

• 基础研究 •

IFN- γ 通过抑制SRC/Areg信号轴减轻克罗恩病肠纤维化

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[摘要] 目的: 系统探讨干扰素- γ (interferon-gamma, IFN- γ) 在克罗恩病 (Crohn's disease, CD) 肠纤维化中的调控作用及机制。方法: 提取分离CD患者肠成纤维细胞, 体外予以不同的细胞因子[20 ng/mL 干扰素- γ (interferon-gamma, IFN- γ)、50 ng/mL 白介素 (interleukin, IL)-17A、10 ng/mL IL-1 β 、100 ng/mL IL-33、200 ng/mL IL-36 α 、20 ng/mL 肿瘤坏死因子 (tumor necrosis factor, TNF)- α] 处理 48 h, RT-PCR 检测人肠成纤维细胞中双调蛋白 (amphiregulin, Areg) 基因表达水平。20 ng/mL IFN- γ 处理人肠成纤维细胞 48 h 后, 进行转录组测序 (RNA-seq), 结合 STRING 数据库构建蛋白质互作 (protein-protein interaction, PPI) 网络。免疫荧光检测 α 平滑肌肌动蛋白 (α -smooth muscle actin, α -SMA)、抗原 Kiel 67 (antigen Kiel 67, Ki67) 等肌成纤维细胞标志物, 评估 IFN- γ 对人肠道成纤维细胞活化、增殖的影响。结果: Areg 显著促进肠成纤维细胞活化、增殖及胶原合成; IFN- γ 可显著抑制肠成纤维细胞 Areg 表达, 并下调 α -SMA、COL1A1 和 COL6A1 等纤维化相关基因, 同时 IFN- γ 抑制成纤维细胞增殖与活化能力。RNA-seq 分析发现 IFN- γ 调控的差异基因显著富集于细胞外基质 (extracellular matrix, ECM) 重构通路, 蛋白互作 (protein-protein interaction, PPI) 网络鉴定出肉瘤原癌基因 (sarcoma proto-oncogene, SRC) 为核心节点, 提示其可能介导 IFN- γ 的抗纤维化作用。结论: Areg 是促 CD 肠纤维化关键介质, IFN- γ 通过转录调控抑制 Areg 表达, 并发现 SRC 可能是 IFN- γ 下游的关键效应分子。IFN- γ 可能通过抑制 SRC/Areg 信号轴来发挥其抗纤维化作用。这些发现为开发靶向 IFN- γ /SRC/Areg 通路的抗纤维化策略提供了理论依据。

[关键词] IFN- γ ; 双调蛋白; 肠成纤维细胞; 克罗恩病; 肠纤维化**[中图分类号]** R574.62**[文献标志码]** A**[文章编号]** 1007-4368(2025)11-1608-09**doi:** 10.7655/NYDXBNSN250631

IFN- γ alleviates intestinal fibrosis in Crohn's disease through inhibiting the SRC/Areg signaling axis

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[Abstract] **Objective:** This study systematically investigates the regulatory role and mechanism of interferon-gamma (IFN- γ) in Crohn's disease (CD)-associated intestinal fibrosis. **Methods:** Intestinal fibroblasts were isolated from CD patients and treated with various cytokines [IFN- γ , interleukin (IL)-17A, IL-1 β , IL-33, IL-36 α , tumor necrosis factor (TNF)- α] *in vitro*. The mRNA expression level of amphiregulin (Areg) in human intestinal fibroblasts was detected using RT-PCR. Following treatment with 20 ng/mL IFN- γ for 48 h, transcriptome sequencing (RNA-seq) was performed, and a protein-protein interaction (PPI) network was constructed using the STRING database. Immunofluorescence was employed to detect myofibroblast markers such as alpha-smooth muscle actin (α -SMA) and antigen Kiel 67 (Ki67), evaluating the effects of IFN- γ on the activation and proliferation of human intestinal fibroblasts. **Results:** Areg significantly promoted the activation, proliferation, and collagen synthesis of intestinal fibroblasts, whereas IFN- γ markedly suppressed Areg expression in intestinal fibroblasts and downregulated fibrosis-related genes including α -SMA, COL1A1, and COL6A1, while also inhibiting fibroblast proliferation and activation. RNA-seq analysis revealed that differentially expressed genes regulated by IFN- γ were significantly enriched in the extracellular matrix (ECM) remodeling pathway. PPI network analysis identified SRC as a core node, suggesting its potential role in mediating the anti-fibrotic effects of IFN- γ . **Conclusion:** Areg is a key mediator promoting intestinal fibrosis in CD. IFN- γ inhibits Areg expression through transcriptional regulation, and it is found that SRC may be a key effector molecule downstream of IFN- γ . IFN- γ may exert its anti-fibrotic effect by inhibiting the SRC/Areg signaling axis. These findings provide a theoretical basis for the development of anti-fibrotic strategies targeting the IFN- γ /SRC/Areg pathway.

[Key words] IFN- γ ; amphiregulin; intestinal fibroblast; Crohn's disease; intestinal fibrosis

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克罗恩病(Crohn's disease, CD)是一种反复发作的慢性消化道炎症性疾病^[1]。随着病程进展,肠道狭窄发生率逐渐升高,约60%的CD患者在确诊后的20年内会出现一次肠道狭窄,引发肠梗阻甚至肠穿孔^[2-5]。肠道狭窄的病理基础是肠道纤维化,尽管近年来临床出现了多种生物制剂,但目前尚无证据表明生物制剂可逆转肠道纤维化^[3,6]。肠狭窄及其所引发的肠穿孔等一系列并发症已成为CD诊治的难点^[1,7]。

目前,CD肠道纤维化的发病机制仍未完全明确,通常认为是遗传易感性、环境因素、肠道菌群失衡以及免疫调节异常等多种因素共同作用的结果^[8-11]。在分子层面,慢性炎症微环境中免疫细胞与间质细胞之间的相互作用被认为是推动纤维化进程的关键环节。多种免疫细胞,如辅助型T细胞2(T helper 2 cell, Th2)、调节性T细胞(regulatory T cell, Treg)细胞、巨噬细胞等,通过分泌特定细胞因子,持续激活肠道成纤维细胞,进而导致细胞外基质(extracellular matrix, ECM)过度沉积以及组织结构重塑^[12-13]。在这一复杂的细胞因子网络中,转化生长因子- β (transforming growth factor beta, TGF- β)被广泛认为是最重要的促纤维化因子,然而对于其他细胞因子的研究相对较少^[14]。

本课题组前期研究发现,双调蛋白(amphiregulin, Areg)作为表皮生长因子家族的一员,在CD肠纤维化组织中表达显著上调,并能显著促进肠成纤维细胞的活化和胶原合成^[14-15]。这一发现提示Areg可能是连接炎症反应与纤维化进程的关键介质。值得注意的是,干扰素- γ (interferon-gamma, IFN- γ)作为典型的Th1型细胞因子,在CD发病中具有双重作用:既参与炎症反应,又具有抗纤维化潜能^[16-17]。然而,IFN- γ 是否通过调控Areg表达影响CD肠纤维化进程目前并未有研究。

基于以上研究背景和科学问题,本研究拟通过不同细胞因子处理CD肠成纤维细胞,探究IFN- γ 在肠纤维化中的作用及机制。这些研究不仅有助于阐明IFN- γ 在肠纤维化中的复杂作用,还可能为开发靶向干预策略提供新的理论依据和治疗靶点。

1 材料和方法

1.1 材料

1.1.1 组织样本

选取2024年3月—2025年3月在南京医科大学

第一附属医院普外科接受手术治疗的6例CD合并肠纤维性狭窄患者。所有患者均经临床确诊并接受病变肠段切除术^[18],且研究方案已获得医院伦理委员会批准(批件号:2023-SR-852)。手术过程中,分别采集狭窄段肠管及其近端配对样本的非狭窄段全层肠壁组织,每例患者至少获取2组配对样本。组织标本经生理盐水冲洗后,分为两部分处理:第一部分用于分离肠道成纤维细胞,第二部分立即投入液氮速冻并转移至-80℃超低温冰箱保存。样本采集全程严格遵循无菌操作规范,并建立了完整的临床资料数据库,涵盖患者基本资料、病程、药物治疗史等关键信息。

1.1.2 实验试剂

磷酸盐缓冲液(phosphate-buffered saline, PBS)、Hochest33342(上海碧云天);封闭用山羊血清(上海Biosharp公司);胎牛血清(浙江天杭);DMEM高糖基础培养基(Gibco公司,美国);Hank's平衡盐溶液(HBSS,南京凯基);双抗(青霉素-链霉素混合液)、三抗(青霉素-链霉素-庆大霉素混合液,Invitrogen公司,美国);二硫苏糖醇(DTT)、EdU检测试剂盒(上海翌圣公司);总RNA提取试剂TRIzol(Thermo Fisher公司,美国);实时荧光定量PCR试剂盒(南京诺唯赞公司);I型胶原蛋白(collagen I, COL1A1)、VI型胶原蛋白抗体(collagen VI, COL6A1)、Ki67抗体(Abcam公司,美国)、 α -平滑肌肌动蛋白(α -smooth muscle actin, α -SMA)抗体(Santa Cruz公司,美国)。

1.2 方法

1.2.1 原代人肠道成纤维细胞提取

本研究采用组织块贴壁法分离培养原代人肠道成纤维细胞^[14,19-20]。具体操作如下:手术切除的狭窄段和非狭窄段组织迅速置于含1%三抗的PBS中保存;超净工作台内用HBSS充分冲洗标本,精细分离并去除黏膜上皮层、肌层及浆膜层,保留黏膜固有层并制备成2~3 cm条状组织(每次3~4条);组织块依次经DTT溶液(75 mg/50 mL HBSS)处理10~15 min、HBSS漂洗后,置于2.5%三抗-HBSS溶液中孵育3 h(每20 min翻转1次);处理后的1 mm \times 1 mm组织块均匀贴附于划格培养皿,滴加少量DMEM完全培养基37℃过夜培养促进贴壁后补足10 mL完全培养基,每3 d更换5 mL培养基;培养4~7 d后组织边缘可见细胞迁移,待成纤维细胞形成后移除组织块,继续培养3~4周后采用0.25%胰酶消化传代。全部实验过程均在生物安全柜中完成,使用含10%胎牛血清和1%双抗的培养基维持培养,并定期通过显微镜观察细胞形态变化。

1.2.2 细胞培养及处理

人肠成纤维细胞分为7组,分别为空白对照组及6种细胞因子处理组,处理组分别使用以下细胞因子刺激人肠道成纤维细胞48 h: IFN- γ (20 ng/mL)、白介素(interleukin, IL)-17A(50 ng/mL)、IL-1 β (10 ng/mL)、IL-33(100 ng/mL)、IL-36 α (200 ng/mL)、肿瘤坏死因子(tumor necrosis factor, TNF)- α (20 ng/mL)。

1.2.3 Real-time PCR

采用TRIzol法提取各组细胞总RNA后,经Nanodrop检测RNA浓度及纯度[$D(260\text{ nm})/D(280\text{ nm})$:1.8~2.0]。参照逆转录试剂盒操作流程,取1 μg 总RNA进行cDNA合成。qPCR反应使用SYBR Green法在ABI 7500系统上完成,引物序列由擎科生物合成:COL1A1(F:5'-GAGGGCCAAGACGAAGACATC-3', R:5'-CAGATCACGTCATCGCAAC-3');COL6A1(F:5'-ACAGTGACGAGGTGGAGATCA-3', R:5'-GATAGCGCAGTCGGTGTAGG-3');COL6A3(F:5'-ATGAGGAAACATCGGCACTTG-3', R:5'-GGGCATGAGTTGTAGGAAAGC-3');Acta2(F:5'-AAAAGACAGCTACGTGGGTGA-3', R:5'-GC-CATGTTCTATCGGGTACTTC-3');GAPDH(F:5'-GGAGCGAGATCCCTCCAAAAT-3', R:5'-GGCTGTTGTCATACT-TCTCATGG-3')。PCR反应程序:95 $^{\circ}\text{C}$ 预变性30 s;95 $^{\circ}\text{C}$ 5 s,60 $^{\circ}\text{C}$ 34 s,40个循环。采用 $2^{-\Delta\Delta\text{Ct}}$ 法分析基因相对表达量,GAPDH作为内参基因进行标准化。

1.2.4 Western blot

将蛋白样品加入预制好的SDS-PAGE凝胶上样孔中,以100 V恒压电泳至分离胶底部。电泳完成后,用无水乙醇激活PVDF膜,采用湿转法(250 mA恒流,冰浴冷却)将蛋白转移至PVDF膜上。转膜后,用5%脱脂牛奶室温封闭1 h, TBST漂洗后加入一抗,4 $^{\circ}\text{C}$ 孵育过夜($\geq 12\text{ h}$)。次日, TBST洗膜3次(10 min/次),加入HRP标记的二抗室温孵育1 h,再次TBST漂洗3次。滴加ECL显影液,使用凝胶成像系统检测信号。

1.2.5 EdU染色

采用EdU标记法检测细胞增殖活性。待测细胞以含20 $\mu\text{mol/L}$ EdU的培养基孵育4~6 h(依细胞增殖速率优化),4%多聚甲醛固定15 min,0.5% Triton X-100透化20 min后,通过铜催化的点击化学反应(Click-iT[®])将荧光标记叠氮化物(Alexa Fluor 594)与EdU共价结合,避光反应30 min。细胞核以Hochest33342复染,荧光显微镜观察并计算EdU阳

性细胞比例(EdU阳性细胞数/总细胞数 $\times 100\%$)。

1.2.6 细胞免疫荧光染色及Ki67染色

当细胞融合度达到90%~95%时,予0.25%胰酶消化并计数,按 1×10^4 个/孔接种于12孔板,37 $^{\circ}\text{C}$ 培养过夜。待细胞贴壁后,更换无血清培养基进行6~12 h饥饿处理,随后分别加入重组IFN- γ (20 ng/mL)和TGF- β (5 ng/mL)刺激48 h。实验过程中,先用PBS洗涤3次,4%多聚甲醛室温固定20 min, PBS再次洗涤后,0.1% Triton X-100处理20 min实现细胞膜通透,10%山羊血清封闭2 h。采用 α -SMA抗体(1:100)和Ki67抗体(1:100)4 $^{\circ}\text{C}$ 过夜孵育, Alexa Fluor 594标记的二抗37 $^{\circ}\text{C}$ 避光孵育1 h, DAPI染色细胞核10 min,所有洗涤步骤均采用PBS浸洗3次,每次5 min。最后封片并在Leica THUNDER高分辨荧光显微镜下观察并获取图像。

1.2.7 转录组测序(RNA sequencing, RNA-seq)

本研究采用20 ng/mL IFN- γ 处理人肠成纤维细胞48 h(每组设3个生物学重复),随后使用TRIzol试剂同步裂解IFN- γ 处理组和对照组细胞并提取总RNA,样本送交华大基因进行RNA-seq测序及生物信息学分析。

1.3 统计学方法

使用GraphPad Prism 8.0软件进行统计学处理。数据以均数 \pm 标准差($\bar{x}\pm s$)表示,两组间比较采用独立样本 t 检验;多组间比较采用单因素方差分析,方差齐性时选用Tukey检验,方差不齐时采用Games-Howell检验进行事后分析。所有统计检验均采用双侧检验,显著性水平设定为 $\alpha=0.05$ 。

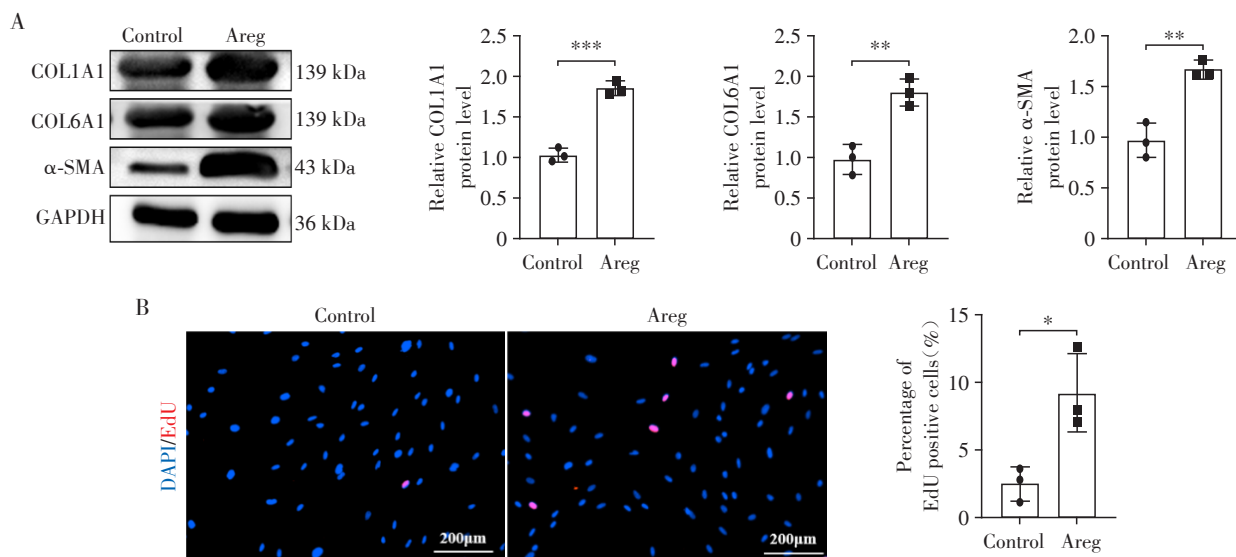
2 结果

2.1 Areg促进肠道成纤维细胞活化及增殖

Western blot检测结果显示,经Areg处理48 h后,肠道成纤维细胞中胶原蛋白COL1A1、COL6A1及活化标志物 α -SMA的表达水平较对照组均显著上调(图1A)。EdU细胞增殖实验进一步证实,Areg可显著增强肠道成纤维细胞的增殖能力(图1B)。上述结果表明,Areg通过促进肠道成纤维细胞的胶原蛋白合成、细胞活化和增殖,在CD相关肠纤维化进程中发挥重要作用。

2.2 IFN- γ 抑制肠成纤维细胞Areg表达

肠道免疫功能紊乱是CD患者肠纤维化发生发展的关键因素,多种细胞因子参与这一病理进程^[10]。TGF- β 作为重要的促纤维化因子,可通过促进细胞外基质合成参与组织修复^[21]。课题组前期研究发



A: Intestinal fibroblasts derived from CD patients were treated with Areg for 48 h, and the expression levels of collagen proteins (COL1A1, COL6A1) and the activation marker α -SMA were analyzed by Western blot. B: The proliferation of intestinal fibroblasts derived from CD patients was assessed using the EdU assay (scale bar=200 μ m). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ ($n=3$).

图1 Areg促进CD患者来源的肠道成纤维细胞活化及增殖

Figure 1 Areg promoted activation and proliferation of intestinal fibroblasts derived from CD patients

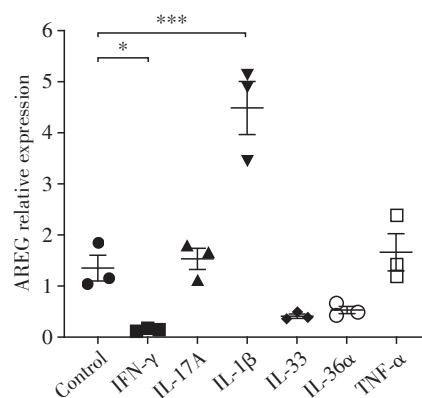
现, TGF- β 可通过促进Treg细胞表达Areg介导CD肠纤维化^[14]。为系统评估不同细胞因子对Areg表达的调控作用, 本研究采用CD合并肠纤维性狭窄患者的人肠道成纤维细胞模型, 设置了空白对照组及6种细胞因子处理组(IFN- γ 、IL-17A、IL-1 β 、IL-33、IL-36 α 、TNF- α)。RT-PCR结果显示, 不同细胞因子对Areg表达的调控呈现差异性: IL-1 β 显著促进肠成纤维细胞Areg的表达, 而IFN- γ 则表现出明显的抑制作用(图2)。这一发现不仅验证了课题组先前的研究结果^[14-15], 更揭示了促炎因子在纤维化调控中的复杂作用。鉴于IFN- γ 对Areg的特异性抑制作用, 推测其可能通过下调Areg表达来减缓纤维化进程。

2.3 IFN- γ 抑制肠成纤维细胞胶原蛋白表达

基于前期研究发现Areg可显著促进肠成纤维细胞胶原蛋白表达^[14]。本研究进一步探讨IFN- γ 是否通过调控Areg影响胶原蛋白合成。提取CD合并肠纤维性狭窄患者的人肠道成纤维细胞, 设置空白对照组、TGF- β (阳性对照)组、IFN- γ 组及TGF- β +IFN- γ 处理组。RT-PCR结果显示, IFN- γ 不仅抑制Areg表达(图3A), 还显著下调 α -SMA(ACTA2)、COL1A1、COL6A1和COL6A3的mRNA水平(图3B~E), 表明IFN- γ 可能通过抑制Areg表达参与调控肠成纤维细胞胶原蛋白合成。

2.4 IFN- γ 抑制肠成纤维细胞活化及增殖

成纤维细胞的活化与增殖是肠纤维化进程中

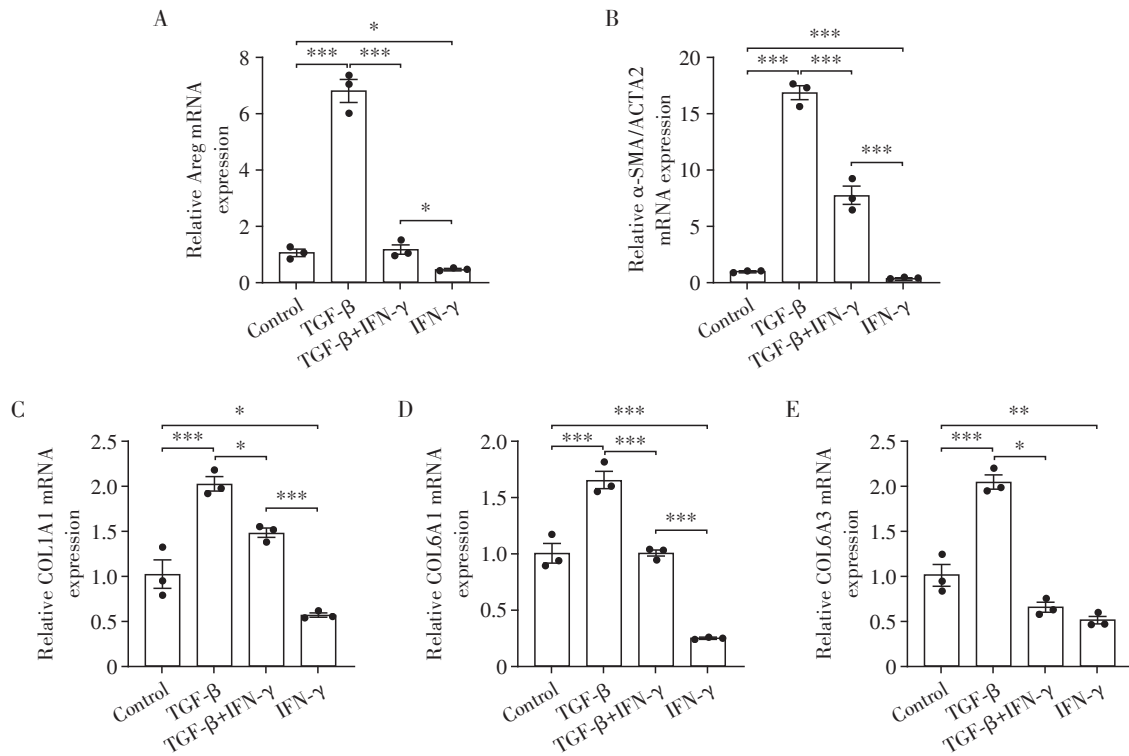


The mRNA expression levels of Areg in intestinal fibroblasts treated with various cytokines (IFN- γ , IL-17A, IL-1 β , IL-33, IL-36 α , and TNF- α) were assessed by RT-PCR. * $P < 0.05$ and *** $P < 0.001$ ($n=3$).

图2 IFN- γ 抑制肠成纤维细胞Areg表达

Figure 2 IFN- γ inhibited Areg expression in intestinal fibroblasts

的核心环节, 涉及 α -SMA等标志物的表达以及细胞运动能力的改变。通过Ki67免疫荧光染色特异性检测增殖期细胞, 以评估IFN- γ 对人肠成纤维细胞增殖能力的作用。结果显示, IFN- γ 处理组中Ki67阳性细胞比例显著低于对照组(图4A、B)。同时, 利用免疫荧光检测细胞活化指标 α -SMA, 发现IFN- γ 处理后显著抑制了CD肠成纤维细胞中 α -SMA的表达(图4A、C), 提示IFN- γ 可能抑制成纤维细胞向肌成纤维细胞的转化。综上, IFN- γ 通过抑制



Human intestinal fibroblasts were treated with TGF-β(5 ng/mL), IFN-γ(20 ng/mL), and the combination of TGF-β + IFN-γ for 48 h. Subsequently, the mRNA expression levels of Areg(A), α-SMA/ACTA2(B), COL1A1(C), COL6A1(D), and COL6A3(E) were detected by RT-PCR. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ ($n=3$).

图3 IFN-γ抑制肠成纤维细胞胶原蛋白表达

Figure 3 IFN-γ suppressed the expression of collagen in intestinal fibroblasts

人肠成纤维细胞的增殖、活化从而减轻CD肠纤维化。

2.5 IFN-γ可能通过抑制SRC下调Areg表达调控肠成纤维细胞活化

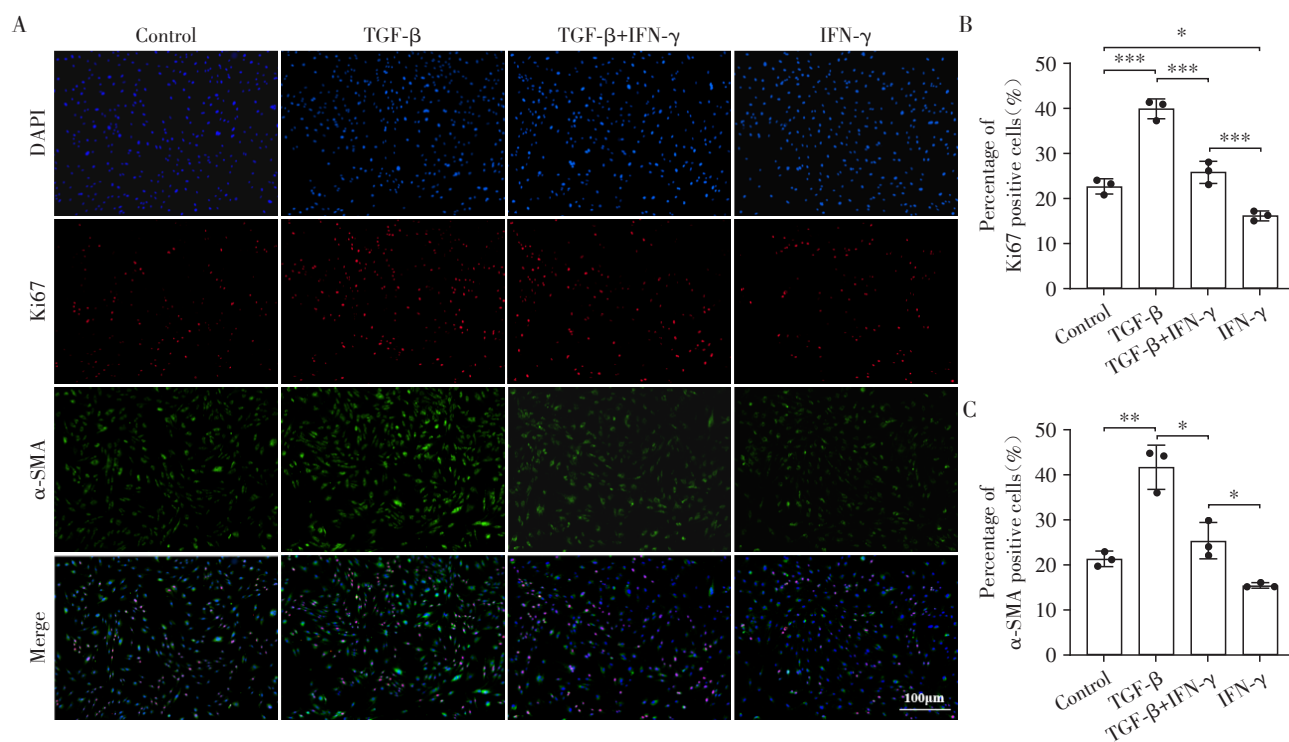
20 ng/mL IFN-γ处理人肠道成纤维细胞48 h后,进行RNA-Seq转录组测序,结果显示,人肠道成纤维细胞中共有650个基因表达显著上调,795个基因显著下调(图5A)。进一步通过STRING数据库构建差异表达基因的蛋白质互作(protein-protein interaction, PPI)网络,并利用Cytoscape软件进行可视化分析。关键枢纽节点分析鉴定出4个连接度>2的核心蛋白,分别为Stat1、Stat3、SRC和Gja1(图5B)。研究表明,Stat1和Stat3是IFN-γ信号通路的关键效应分子,广泛参与细胞分化等生物学过程,其作用机制已较为明确。相比之下,SRC激酶在IFN-γ通路中的功能研究相对较少。值得注意的是,已有研究表明SRC能够与多种膜受体(包括G蛋白偶联受体GPCR、受体酪氨酸激酶RTK如MET/PDGFR/EGFR以及整合素家族)相互作用,进而激活PI3K、RAS/ERK和STAT3等下游信号通路,调控细胞增殖、存

活及血管生成。此外,SRC-FAK信号轴可通过磷酸化p130CAS、桩蛋白和RhoA等底物,协同整合素调控ECM重塑,最终促进细胞迁移、侵袭和转移过程^[22]。有研究报道SRC激酶依赖的Areg自分泌刺激正常人角质形成细胞的增殖与迁移^[23]。因此,推测IFN-γ可能通过抑制SRC激酶的活性降低Areg表达,进而阻断成纤维细胞的活化过程,最终减轻肠道纤维化的发生发展。

3 讨论

本研究系统探讨了IFN-γ-Areg在CD相关肠纤维化中的调控作用,揭示了Areg促进成纤维细胞活化及胶原沉积的促纤维化机制,同时发现IFN-γ可通过抑制Areg表达及下游纤维化相关基因发挥抗纤维化作用。这些发现不仅深化了对CD肠纤维化分子机制的理解,也为靶向干预纤维化进程提供了潜在策略。

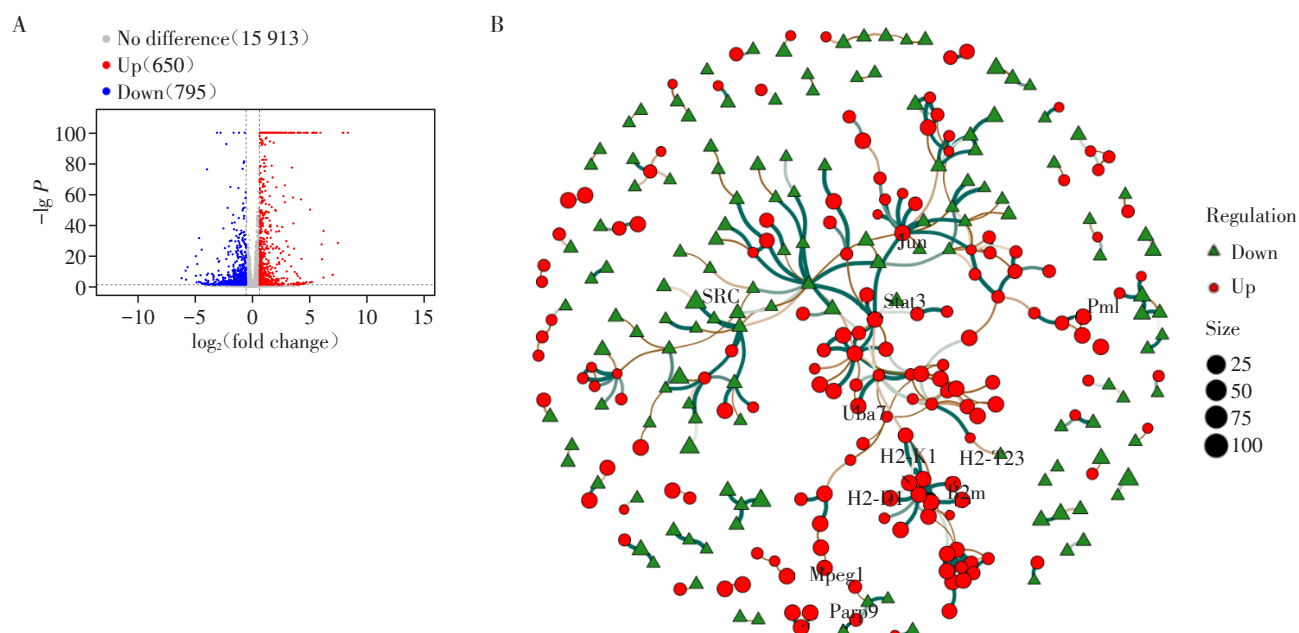
本研究证实,Areg能显著促进肠道成纤维细胞的胶原(COL1A1、COL6A1)合成及活化标志物(α-SMA)的表达,并增强其增殖能力,这与既往研究报道的Areg在组织修复和纤维化中的作用一致^[24]。Areg



A: Representative immunofluorescence images showing the expression of the proliferation marker Ki67 (red) and the activation marker α -SMA (green) in human intestinal fibroblasts treated with or without TGF- β and IFN- γ . Nuclei were counterstained with DAPI (blue, scale bar=100 μ m). B: Quantitative analysis of the percentage of Ki67-positive cells. C: Quantitative analysis of the percentage of α -SMA positive cells. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ ($n=3$).

图4 IFN- γ 抑制肠成纤维细胞活化及增殖

Figure 4 IFN- γ inhibited activation and proliferation of intestinal fibroblasts



A: Transcriptomic profiling by RNA-seq revealed the quantity of differentially expressed genes in fibroblasts. B: Protein-protein interaction network depicting genes (as nodes) and their interactions (edges). Node size corresponds to the degree of connectivity. “Up” and “Down” indicate upregulated and downregulated genes, respectively.

图5 IFN- γ 可能通过抑制SRC下调Areg表达调控肠成纤维细胞活化

Figure 5 IFN- γ regulated the activation of intestinal fibroblasts by downregulating Areg expression through inhibiting SRC

作为表皮生长因子受体(epidermal growth factor receptor, EGFR)的配体,可能通过激活下游信号通路(如MAPK/ERK或PI3K/AKT)促进成纤维细胞向肌成纤维细胞转化,从而驱动纤维化进程^[25]。此外,Areg在多种纤维化疾病(如肺纤维化、肝纤维化)中均被报道可促进基质沉积^[26-27],本研究进一步拓展了其在CD肠纤维化中的关键角色,提示靶向Areg/EGFR通路可能成为缓解纤维化的潜在途径。

本研究首次发现IFN- γ 能显著抑制CD肠成纤维细胞中Areg的表达,并进一步下调 α -SMA、COL1A1和COL6A1等纤维化标志物。这一发现与既往研究报道的IFN- γ 在肝纤维化中抑制肝星状细胞活化^[28]及在系统性硬化症中减少胶原沉积^[29]的抗纤维化作用相吻合。RNA-Seq分析显示肉瘤原癌基因(sarcoma proto-oncogene, SRC)处于差异表达基因网络的核心位置,同时文献支持SRC通过FAK/p130CAS/RhoA等多条通路调控ECM重塑^[22]。并且文献报道SRC激酶依赖的Areg自分泌刺激正常人角质形成细胞的增殖与迁移^[23]。IFN- γ 与SRC家族激酶之间存在复杂的双向调控关系。一方面,SRC通过磷酸化STAT1或修饰IFNGR受体增强IFN- γ 信号传导,促进MHC-II表达和免疫应答^[30];在T/NK细胞中,SRC-MAPK轴正向促进IFN- γ 合成,形成促炎循环^[31-33]。另一方面,IFN- γ 可通过上调SOCS1负调控JAK-STAT信号通路,从而影响下游的细胞增殖和免疫反应^[34],SRC可与JAK-STAT通路相互作用^[35],而SOCS1作为JAK-STAT的负调控因子,可能间接抑制SRC活性。基于这些发现,推测IFN- γ 可能通过抑制“SRC-AREG”信号轴阻断成纤维细胞活化。此外,IFN- γ 与TGF- β 的拮抗作用(TGF- β +Areg组 vs. TGF- β +IFN- γ 组)进一步支持其在调节纤维化微环境中的关键地位。值得注意的是,IFN- γ 在CD中的双重角色仍需谨慎评估。尽管本研究聚焦其抗纤维化潜能,但临床观察显示IFN- γ 在CD炎症期可能加剧Th1型免疫反应^[36]。因此,未来研究需明确IFN- γ 在疾病不同阶段(急性炎症 vs. 慢性纤维化)的时空特异性作用,以优化其治疗应用,深入探讨IFN- γ 可能通过抑制SRC激酶活性发挥抗纤维化作用的机制。

本研究存在一定的局限性。首先,目前的研究结果主要基于体外实验,未来需要通过动物模型,如葡聚糖硫酸钠或2,4,6-三硝基苯磺酸诱导的肠纤维化模型以及临床样本进行进一步验证,以提高结论的可靠性。其次,IFN- γ 调控Areg的具体分子机制尚未完全阐明,例如STAT3是否直接抑制Areg

启动子活性,仍需通过更深入的实验(如ChIP-seq、报告基因检测等)加以探索。

综上所述,Areg通过促进肠道成纤维细胞活化、增殖及胶原合成驱动CD肠纤维化,而IFN- γ 可拮抗这一过程。这些发现为CD纤维化的机制研究和靶向治疗提供了新思路。

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林俊杰负责实验设计、研究实施、数据分析,论文撰写;孙君健负责研究实施、数据分析;王璐、王舒负责方法学、数据分析;张红杰、赵小静提供研究思路,指导数据分析,修改论文修改及提供经费支持。

Author's Contributions:

LIN Junjie was responsible for experimental design, research implementation, data analysis, and manuscript writing; SUN Junjian participated in research implementation and data analysis; WANG Lu and WANG Shu contributed to methodology development and data analysis; ZHANG Hongjie and ZHAO Xiaojing provided research conceptualization, guided data analysis, revised the manuscript, and secured funding support.

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