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· 论 著 ·

lncRNA FAIF1 调控 miRNA-424-5p/Smad7 轴抑制晚期糖基化终产物诱导的心肌成纤维细胞功能紊乱

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[摘要] **目的** 探讨 lncRNA 成纤维细胞活化抑制因子 1 (FAIF1) 调控晚期糖基化终产物 (AGE) 诱导的人心肌成纤维细胞增殖、活化及纤维化的机制。**方法** 将人心肌成纤维细胞分为对照组、AGE 组、FAIF1 重组慢病毒 (Lv-FAIF1) + AGE 组和对对照慢病毒 (Lv-control) + AGE 组。通过 qPCR 和蛋白质印迹法检测 AGE 诱导下人心肌成纤维细胞中 miRNA-424-5p、FAIF1 及 Smad7 的表达水平。通过生物信息学分析预测 miRNA-424-5p、FAIF1 和 Smad7 的相互关系, 并用萤光素酶报告基因实验验证。利用 CCK-8 检测细胞增殖活性, 免疫荧光染色观察 I / III 型胶原蛋白的表达分泌情况, qPCR 评估 Lv-FAIF1 对 AGE 诱导细胞活化标志物 α -平滑肌肌动蛋白 (α -SMA) 和基质金属蛋白酶 9 (MMP9) 表达的影响。**结果** qPCR 及蛋白质印迹法检测结果显示, AGE 诱导后人心肌成纤维细胞中 FAIF1 和 Smad7 表达降低, miRNA-424-5p 表达升高 (与对照组比较, 均 $P < 0.05$)。生物信息学分析显示, Smad7 mRNA 3'-非翻译区存在 miRNA-424-5p 结合位点 (序列为 UGCUGCU), 而 FAIF1 序列中存在 3 个相同的结合位点。萤光素酶报告基因实验显示, miRNA-424-5p 抑制 Smad7 的表达, 而 FAIF1 通过竞争性结合 miRNA-424-5p 解除 miRNA-424-5p 对 Smad7 mRNA 的抑制作用。功能实验显示, Lv-FAIF1 能够抑制 AGE 诱导的细胞增殖、I / III 型胶原蛋白表达分泌及 α -SMA 和 MMP9 表达 (与 AGE 组比较, 均 $P < 0.01$), 促进 Smad7 的表达 (与 AGE 组比较, $P < 0.01$)。**结论** miRNA-424-5p 抑制 Smad7 的表达, FAIF1 通过调控 miRNA-424-5p/Smad7 轴有效抑制 AGE 诱导的人心肌成纤维细胞过度活化。本研究为糖尿病心肌病的防治提供了新的分子靶点。

[关键词] 长链非编码 RNA; 微 RNA-424-5p; Smad7; 心肌成纤维细胞; 晚期糖基化终产物

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lncRNA FAIF1 regulates the inhibitory effect of miRNA-424-5p/Smad7 axis against cardiac fibroblast dysfunction induced by advanced glycation end products

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[Abstract] **Objective** To explore the mechanism of long non-coding RNA fibroblast activation inhibitory factor 1 (FAIF1) regulates the proliferation, activation, and fibrosis of human cardiac fibroblasts induced by advanced glycation end products (AGEs). **Methods** Human cardiac fibroblasts were assigned to control group, AGE group, FAIF1 recombinant lentivirus (Lv-FAIF1) + AGE group or control lentivirus (Lv control) + AGE group. The expression levels of miRNA-424-5p, FAIF1, and Smad7 in myocardial fibroblasts induced by AGEs were detected by quantitative polymerase chain reaction (qPCR) and Western blotting. Bioinformatics analysis was used to predict the interactions between miRNA-424-5p, FAIF1, and Smad7; and luciferase reporter assays were used for verification. Cell proliferation activity was measured by cell counting kit 8 assay, the expression and secretion of collagen I / III were observed by immunofluorescence staining, and the effect of Lv-FAIF1 on cell activation markers α -smooth muscle actin (α -SMA) and migration proteins matrix metalloproteinase 9 (MMP9) induced by AGEs was evaluated by qPCR. **Results** qPCR and Western blotting results showed that AGEs significantly reduced the expression of FAIF1 and Smad7 in myocardial fibroblasts and upregulated the level of miRNA-424-5p (compared with the control group, all $P < 0.05$). Bioinformatics analysis revealed that the 3'-untranslated region of Smad7

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mRNA contained a binding site for the miRNA-424-5p seed sequence “UGCUGCU”, and FAIF1 sequence contained 3 identical binding sites. Luciferase assays showed that miRNA-424-5p inhibited the expression of *Smad7*, while FAIF1 competed with miRNA-424-5p for binding, thereby relieving the inhibitory effect of miRNA-424-5p on *Smad7* mRNA. Functional experiments showed that Lv-FAIF1 significantly inhibited AGEs-induced cell proliferation, collagen I/III expression and secretion, as well as α -SMA and *MMP9* expression (compared with AGE group, all $P < 0.01$); and it promoted the expression of *Smad7* (compared with AGE group, $P < 0.01$). **Conclusion** miRNA-424-5p can inhibit the expression of *Smad7*, and FAIF1 effectively suppresses AGEs-induced over-activation of cardiac fibroblasts by regulating the miRNA-424-5p/*Smad7* axis, which provides a new molecular target for the prevention and treatment of diabetic cardiomyopathy.

[Key words] long non-coding RNA; microRNA-424-5p; *Smad7*; cardiac fibroblasts; advanced glycation end products

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心肌纤维化是心血管疾病的重要病理特征之一,在糖尿病心脏病中尤为突出^[1-2]。糖尿病引发的心肌纤维化主要由代谢紊乱^[3]、氧化应激^[4-5]、晚期糖基化终产物(advanced glycation end product, AGE)的积累^[6-7]及相关炎症反应驱动,这些因素共同导致心肌结构的异常重塑和功能损伤。AGE是由糖分子与蛋白质、脂质或核酸的非酶促反应(糖基化反应)生成的一类复杂化合物^[8]。AGE在正常代谢中少量生成,但在高血糖、衰老和氧化应激状态下,其生成速度显著加快^[9]。AGE通过与其受体结合激活纤维化相关的信号通路,在糖尿病心肌纤维化中发挥重要作用^[6-7],但其机制尚未阐明。

近年来,随着分子生物学技术的发展,非编码RNA尤其是lncRNA在心肌纤维化中的作用受到广泛关注^[10-11]。lncRNA通过与miRNA竞争性结合调控基因表达,这一竞争性内源RNA网络在心肌纤维化的调控中扮演着重要角色^[12]。本课题组在前期研究中通过高通量筛选得到1个在AGE诱导的人心肌成纤维细胞中表达明显降低的lncRNA,命名为成纤维细胞活化抑制因子1(fibroblast activation inhibitory factor 1, FAIF1)。FAIF1定位于人类第21号染色体,此前未见关于其与心肌纤维化关系的研究报道。通路富集分析显示,FAIF1是心肌纤维化调控通路TGF- β /*Smad*通路的抑制因子,且其作用途径与miRNA-424-5p/*Smad7*调控轴的失活有关。心肌成纤维细胞过度活化是心肌纤维化进展的重要病理基础和推动因素^[13],本研究旨在明确FAIF1在AGE诱导的心肌成纤维细胞活化中的作用并探讨其作用机制,为糖尿病心脏病的干预提供新的理论依据和潜在治疗靶点。

1 材料和方法

1.1 主要试剂和材料 人心肌成纤维细胞(上海经科化学科技有限公司),成纤维细胞培养基(美国Invitrogen公司),AGE和DAPI染色液(美国Sigma-Aldrich公司),TRIzol RNA提取试剂(美国Invitrogen公司),SYBR Green PCR试剂盒(日本Takara公司),M-PER蛋白质提取试剂盒(美国ThermoFisher Scientific公司),BCA蛋白定量试剂盒(美国Pierce公司),CCK-8试剂盒(日本同仁化学研究所);所有抗体均购自美国Cell Signaling Technology公司;重组慢病毒Lv-FAIF1和Lv-control购自山东维真生物科技有限公司;ABI 7500实时荧光定量PCR仪为美国ThermoFisher Scientific公司产品;pGL3萤光素酶报告质粒和萤光素酶检测系统均购自德国普洛麦格公司。miRNA-424-5p mimic、miRNA-424-5p inhibitor、miRNA-424-5p阴性对照(negative control, NC),以及各种引物均由上海吉玛制药技术有限公司设计、合成。

1.2 细胞培养与处理 人心肌成纤维细胞培养于专用培养基中,37℃、5% CO₂条件下维持生长。将细胞分为对照组、AGE组、重组慢病毒Lv-FAIF1干预+AGE组(Lv-FAIF1+AGE组)、对照慢病毒Lv-control干预+AGE组(Lv-control+AGE组)。对照组予以与AGE组等体积的PBS处理;AGE组予以200 μ g/mL的AGE处理48 h;Lv-FAIF1+AGE组予以感染复数为10的重组慢病毒Lv-FAIF1感染72 h,随后加入200 μ g/mL的AGE处理48 h;Lv-control+AGE组予以感染复数为10的重组慢病毒Lv-control感染72 h,随后加

入 200 $\mu\text{g/mL}$ 的 AGE 处理 48 h。

1.3 lncRNA 转录组测序 采用 TRIzol RNA 提取试剂提取细胞总 RNA, 进行 lncRNA 转录组测序。lncRNA 转录组测序由北京百迈客生物科技有限公司完成。使用 DESeq2 软件进行差异表达分析。差异表达标准: $|\log_2(\text{FC})| > 3$ [FC 为差异表达倍数 (fold change)] 且 $P < 0.05$ 。

1.4 qPCR 检测 采用 TRIzol RNA 提取试剂提取细胞总 RNA, 进行 qPCR 检测。qPCR 使用 SYBR Green 试剂盒, 反应体系总量 20 μL , 采用 ABI 7500 实时荧光定量 PCR 仪进行扩增。通过 $2^{-\Delta\Delta C_t}$ 法计算目的 RNA 的相对表达量。引物序列: miRNA-424-5p 特异反转录引物为 5'-GTCG-TATCCAGTGCGTGTCTGAGTCGGCAATTG-CACTGGATACGAGTCGT-3', U6 特异反转录引物为 5'-TACCTTGCGAAGTGCTTAAAC-3'。U6 正向 PCR 引物为 5'-GTGCTCGCTTCGGCAGCACA-T-3', 反向 PCR 引物为 5'-TACCTTGCGAAGTGCT-TAAAC-3'; miRNA-424-5p 正向 PCR 引物为 5'-GCCGGCGCCCGAGCTCTGGCTC-3', 反向 PCR 引物为 5'-AAGTTTTGTACTTAACGACGAC-3'; Smad7 正向 PCR 引物为 5'-GGAGGAGGCGAGCT-GCGGGGAGAA-3', 反向 PCR 引物为 5'-CTGGCG-GGTGGGGATGGTGGTGAC-3'; β -actin 正向 PCR 引物为 5'-CCTGTACGCCAACACAGTGC-3', 反向 PCR 引物为 5'-ATACTCTGCTTGCTGATCC-3'; α -平滑肌肌动蛋白 (α -smooth muscle actin, α -SMA) 正向 PCR 引物为 5'-GCACAGGCCAGAGCGATG-AT-3', 反向 PCR 引物为 5'-GTTTTTGGTTTACCCGA-AGTTT-3'; 基质金属蛋白酶 9 (matrix metalloproteinase 9, MMP9) 正向 PCR 引物为 5'-CTGGGCAAGGGC-GTCGTGGTTC-3', 反向 PCR 引物为 5'-TCGGTGG-TGCAGGCAGAGTAGGAG-3'。PCR 反应条件: 95 $^{\circ}\text{C}$ 预变性 10 min; 95 $^{\circ}\text{C}$ 变性 5 s, 56 $^{\circ}\text{C}$ 退火 10 s, 72 $^{\circ}\text{C}$ 延伸 15 s, 共 42 个循环。

1.5 蛋白质印迹法 使用 M-PER 蛋白质提取试剂盒提取细胞总蛋白, 用 BCA 法定量后, 每孔加入等量蛋白样品进行 SDS-PAGE, 并转移至 PVDF 膜。分别用一抗 (Smad7 抗体、I / III 型胶原蛋白抗体) 和二抗孵育, 随后用 ECL 显影液显影, 利用凝胶成像系统分析蛋白条带, 以 β -actin 作为内参。

1.6 生物信息学分析和萤光素酶报告基因实验 先从

美国国家生物技术信息中心数据库查找人 Smad7 mRNA 3'-非翻译区 (3'-untranslated region, 3'-UTR) 序列, 然后用 TargetScanHuman 7.1 软件预测序列中的 miRNA-424-5p 种子区结合位点。通过萤光素酶报告基因实验验证 miRNA-424-5p 与 Smad7 mRNA 3'-UTR 的结合及 FAIF1 对 miRNA-424-5p/Smad7 通路的调控作用。构建含 Smad7 3'-UTR 野生型 (wild type, wt) 或突变型 (mutant type, mt) 结合位点的 pGL3 萤光素酶报告质粒 (pGL3-wt-Smad7 和 pGL3-mt-Smad7) 并转染细胞, 或将质粒分别与 miRNA-424-5p mimic、miRNA-424-5p inhibitor、miRNA-424-5p NC 及 Lv-FAIF1 或 Lv-control 共转染细胞。慢病毒感染复数值设定为 10, miRNA-424-5p mimic、miRNA-424-5p inhibitor、miRNA-424-5p NC 及转染试剂用量均参照转染试剂说明书。48 h 后, 使用萤光素酶检测系统测量荧光强度。

1.7 细胞增殖检测 利用 CCK-8 试剂盒检测细胞增殖活性。将细胞接种于 96 孔板, 每孔加入 10 μL CCK-8 试剂后, 37 $^{\circ}\text{C}$ 孵育 2 h, 随后在 450 nm 波长下用酶标仪测定光密度值, 并计算细胞相对增殖率。

1.8 免疫荧光染色 通过免疫荧光染色检测 I / III 型胶原蛋白表达。细胞于 4% 多聚甲醛溶液中固定, 封闭后与一抗 (I / III 型胶原蛋白抗体) 孵育过夜, 随后用 Alexa Fluor 488/594 荧光标记的二抗分别孵育, DAPI 染核。在荧光显微镜下观察结果。

1.9 统计学处理 使用 GraphPad Prism 8.0 软件进行数据分析。首先运用 Shapiro-Wilk 检验对数据分布进行联合正态性评估, 符合正态分布的数据以 $\bar{x} \pm s$ 表示, 通过 Bartlett 检验确认方差齐性后, 两组间比较采用非配对双尾 Student t 检验, 多组间比较采用单因素方差分析结合 Tukey 多重比较进行事后检验; 不符合正态分布的数据则以中位数及四分位距 (interquartile range, IQR) 形式描述。检验水准 (α) 为 0.05。

2 结果

2.1 AGE 诱导下人心肌成纤维细胞 lncRNA 表达的差异分析及验证 lncRNA 测序分析结果显示, 在 AGE 诱导下人心肌成纤维细胞有 108 个 lncRNA 存在差异表达 ($|\log_2(\text{FC})| > 3$, $P < 0.05$), 其中 43 个上调、65 个下调 (图 1)。选择下调 lncRNA

中差异表达最显著的前5个lncRNA,通qPCR检测其在AGE诱导下人心肌成纤维细胞的表达水平,结果显示FAIF1的表达与测序数据一致。

2.2 AGE抑制人心肌成纤维细胞中FAIF1及Smad7表达并上调miRNA-424-5p qPCR及蛋白质印迹法检测结果表明,与对照组比较,AGE组人心肌成纤维细胞在诱导后的24、48和72 h时miRNA-424-5p含量升高(均 $P<0.01$)、FAIF1含量降低($P<0.01$ 或 $P<0.05$)、Smad7蛋白表达降低(均 $P<0.01$)。在AGE组中,与诱导后24 h比较,诱导后48 h和72 h人心肌成纤维细胞中miRNA-424-5p含量升高($P<0.01$),而FAIF1含量和Smad7蛋白表达降低($P<0.01$ 或 $P<0.05$);与诱导后48 h比较,诱导后72 h人心肌成纤维细胞中miRNA-424-5p含量升高($P<0.01$),而FAIF1含量和Smad7蛋白表达进一步降低($P<0.01$ 或 $P<0.05$)。见图2。

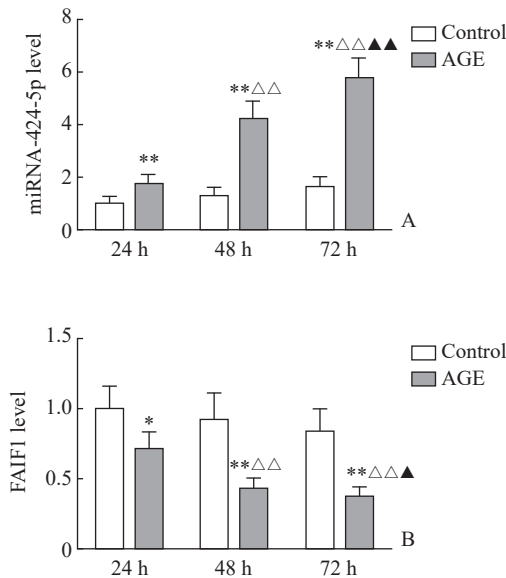


图2 AGE诱导人心肌成纤维细胞中miRNA-424-5p、FAIF1和Smad7的表达

Fig 2 Expression of miRNA-424-5p, FAIF1, and Smad7 in cardiac fibroblasts induced by AGEs

A: miRNA-424-5p expression (normalized to *U6*) detected by qPCR; B: FAIF1 expression (normalized to *U6*) detected by qPCR; C: Smad7 protein expression (normalized to β -actin) detected by Western blotting. The final concentration of AGEs used was 200 μ g/mL. * $P<0.05$, ** $P<0.01$ vs control group; $\Delta P<0.05$, $\Delta\Delta P<0.01$ vs AGE group treated for 24 h; $\blacktriangle P<0.05$, $\blacktriangle\blacktriangle P<0.01$ vs AGE group treated for 48 h. $n=3$, $\bar{x}\pm s$. AGE: Advanced glycation end product; miRNA-424-5p: MicroRNA-424-5p; FAIF1: Fibroblast activation inhibitory factor 1; Smad7: SMAD family member 7; qPCR: Quantitative polymerase chain reaction.

2.3 FAIF1能够抑制miRNA-424-5p与Smad7 mRNA 3'-UTR结合 生物信息学分析显示,Smad7 mRNA 3'-UTR有1个miRNA-424-5p种子区结合位点,序列为UGCUGCU,而FAIF1序列中存在3个相同

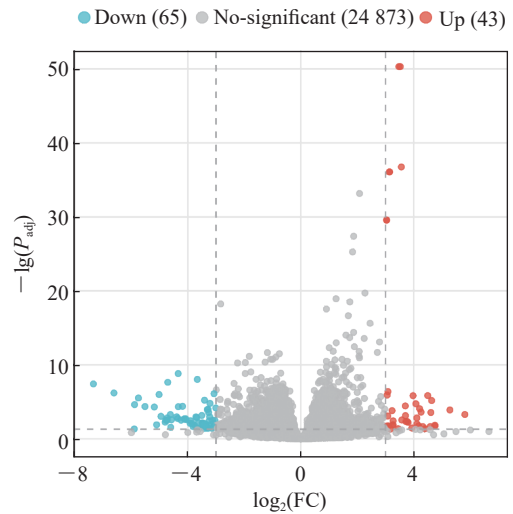
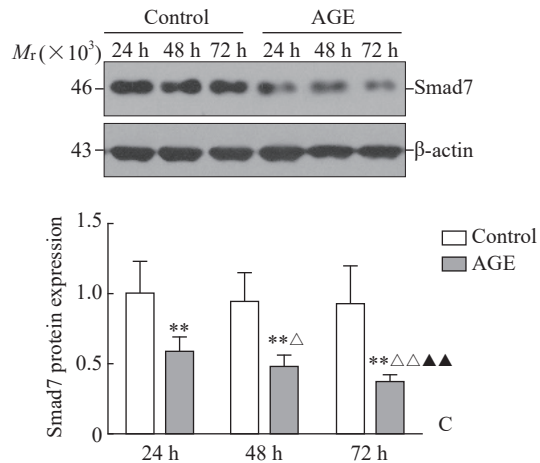


图1 AGE诱导72 h后人心肌成纤维细胞中差异表达lncRNA火山图

Fig 1 Volcano plot of differentially expressed lncRNAs in human cardiac fibroblasts induced by AGEs for 72 h AGE: Advanced glycation end product; lncRNA: Long non-coding RNA; FC: Fold change; P_{adj} : Adjusted P value.



的结合位点(图3A)。

萤光素酶报告基因实验结果(图3B、3C)显示,与对照组比较,pGL3-wt-Smad7和pGL3-mt-Smad7组人心肌成纤维细胞中萤光素酶活性均增

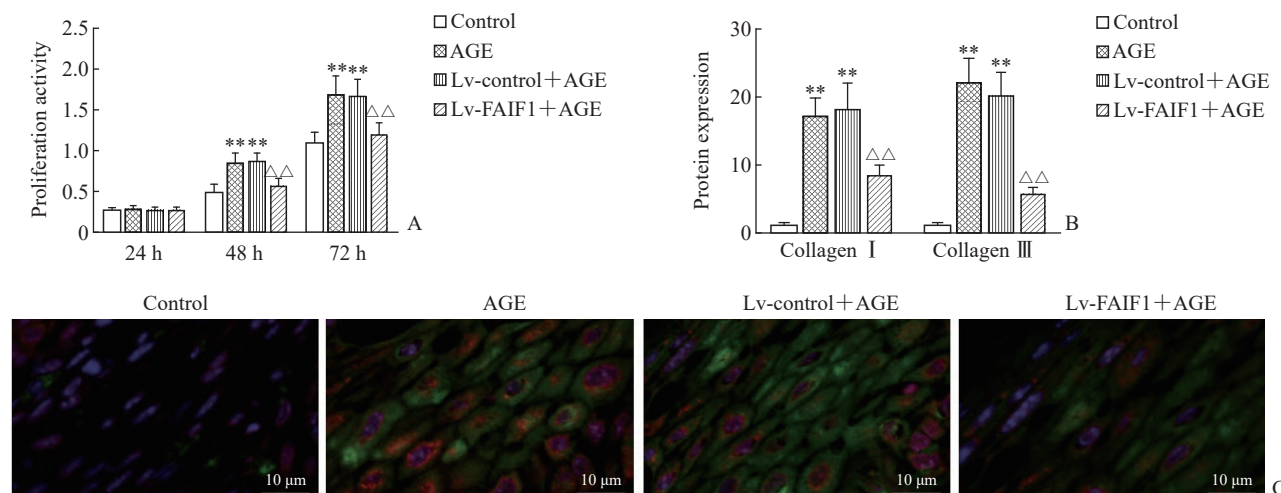


图4 不同处理组人心肌成纤维细胞增殖活性及I/III型胶原蛋白的表达和分泌

Fig 4 Proliferation activity and collagen I/III expression and secretion in cardiac fibroblasts under different treatments

A: CCK-8 assay for cell proliferation activity with the AGE induction for 24, 48, and 72 h; B, C: Immunofluorescence staining to detect collagen I/III expression and secretion in cardiac fibroblasts under different treatments with an AGE induction time of 48 h. Blue fluorescence represents DAPI staining for nuclei, red fluorescence indicates collagen I protein (Alexa Fluor 594), and green fluorescence indicates collagen III protein (Alexa Fluor 488). The final concentration of AGEs used was 200 μg/mL. Recombinant virus treatment was conducted 72 h before AGE induction. ** $P < 0.01$ vs control group; $\Delta\Delta P < 0.01$ vs AGE group. $n = 3$, $\bar{x} \pm s$. CCK-8: Cell counting kit 8; AGE: Advanced glycation end product; Lv: Lentivirus; FAIF1: Fibroblast activation inhibitory factor 1; DAPI: 4',6-diamidino-2-phenylindole.

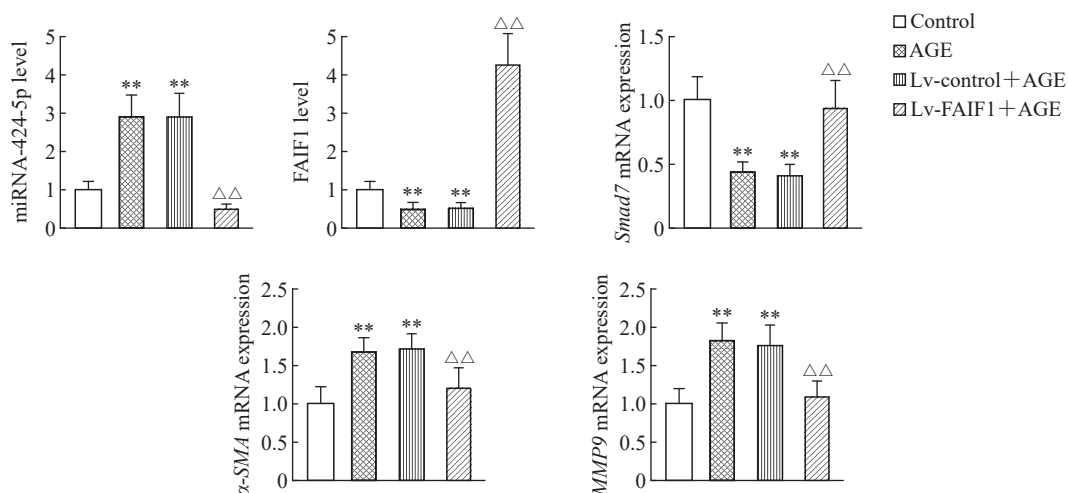


图5 不同处理组人心肌成纤维细胞中miRNA-424-5p、FAIF1、Smad7 mRNA、α-SMA mRNA和MMP9 mRNA水平

Fig 5 miRNA-424-5p, FAIF1, Smad7 mRNA, α-SMA mRNA and MMP9 mRNA levels in cardiac fibroblasts under different treatments

The miRNA-424-5p, FAIF1, Smad7 mRNA, α-SMA mRNA and MMP9 mRNA levels were detected by qPCR, and U6 and β-actin were used as internal controls. The final concentration of AGEs used was 200 μg/mL, with an induction time of 48 h. Recombinant virus treatment was conducted 72 h before AGE induction. ** $P < 0.01$ vs control group; $\Delta\Delta P < 0.01$ vs AGE group. $n = 3$, $\bar{x} \pm s$. miRNA-424-5p: MicroRNA-424-5p; FAIF1: Fibroblast activation inhibitory factor 1; Smad7: SMAD family member 7; qPCR: Quantitative polymerase chain reaction; AGE: Advanced glycation end product; Lv: Lentivirus; α-SMA: α-smooth muscle actin; MMP9: Matrix metalloproteinase 9.

3 讨 论

糖尿病心肌纤维化是糖尿病心肌病的主要特征之一,其病理特征包括心肌成纤维细胞的活化^[14]、心肌细胞外基质(extracellular matrix, ECM)过度沉积及心肌僵硬度增加。心肌成纤维细胞活化在糖尿病心肌纤维化中发挥核心作用^[15],在AGE的刺激下,糖尿病心肌成纤维细胞增殖并转化为肌成纤维细胞,激活的成纤维细胞分泌大量ECM成分(如I型和Ⅲ型胶原蛋白),导致ECM过度沉积^[16]。目前非编码RNA在糖尿病心肌成纤维细胞活化中的调控作用日益受到关注。本研究系统探讨了lncRNA FAIF1在AGE诱导的心肌纤维化中的作用及其潜在的分子机制,结果表明,AGE显著下调了人心肌成纤维细胞中FAIF1和Smad7的表达,同时上调了miRNA-424-5p的水平。

值得注意的是,Smad7可通过直接竞争性结合TGF- β I型受体,阻止受体调节型Smad(R-Smad,如Smad2和Smad3)的磷酸化,从而抑制信号的向下传递,是TGF- β /Smad通路的重要负调控因子^[17-18]。为了进一步探讨miRNA-424-5p、FAIF1和Smad7三者之间的关系,本研究采用萤光素酶报告基因实验进行验证,结果进一步证实了miRNA-424-5p/Smad7轴中miRNA-424-5p负性调控Smad7的表达,而FAIF1通过竞争性结合miRNA-424-5p解除了miRNA-424-5p对Smad7的负调控作用,进而有效抑制AGE诱导的人心肌成纤维细胞过度活化。目前有关miRNA-424-5p的研究主要集中在肿瘤领域,其表现出不同的生物学功能。在肝细胞癌中,它通过靶向CHEK1抑制肿瘤生长,可能作为治疗靶点^[19];在胃癌中,其表达上调,促进细胞增殖、迁移和侵袭,可能是促癌因子^[20];在肾透明细胞癌中,过表达miRNA-424-5p可抑制癌细胞的增殖和侵袭,表现出抑癌作用^[21]。尽管目前尚未见有关miRNA-424-5p参与心肌病或纤维化进程的报道,但鉴于miRNA-424-5p在细胞周期、凋亡和炎症调控方面的影响,探索其在心血管疾病及心肌重塑中的潜在作用对于挖掘糖尿病心肌病的治疗靶点具有重要意义。

最后,本研究通过FAIF1过表达细胞模型验证其对AGE诱导的人心肌成纤维细胞活化的影响,结果显示FAIF1可以显著抑制细胞增殖、I/Ⅲ

型胶原蛋白的表达和分泌及纤维化相关基因(如 α -SMA、MMP9)的表达,促进纤维化抑制基因Smad7的表达。这表明FAIF1能够明显缓解AGE诱导的纤维化过程。值得注意的是,目前关于miRNA-424-5p/Smad7轴的上下游关系的证据仍不充分,后续将通过敲除miRNA-424-5p实验进行进一步验证。此外,本研究主要基于体外细胞模型,缺乏体内实验验证。未来研究应结合动物模型进一步验证FAIF1在心肌纤维化中的治疗效果。

综上所述,本研究揭示了lncRNA FAIF1通过调控miRNA-424-5p/Smad7轴抑制AGE诱导的心肌纤维化的作用机制。这一结果不仅加深了对AGE相关纤维化分子机制的理解,也为糖尿病相关心肌纤维化的治疗提供了新的潜在靶点。未来通过开发靶向FAIF1的干预策略,可能为糖尿病心肌病的治疗开辟新途径。

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