

G3BP2对肝星状细胞活化增殖与迁移能力的影响

董琪琪^{1,2}, 孙文杰^{1,2}, 李明慧^{1,2}, 杨晶晶², 周仁鹏^{1,2}, 胡伟^{1,2}, 鲁超^{1,3}

(¹安徽医科大学药学科学学院, 合肥 230032; ²安徽医科大学第二附属医院药物临床试验研究中心, 合肥 230601; ³安徽理工大学第一附属医院药物临床试验研究中心, 淮南 232007)

摘要 目的 探究 Ras-GAP SH3 结构域结合蛋白家族 2 (G3BP2) 在调控肝星状细胞 (HSCs) 活化、增殖及迁移中的作用。方法 采用 5 μg/L 转化生长因子-β1 (TGF-β1) 处理小鼠 HSCs (JS-1 细胞系) 24 h 建立 HSCs 活化增殖模型, 通过 siRNA 干扰技术构建 G3BP2 敲低体系, 实验设置对照 (Control) 组、TGF-β1 处理组、TGF-β1+si-NC 组及 TGF-β1+G3BP2-siRNA 4 组。通过 Western blot 和 RT-qPCR 检测纤维化关键指标 I 型胶原蛋白 (Collagen I)、α-平滑肌肌动蛋白 (α-SMA) 及 G3BP2 的表达; 应用 CCK-8 增殖检测试剂盒和 EdU 荧光标记技术评估细胞增殖活性; 采用划痕愈合实验和 Transwell 迁移模型分析细胞迁移能力; 借助免疫荧光显微技术定量应激颗粒形成水平, 研究活化 HSCs 中 G3BP2 对应激颗粒形成的影响。结果 TGF-β1 刺激上调 JS-1 细胞中 G3BP2 表达 (RT-qPCR: $P < 0.0001$; Western blot: $P < 0.0001$), 而在 G3BP2 基因沉默组中表达量呈降低趋势 (RT-qPCR: $P < 0.01$; Western blot: $P < 0.0001$)。与对照组相比, TGF-β1 组 α-SMA 和 Collagen I 的蛋白表达水平平均升高 (RT-qPCR: 均 $P < 0.01$; Western blot: $P < 0.01$, $P < 0.05$), 同时伴随应激颗粒数量增加和细胞增殖迁移能力增强 (均 $P < 0.001$)。实验显示, G3BP2 敲除有效逆转上述表型, 与阴性对照组相比, G3BP2 基因沉默组纤维化指标表达降低 (均 $P < 0.01$), 应激颗粒形成减少 ($P < 0.01$), 且细胞增殖迁移能力下降 (均 $P < 0.05$)。结论 G3BP2 通过促进应激颗粒的生成, 增强 HSCs 的活化增殖与迁移能力, 进而加速肝纤维化的病理进程。提示应激颗粒可能是参与调控 HSCs 活化、增殖和迁移的重要调节因子。

关键词 G3BP2; HSCs; 应激颗粒; 增殖; 迁移; 肝纤维化

中图分类号 R 966

文献标志码 A **文章编号** 1000-1492(2026)03-0501-08

doi:10.19405/j.cnki.issn1000-1492.2026.03.016

2025-11-20 接收

基金项目: 安徽省自然科学基金项目 (编号: 2408085MH213); 安徽省卫生健康科研项目 (编号: 2024Aa40016); 安徽省高校自然科学基金研究重点项目 (编号: 2024AH050803)

作者简介: 董琪琪, 男, 硕士研究生;

鲁超, 男, 研究员, 博士生导师, 通信作者, E-mail: chaolu@aust.edu.cn

肝纤维化是由不同程度损伤和纤维沉积引起的一类肝病, 仍是未解决的医学难题^[1-2]。其核心病理特征为肝星状细胞 (hepatic stellate cells, HSCs) 的异常增殖、迁移及胶原蛋白过度累积^[3-6]。在真核细胞中, 外界刺激可诱导胞质形成无膜细胞器——应激颗粒。研究^[7]表明, 线粒体来源双链

model of post-traumatic stress disorder (PTSD). **Methods** A PTSD mouse model was established using a single prolonged stress and foot shock stimulation (SPS&S) method. The despair, anxiety, and learning and memory functions of PTSD mice were assessed through the open field test, Y-maze test, and forced swimming test. Neuronal damage was detected *via* HE and Nissl staining. The expression levels of METTL3, FTO, ALKBH5, and neuronal nuclear protein (NEUN) were assessed by Western blot and immunofluorescence staining. **Results** Compared to control group, PTSD mice subjected to SPS&S exhibited signs of despair, anxiety, and impaired learning and memory. HE and Nissl staining results showed neuronal damage in the prefrontal cortex of PTSD mice. Western blot and immunofluorescence staining results showed that the expression of the m6A-related proteins METTL3 and FTO decreased, while the expression of ALKBH5 increased in the prefrontal cortex. Additionally, NEUN protein levels showed a declining trend. **Conclusion** The pathogenesis of PTSD may be associated with neuronal damage in the prefrontal cortex and alterations in m6A methylation proteins.

Key words post-traumatic stress disorder; prefrontal cortex; SPS&S model; m6A-related proteins; neurons

Fund program National Natural Science Foundation of China (No. 32260208)

Corresponding author Zhang Guiqing, E-mail: firstli@126.com

RNA 可在 HSCs 中诱导含干扰素调节因子 3 (interferon regulatory factor 3, IRF3) 的应激颗粒生成, 促进纤维化。在宫颈癌、乳腺癌等多种肿瘤中, 应激颗粒通过招募活化 C 激酶 1 受体 (receptor for activated C kinase 1, RACK1) 蛋白, 抑制丝裂原活化蛋白激酶激酶 4 - c-Jun 氨基末端激酶通路活化, 从而抑制细胞凋亡、促进肿瘤发展^[8-9]。

Ras GTP 酶激活蛋白结合蛋白 2 (Ras GTPase-activating protein-binding protein 2, G3BP2) 是应激颗粒形成的关键调控因子, 凭借多价 RNA 结合能力介导其组装与动态平衡, 并在氧化应激下调控 mRNA 稳定性与翻译暂停^[10-11]。在动脉粥样硬化中, G3BP2 通过整合素 β 3-Yes 相关蛋白信号通路促进内皮炎症和屏障破坏, 提示其在炎症环境中的重要作用^[12]。鉴于肝纤维化微环境中存在持续氧化应激, HSCs 中可能通过 G3BP2 依赖的应激颗粒形成影响疾病进展^[13-14]。深入探究 G3BP2 调控应激颗粒形成对肝纤维化发生发展的影响, 有助于揭示肝纤维化治疗的新靶点, 开发新的抗肝纤维化治疗策略。

1 材料与方法

1.1 材料

1.1.1 细胞株 小鼠 HSCs 系 (JS-1) 购自深圳豪地华拓生物科技有限公司, 货号: HTX2182。

1.1.2 试剂 CCK-8 试剂盒 (中国 Biosharp 公司, BS350A); DMEM 培养基 (南京森贝伽生物科技有限公司, BC-M-004); 胎牛血清 (美国 Gibco 公司, 10270-106); TGF- β 1 (苏州近岸蛋白科技有限公司, CK33); 胰蛋白酶、BeyoClick™ EdU-488 试剂盒、BCA 试剂盒、RIPA 裂解液 (上海碧云天生物技术有限公司, C0201、C0038、P0012、P0013B); Lipofectamine 3000 (美国赛默飞世尔科技公司, L3000001); TRIzol (北京索莱宝生物科技有限公司, 15596018); 细胞培养小室 (中国 LABSELECT 公司, 14341); 一步法逆转录试剂盒、qPCR 染料法 (长沙艾克瑞生物工程有限公司, AG11706、AG11701); Triton X-100 (北京索莱宝生物科技有限公司, T8200); Goat anti-Rabbit IgG (H+L) HRP、Anti-GAPDH (美国 Immunoway 公司, RS0002、YM8394); Anti-G3BP1、Anti-G3BP2、Anti-Collagen I (美国 Proteintech 公司, 13057-2-AP、16276-1-AP、14695-1-AP); α -平滑肌肌动蛋白多克隆抗体 (alpha-smooth muscle actin polyclonal an-

tibody, α -SMA pAb) (美国 Sigam 公司, A5228); RT-qPCR 引物: 上海生工生物工程有限公司, 表 1 均为 C57 小鼠基因序列。

表 1 RT-qPCR 中目的基因引物

Tab. 1 Target gene primers for RT-qPCR

Gene	Primer sequences (5'-3')
<i>G3BP2</i>	F: AAAGCTCCCGAGTATTTCAC R: GAGCATCCACATGACGAATTTG
<i>Collagen I</i>	F: GCTCCTCTTAGGGGCCACT R: ATTGGGGACCTTAGGCCAT
α -SMA	F: GTCCTCTATGCCTCTGGAC R: AAGGAATAGCCACGCTCAGT
<i>GAPDH</i>	F: GGTGTCTCTCTCGGACTCA R: TGGTCCAGGTTTCTTACTCC
<i>si-G3BP2</i>	F: CCAGUUCAGAGAAUCUUAATT R: UUAAGAUCUCUGAACUGGTT

1.1.3 仪器设备 无菌工作台 (上海海尔医疗科技有限公司); 高压灭菌锅 (上海申安医疗器械有限公司); 细胞培养箱 (深圳瑞沃德生命科技有限公司); 低温离心机 (德国艾本德); 酶标仪 (美国伯腾仪器有限公司); PCR 扩增仪 (上海山富科学仪器有限公司); 实时荧光定量 PCR 检测仪 (上海宏石医疗科技有限公司); 多功能图像工作站 (广州博鹭腾生物科技有限公司); 研究级电动显微镜 (德国蔡司)。

1.2 方法

1.2.1 细胞培养 JS-1 细胞于高糖 DMEM 培养基 (含 1% 双抗、10% FBS) 中培养。待细胞达 80% ~ 90% 后, 将细胞消化, 传代用于后续实验。

1.2.2 HSCs 活化 将细胞按 30% 密度铺板, 待细胞达 60% ~ 70% 时, 更换高糖 DMEM 培养基 (含 5 μ g/L TGF- β 1、不含 FBS), 于标准培养条件下诱导 24 h 激活 HSCs。

1.2.3 G3BP2-siRNA 转染及实验分组 JS-1 细胞活化后, 采用 Lipofectamine 3000 转染阴性对照 siRNA、G3BP2 siRNA, 继续培养 24 h, 进行实验检测。实验设 4 组: Control 组 (基础培养); TGF- β 1 组 (TGF- β 1 24 h 诱导活化); TGF- β 1+si-NC 组; TGF- β 1+si-G3BP2 组 (TGF- β 1 24 h 诱导活化, G3BP2-siRNA 构建 G3BP2 沉默模型)。

1.2.4 Western blot 实验 JS-1 细胞经分组处理, RIPA 裂解缓冲液提取蛋白, 蛋白样用 BCA 法定量后等量上样。SDS-PAGE 分离蛋白并湿转至 PVDF 膜, 封闭后 4 $^{\circ}$ C 过夜孵育一抗, TBST 漂洗后室温孵育二抗 1 h, TBST 漂洗后显影, ImageJ 分析条带灰度

值,实验重复3次。

1.2.5 RT-qPCR实验 JS-1细胞经分组干预后,通过TRIzol法提取RNA。采用试剂盒推荐方法进行逆转录,合成cDNA,基于SYBR Green预混体系配制20 μ L qPCR反应体系,引物见表1。CFX96 Touch™平台完成扩增。GAPDH为内参作归一化处理,采用 $2^{-\Delta\Delta C_T}$ 法进行计算,实验均设3个独立重复。

1.2.6 CCK-8实验 JS-1细胞以每孔2 000个种于96孔板,边缘孔加入PBS,在细胞培养箱中培养4 h,更换为含10% CCK-8的培养基,避光孵育1~4 h。酶标仪检测450 nm波长处吸光度,计算增殖率,每组6个复孔。

1.2.7 EdU细胞增殖实验 JS-1细胞以 2×10^3 个/孔密度接种至24孔培养板并进行分组处理。每孔加入EdU工作液500 μ L,细胞培养箱中孵育2 h。依次进行以下操作:4%多聚甲醛固定15 min;0.3% Triton X-100通透15 min;避光条件中加入Click反应液避光静置30 min;加入Hoechst 33342染细胞核;最终应用激光共聚焦显微镜系统进行荧光信号定量分析。

1.2.8 细胞划痕实验 在6孔培养板基底面均匀绘制3条垂直标记线界定观察区域,铺种细胞悬液至约90%汇合度后,用无菌200 μ L移液器吸头垂直于标记线进行划痕处理。PBS洗3次,清除脱落细胞残骸。更换为无血清DMEM培养基。在划痕后0 h和48 h时采集图像,ImageJ软件统计划痕面积,实验独立重复6次。

1.2.9 Transwell细胞迁移实验 Transwell小室加500 μ L DMEM培养基,置于培养箱进行1 h平衡处理以激活膜表面特性。预处理完成后,上室加 2×10^4 细胞悬液(体积100 μ L),下室加入1 mL完全培养基,孵育24 h。固定后加入0.1%结晶紫溶液染色。用棉签移除上室未迁移细胞;随机选取5个不同视野观察并拍摄。用ImageJ计算细胞数量,每组重复6次。

1.2.10 免疫荧光染色 药物处理后的细胞分组接种于含14 mm爬片的24孔板,细胞贴壁后处理,使用冷甲醛处理5 min;PBS漂洗3次(5 min/次);5% BSA封闭60 min;G3BP1一抗4 $^{\circ}$ C过夜孵育,荧光二抗避光孵育60 min,封片处理。共聚焦显微系统采集图像,ImageJ分析共定位,独立生物学重复3次。

1.3 统计学处理 所有的数据用GraphPad Prism

9.0进行统计分析,多组组间差异均采用单因素方差分析结合Tukey多重比较检验。 $P < 0.05$ 表示差异有统计学意义,所有实验均重复3次以上。

2 结果

2.1 G3BP2在活化JS-1细胞中的表达 如图1显示,经TGF- β 1处理的JS-1细胞中G3BP2表达量高于对照组(RT-qPCR: $t=8.274$, $P < 0.0001$; Western blot: $t=8.486$, $P < 0.0001$)。通过si-G3BP2转染技术干预后,实验组JS-1细胞中的G3BP2 mRNA及蛋白表达量较阴性对照组均呈现降低趋势(RT-qPCR: $t=4.975$, $P < 0.01$; Western blot: $t=8.564$, $P < 0.0001$),这一结果表明G3BP2基因被沉默。

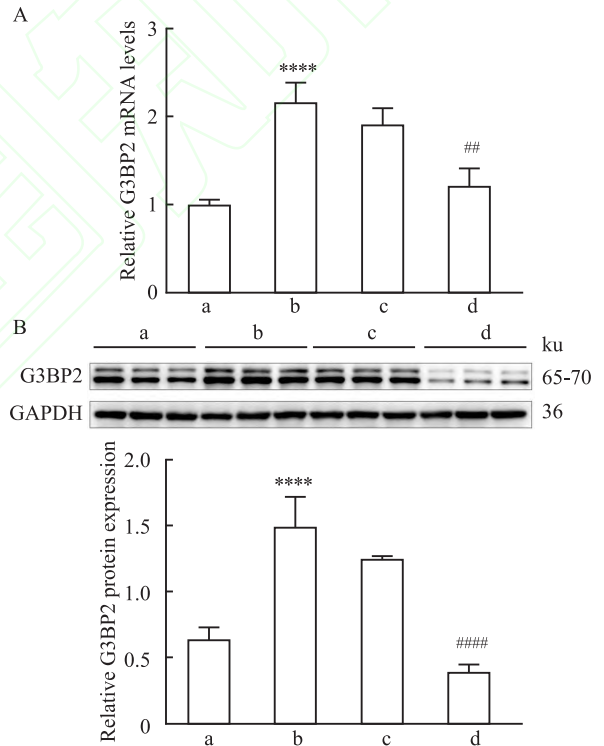


图1 各组中G3BP2的表达情况

Fig. 1 The expression of G3BP2 in each group

A: Changes of the expression level of G3BP2 mRNA in each group detected by RT-qPCR; B: Changes of the expression level of G3BP2 protein in each group detected by Western blot; a: Control; b: TGF- β 1; c: TGF- β 1+si-NC; d: TGF- β 1+si-G3BP2; **** $P < 0.0001$ vs Control group; ## $P < 0.01$, #### $P < 0.0001$ vs TGF- β 1+si-NC group.

2.2 应激颗粒水平在JS-1细胞中的变化 如图2所示,TGF- β 1组较对照组细胞中的G3BP1表达水平上升($t=8.019$, $P < 0.0001$),这一应激颗粒的标志性蛋白变化表明细胞内应激颗粒含量上升。同时,与阴性对照组相比,当G3BP2表达被抑制时,细胞

内的应激颗粒水平下降($t=3.860, P<0.01$)。这一现象表明,在JS-1细胞活化过程中,应激颗粒水平呈现上升趋势,而G3BP2的沉默能够有效抑制这一过程。

2.3 G3BP2对Collagen I、 α -SMA表达的影响 如图3所示,RT-qPCR和Western blot分析显示,TGF- β 1激活的JS-1细胞中纤维化标志物Collagen I和 α -SMA表达显著上调(RT-qPCR: $t=4.53, P<0.01; t=4.89, P<0.01$; Western blot: $t=4.49, P<0.01; t=3.38, P<0.05$);通过免疫荧光显示,TGF- β 1处理过程中的应激颗粒的形成早于Collagen I和 α -SMA蛋白的表达。当采用基因沉默技术抑制G3BP2表达时,可观察到上述两种关键蛋白的mRNA和蛋白水平均出现明显下降(RT-qPCR: $t=5.78, P<0.001; t=5.19, P<0.01$; Western blot: $t=16.68, P<0.0001; t=13.01, P<0.0001$)。

2.4 G3BP2对JS-1细胞增殖活性的影响 通过CCK-8与EdU实验方法对细胞增殖能力进行评估。如图4所示,实验数据表明,相较于Control组,TGF- β 1处理增强了JS-1细胞的增殖能力(CCK-8: $t=7.032, P<0.0001$; EdU: $t=13.60, P<0.0001$)。同时,与TGF- β 1+si-NC组相比,TGF- β 1+si-G3BP2组的JS-1细胞增殖水平呈现降低趋势(CCK-8: $t=5.917, P<0.0001$; EdU: $t=6.796, P<0.001$),表明G3BP2的沉默有效抑制了细胞增殖。这些结果表明,G3BP2基因表达的抑制能够拮抗TGF- β 1诱导的细胞增殖

效应,从而影响JS-1细胞的增殖活性。

2.5 G3BP2对JS-1细胞迁移能力的影响 划痕愈合与Transwell迁移实验评估细胞的迁移能力。如图5所示,经TGF- β 1处理的实验组较未处理对照组展现出更高的细胞迁移活性($P<0.05$)。此外,相较于转染si-NC的阴性对照组,si-G3BP2转染组表现出明显的迁移抑制效应($P<0.05$)。上述结果表明,敲低G3BP2可有效降低JS-1细胞的迁移潜能。

3 讨论

G3BP2作为多功能RNA结合蛋白及应激颗粒的核心组分,在细胞应激条件下通过特异性结合RNA分子介导应激颗粒的动态组装,其功能调控对细胞应激应答及疾病病理进程具有重要影响。G3BP2通过影响mRNA的稳定性来调控靶基因的表达,这种调控影响着包括肿瘤在内的多种疾病的发展^[15]。相关文献^[16]表明,G3BP1作为应激颗粒的核心标志蛋白,通常用其表达水平反映细胞内应激颗粒的表达水平,通过TGF- β 1刺激HSCs成功构建活化模型,在TGF- β 1组中G3BP1的表达增加,提示细胞内应激颗粒数量增多。通过实验显示,在TGF- β 1刺激下,细胞内G3BP2表达显著上升,表明G3BP2可能通过参与应激颗粒的形成促进HSCs的活化增殖。

为了进一步探讨G3BP2与肝纤维化之间的关系,敲低G3BP2后进行相关实验。免疫荧光结果显

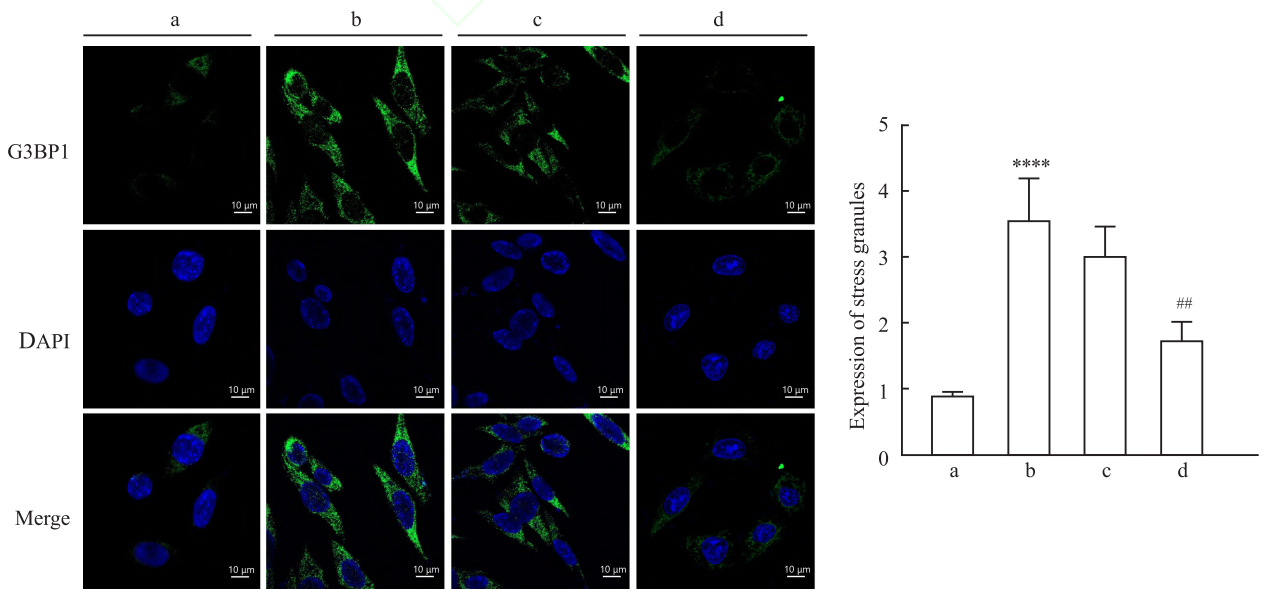


图2 免疫荧光染色实验检测G3BP2对应激颗粒的影响 $\times 1000$

Fig. 2 Immunofluorescence staining analyzed the regulation of stress granule formation by G3BP2 $\times 1000$

a: Control; b: TGF- β 1; c: TGF- β 1+si-NC; d: TGF- β 1+si-G3BP2; **** $P<0.0001$ vs Control group; ## $P<0.01$ vs TGF- β 1+si-NC group.

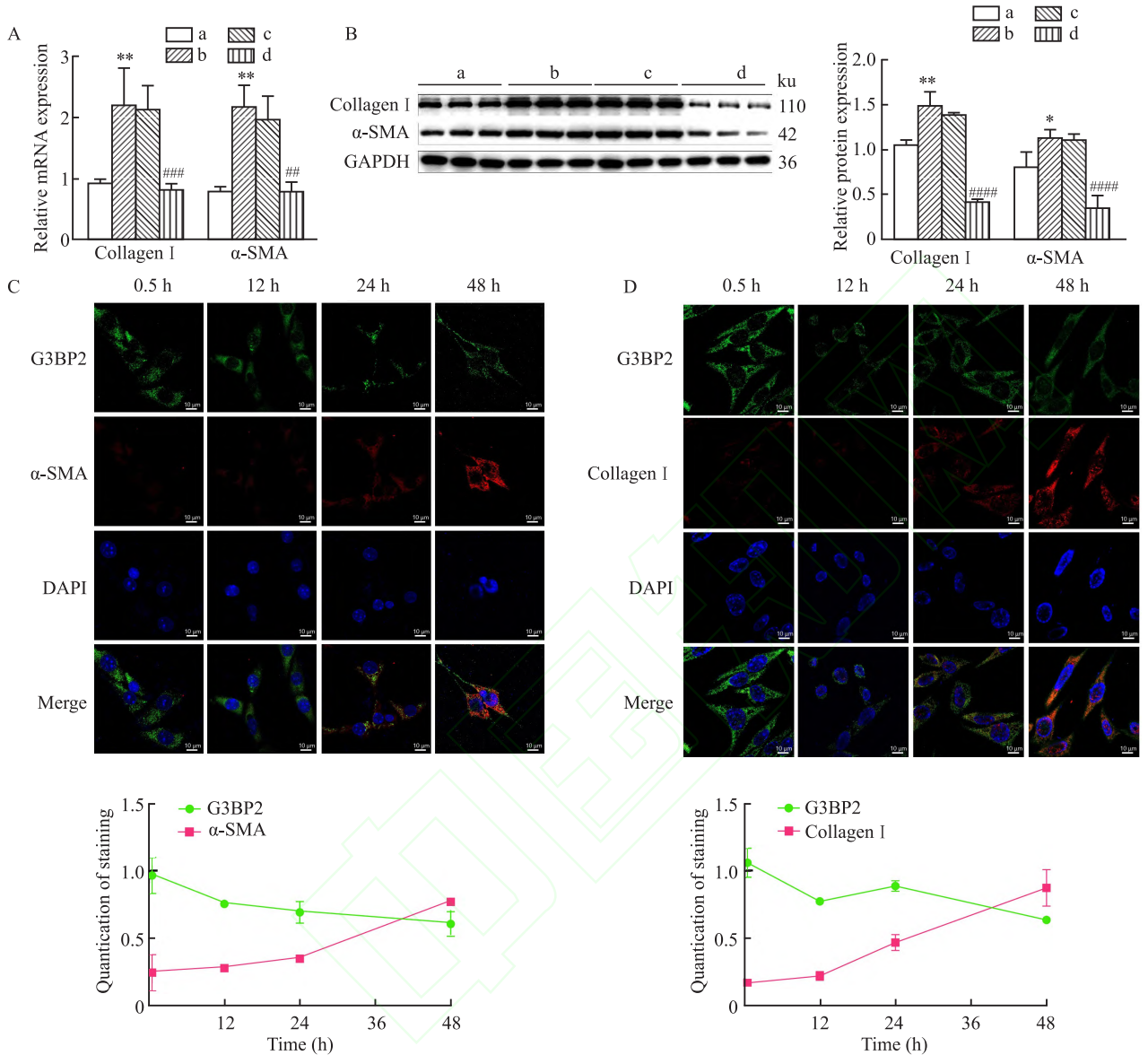


图3 G3BP2对Collagen I和 α -SMA表达的影响及TGF- β 1处理下应激颗粒形成和Collagen I、 α -SMA表达情况

Fig. 3 The effects of G3BP2 on the expression of collagen I and α -SMA, and the formation of stress granules and the expression of collagen I and α -SMA under TGF- β 1 treatment

A: The effects of G3BP2 on the expression of Collagen I and α -SMA mRNA in each group detected by RT-qPCR; B: The effects of G3BP2 on Collagen I and α -SMA protein expression in each group detected by Western blot; C: Immunofluorescence detection of stress granule and α -SMA formation in JS-1 cells under TGF- β 1 stimulation $\times 1000$; D: Immunofluorescence detection of stress granule and Collagen I formation in JS-1 cells under TGF- β 1 stimulation $\times 1000$; a: Control; b: TGF- β 1; c: TGF- β 1+si-NC; d: TGF- β 1+si-G3BP2. * $P < 0.05$, ** $P < 0.01$ vs Control group; ## $P < 0.01$, ### $P < 0.001$, #### $P < 0.0001$ vs TGF- β 1+si-NC group.

示,与阴性对照组相比,TGF- β 1+si-G3BP2组中G3BP1的表达显著下降,细胞内应激颗粒数量显著下降。CCK-8、划痕实验及Transwell检测结果表明,经TGF- β 1处理的HSCs在增殖与迁移能力上显著提升,而通过si-G3BP2干预后,这一效应被有效抑制。在TGF- β 1+si-G3BP2处理组中,与TGF- β 1+si-NC组相比Collagen I与 α -SMA的表达水平明显降低,这进一步验证了G3BP2在HSCs活化中的促进作用,

但其介导HSCs增殖迁移的潜在分子通路仍有待深入研究。G3BP2作为多效性RNA结合蛋白,不仅介导多种RNA分子的互作,同时调控应激颗粒的动态组装过程,在细胞增殖、代谢调控及迁移过程中发挥重要作用^[17]。

应激颗粒最为人所知的是通过隔离mRNA和翻译起始组分来调节mRNA翻译,在细胞受到外界刺激后,通过暂停非必需mRNA翻译,减少错误折

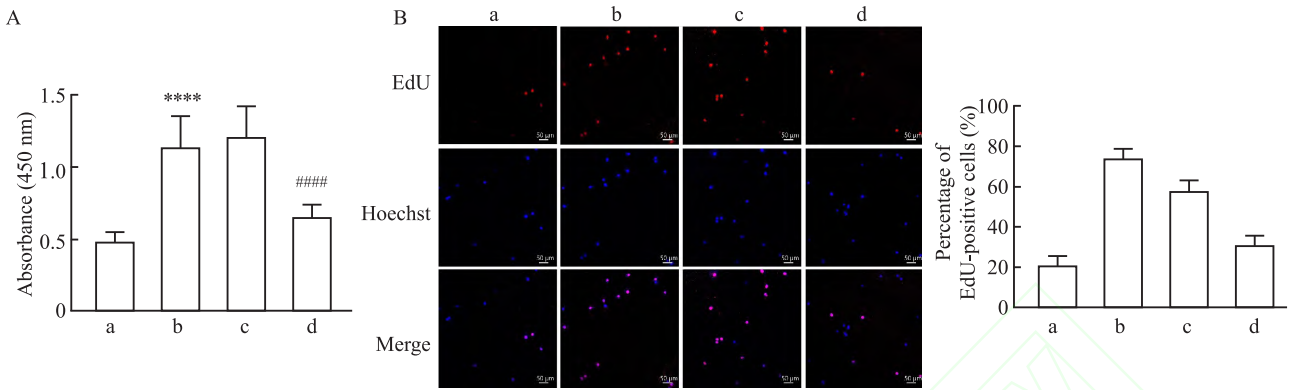


图4 G3BP2对JS-1细胞增殖的影响

Fig. 4 The effects of G3BP2 on the proliferation of JS-1

A: G3BP2-dependent modulation of the proliferative activity of JS-1 cells evaluated via CCK-8 colorimetric assay ($n=6$); B: Representative EdU images of JS-1 ($n=3$) and statistical analysis of them; a: Control; b: TGF-β1; c: TGF-β1+si-NC; d: TGF-β1+si-G3BP2. **** $P<0.0001$ vs control group; ### $P<0.001$, #### $P<0.0001$ vs TGF-β1+si-NC group.

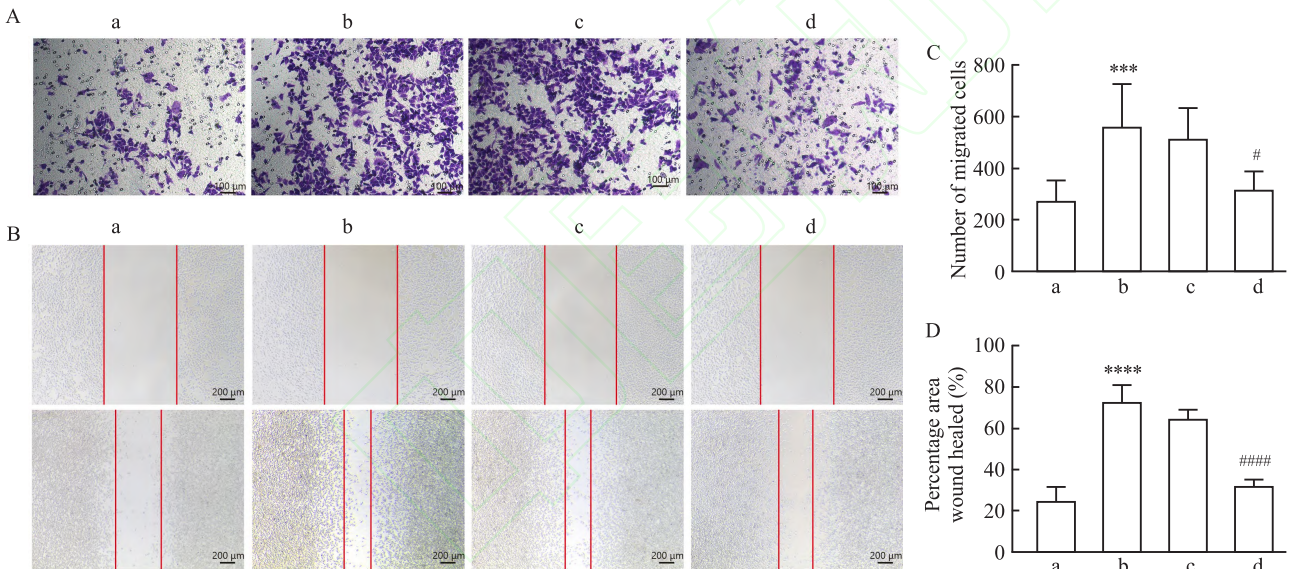


图5 G3BP2对JS-1细胞迁移能力的影响 ×200

Fig. 5 Modulatory influence of G3BP2 on the motility potential of JS-1 ×200

A, C: Transwell migration assay evaluating G3BP2-mediated regulation of JS-1 cells motility; B, D: Scratch healing assay assessing the effects of G3BP2 on the migration ability of JS-1 cells; a: Control; b: TGF-β1; c: TGF-β1+si-NC; d: TGF-β1+si-G3BP2; *** $P<0.001$, **** $P<0.0001$ vs Control group; # $P<0.05$, #### $P<0.0001$ vs TGF-β1+si-NC group.

叠蛋白积累,缓解内质网应激(endoplasmic reticulum stress, ERS),阻断应激颗粒形成后,ERS水平显著升高,ERS持续激活上调转录因子4(activating transcription factor 4, ATF4),而上调CCAAT/增强子结合蛋白同源蛋白(CCAAT/Enhancer-binding protein homologous protein, CHOP),促凋亡信号增强,导致肝星状细胞持续活化,促进纤维化进展^[18]。相关研究^[19]报道显示,应激颗粒可保护人牙周膜细胞免受应激诱导的细胞凋亡,并抑制脂多糖触发的炎症因子的表达及活性氧生成,抑制NOD样受体热蛋

白结构域相关蛋白3(NOD-like receptor protein 3, NLRP3)炎症小体形成,减少炎症因子的分泌。推测在肝星状细胞中,SGs缺失导致活性氧清除能力下降,线粒体功能障碍,激活NLRP3炎症小体,促进白细胞介素-1β(interleukin-1 beta, IL-1β)释放,驱动肝星状细胞活化向肌成纤维细胞转化。据此推测,G3BP2的上调或可通过与特定纤维化相关mRNA结合,影响其稳定状态及表达水平,从而调控应激颗粒的形成过程。该机制可能进一步促进HSCs增殖活性和迁移能力,进而加剧肝纤维化病理进展。

参考文献

- [1] Kisseleva T, Brenner D. Molecular and cellular mechanisms of liver fibrosis and its regression [J]. *Nat Rev Gastroenterol Hepatol*, 2021, 18 (3) : 151-66. doi: 10.1038/s41575-020-00372-7.
- [2] 贾琳, 孙峰, 董琪琪, 等. YTHDF1调控Fis1对肝星状细胞活化、增殖及迁移能力的影响[J]. *安徽医科大学学报*, 2025, 60 (1) : 49-58. doi: 10.19405/j. cnki. issn1000-1492. 2025. 01. 007.
- [2] Jia L, Sun F, Dong Q Q, et al. YTHDF1 regulation of Fis1 on the activation and proliferation and migration ability of hepatic stellate cells [J]. *Acta Univ Med Anhui*, 2025, 60(1) : 49-58. doi: 10.19405/j. cnki. issn1000-1492. 2025. 01. 007.
- [3] Tong G, Chen X, Lee J, et al. Fibroblast growth factor 18 attenuates liver fibrosis and HSCs activation *via* the SMO-LATS1-YAP pathway [J]. *Pharmacol Res*, 2022, 178: 106139. doi: 10.1016/j. phrs. 2022. 106139.
- [4] Zhang M, Serna-Salas S, Damba T, et al. Hepatic stellate cell senescence in liver fibrosis: characteristics, mechanisms and perspectives [J]. *Mech Ageing Dev*, 2021, 199: 111572. doi: 10.1016/j. mad. 2021. 111572.
- [5] Horn P, Tacke F. Metabolic reprogramming in liver fibrosis [J]. *Cell Metab*, 2024, 36 (7) : 1439-55. doi: 10.1016/j. cmet. 2024. 05. 003.
- [6] 尤鸿美, 王凌, 卜芳田, 等. microRNAs在肝纤维化进展中的作用[J]. *中国药理学通报*, 2021, 37(2) : 171-5. doi: 10.3969/j. issn. 1001-1978. 2021. 02. 005.
- [6] You H M, Wang L, Bu F T, et al. Role of microRNAs in progress of liver fibrosis [J]. *Chin Pharmacol Bull*, 2021, 37 (2) : 171-5. doi: 10.3969/j. issn. 1001-1978. 2021. 02. 005.
- [7] Travers J, Huang E, McMullen M R, et al. Mitochondria-derived dsRNA-induced stress granules promote IRF3-mediated fibrotic responses [J]. *bioRxiv*, 2025, 5 (14) : 654103. doi: 10.1101/2025. 05. 14. 654103.
- [8] 任翎璇, 王维蓉, 林蓉. 应激颗粒在心脑血管疾病、肿瘤和神经退行性疾病中的研究进展[J]. *生命科学*, 2024, 36(8) : 1089-97. doi: 10.13376/j. cbls/20240110.
- [8] Ren L X, Wang W R, Lin R. Research progress of stress granule in cardiovascular and cerebrovascular diseases, tumor and neurodegenerative diseases [J]. *Chin Bull Life Sci*, 2024, 36 (8) : 1089-97. doi: 10.13376/j. cbls/20240110.
- [9] 金志刚, 蒋莹佩, 张晶晶. 应激颗粒在细胞衰老中的作用 [J]. *浙江师范大学学报(自然科学版)*, 2022, 45(2) : 184-93. doi: 10.16218/j. issn. 1001-5051. 2022. 02. 009.
- [9] Jin Z G, Jiang Y P, Zhang J J. The role of stress granules in cellular senescence [J]. *J Zhejiang Norm Univ Nat Sci*, 2022, 45 (2) : 184-93. doi: 10.16218/j. issn. 1001-5051. 2022. 02. 009.
- [10] Jin G, Zhang Z, Wan J, et al. G3BP2: structure and function [J]. *Pharmacol Res*, 2022, 186: 106548. doi: 10.1016/j. phrs. 2022. 106548.
- [11] Hao Q, Zhang M, Wu Y, et al. Hsa_circRNA_001676 accelerates the proliferation, migration and stemness in colorectal cancer through regulating miR-556-3p/G3BP2 axis [J]. *Sci Rep*, 2023, 13(1) : 18353. doi: 10.1038/s41598-023-45164-6.
- [12] Li T, Qiu J, Jia T, et al. G3BP2 regulates oscillatory shear stress-induced endothelial dysfunction [J]. *Genes Dis*, 2022, 9 (6) : 1701-15. doi: 10.1016/j. gendis. 2021. 11. 003.
- [13] Song L J, Yin X R, Guan S W, et al. RIP3 deficiency attenuated hepatic stellate cell activation and liver fibrosis in schistosomiasis through JNK-cJUN/Egr1 downregulation [J]. *Sig Transduct Target Ther*, 2022, 7(1) : 193. doi: 10.1038/s41392-022-01019-6.
- [14] Luo T, Yang S, Zhao T, et al. Hepatocyte DDX3X protects against drug-induced acute liver injury *via* controlling stress granule formation and oxidative stress [J]. *Cell Death Dis*, 2023, 14(7) : 400. doi: 10.1038/s41419-023-05913-x.
- [15] Liu B, Zhang J, Meng X, et al. HDAC6-G3BP2 promotes lysosomal-TSC2 and suppresses mTORC1 under ETV4 targeting-induced low-lactate stress in non-small cell lung cancer [J]. *Oncogene*, 2023, 42(15) : 1181-95. doi: 10.1038/s41388-023-02641-6.
- [16] Lee S, Kim S Y, Kwon E, et al. A novel G3BP1-GFP reporter human lung cell system enabling real-time monitoring of stress granule dynamics for *in vitro* lung toxicity assessment [J]. *Ecotoxicol Environ Saf*, 2024, 269: 115755. doi: 10.1016/j. ecoenv. 2023. 115755.
- [17] Zhang H, Wang Y, Zhang W, et al. BAALC-AS1/G3BP2/c-Myc feedback loop promotes cell proliferation in esophageal squamous cell carcinoma [J]. *Cancer Commun (Lond)*, 2021, 41(3) : 240-57. doi: 10.1002/cac2. 12127.
- [18] Li W Y, Yang F, Li X, et al. Stress granules inhibit endoplasmic reticulum stress-mediated apoptosis during hypoxia-induced injury in acute liver failure [J]. *World J Gastroenterol*, 2023, 29 (8) : 1315-29. doi: 10.3748/wjg. v29. i8. 1315.
- [19] 徐辉, 乔广艳, 苏俭生. 应激颗粒形成对人对牙周膜细胞凋亡及对LPS刺激下炎症相关因子表达及ROS生成的影响 [J]. *口腔颌面外科杂志*, 2025, 35 (3) : 181-9. doi: 10.12439/kqhm. 1005-4979. 2025. 03. 003.
- [19] Xu H, Qiao G Y, Su J S. Effects of stress granule formation on apoptosis of human periodontal ligament cells and the expression of inflammatory-related factors and ROS generation under LPS stimulation [J]. *J Oral Maxillofac Surg*, 2025, 35 (3) : 181-9. doi: 10.12439/kqhm. 1005-4979. 2025. 03. 003.

Regulatory effects of G3BP2 on activation, proliferation, and migratory capacity in hepatic stellate cells

Dong Qiqi^{1,2}, Sun Wenjie^{1,2}, Li Minghui^{1,2}, Yang Jingjing², Zhou Renpeng^{1,2}, Hu Wei^{1,2}, Lu Chao^{1,3}

(¹ School of Pharmaceutical Sciences, Anhui Medical University, Hefei 230032; ² Dept. of Clinical Pharmacology, The Second Affiliated Hospital of Anhui Medical University, Hefei 230601; ³ Dept. of Clinical Pharmacology, The First Affiliated Hospital, Anhui University of Science and Technology, Huainan 232007)

Abstract Objective To investigate the role of Ras-GTPase-activating protein SH3 domain-binding protein 2 (G3BP2) in regulating the activation, proliferation, and migration of hepatic stellate cells (HSCs). **Methods** The mouse HSCs (JS-1 cell line) were treated with 5 µg/L transforming growth factor-beta 1 (TGF-β1) for 24 hours to establish an HSC activation and proliferation model. A G3BP2 knockdown system was constructed using siRNA interference technology. The experiment was divided into four groups: Control, TGF-β1 treatment, TGF-β1+si-NC, and TGF-β1+ G3BP2-siRNA. The expression levels of key fibrosis indicators, including type I collagen (Collagen I), α-smooth muscle actin (α-SMA), and G3BP2, were detected by Western blot and RT-qPCR. Cell proliferation activity was assessed using the CCK-8 proliferation assay kit and EdU fluorescence labeling technology. Cell migration ability was analyzed by scratch wound healing assay and Transwell migration assay. The formation level of stress granules was quantified by immunofluorescence microscopy to investigate the effects of G3BP2 on stress granule formation in activated HSCs. **Results** Stimulation with TGF-β1 upregulated the expression of G3BP2 in JS-1 cells (RT-qPCR: $P < 0.0001$; Western blot: $P < 0.0001$), while a downward trend in its expression was observed in the G3BP2-silenced group (RT-qPCR: $P < 0.01$; Western blot: $P < 0.0001$). Compared with the control group, the TGF-β1 group exhibited increased protein expression levels of α-SMA and Collagen I (RT-qPCR: both $P < 0.01$; Western blot: $P < 0.01$ and $P < 0.05$, respectively), concomitant with an increased number of stress granules and enhanced cell proliferation and migration capacity (all $P < 0.001$). The experimental results demonstrated that G3BP2 knockout effectively reversed the aforementioned phenotypes, with the G3BP2-silenced group showing reduced expression of fibrotic markers (all $P < 0.01$), decreased stress granule formation ($P < 0.01$), and reduced cell proliferation and migration capacity (all $P < 0.05$), compared to the negative control group. **Conclusion** G3BP2 enhances the activation, proliferation, and migration of HSCs by promoting the formation of stress granules, thereby accelerating the pathological progression of liver fibrosis. This suggests that stress granules may serve as important regulators in controlling the activation, proliferation, and migration of HSCs.

Key words G3BP2; HSCs; stress granules; proliferation; migration; hepatic fibrosis

Fund program Natural Science Foundation of Anhui Province (No. 2408085MH213); Health Research Project of Anhui Province (No. 2024Aa40016); Natural Science Research Project of Anhui Educational Committee (No. 2024AH050803)

Corresponding author Lu Chao, E-mail: chaolu@aust.edu.cn