-3CT 未引起肺组织纤维化改变(图 4E、F)。这些结果表明,MMP9 的上调在 BLM 诱导的肺纤维化进程中发挥关键作用。

2.5 NF- κ B 激活介导 BLM 诱导的 MMP9 上调

NF- κ B 是一种重要的转录因子,在调控基因表达中起重要作用。为探究 NF- κ B 激活是否促进 BLM 诱导的 MMP9 上调,我们首先检测了 BLM 给药后 NF- κ B p65 的磷酸化水平。Western blot 结果经双因素 ANOVA 分析后显示,BLM 处理第 14 天,肺组织中 p-NF- κ B p65(Ser311)水平显著升高,而总 NF- κ B p65 蛋白表达无显著变化(图 5A)。进一步,我们使用 NF- κ B 抑制剂 PDTC 进行干预,经单因素方差分析结果显示,PDTC 处理显著抑制了 BLM 诱导的 MMP9 在 mRNA 和蛋白水平的表达上调(图 5B、C)。以上结果表明,NF- κ B 通路的激活在 BLM 诱导的肺纤维化中促进了 MMP9 的表达。

2.6 EA 抑制 IL-17/NF- κ B/MMP9 通路介导的肺纤维化

为探讨 EA 是否通过调控 IL-17/NF- κ B/MMP9 通路改善肺功能,首先观察了 IL-17 中和抗体对该通路的影响。结果经双因素方差分析显示,气管内给予 IL-17 中和抗体可显著抑制 BLM 诱导的 NF- κ B p65 磷酸化(图 6A)。进一步研究发现,EA 干预能明显降低 BLM 引起的 *IL17* mRNA 升高,并抑制 p-NF- κ B p65 及 MMP9 的表达上调(图 6B - D)。同时,Western blot 表明 EA 治疗可显著下调纤维化

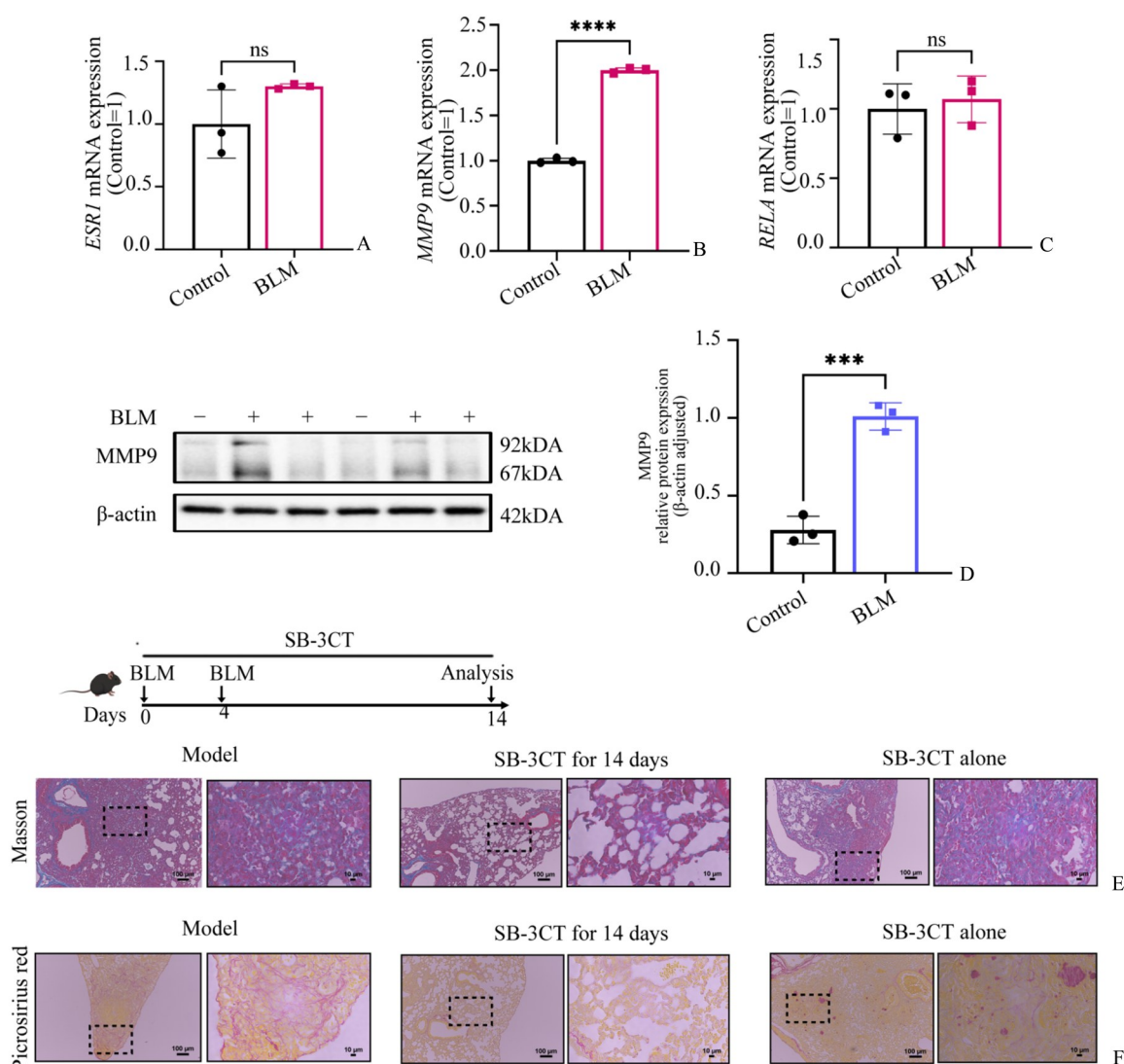
标志物 α -SMA 和 Collagen-I 的蛋白水平(图 6E、F)。结果提示,EA 可通过抑制 IL-17/NF- κ B/MMP9 信号通路减轻肺纤维化。

2.7 EA 改善 BLM 诱导肺纤维化的肺功能与病理改变

与上述分子变化一致,EA 处理显著改善了肺功能,表现为 IC、Cdyn、FVC、PEF 和 FEV₁₀₀ 等指标经单因素方差分析结果显示均较 BLM 组显著回升,PIF 和 FEV₂₀₀ 两指标无显著性但有回升趋势(图 7A-G)。HE 染色结果显示,EA 处理后肺泡腔与肺间质中的巨噬细胞及中性粒细胞数量明显减少(图 7H)。以上结果提示,EA 可通过抑制 IL-17/NF- κ B/MMP9 信号通路减轻肺纤维化,进而改善 BLM 诱导的肺功能障碍与病理改变。

3 讨论

肺纤维化是一种慢性进行性肺部疾病,常导致患者出现呼吸衰竭^[26]。本研究通过气管内注射 BLM 成功构建了小鼠肺纤维化模型,并证实其伴随显著的肺功能损伤。组织病理学分析显示,BLM 处理后肺泡腔和肺间质中有明显的巨噬细胞与中性粒细胞浸润。研究显示,IL-17 在自身免疫性疾病、过敏及感染过程中发挥关键炎症介质作用^[27]。本研究中 BLM 处理第 14 天肺组织内 IL-17 表达显著升高,且使用 IL-17 中和抗体或受体拮抗剂 Ixekizumab 抑制 IL-17 信号后,纤维化进程明显延缓,进一步支持 IL-17 在该过程中的促纤维化作用,与既往报道一致^[28]。除了巨噬细胞与中性粒细胞浸润以外,本研究中,我们虽然并没有检测结构细胞(内皮细胞和肺泡上皮细胞)和免疫细胞是否合成并释放 IL-17,但既往的同行研究表明,多种免疫细胞及结构细胞均可产生 IL-17^[29]。由此,我们不排除这些结构细胞可能是 IL-17 的来源,进而促进免疫细胞聚集,加剧持续性炎症反应。此外,在肺纤维化进程中,成纤维细胞与肌成纤维细胞通过过量产生胶原蛋白等细胞外基质(ECM)成分发挥核心作用。MMPs 与其抑制剂 TIMPs 之间的平衡失调将破坏 ECM 稳态,导致异常沉积及不可逆的瘢痕形成^[30-31]。与此一致的是,本研究也观察到



A-C: The mRNA levels of *ESRI*, *MMP9* and *RELA* in lung tissue were examined by using qPCR on day 14 after BLM administration (A: $t=1.906$, $P=0.1293$ vs. Control; B: $t=46.29$, $P<0.0001$ vs. Control; C: $t=0.4893$, $P=0.6502$ vs. Control; $n=3$ per group). D: MMP9 protein expression in lung tissue detected by Western blot on day 14. ($t=10.18$, $P=0.0005$ vs. Control; $n=3$ per group). E-F: Masson's trichrome and Sirius red staining showing that MMP9 inhibitor SB-3CT attenuates BLM-induced pulmonary fibrosis. SB-3CT alone caused no fibrotic changes ($n=3$ per group). Data was presented as the means \pm SEM. *** $P<0.001$.

图4 MMP9上调促进BLM诱导的肺纤维化

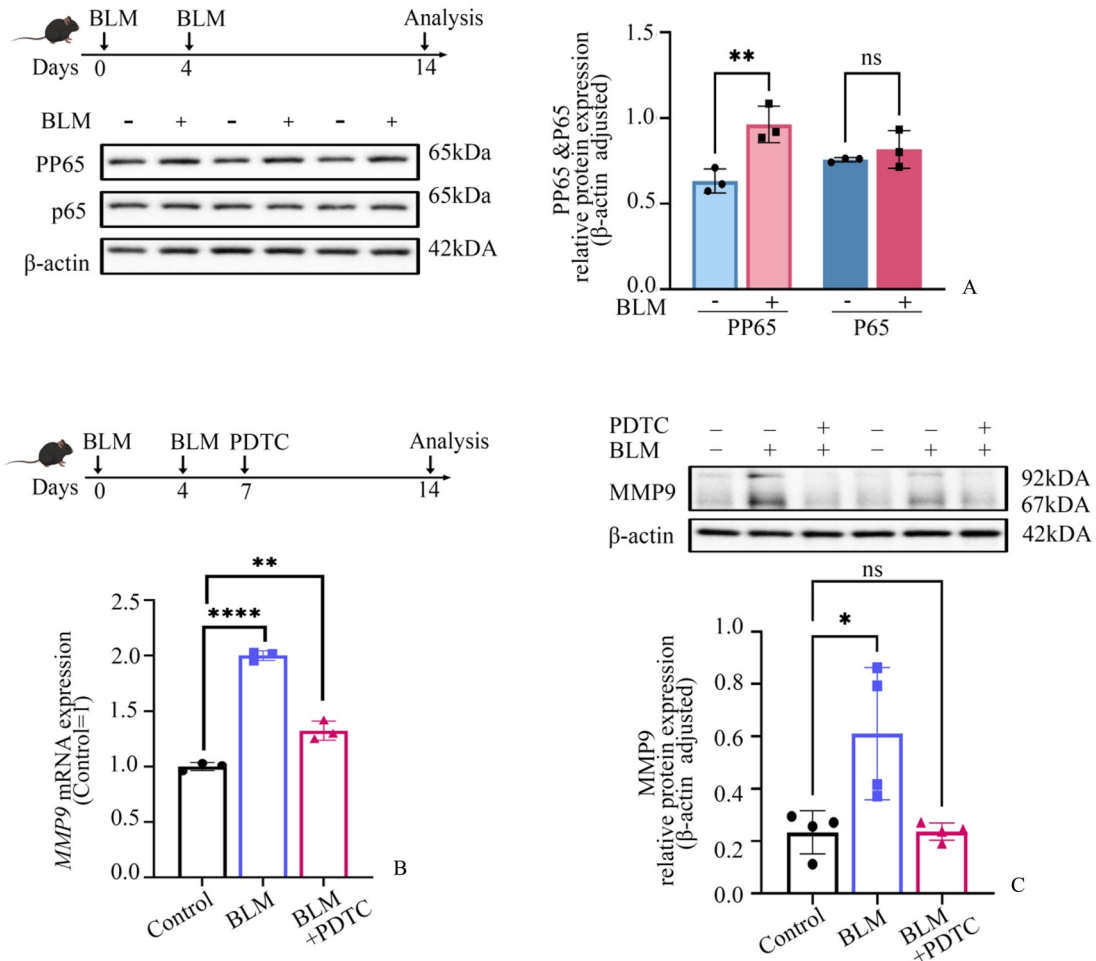
Fig. 4 MMP9 upregulation contributes to BLM-induced pulmonary fibrosis

BLM处理组肺组织中 α -SMA与Collagen-I表达升高,同时MMP9水平显著增加。

转录因子NF- κ B是炎症及纤维化相关基因的关键调控因子,可调节包括MMPs和胶原蛋白在内的多种促纤维化介质,促进不同器官的纤维化^[32-34]。我们的研究显示,BLM能够激活NF- κ B p65通路;而采用PDTC抑制NF- κ B后,MMP9的上调受到抑制,纤维化程度减轻,说明该通路在肺纤维化进展中具有重要作用。同样,使用MMP9

抑制剂SB-3CT也能缓解BLM诱导的纤维化表型。既往研究提示,IL-17可与成纤维细胞表面的IL-17受体结合并激活NF- κ B信号,从而促进结肠纤维化的发生^[35]。本研究在肺纤维化中揭示了类似的机制,即IL-17通过驱动NF- κ B介导的MMP9表达,促进ECM积累,加速纤维化进程。

最后,通过网络药理学分析,我们从传统药物诃子(已知具有免疫调节与抗感染特性^[36-37])中筛选出生物活性成分EA,并证实其可作为肺纤维化



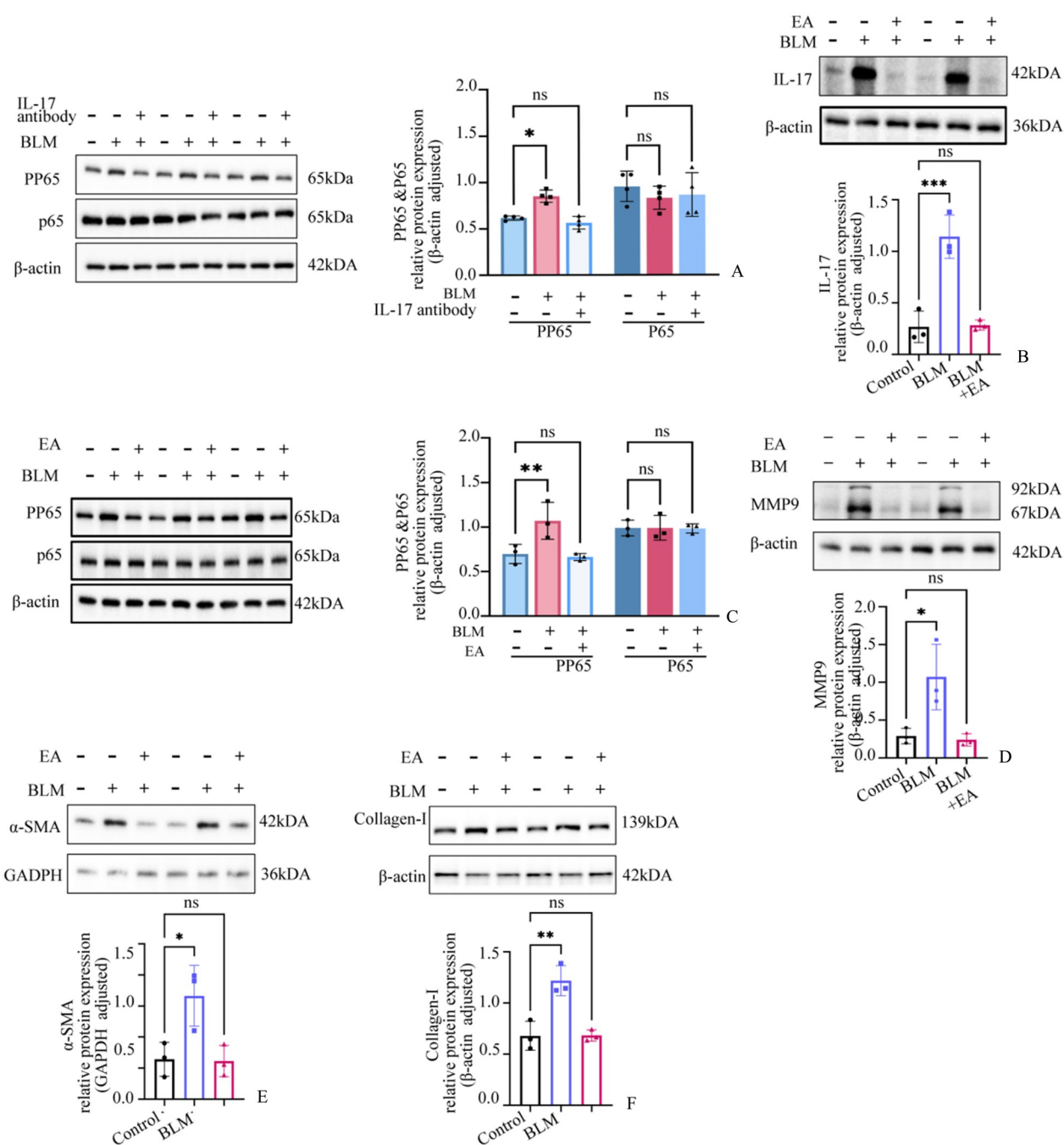
A: Levels of phosphorylated NF- κ B p65 (Ser311) in lung tissues on day 14 after BLM treatment (pP65: $t=4.797$, $**P=0.0014$ vs. Control; P65: $t=0.8799$, $P=0.4046$ vs. Control; $n=3$ per group). B-C: The NF- κ B inhibitor PDTC suppressed BLM-induced upregulation of MMP9 at both mRNA and protein levels (B: $F=216.3$, $P<0.0001$, $****P<0.0001$ vs. Control, $**P=0.0011$ vs. Control; C: $F=7.882$, $P=0.0105$, $*P=0.0132$ vs. Control, $P=0.9993$ vs. Control; $n=3$ per group). Data are presented as the mean \pm SEM. $*P<0.05$, $**P<0.01$, $***P<0.001$.

图5 NF- κ B p65 激活介导 BLM 诱导的 MMP9 上调

Fig. 5 NF- κ B p65 activation mediates BLM-induced MMP9 upregulation in pulmonary fibrosis

的潜在治疗候选物。先前研究显示 EA 可以通过多种机制发挥抗纤维化作用,具有广泛的生物学功能:例如,可以通过增强蛋白酶体依赖性途径中突触小泡蛋白 2 的降解,诱导肝星状细胞发生铁转运蛋白依赖性铁死亡,进而抑制肝纤维化的发生^[38]。在细胞模型中 EA 通过激活 Nrf2 并抑制 TGF β 1-Smad2/3-MMP2/9 信号通路从而抑制纤维化的发生^[39]。本研究表明,EA 干预能够抑制 BLM 诱导的 IL-17/NF- κ B p65/MMP9 信号轴激活,下调 α -SMA 与 Collagen-I 表达,并改善肺功能。尽管先前研究将 EA 的抗纤维化作用归因于对 Wnt 信号通路的抑制^[21],但本研究则揭示了一条新的作用机制

——即通过抑制 IL-17/NF- κ B/MMP9 级联反应发挥疗效。EA 的抗纤维化作用机制可能是双重的:一方面它可能通过干预抑制 IL-17 的表达或抑制其受体的激活,减少 NF- κ B 的激活,从而抑制上游信号传导,减缓纤维化的进程;另一方面它也可能通过直接抑制 NF- κ B 的转录作用或减少 MMP9 等纤维化相关因子的表达,从而减少细胞外基质的积累,减轻纤维化^[40]。这些发现凸显了 EA 作为多靶点抗肺纤维化药物的潜力,相比传统药物通常通过单一机制起效的模式,EA 能够同时作用于多个相关通路,从而实现更全面的治疗效果。近年来的研究也已证实 EA 在不同类型的纤维化疾病中具有



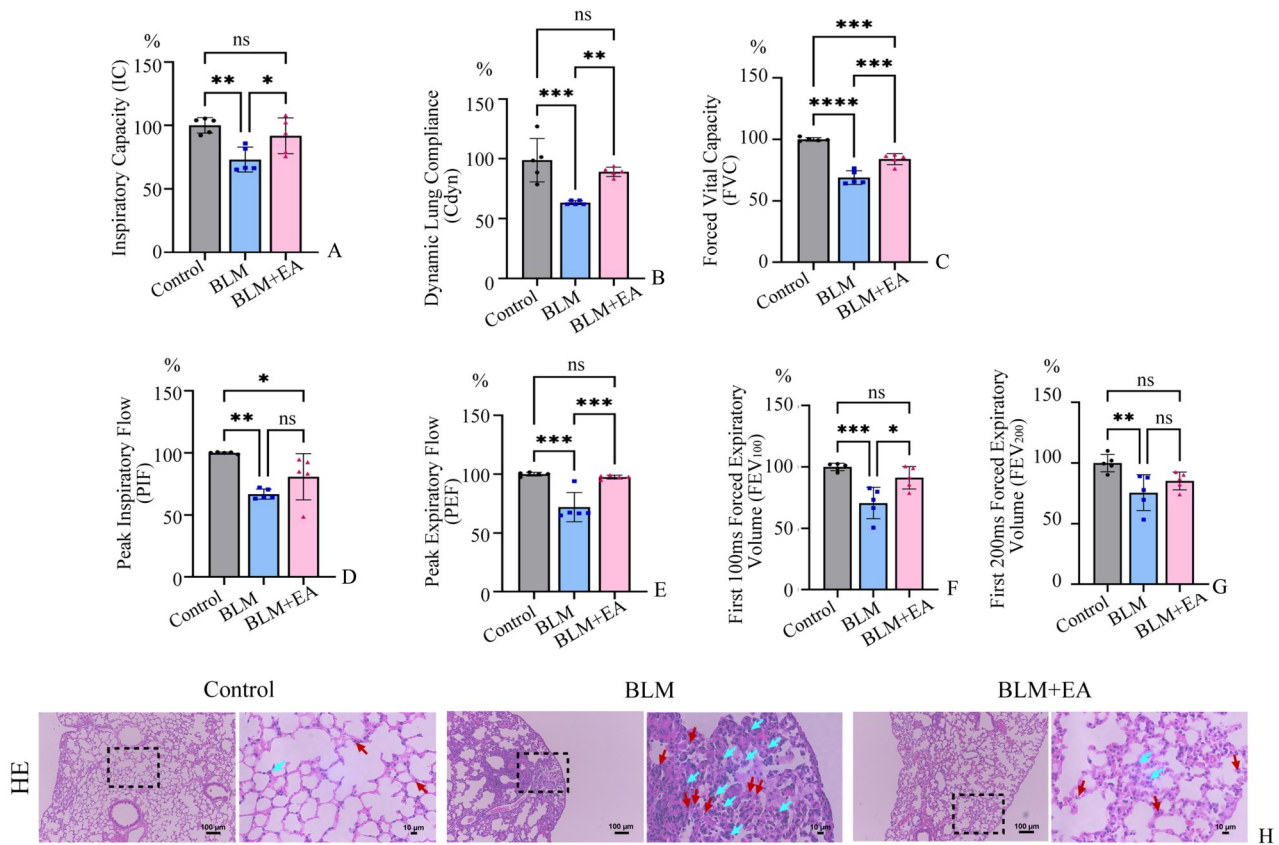
A: IL-17 neutralizing antibody suppressed p-NF- κ B p65 (Ser311) expression in BLM-treated mice (pP65: $F=4.239$, $P=0.029$, $*P=0.041$ vs. Control). B: Continuous application of EA inhibited BLM-induced upregulation of IL-17 protein ($F=32.34$, $P=0.000$, $***P=0.000$ vs. Control, $n=3$ per group). C-D: EA suppressed the upregulation of p-NF- κ B p65 and MMP9 protein in BLM-treated mice (C: pP65: $F=5.226$, $P=0.023$, $*P=0.0045$ vs. Control, $n=3$ per group; D: $F=9.479$, $P=0.013$, $*P=0.019$ vs. Control, $n=3$ per group). E-F: EA attenuated BLM-induced elevation of fibrosis markers α -SMA and Collagen I (E: $F=8.541$, $P=0.017$, $*P=0.021$ vs. Control, $n=3$ per group; F: $F=19.39$, $P=0.002$, $**P=0.002$ vs. Control, $n=3$ per group)

图6 EA抑制IL-17/NF- κ B/MMP9通路减轻BLM诱导的肺纤维化

Fig. 6 EA attenuates BLM-induced pulmonary fibrosis via suppression of the IL-17/NF- κ B/MMP9 pathway

良好的疗效,例如在肝纤维化、肾脏纤维化等方面表现出显著的抗纤维化效果^[38,40-41],这些研究为EA

的临床应用提供了有力支持。另外,EA存在于珠子等多种藏药植物中,具有良好的安全性和生物相



A-G: EA improved pulmonary function parameters (IC, C_{dyn}, FVC, PIF, PEF, FEV₁₀₀, FEV₂₀₀) as measured by whole-body PFT system (A: $F=8.598$, $P=0.0048$, $**P=0.0043$ vs. Control, $*P=0.0388$ vs. BLM; B: $F=14.45$, $P=0.0006$, $***P=0.0006$ vs. Control, $**P=0.0069$ vs. BLM; C: $F=67.96$, $P<0.0001$, $****P<0.0001$ vs. Control, $***P=0.0002$ vs. Control, $***P=0.0003$ vs. BLM; D: $F=11.38$, $P=0.0017$, $**P=0.0013$ vs. Control, $*P=0.0431$ vs. Control; E: $F=22.77$, $P<0.0001$, $***P=0.0001$ vs. Control, $***P=0.0003$ vs. BLM; F: $F=13.32$, $P=0.0009$, $***P=0.0008$ vs. Control, $*P=0.0108$ vs. BLM; G: $F=6.959$, $P<0.01$, $**P=0.0078$ vs. Control; $n=5$ per group). H: HE staining of lung tissues after BLM and EA treatment (Red arrows indicate neutrophils, and blue arrows indicate macrophages, $n=3$ per group). Data are presented as the mean \pm SEM. $*P<0.05$, $**P<0.01$, $***P<0.001$.

图7 EA抑制BLM诱导肺纤维化的功能障碍及病理改变

Fig. 7 EA attenuates BLM-induced pulmonary fibrosis dysfunction and pathological changes

容性^[42],其副作用远低于类固醇和免疫抑制剂等抗纤维化传统药物,这使其更容易被患者接受,尤其

适合长期使用^[43],也为藏药来源的化合物在治疗纤维化性肺疾病中的应用提供了机制依据。

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(编辑 孙慧兰)