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· 研究论文 ·

巨噬细胞移动抑制因子通过 Scr激酶信号通路调控心房成纤维细胞增殖与活化

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摘要 该研究旨在探讨巨噬细胞移动抑制因子(macrophage migration inhibitory factor, MIF)通过酪氨酸(Src)激酶调节心房成纤维细胞增殖和活化的作用。首先, 采用血管紧张素II(angiotensin II, Ang II)构建小鼠心房颤动易感模型, 通过Western blot检测MIF表达和Src磷酸化水平, 并利用天狼星红观察*MIF*基因敲除对心房纤维化的影响。其次, 分离成年小鼠心房组织原代细胞, 并采用免疫荧光对所分离的细胞进行鉴定。使用重组MIF(rMIF, 20 ng/mL)及Src激酶抑制剂PP1分别处理细胞24和48 h, CCK-8和EdU实验检测细胞增殖和活化; 免疫荧光和Western blot检测纤维相关蛋白的表达情况。结果显示, 房颤小鼠中MIF表达及Src磷酸化水平均显著升高; *MIF*基因敲除可降低Src磷酸化水平并减轻纤维化。体外实验结果表明, 分离所得的细胞呈现波形蛋白(vimentin)高表达与α-平滑肌动蛋白(α-smooth muscle actin, α-SMA)低表达的特征, 鉴定为心房成纤维细胞。MIF可显著促进心房成纤维细胞的增殖与活化, 而PP1能够有效抑制该效应。MIF通过激活Src激酶, 上调包括COL3A1、COL1A1、MMP-2和TGF-β1在内的纤维化相关蛋白表达, 并诱导Smad2/3磷酸化; 而加入PP1处理则可减弱上述现象。MIF通过Src-Smad2/3信号轴在心房成纤维细胞活化过程中发挥关键作用。

关键词 巨噬细胞移动抑制因子; Src激酶; 心房成纤维细胞; 细胞增殖

Macrophage Migration Inhibitory Factor Promotes the Proliferation and Activation of Atrial Fibroblasts through the Src Kinase Signaling Pathway

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Abstract This study aimed to investigate the role of MIF (macrophage migration inhibitory factor) in regulating the proliferation and activation of atrial fibroblasts through Src kinase. An atrial fibrillation-susceptible

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mouse model was first established using Ang II (angiotensin II). Western blot was performed to detect MIF expression and Src phosphorylation levels, and Sirius red staining was used to evaluate the effect of MIF knockout on atrial fibrosis. Subsequently, primary cells were isolated from the atrial tissue of adult mice and characterized by immunofluorescence. Cells were treated with recombinant MIF (rMIF, 20 ng/mL) and the Src kinase inhibitor PP1 for 24 and 48 h. Cell proliferation and activation were assessed using CCK-8 and EdU assays, while the expression of fibrosis-related proteins was detected via immunofluorescence and Western blot. The results showed that MIF expression and Src phosphorylation were significantly elevated in atrial fibrillation mice. MIF knockout reduced Src phosphorylation and attenuated fibrosis. *In vitro* experiments showed that the isolated cells exhibited a characteristic profile of high vimentin expression and low α-SMA (α-smooth muscle actin) expression, and were identified as atrial fibroblasts. rMIF significantly promoted the proliferation and activation of atrial fibroblasts, whereas PP1 effectively inhibited these effects. Furthermore, MIF activated Src kinase, upregulating the expression of fibrosis-related proteins including COL3A1, COL1A1, MMP-2, and TGF-β1, and induced Smad2/3 phosphorylation. These effects were attenuated by PP1 treatment. In conclusion, MIF plays a key role in atrial fibroblast activation through the Src-Smad2/3 signaling axis.

Keywords macrophage migration inhibitory factor; Src kinase; atrial fibroblasts; cell proliferation

心房颤动(atrial fibrillation, AF)是临床上最常见的心律失常之一, 发病机制复杂, 主要包括心房结构重构和电重构[1]。心房纤维化是心房颤动显著性的特征之一, 亦是心房颤动发生和发展的重要病理基础[2-4]。心房纤维化的主要特征是心房成纤维细胞的异常激活、增殖和活化, 伴随着细胞外基质(extracellular matrix, ECM)蛋白的过度合成和不规则沉积[5]。正常组织中心房成纤维细胞处于静止状态, 产生少量的胶原蛋白和细胞外基质蛋白[6]。然而, 在持续的应激刺激下, 心房成纤维细胞转化为肌成纤维细胞, 并显著促进细胞外基质的产生[7]。尽管纤维化与房颤有关, 但纤维化在房颤发病机制中的确切作用仍有待充分阐明。因此, 深入研究心房成纤维细胞的调控机制对于心房颤动的防治具有重要意义。

近年来, 巨噬细胞移动抑制因子(macrophage migration inhibitory factor, MIF)作为一种多效性细胞因子, 调节免疫、炎症、伤口愈合以及细胞增殖和迁移, 参与许多炎症和纤维化病理生理过程, 在多种疾病中显示出重要的调控作用[8-10]。在心肌梗死、心房颤动等心血管疾病中, MIF已被证实参与炎症反应和组织纤维化过程[11]。然而, MIF在心房成纤维细胞增殖和分泌功能中的具体作用及其潜在机制尚不清楚。Src激酶是一种非受体酪氨酸激酶, 广泛参与细胞增殖、活化和炎症反应的调控[12]。炎症因子可以激活Src激酶在心肌纤维化中发挥重要作用, 促进成纤维细胞的增殖和细胞外基质的合成[13]。推测, MIF与Src激酶之间存在潜在的信号通路联系, MIF可能通过激活Src激酶来影响心房成纤维细胞的生物学行为。

本研究旨在探讨MIF对心房成纤维细胞增殖和分泌功能的影响, 并进一步研究MIF/Src信号通路在心房成纤维细胞中的作用机制。通过靶向干预MIF/Src信号通路, 抑制心房成纤维细胞的增殖和分泌, 为房颤纤维化的治疗提供新的靶点。

1 材料和方法

1.1 材料

胎牛血清、DMEM培养基购自美国Gibco公司; Vimentin单克隆抗体、α-SMA单克隆抗体、Src单克隆抗体、p-Src单克隆抗体、Smad单克隆抗体购自美国Cell Signaling Technology公司; I型胶原蛋白(collage type I, COL1A1)多克隆抗体、III型胶原蛋白(collage type III, COL3A1)多克隆抗体、转化生长因子-β1(transforming growth factor beta 1, TGF-β1)、碱性磷酸酶(alkaline phosphatase, ALP)化学发光试剂盒购自武汉三鹰生物技术有限公司; 基质金属蛋白酶2(matrix metalloproteinase 2, MMP2)多克隆抗体购自北京博奥森生物技术有限公司; 小鼠重组巨噬细胞移动抑制因子(rMIF)购自美国R&D Systems公司; Scr抑制剂PP1购自美国MCE公司; CCK-8试剂盒和EdU-488细胞增殖检测试剂盒购自上海碧云天生物技术有限公司; 山羊抗兔IgG(Alexa Fluor594)和山羊抗兔IgG(Alexa Fluor488)购自英国Abcam公司; BCA蛋白定量试剂盒购自美国ThermoFisher Scientific公司; 辣根过氧化物酶(HRP)标记的山羊抗兔IgG和山

羊抗鼠IgG购自北京中杉金桥生物技术有限公司。

1.2 实验动物

雄性健康成年SPF级WT小鼠30只, 5周龄, 体质量(20±0.5) mg, 购自新疆医科大学实验动物中心, 动物许可证号: SCXK(新)2016-0001。饲养条件: 室温(18~22) °C, 昼夜12 h节律, 自由饮水进食。运用Ang II持续微量灌注野生型C57BL/6小鼠(WT)和*MIF*基因敲除(MIFKO)小鼠, 持续三周建立小鼠心房颤动易感模型, 对照组灌注生理盐水。实验动物分组如下: WT-NaCl、WT-Ang II、MIFKO-NaCl、MIFKO-Ang II。动物实验经新疆医科大学动物实验医学伦理委员会审核通过(批准号: IACUC-JT-20230110-22)。

1.3 小鼠心房成纤维细胞提取、培养及实验分组

小鼠经腹腔注射1%戊巴比妥钠(0.03 mL/kg)麻醉后, 快速取心房组织, 置于PBS中。将心房组织剪成1 mm×1 mm×1 mm的小块, 加入终浓度0.6 g/L胰蛋白酶和0.3 g/L胶原酶II的混合酶液, 37 ℃消化10 min后弃去细胞悬液(含大量血细胞), 加入混合酶液继续消化5 min, 消化至心房组织消失为止(大约9次)。1 500 r/min室温离心10 min后收集细胞, 加入完全培养基(含10% FBS、1%青–链霉素)重悬细胞, 接种于培养皿中, 于37 ℃、5% CO_2培养箱中培养。细胞贴壁70 min后更换DMEM完全培养液, 细胞生长至90%左右时进行传代。细胞分组: Control组、rMIF组、rMIF+PP1组、PP1组。

1.4 CCK-8实验

心房成纤维细胞(密度为1×10⁴/孔)接种到96孔板, 待贴壁后换用含2%血清、且rMIF终浓度分别为0、5、10、20、30、50 ng/mL的新鲜培养基继续培养。培养24 h和48 h后, 每孔加入10 μL CCK-8试剂, 于37 ℃、5% CO_2的条件下孵育2~4 h, 使用酶标仪在450 nm波长下测量吸光度(*D*)值。

1.5 EdU增殖实验

细胞接种到48孔板中, rMIF组中加入20 ng/mL的rMIF, rMIF+PP1组中加入20 ng/mL rMIF和5 μmol/L的PP, PP1组中加入5 μmol/L PPI处理48 h, 细胞与EdU试剂共同室温孵育2 h, 随后用4%多聚甲醛室温固定15 min, Hoechst 33342对细胞核进行复染, 用荧光显微镜拍摄图像。

1.6 细胞免疫荧光

弃去细胞培养基, 在室温下用4%多聚甲醛固定细胞15 min。洗涤后用0.25% Triton进行破膜处理10 min, 经过PBS洗涤后, 含3%牛血清白蛋白(bovine serum albumin, BSA)室温封闭30 min。按照比例用3% BSA稀释一抗, α-SMA(1:200)和Vimentin(1:100), 4 °C条件下孵育过夜。3% BSA稀释Alexa Fluor 488/594标记的二抗(1:400), 室温下孵育60 min。细胞核用DAPI室温孵育10 min, 荧光显微镜观察并拍摄了染色情况。

1.7 蛋白质免疫印迹(Western blot)

细胞培养皿中去除培养基后加入预冷的PBS润洗, 加入RIPA裂解液提取细胞总蛋白。BCA试剂盒进行蛋白定量, 煮沸蛋白变性。10%的SDS-PAGE电泳分离, 湿转法将蛋白转移至PVDF膜上, 5%脱脂牛奶室温封闭1 h, 加入一抗4 ℃孵育过夜, 一抗包括COL1A1(1:1 000)、COL3A1(1:1 000)、Src(1:1 000)、p-Src Tyr416(1:1 000)、α-SMA(1:2 000), TGF-β1(1:500)、MMP-2(1:500)、Smad2(1:1 000)、p-Smad2(1:1 000)、Smad3(1:1 000)、p-Smad3(1:1 000)、MIF(1:1 000)和GAPDH(1:2 500)。加入对应的辣根过氧化物酶二抗(1:5 000), 室温孵育1 h。TBST洗3次, 化学发光显影, 化学发光成像系统采集目的蛋白条带图像并进行分析。

1.8 统计分析

数据分析通过SPSS 22.0统计软件完成, 使用GraphPad Prism 8.0软件作图。所有数据以均值±标准差($\bar{x}\pm s$)表示。两组间均值的比较采用Student's *t*检验; 同组内不同时间点的比较则使用重复测量方差分析。多组间比较采用单因素方差分析(One-Way ANOVA), 并辅以图基检验(Tukey test)进行事后分析。每项实验重复3次, $P<0.05$表示差异具有统计学意义。

2 结果

2.1 *MIF*促进房颤心房纤维化的发展

为了研究*MIF*在心房颤动发生中的作用, 我们检测Ang II灌注后野生小鼠心房中MIF的表达情况, 结果提示与WT-NaCl组相比, WT-Ang II组中MIF蛋白表达水平显著增加($P<0.05$)(图1A)。我们还发现与WT-NaCl组和MIFKO-NaCl组相比, WT-Ang II组中Src蛋白磷酸化水平显著升高($P<0.05$)(图1B); 与MIFKO-NaCl组比较发现, MIFKO-Ang II组中Src蛋白磷酸化水平有升高的趋势, 但无明显差异(图1B)。接下来我们检测*MIF*敲除是否影响心房纤维化, 与

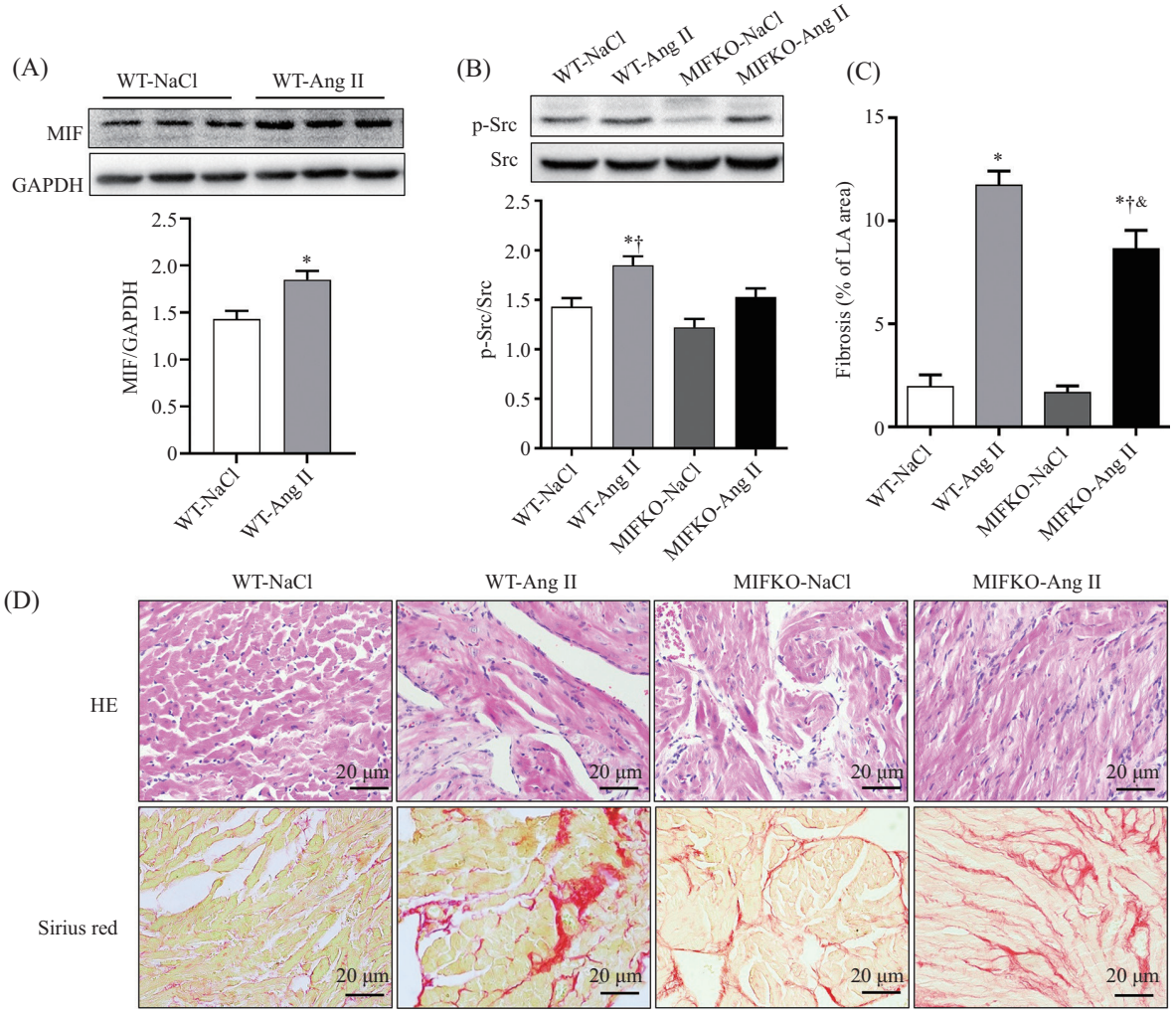
生理盐水灌注组相比，Ang II处理能够显著增加野生小鼠心房纤维化面积(图1C和图1D)，在Ang II灌注的*MIF*基因敲除小鼠中心房纤维化面积显著缩小(P<0.05)(图1C和图1D)，提示敲除*MIF*基因可以减轻心房纤维化，说明*MIF*在心房纤维化中起到重要作用。

2.2 成年小鼠心房成纤维细胞培养及鉴定

原代细胞培养5天后，显微镜下可见成年小鼠心房成纤维细胞呈漩涡状或纵横交错状排列(图2A)。免疫荧光结果显示，这些细胞不表达α-SMA，而Vimentin呈阳性表达(图2B)。这证实分离培养的细胞为高纯度的心房成纤维细胞。

2.3 Src激酶抑制剂PP1抑制MIF诱导的心房成纤维细胞增殖

为明确MIF对心房成纤维细胞的增殖的影响，通过CCK-8检测心房成纤维细胞增殖情况。结果显示24 h时，rMIF浓度从5 ng/mL到30 ng/mL对心房成纤维细胞有增殖作用，但增殖作用不显著(图3A)；48 h时，20 ng/mL rMIF组与其他组比较有显著性差异(P<0.05)，说明此浓度可以促进心房成纤维细胞的增殖(图3B)。在rMIF刺激前，用5 μmol/L PP1预处理心房成纤维细胞1 h，CCK-8实验结果提示PP1完全抑制了MIF诱导的心房成纤维细胞增殖(图3C)。同时EdU实验结果提示MIF诱导心房成纤维细胞增殖

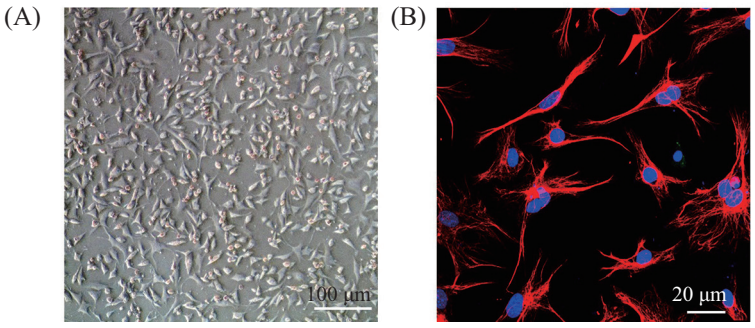


A: Western blot检测*MIF*蛋白在心房中的表达情况; B: Western blot检测p-Src蛋白在心房中的表达情况; C: 左心房纤维化面积; D: HE染色和天狼星红染色。*P<0.05, 与WT-NaCl组比较; &P<0.05, 与WT-Ang II组比较; †P<0.05, 与MIFKO-NaCl组比较。

A: Western blot was used to detect the expression of MIF in the atrial tissue; B: Western blot was used to detect the expression of p-Src in the the atrial tissue; C: left atrial fibrosis area; D: HE staining and Sirius Red staining. *P<0.05 compared with WT-NaCl, &P<0.05 compared with WT-Ang II, †P<0.05 compared with MIFKO-NaCl.

图1 *MIF*基因敲除减轻心房中的纤维化

Fig.1 The knockout of the *MIF* gene alleviates fibrosis in the atria



A: 显微镜观察心房成纤维细胞的形态; B: 免疫荧光检测心房成纤维细胞表面标志物α-SMA和Vimentin的表达情况。

A: observation of the morphology of atrial fibroblasts with microscope; B: immunofluorescence was used to detect the expression of the fibroblast surface markers α-SMA and Vimentin in atrial fibroblasts.

图2 成年小鼠心房成纤维细胞原代培养及鉴定

Fig.2 Primary culture and identification of atrial fibroblasts from adult mice

明显, PP1抑制了MIF诱导的心房成纤维细胞增殖(图3D)。这些结果表明, MIF通过Src激酶依赖机制诱导心房成纤维细胞细胞增殖, 特异性Src拮抗剂PP1抑制细胞增殖。

2.4 MIF促进心房纤维细胞活化

成纤维细胞活化的关键标志是其α-平滑肌肌动蛋白(α-SMA)表达的上调。免疫荧光与Western blot结果一致表明, rMIF处理组中α-SMA蛋白表达水平显著升高(图4A和图4B), 说明MIF能够促进成纤维细胞活化为肌成纤维细胞。

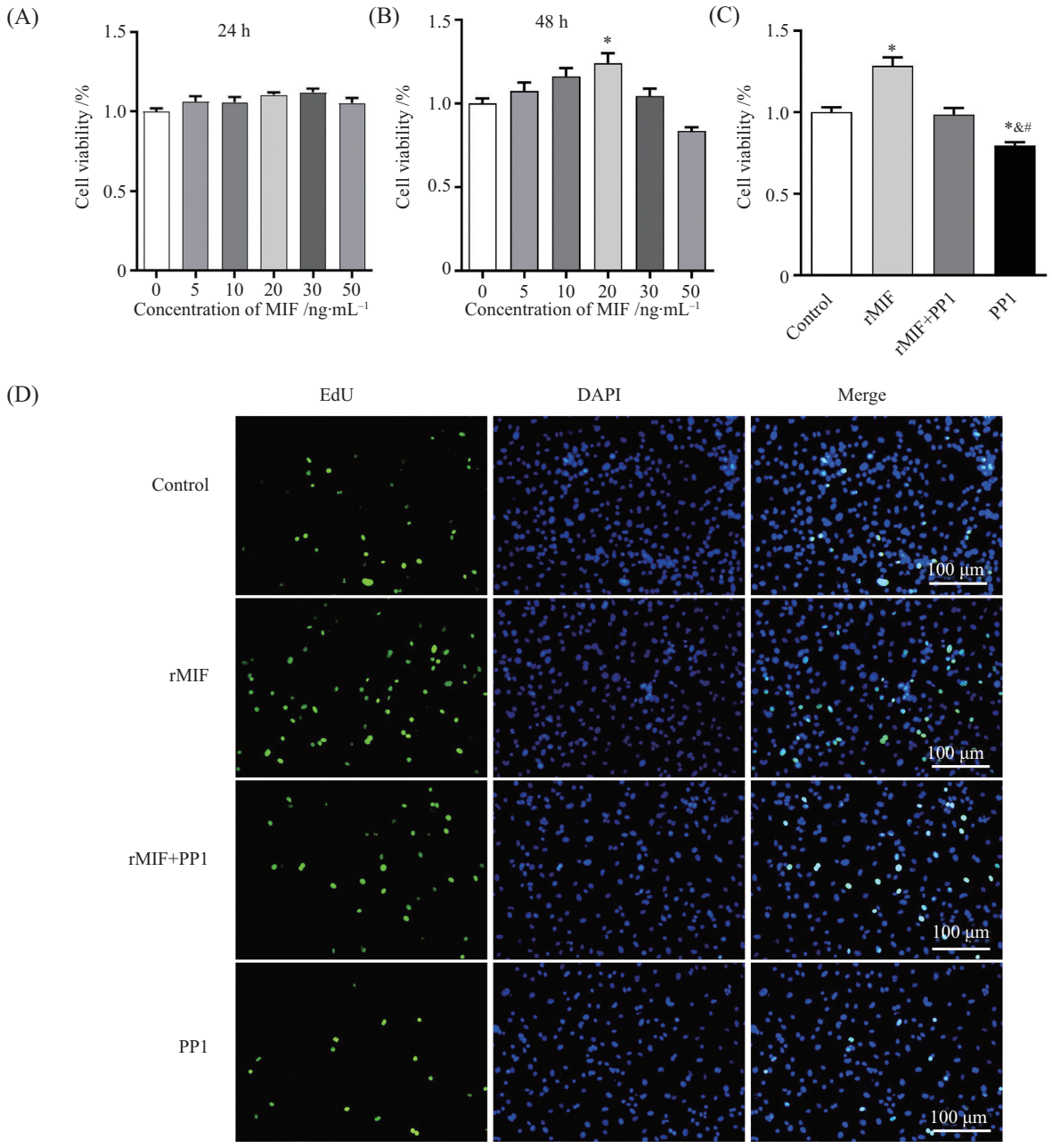
2.5 MIF通过Scr激酶信号通路调控心房成纤维细胞的活化

纤维化发展中的核心环节是成纤维细胞向肌成纤维细胞转化, 后者可分泌多种促纤维化因子。免疫荧光结果显示, 与对照组相比, rMIF组中α-SMA表达水平显著升高, 而PP1干预组中其表达水平较rMIF组明显降低(P<0.05)(图5A)。Western blot结果进一步表明, 与对照组相比, rMIF组的COL1A1、COL3A1、MMP-2、α-SMA、TGF-β1、p-Src及p-Smad2/3等纤维化相关蛋白的表达水平显著升高(P<0.05)(图5B和图5C); PP1组与rMIF组比较发现, PP1干预则逆转了上述升高趋势(图5B和图5C), 由此表明, MIF通过激活Src激酶信号通路促进心房成纤维细胞活化及胶原分泌, 而特异性阻断Src可有效抑制MIF介导的促纤维化效应。

3 讨论

心房颤动发生和持续可加重心肌重塑, 最终发展为心力衰竭[14]。心房纤维化是心房心肌病的标志, 在心房颤动的发生和进展过程中发挥关键作用, 其机制涉及牵张诱导的成纤维细胞活化、氧化应激、炎症和凝血途径等多方面[15]。心房纤维化主要特征是成纤维细胞的异常激活、增殖和活化, 以及细胞外基质蛋白的过度合成和不规则沉积, 其本质是胶原蛋白I和III合成与其降解失衡, 从而促进心房颤动的发展[5,16-17]。最近的研究表明吡非尼酮、吡格列酮和白藜芦醇等药物可减轻心肌纤维化并降低心房颤动的发病率[18]。丹参酮可通过抑制TGF-β1/Smad2/3和TXNIP/NLRP3炎症体信号通路来减轻心房间质纤维化, 进一步证实抗纤维化策略有治疗潜力[19]。我们体内实验显示, 在Ang II诱导的野生小鼠模型中*MIF*蛋白表达水平升高且心房纤维化加重; 而在*MIF*基因敲除小鼠中, 相同刺激下心房纤维化显著减轻, 说明*MIF*通过促进Src磷酸化加速心房纤维化, 有望成为新的抗纤维化干预靶点。

炎症是机体对感染或损伤的保护性反应, 如果不加以控制, 炎症会导致细胞因子和活性氧的过度产生, 从而导致疾病发生[20]。近年研究证实, 心肌局部炎症可造成心房损伤并驱动纤维化, 进而促进心房颤动(AF)的发生与维持; 因此, 抑制炎症–纤维化轴有望成为干预AF的新策略[21]。研究表明炎症促进房颤的发生, C-反应蛋白(C-reactive protein, CRP)、白细胞介素6(interleukin 6, IL-6)、白细胞介素8(interleukin 8, IL-8)和肿瘤坏死因子-α(tumor necrosis factor-alpha, TNF-α)与房颤的发病率呈正相关, 且这可以预测AF消融术后复发的风险[22]。细胞受到炎症刺激时, 成纤维细胞增殖并活化为分泌表型的肌成纤维细胞。肌成纤维细胞具有收缩特性, 有



A、B: 不同浓度的rMIF处理24 h和48 h心房成纤维细胞的增殖情况; C: CCK-8检测PP1对rMIF诱导的心房成纤维细胞增殖的影响; D: EdU检测显示PP1抑制了rMIF诱导的心房成纤维细胞的增殖。*P<0.05, 与control组比较; &P<0.05, 与rMIF组比较; #P<0.05, 与rMIF+PP1组比较。

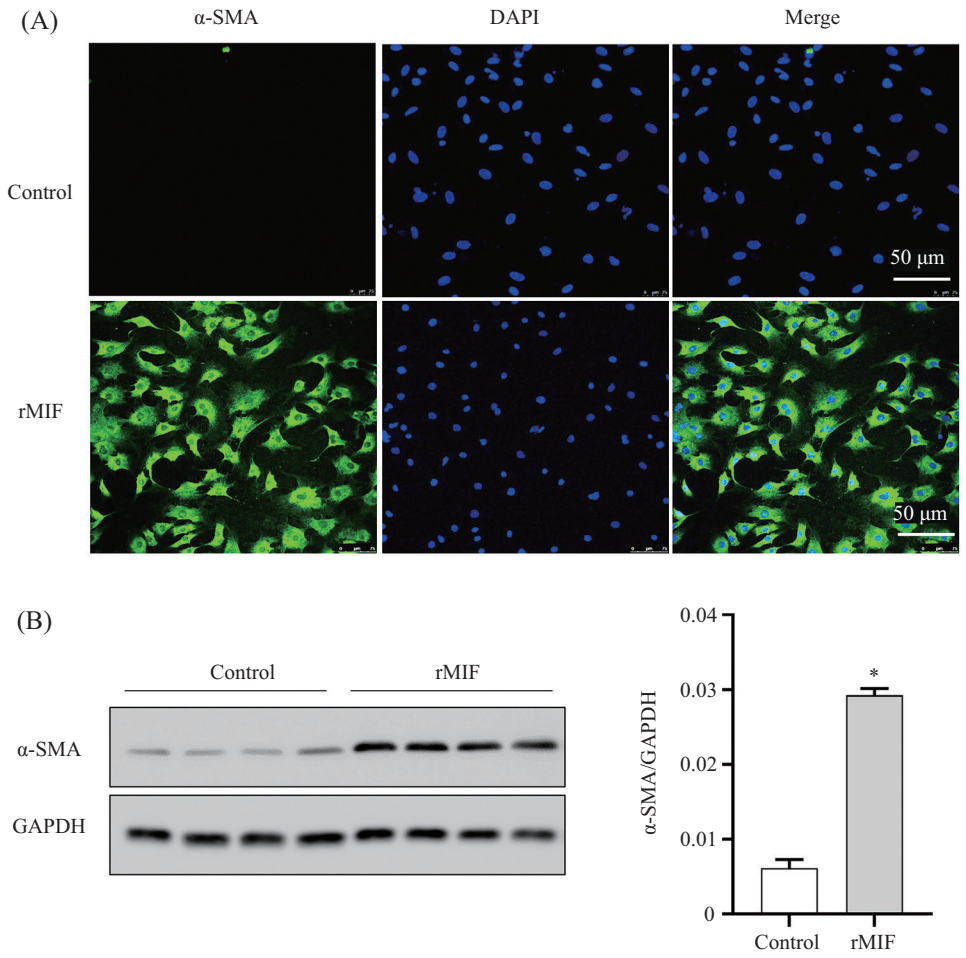
A,B: the proliferation of atrial fibroblasts after treatment with different concentrations of rMIF for 24 h and 48 h; C: the effect of PP1 on rMIF-induced proliferation of atrial fibroblasts was assessed by CCK-8 assay; D: the EdU assay demonstrated that PP1 inhibited the proliferation of atrial fibroblasts induced by rMIF. *P<0.05 compared with control group; &P<0.05 compared with rMIF group; #P<0.05 compared with rMIF+PP1 group.

图3 Src激酶抑制剂PP1抑制rMIF促进心房成纤维细胞增殖

Fig.3 The Src kinase inhibitor PP1 suppresses rMIF-induced atrial fibroblasts proliferation

助于瘢痕收缩, 而α-平滑肌肌动蛋白(α-SMA)的表达是其关键的标志性表型[23]。心脏成纤维细胞体积大且形态多样多变, 主要以梭形或星形为主。Vimentin是心脏成纤维细胞的主要标记物, 通过与其中间丝结合而发挥作用[24]; 而α-SMA则是肌成纤维细胞的特异性表达蛋白, 二者可用于鉴别成纤维细胞与肌成纤维细胞。我们的研究结果表明分离的原代细胞为心房成纤维细胞。

MIF是一种多效性炎性细胞因子, 是多种急性和慢性炎症性疾病的介质[25]。心房颤动患者中检测到MIF水平升高提示MIF可能在心房颤动的发病机制中起重要作用[26]。MIF与离子通道异常和心房纤维化



A: 免疫荧光检测control 组和rMIF组α-SMA的荧光表达情况; B: Western blot实验检测control 组和rMIF组中α-SMA蛋白表达情况。*P<0.05, 与control组相比。

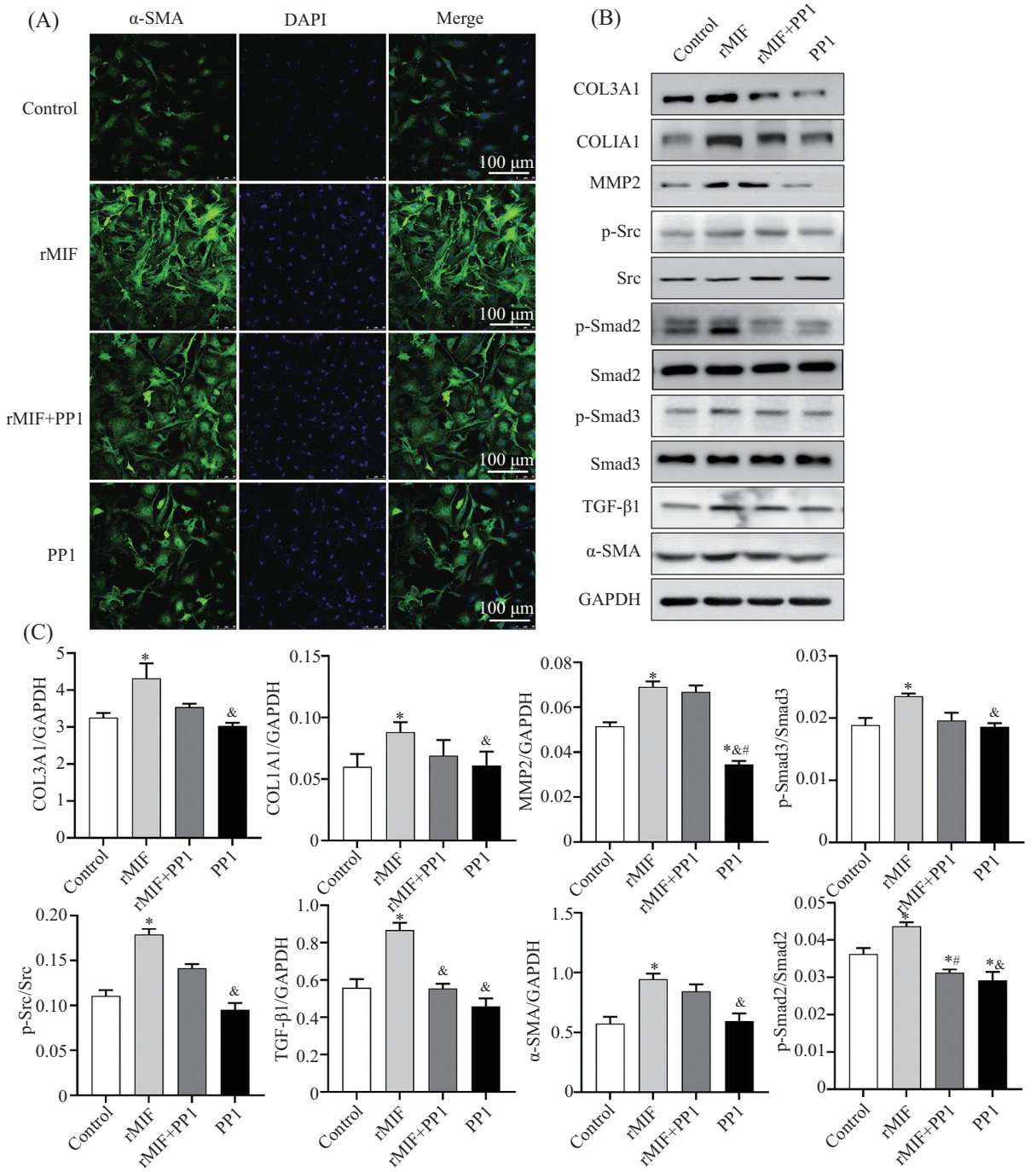
A: immunofluorescence staining was performed to assess α-SMA expression levels in the control and rMIF-treated groups. B: Western blot analysis was performed to detect the expression of α-SMA protein in the control group and the rMIF group. *P<0.05 compared with control group.

图4 MIF促进心房成纤维细胞的活化

Fig.4 MIF promotes the activation of atrial fibroblasts

的风险增加有关[27-28]。MIF的功能取决于其细胞来源, 可发挥促炎或抗炎作用, 从而增加靶向治疗的难度。MIF与败血症、类风湿性关节炎、肾小球肾炎、多发性硬化、动脉粥样硬化等自身免疫和炎症疾病有关[29], MIF调节免疫和炎症反应, 加重组织损伤和加快病理进程。白细胞*MIF*缺失(WT^{KO})小鼠显示出抑制局部炎症反应和减轻心脏重塑的效果, 表明炎症细胞来源的*MIF*主要发挥促炎作用, 而心源性*MIF*(WT^{KO})能够促进成纤维细胞的活化和增殖[30-31]。我们的研究结果显示分离的成纤维细胞给予外源性MIF后促进心房成纤维细胞的增殖和活化。

Src是蛋白酪氨酸激酶(protein tyrosine kinase, PTK)家族的重要成员, 参与细胞增殖、存活和迁移[32]。Src的激活可以促进细胞生存和增殖, 甚至诱发恶性肿瘤[33]。高糖和炎症因子(TNF-α、IFN-γ和MIF)均可激活Src[13,34-35]。MIF可通过激活Src激酶等下游信号通路发挥作用, 而Src激酶作为TGF-β1信号通路中关键的上游信号转导因子, 其激活促进Smad2/3的磷酸化使其从细胞质向细胞核转运, 从而调节下游基因的转录[36-37]。心肌梗死中, MIF的释放量增加, 激活Src激酶, 进一步促进Smad2/3的激活, 导致心肌纤维化和心脏重塑[38]。Src激酶参与心房肌细胞钙离子通道的调节, 在心房电重构中起到重要作用[39]。我们的研究发现, 特异性Src拮抗剂PP1能够抑制MIF诱导的心房成纤维细胞的增殖。此外, Src特异性拮抗剂PP1减弱MIF对心房成纤维细胞功能的影响, 从而抑制MIF诱导的纤维化相关的因子的蛋白表达。



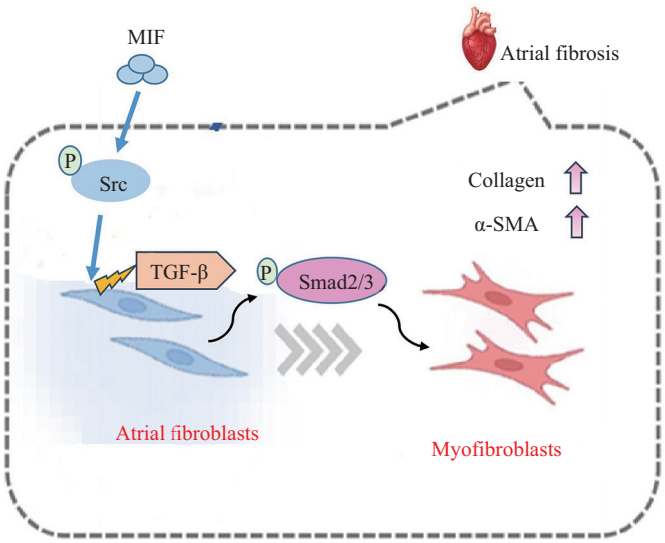
A: 免疫荧光检测不同组α-SMA表达情况; B、C: Western blot 检测COL1A1、COL3A1、MMP2、TGF-β1、α-SMA、p-Src、p-Smad2和p-Smad3在不同组中的表达情况。*P<0.05, 与control相比; $^{\&}P$<0.05, 与 rMIF组相比; $^{\#}P$<0.05, 与rMIF+PP1组相比。

A: immunofluorescence staining was performed to assess α-SMA expression in the control, rMIF, rMIF+PP1, and PP1 groups. B,C: Western blot analysis was performed to detect the expression of COL1A1, COL3A1, MMP2, TGF-β1, α-SMA, p-Src, p-Smad2 and p-Smad3 in the control, rMIF, rMIF+PP1, and PP1 groups. *P<0.05 compared with control group, $^{\&}P$<0.05 compared with rMIF group, $^{\#}P$<0.05 compared with rMIF+PP1 group.

图5 MIF通过Scr激酶信号通路调控心房成纤维细胞的活化

Fig.5 MIF regulates the activation of atrial fibroblasts via the Src kinase signaling pathway

因此，我们研究表明MIF刺激Src激酶磷酸化，从而促进心房成纤维细胞的增殖和纤维化相关因子的产生，激活Src激酶磷酸化和激活TGF-β1/Smad2/3信号通路；而给予Src激酶抑制剂PP1可抑制心房成纤维细胞的增殖和活化，并抑制Src激酶和Smad2/3的磷酸化。这表明MIF-Src-Smad2/3轴在成纤维细胞活化中起关键作用，这为探寻心房纤维化的治疗提供新的潜在靶点(图6)。



MIF激活Src激酶进而激活TGF-β1/Smad2/3信号有助于心房成纤维细胞中纤维化相关基因表达水平增高, 活化为肌成纤维细胞。
MIF activates Src kinase, which subsequently triggers the TGF-β1/Smad2/3 signaling pathway. This cascade upregulates the expression of fibrosis-associated genes in atrial fibroblasts and drives their differentiation into myofibroblasts.

图6 MIF促进心房成纤维细胞活化

Fig.6 MIF promotes atrial fibroblast activation

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