DOI: 10.11844/cjcb.2025.10.0009
中国细胞生物学学报 Chinese Journal of Cell Biology 2025, 47(10): 2526–2538 CSTR: 32200.14.cjcb.2025.10.0009

LncRNA XIST调控miR-7-5p/ADAM10轴对人瘢痕疙瘩成纤维细胞增殖和凋亡的影响

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摘要 该文研究长链非编码RNA-X染色体失活特异性转录因子(LncRNA XIST)调控miR-7-5p/解整合素金属蛋白酶10(ADAM10)轴对人瘢痕疙瘩成纤维细胞(HKF)增殖和凋亡的影响。采用qRT-PCR分析正常皮肤组织、瘢痕疙瘩组织、正常人皮肤成纤维细胞-1(HFF-1)和HKF中LncRNA XIST、miR-7-5p及*ADAM10* mRNA的表达水平。将HKF分为: Control组、si-NC组、si-LncRNA XIST组、si-LncRNA XIST+anti-NC组、si-LncRNA XIST+anti-miR-7-5p组、mimic-NC组、miR-7-5p mimic组、miR-7-5p mimic+pcDNA-NC组、miR-7-5p mimic+pcDNA-ADAM10组。采用qRT-PCR测定各组细胞LncRNA XIST、miR-7-5p和*ADAM10*的表达情况; 双荧光素酶报告实验验证LncRNA XIST与miR-7-5p以及miR-7-5p与*ADAM10*的关系; WST-1和平板克隆形成实验测定各组细胞增殖情况; 流式细胞术测定各组细胞凋亡情况; Western blot测定各组细胞ADAM10、切割型胱天蛋白酶-3(cleaved-Caspase-3)、Caspase-3蛋白表达水平。结果显示, 瘢痕疙瘩组织和HKF中LncRNA XIST、*ADAM10* mRNA表达上调, miR-7-5p表达下调。双荧光素酶报告实验显示, LncRNA XIST和miR-7-5p以及miR-7-5p和*ADAM10*均有靶向作用关系。si-LncRNA XIST组的LncRNA XIST水平、ADAM10 mRNA及蛋白水平、存活率、克隆形成数、Caspase-3表达水平较si-NC组或Control组降低, 而miR-7-5p水平、细胞凋亡率、cleaved-Caspase-3表达水平则升高(P<0.05); miR-7-5p mimic转染对HKF的作用与si-LncRNA XIST一致。与si-LncRNA XIST组或si-LncRNA XIST+anti-NC组相比, si-LncRNA XIST+anti-miR-7-5p组ADAM10 mRNA及蛋白水平、存活率、克隆形成数、Caspase-3表达水平升高, 而miR-7-5p水平、细胞凋亡率、cleaved-Caspase-3表达水平降低(P<0.05)。与miR-7-5p mimic组或miR-7-5p mimic+pcDNA-NC相比, miR-7-5p mimic+pcDNA-ADAM10组ADAM10 mRNA及蛋白水平、存活率、克隆形成数、Caspase-3表达水平升高, 细胞凋亡率、cleaved-Caspase-3表达水平降低(P<0.05)。总之, 沉默LncRNA XIST可能通过调控miR-7-5p/ADAM10轴, 进而抑制HKF增殖, 促进其凋亡, 为瘢痕疙瘩的发病机制研究及靶向治疗提供新思路。

关键词 瘢痕疙瘩; 成纤维细胞; 长链非编码RNA; miR-7-5p; 解整合素金属蛋白酶10; 细胞凋亡

The Effects of LncRNA XIST on the Proliferation and Apoptosis of Human Keloid Fibroblasts by Regulating the miR-7-5p/ADAM10 Axis

收稿日期: 2025-04-28 接受日期: 2025-07-04
四川省区域创新合作项目(批准号: 2024YFHZ0128)和西藏自治区科技计划(批准号: XZ202401ZY0087)资助的课题
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Received: April 28, 2025 Accepted: July 4, 2025
This work was supported by the the Regional Innovation and Cooperation Project of Sichuan Province (Grant No.2024YFHZ0128) and the Science and Technology Plan of Tibet Autonomous Region (Grant No.XZ202401ZY0087)
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Abstract This study investigates the effect of LncRNA XIST (long non-coding RNA X inactive specific transcription factor) on the proliferation and apoptosis of HKF (human keloid fibroblast) by regulating the miR-7-5p/ADAM10 (a disintegrin and metalloproteinase 10) axis. qRT-PCR was used to analyze the expression levels of LncRNA XIST, miR-7-5p, and *ADAM10* mRNA in normal skin tissues, keloid tissues, normal HFF-1 (human foreskin fibroblasts-1) and HKF. HKF were assigned into Control group, si-NC group, si-LncRNA XIST group, si-LncRNA XIST+anti-NC group, si-LncRNA XIST+anti-miR-7-5p group, mimic-NC group, miR-7-5p mimic group, miR-7-5p mimic+pcDNA-NC group, and miR-7-5p mimic+pcDNA-ADAM10 group. qRT-PCR was used to measure the expression of LncRNA XIST, miR-7-5p, and *ADAM10* of cells in each group. Dual luciferase reporter assay was used to verify the relationship between LncRNA XIST and miR-7-5p, as well as between miR-7-5p and *ADAM10*. WST-1 and plate clone formation experiments were used to measure cell proliferation in each group. Flow cytometry was used to measure apoptosis of cells in each group. Western blot was used to measure the protein expression of ADAM10, cleaved-Caspase-3 and Caspase-3 of cells in each group. The results showed that LncRNA XIST and *ADAM10* mRNA expressions were upregulated in keloid tissues and HKF, while miR-7-5p expression was downregulated. The dual luciferase reporter assay showed that LncRNA XIST had a targeted relationship with miR-7-5p, and miR-7-5p had a targeted relationship with *ADAM10*. The LncRNA XIST, ADAM10 mRNA and protein, survival rate, colony formation number of cells, and the expression level of Caspase-3 in the si-LncRNA XIST group were lower than those in the si-NC group or Control group, whlie miR-7-5p, apoptosis rate, and the expression level of cleaved-Caspase-3 were higher (P<0.05). The effect of miR-7-5p mimic transfection on HKF was consistent with that of si-LncRNA XIST. Compared with the si-LncRNA XIST group or the si-LncRNA XIST+anti-NC group, the ADAM10 mRNA and protein, survival rate, clone formation number, and the expression level of Caspase-3 in the si-LncRNA XIST+anti-miR-7-5p group were higher, while miR-7-5p, apoptosis rate, and the expression level of cleaved-Caspase-3 were lower (P<0.05). Compared with miR-7-5p mimic group or miR-7-5p mimic+pcDNA-NC group, the mRNA and protein levels of ADAM10, survival rate, clone formation number, and the expression level of Caspase-3 in the miR-7-5p+pcDNA-ADAM10 group were increased, while the apoptosis rate and the expression level of cleaved-Caspase-3 were decreased (P<0.05). In conclusion, silencing LncRNA XIST may inhibit HKF proliferation and promote HKF apoptosis by regulating miR-7-5p/ADAM10 axis, providing a new idea for studying the pathogenesis and targeted therapy of keloid.

Keywords keloid; fibroblasts; long non-coding RNA; miR-7-5p; a disintegrin and metalloproteinase 10; apoptosis

瘢痕疙瘩是一种良性皮肤纤维增殖性疾病, 其特点在于病灶会超出原始伤口边缘生长, 且不具备恶性转化的潜能。其往往会在个体经历轻微创伤或任何形式的皮肤损伤后发生, 特别是在那些具有遗传易感性的个体中更为常见[1-2]。尽管瘢痕疙瘩的病理生理机制尚未完全明确, 但现有研究表明, 体内的初级基质细胞——成纤维细胞遭遇损伤刺激, 可能会过度增殖并分泌大量细胞外基质, 从而促使瘢痕疙瘩的形成[3]。了解瘢痕疙瘩形成的机制对于确定治疗瘢痕疙瘩的新方案至关重要。近年来, 失调的长链非编码RNA(long non-coding RNA, LncRNA)因其能够调节细胞增殖、迁移、凋亡, 细胞外基质生成以及信号通路, 而被认为在瘢痕疙瘩的发病机制中扮演着重要角色[4]。其中, 长链非编码RNA-X染色体失活特异性转录因子(long non-coding RNA X inactive specific transcription factor, LncRNA XIST)已被证实能够加速烧伤创面的愈合过程, 促进皮肤热损伤后变性真皮的修复, 促进人皮肤成纤维细胞

(human foreskin fibroblasts, HFF)增殖及细胞外基质的合成, 为皮肤损伤的修复提供了新策略[5-6]。然而, 关于LncRNA XIST在瘢痕疙瘩中是否也通过miRNA/mRNA轴发挥作用, 目前尚缺乏深入研究。研究表明, 在瘢痕疙瘩组织和人瘢痕疙瘩成纤维细胞(human keloid fibroblast, HKF)中, miR-7-5p表达下调, 过表达miR-7-5p能够抑制HKF增殖, 诱导细胞凋亡; 而解整合素金属蛋白酶10(a disintegrin and metalloproteinase 10, ADAM10)则显著上调, 沉默ADAM10可抑制HKF增殖、迁移和侵袭[7-9]。生物信息学软件预测进一步提示, LncRNA XIST和miR-7-5p之间存在潜在的结合位点, 同时*ADAM10*的mRNA的3′非翻译区(3′ untranslated region, 3′UTR)也包含有miR-7-5p的结合序列。此外, ADAM10可以促进细胞外基质的沉积和细胞纤维化[10]。因此, 本研究旨在深入探究LncRNA XIST能否通过调控miR-7-5p/ADAM10轴, 进而影响HKF的增殖及凋亡过程, 为瘢痕疙瘩的治疗与预防提供理论依据。

1 材料与方法

1.1 实验材料

所有组织均取自2023年5月至2024年10月于成都市第三人民医院接受手术的患者。手术切除后, 从瘢痕疙瘩患者皮肤组织获得瘢痕疙瘩标本(n=30)。正常皮肤样本(n=30)由整形手术过程中健康志愿者(腹部皮肤)提供。受试者均已签署知情同意书。本研究经成都市第三人民医院伦理委员会批准后开展(2023015)。

正常人皮肤成纤维细胞-1(human foreskin fibroblasts-1, HFF-1)(货号: SCSP-109)购自中国科学院干细胞库; HKF(货号: JSY-CC3650)购自上海金少源生物科技有限公司。

1.2 主要试剂

DMEM培养液(货号: 12430054)购自美国Invitrogen公司; 成纤维细胞培养液(货号: Delf-15764)购自合肥万物生物科技有限公司; 通用型microRNA快速提取试剂盒(货号: R015)购自北京金百特生物技术有限公司; Lipofectamine 3000试剂(货号: L3000001)、Opti-MEM培养基(货号: 31985070)、双荧光素酶检测试剂盒(货号: 16181)购自美国ThermoFisher Scientific公司; WST-1试剂(货号: KGA9304)购自江苏凯基生物技术股份有限公司; Annexin V-FITC凋亡检测试剂盒(货号: ab14085)购自美国Abcam公司; Rabbit Anti-ADAM10(货号: YA3292)购自美国MCE公司; Rabbit Anti-切割型胱天蛋白酶-3(cleaved Caspase-3)(货号: 9664)、Rabbit Anti-GAPDH(货号: 2118)、HRP标记的Anti-rabbit IgG抗体(货号: 7074)购自美国CST公司。

1.3 方法

1.3.1 qRT-PCR测定瘢痕疙瘩组织、正常皮肤组织以及HFF-1和HKF中LncRNA XIST、miR-7-5p及*ADAM10* mRNA的表达水平 提取分别使用DMEM、成纤维细胞完全培养液培养的HFF-1、HKF的RNA, 进行qRT-PCR扩增。扩增程序: 95 ℃预变性 1 min; 95 ℃变性10 s, 60 ℃退火/延伸 20 s, 共40个循环。使用$2^{-\Delta\Delta Ct}$方法, *β-actin*、*U6*为内参, 计算LncRNA XIST、miR-7-5p及*ADAM10* mRNA表达水平。引物见表1。

1.3.2 免疫组化测定瘢痕疙瘩组织、正常皮肤组织ADAM10表达水平 取手术切除的瘢痕疙瘩组织、正常皮肤组织, 用福尔马林4 ℃固定24 h, 并用石蜡包埋, 制备3 μm厚的切片, 按常规程序对切片进行脱蜡和脱水。抗原修复后, 用山羊血清室温封闭30 min, 添加兔ADAM10(1:100)抗体, 4 ℃摇床过夜。次日, 加二抗(1:500)室温孵育30 min。DBA室温染色30 min,

表1 qRT-PCR引物序列

Table 1 Primer sequences of qRT-PCR

基因 Gene	上游引物(5′→3′) Upstream primers (5′→3′)	下游引物(5′→3′) Downstream primers (5′→3′)
LncRNA XIST	CTT GGA TGG GTT GCC AGC TA	TCA TGC CCC ATC TCC ACC TA
ADAM10	ATT TAG CAG CCA TCC CCA	CGA TCC CGG ACA TCT TGA
β-actin	GCC GGG ACC TGA CTG ACT AC	TCT CCT TAA TGT CAC GCA CGA T
miR-7-5p	GCC GAG TGG AAG ACT AGT GAT T	CAG TGC GTG TCG TGG AGT
U6	CTC GCT TCG GCA GCA CAT A	AAC GCT TCA CGA ATT TGC GT

苏木素室温复染5 min, 流水冲洗15 min, 梯度乙醇室温脱水(75%乙醇2 min、85%乙醇12 min、95%乙醇I 3 min、95%乙醇II 3 min、100%乙醇I 5 min、100%乙醇II 5 min)、二甲苯室温透明(二甲苯I 5 min、二甲苯II 5 min)后封片。显微镜下观察染色情况。ADAM10染色为棕黄色。由2位经验丰富的病理学专家采用双盲法进行染色结果判断。细胞内染色百分比评分在0~3之间: 0分(未染色); 1分[1%~25%(局部)]; 2分[26%~50%(中等)]; 3分[>50%(弥散)]。0分和1分为低表达, 2分和3分为高表达。

1.3.3 细胞转染及分组 Control组常规培养HKF, si-NC组在HKF中转染si-NC, si-LncRNA XIST组在HKF中转染si-LncRNA XIST, si-LncRNA XIST+anti-NC组在HKF中共转染si-LncRNA XIST、anti-NC, si-LncRNA XIST+anti-miR-7-5p组在HKF中共转染si-LncRNA XIST、anti-miR-7-5p, mimic-NC组在HKF中转染mimic-NC, miR-7-5p mimic组在HKF中转染miR-7-5p mimic, miR-7-5p mimic+pcDNA-NC组在HKF中共转染miR-7-5p mimic、pcDNA-NC, miR-7-5p mimic+pcDNA-ADAM10组在HKF中共转染miR-7-5p mimic、pcDNA-ADAM10。具体转染步骤为: HKF以1×10^5/孔接种于6孔板, 待细胞融合度达60%~70%时进行转染(约24 h); 将5 μg上述转染质粒与10 μL Lipofectamine 3000试剂(2 μL/μg DNA)分别用Opti-MEM培养基稀释, 混合后室温孵育15 min, 加入孔中; 转染后6 h更换完全培养基, 继续培养48 h。然后收集细胞, 依照1.3.1中qRT-PCR步骤检测各组细胞中LncRNA XIST、miR-7-5p、*ADAM10* mRNA相对表达水平。

1.3.4 双荧光素酶报告实验测定LncRNA XIST与miR-7-5p以及miR-7-5p与*ADAM10*之间的关系 LncRNA XIST、*ADAM10*的野生型载体(WT-LncRNA XIST、WT-ADAM10)、突变型载体(MUT-LncRNA XIST、MUT-ADAM10)分别与mimic-NC或miR-7-5p mimic共转染。48 h后参考双荧光素酶检测试剂盒说明书检测其荧光素酶活性。

1.3.5 WST-1与平板克隆形成实验测定各组细胞增殖 ①WST-1实验: HKF依据1.3.3细胞分组处理, 培养于96孔板中。与WST-1试剂反应2 h后, 检测波长为450 nm处的吸光度(D_{450})值, 分析细胞存活率。②平板克隆形成实验: 将HKF培养在24孔板中, 并参照1.3.3细胞分组进行处理, 约每3天更换1次培养液, 约1周后用4%多聚甲醛室温固定细胞30 min, 并用0.1%结晶紫染液室温染色30 min。统计HKF的克隆形成数。

1.3.6 流式细胞术测定各组细胞凋亡 预冷的PBS洗涤收集的细胞后, 根据Annexin V-FITC凋亡检测试剂盒说明书处理细胞, 并在流式细胞仪上分析、检测HKF的凋亡率。

1.3.7 Western blot测定各组细胞ADAM10、cleaved-Caspase-3蛋白表达情况 检测使用RIPA缓冲液提取的总蛋白浓度, 并将经SDS-PAGE凝胶电泳分离后的蛋白转至PVDF膜上。随后在室温下与封闭液孵育1.5 h, 加入一抗ADAM10(1:500)、cleaved-Caspase-3(1:1 000)、GAPDH(1:3 000) 4 °C过夜处理。再将膜与HRP标记的兔二抗(1:6 000)室温孵育1 h, 加入ECL试剂, 使用凝胶成像系统收集蛋白条带图像, ImageJ软件进行评估蛋白条带的灰度值, 并使用GAPDH归一处理。

1.4 统计与分析

采用SPSS 26.0软件进行统计分析, 计量资料表示为平均值±标准差($\bar{x}\pm s$)。采用独立样本t检验分析两组间差异; 采用单因素方差进行多组间比较, 并采用SNK-q检验进一步两两相比。计数资料以n(%)表示, 采用卡方检验比较。P<0.05表示差异有统计学意义。

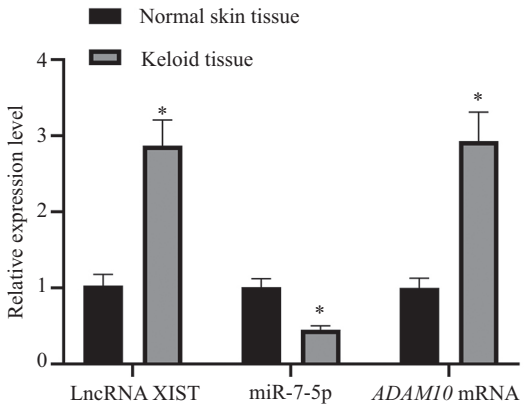
2 结果

2.1 正常皮肤组织和瘢痕疙瘩组织中LncRNA XIST、miR-7-5p及*ADAM10* mRNA的表达情况

与正常皮肤组织相比, 瘢痕疙瘩组织中LncRNA XIST、*ADAM10* mRNA表达量增多, 而miR-7-5p表达量减少(P<0.05, 图1)。瘢痕疙瘩组织中LncRNA XIST和miR-7-5p水平以及miR-7-5p和*ADAM10* mRNA水平均呈负相关(P<0.05, 图2)。免疫组化结果(图3)显示, 瘢痕疙瘩组织ADAM10阳性23例(76.67%)、阴性7例(23.33%), 正常皮肤组织ADAM10阳性5例(16.67%)、阴性25例(83.33%), 卡方检验显示, 瘢痕疙瘩组织中ADAM10阳性率高于正常皮肤组织(P<0.05)。

2.2 HFF-1和HKF中LncRNA XIST、miR-7-5p及*ADAM10* mRNA的表达情况

与HFF-1细胞相比, HKF中LncRNA XIST、*ADAM10* mRNA表达量增多, 而miR-7-5p表达量减

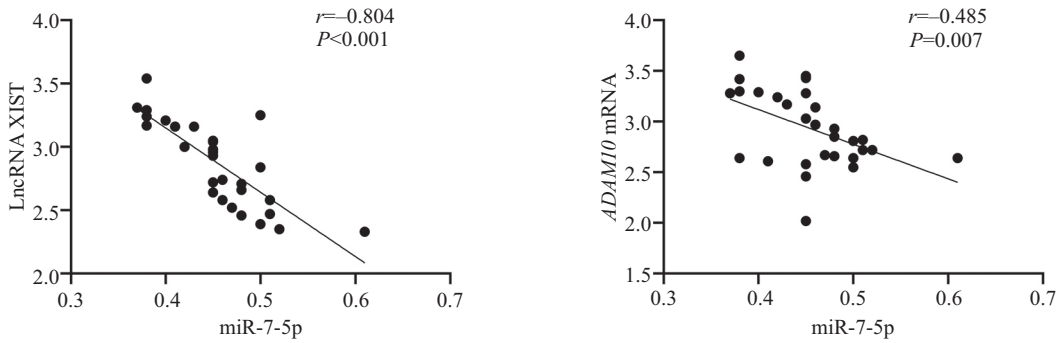


$\bar{x}\pm s$, n=30; *P<0.05, 与正常皮肤组织相比较。

$\bar{x}\pm s$, n=30; *P<0.05 compared with normal skin tissue.

图1 正常皮肤组织和瘢痕疙瘩组织中LncRNA XIST、miR-7-5p及*ADAM10* mRNA的表达水平

Fig.1 Expression of LncRNA XIST, miR-7-5p and *ADAM10* mRNA in normal skin tissue and keloid tissue



A: LncRNA XIST与miR-7-5p表达相关性分析; B: *ADAM10* mRNA与miR-7-5p表达相关性分析。

A: correlation analysis between LncRNA XIST and miR-7-5p expression; B: correlation analysis between *ADAM10* mRNA and miR-7-5p expression.

图2 瘢痕疙瘩组织中LncRNA XIST、miR-7-5p及*ADAM10* mRNA表达的相关性分析

Fig.2 Correlation analysis of LncRNA XIST and miR-7-5p, as well as miR-7-5p and *ADAM10* mRNA expressions in keloid tissues

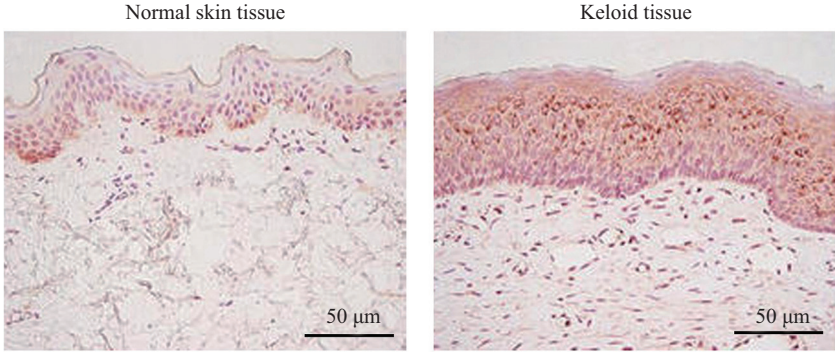


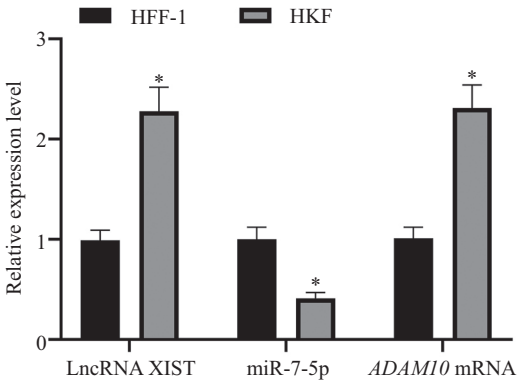
图3 正常皮肤组织和瘢痕疙瘩组织中ADAM10免疫组化图

Fig.3 Immunohistochemical plots of ADAM10 in normal skin tissue and keloid tissue

少(P<0.05, 图4)。

2.3 双荧光素酶报告实验验证LncRNA XIST与miR-7-5p以及miR-7-5p与*ADAM10*关系

生物信息学预测，miR-7-5p分别与LncRNA XIST、*ADAM10*存在互补结合位点(图5和图6)。在HKF中转染WT-LncRNA XIST、WT-ADAM10后，miR-7-5p mimic组比mimic-NC组的荧光素酶活性低(P<0.05, 图7)。



$\bar{x}\pm s$, n=6; *P<0.05, 与HFF-1细胞相比较。

$\bar{x}\pm s$, n=6; *P<0.05 compared with HFF-1 cells.

图4 HFF-1、HKF细胞中LncRNA XIST、miR-7-5p及*ADAM10* mRNA的表达情况

Fig.4 Expression of LncRNA XIST, miR-7-5p and *ADAM10* mRNA in HFF-1 and HKF cells

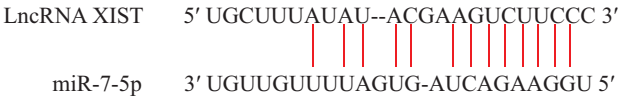


图5 LncRNA XIST与miR-7-5p的结合位点

Fig.5 The binding sites of LncRNA XIST and miR-7-5p

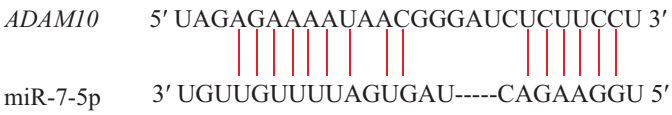
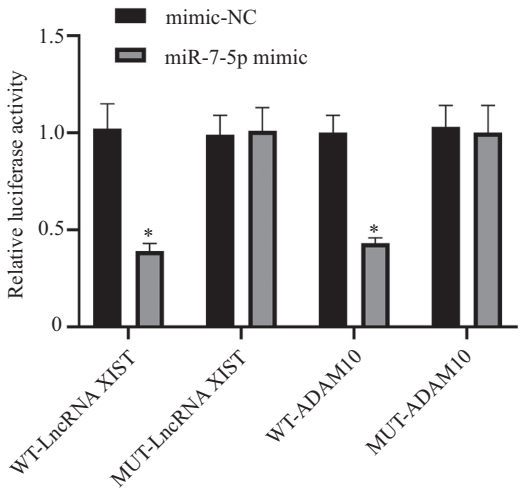


图6 miR-7-5p与*ADAM10*的结合位点

Fig.6 The binding sites of miR-7-5p and *ADAM10*



$\bar{x}\pm s$, n=6; *P<0.05, 与mimic-NC组相比较。

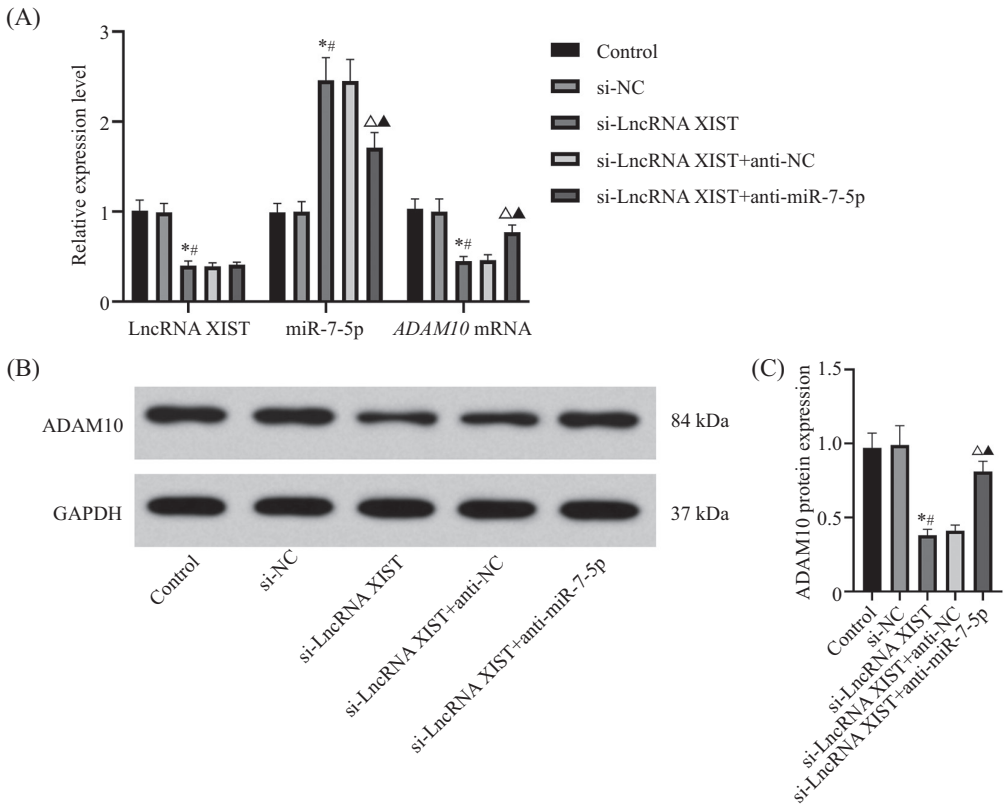
$\bar{x}\pm s$, n=6; *P<0.05 compared with the mimic-NC group.

图7 miR-7-5p对WT-LncRNA XIST、WT-ADAM10荧光素酶活性的影响

Fig.7 Effects of miR-7-5p on the luciferase activities of WT-LncRNA XIST and WT-ADAM10

2.4 各组HKF中LncRNA XIST、miR-7-5p以及ADAM10 mRNA和蛋白的表达情况

si-LncRNA XIST组的LncRNA XIST、ADAM10 mRNA和蛋白水平较si-NC组或Control组降低，而miR-7-5p表达水平则升高(P<0.05)；与si-LncRNA XIST组或si-LncRNA XIST+anti-NC组相比，si-Ln-



A: LncRNA XIST、miR-7-5p及*ADAM10* mRNA表达水平比较; B: ADAM10蛋白表达条带; C: ADAM10蛋白表达情况比较; *P<0.05, 与Control组相比较; #P<0.05, 与si-NC组相比较; △P<0.05, 与si-LncRNA XIST组相比较; ▲P<0.05, 与si-LncRNA XIST+anti-NC组相比较。$\bar{x}\pm s$, n=6。

A: comparison of LncRNA XIST, miR-7-5p and *ADAM10* mRNA expressions; B: ADAM10 protein expression bands; C: comparison of ADAM10 protein expression; *P<0.05 compared with the Control group; #P<0.05 compared with the si-NC group; △P<0.05 compared with the si-LncRNA XIST group; ▲P<0.05 compared with the si-LncRNA XIST+anti-NC group. $\bar{x}\pm s$, n=6.

图8 各组HKF中LncRNA XIST、miR-7-5p以及*ADAM10* mRNA和ADAM10蛋白的表达情况

Fig.8 Expression of LncRNA XIST, miR-7-5p, *ADAM10* mRNA and ADAM10 protein in HKF of each group

cRNA XIST+anti-miR-7-5p组细胞的LncRNA XIST水平无显著变化, 而ADAM10 mRNA和蛋白水平升高, miR-7-5p表达水平降低(P<0.05)。见图8。

2.5 沉默LncRNA XIST对HKF增殖的影响

si-LncRNA XIST组细胞的存活率及克隆形成数较si-NC组或Control组降低(P<0.05); 与si-LncRNA XIST组或si-LncRNA XIST+anti-NC组相比, si-LncRNA XIST+anti-miR-7-5p组细胞的存活率及克隆形成数升高(P<0.05)。见图9。

2.6 沉默LncRNA XIST对HKF凋亡的影响

si-LncRNA XIST组细胞凋亡率、cleaved-Caspase-3表达水平较si-NC组或Control组升高, Caspase-3表达水平较si-NC组或Control组降低(P<0.05); 与si-LncRNA XIST组或si-LncRNA XIST+anti-NC组相比, si-LncRNA XIST+anti-miR-7-5p组细胞的细胞凋亡率、cleaved-Caspase-3表达水平降低, Caspase-3表达水平升高(P<0.05)。见图10和图11。

2.7 各组HKF中miR-7-5p以及ADAM10 mRNA和蛋白的表达情况

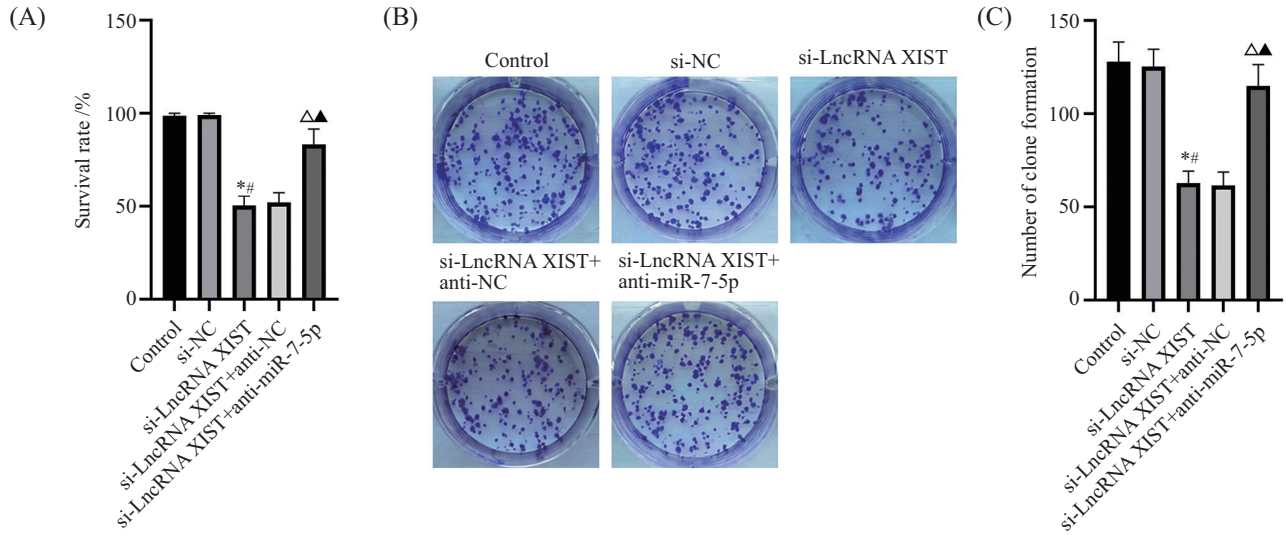
miR-7-5p mimic组的ADAM10 mRNA和蛋白水平较mimic-NC组或Control组降低, 而miR-7-5p表达水平则升高(P<0.05); 与miR-7-5p mimic组或miR-7-5p mimic+pcDNA-NC相比, miR-7-5p mimic+pcDNA-ADAM10组细胞的miR-7-5p水平无显著变化, 而ADAM10 mRNA和蛋白水平升高(P<0.05)。见图12。

2.8 过表达miR-7-5p对HKF增殖的影响

miR-7-5p mimic组的存活率及克隆形成数较mimic-NC组或Control组降低(P<0.05); 与miR-7-5p mimic组或miR-7-5p mimic+pcDNA-NC组相比, miR-7-5p mimic+pcDNA-ADAM10组细胞的存活率及克隆形成数升高(P<0.05)。见图13。

2.9 过表达miR-7-5p对HKF凋亡的影响

miR-7-5p mimic组的细胞凋亡率、cleaved-



A: 细胞存活率比较; B: 细胞克隆结果; C: 克隆形成数比较; *P<0.05, 与Control组相比较; #P<0.05, 与si-NC组相比较; △P<0.05, 与si-LncRNA XIST组相比较; ▲P<0.05, 与si-LncRNA XIST+anti-NC组相比较。$\bar{x}\pm s$, n=6。

A: comparison of cell survival rates; B: cell cloning results; C: comparison of the number of clone formations; *P<0.05 compared with the Control group; #P<0.05 compared with the si-NC group; △P<0.05 compared with the si-LncRNA XIST group; ▲P<0.05 compared with the si-LncRNA XIST+anti-NC group. $\bar{x}\pm s$, n=6.

图9 沉默LncRNA XIST对HKF增殖的影响

Fig.9 The effect of silencing LncRNA XIST on the proliferation of HKF

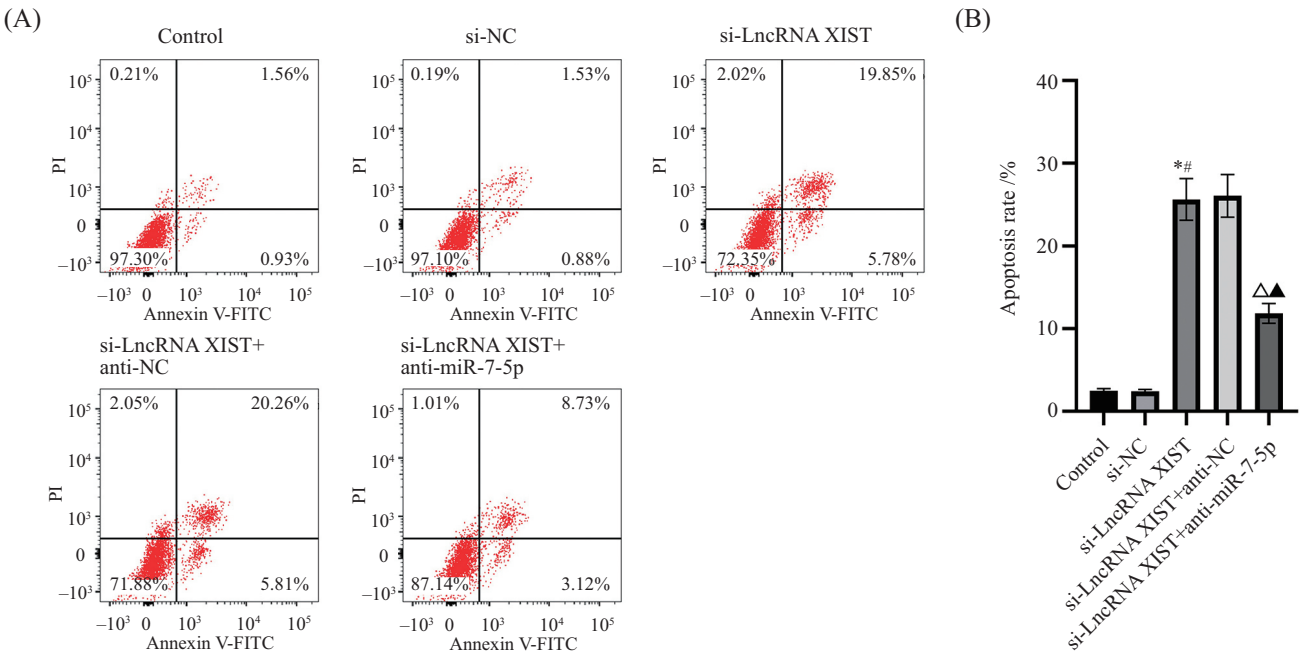
Caspase-3表达水平较mimic-NC组或Control组升高, Caspase-3表达水平较mimic-NC组或Control组下降(P<0.05); 与miR-7-5p mimic组或miR-7-5p mimic+pcDNA-NC组相比, miR-7-5p mimic+pcDNA-ADAM10组细胞的细胞凋亡率、cleaved-Caspase-3表达水平降低, Caspase-3表达水平升高(P<0.05)。见图14和图15。

3 讨论

瘢痕组织形成是皮肤损伤后生理性愈合的关键过程, 但过度反应可形成瘢痕疙瘩, 其组织病理特征为表皮增厚变平、真皮细胞数量增加及大量细胞外基质沉积, 不仅会引起疼痛、瘙痒和美观上的问题, 还会影响组织生长、体温调节及运动等正常的生物学功能[11-13]。成纤维细胞因具有较高的增殖速率和较强的细胞外基质生成能力, 导致细胞密度增加和组织质地变得致密, 在瘢痕疙瘩的形成中扮演着至关重要的角色[14]。因此, 深入了解瘢痕疙瘩成纤维细胞的增殖、凋亡病理生理学机制对于指导药物治疗以及减少或预防瘢痕疙瘩的形成或复发具有重要意义。

研究表明, 过表达LncRNA XIST除了能够促进癌细胞进展外, 还能促进肌腱损伤小鼠成纤维细胞的增殖, 而敲低LncRNA XIST可抑制类风湿性关节炎滑膜成纤维细胞的迁移、增殖、侵袭, 并降低血管生成活性[15-17]。本研究首次发现, LncRNA XIST在瘢痕疙瘩组织和HKF中过表达, 沉默LncRNA XIST可降低HKF的存活率及细胞克隆形成数量, 并促进细胞凋亡的发生。这样的结果提示, 沉默LncRNA XIST不仅能够抑制HKF增殖, 还能促进其凋亡, 从而在分子层面为抑制瘢痕疙瘩的形成提供了新的线索和潜在的治疗策略。

基于竞争性内源RNA相互作用所构成的基因调控网络, 在揭示瘢痕疙瘩发病机制的过程中正日益受到学术界的广泛关注[18]。LÜ等[19]研究报道, 在瘢痕疙瘩组织及HKF中, miR-7-5p显著下调, 而circ-COL5A1作为竞争性内源RNA, 通过特异性地吸附并海绵化miR-7-5p, 释放环磷酸腺苷调节鸟嘌呤核苷酸交换因子1, 进而激活PI3K/Akt信号通路, 促进瘢痕疙瘩的病理性增生。此外, LncRNA也被证实能够通过调控miR-7-5p与靶mRNA之间的相互作用, 来影响细胞的增殖与凋亡动态平衡[20]。本研究借助生物信息学分析手段, 预测并发现了LncRNA XIST与miR-7-5p之间存在互补结合位点。本研究发现, miR-7-5p在瘢痕疙瘩组织和HKF中表达下调, 与LÜ

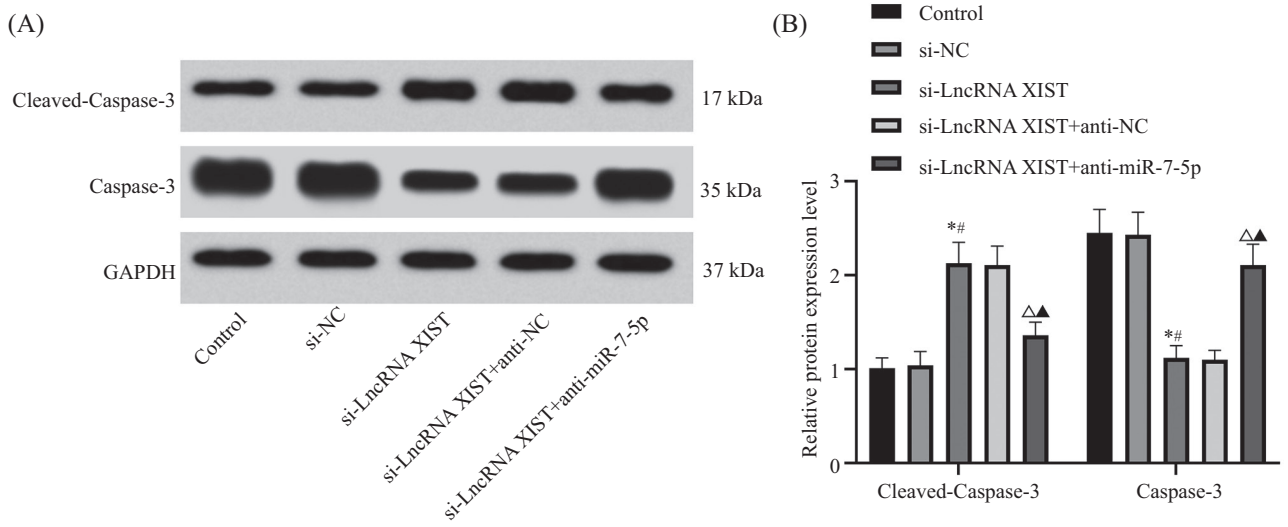


A: 流式细胞仪检测细胞凋亡情况; B: 细胞凋亡率比较; *P<0.05, 与Control组相比较; #P<0.05, 与si-NC组相比较; △P<0.05, 与si-LncRNA XIST组相比较; ▲P<0.05, 与si-LncRNA XIST+anti-NC组相比较。$\bar{x}\pm s$, n=6。

A: flow cytometry was used to detect cell apoptosis; B: comparison of cell apoptosis rates; *P<0.05 compared with the Control group; #P<0.05 compared with the si-NC group; △P<0.05 compared with the si-LncRNA XIST group; ▲P<0.05 compared with the si-LncRNA XIST+anti-NC group. $\bar{x}\pm s$, n=6.

图10　沉默LncRNA XIST对HKF凋亡的影响

Fig.10　The effect of silencing LncRNA XIST on the apoptosis of HKF

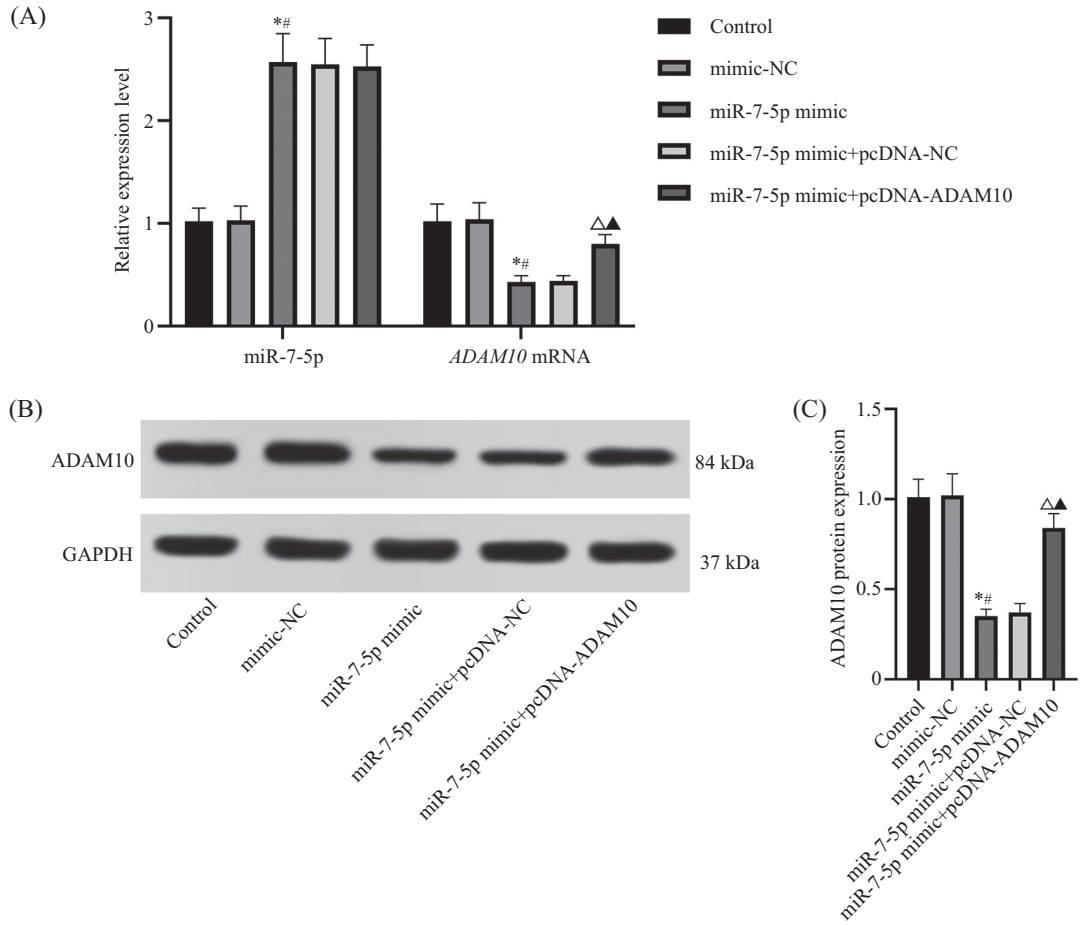


A: cleaved-Caspase-3、Caspase-3蛋白表达条带; B: cleaved-Caspase-3、Caspase-3蛋白表达水平比较; *P<0.05, 与Control组相比较; #P<0.05, 与si-NC组相比较; △P<0.05, 与si-LncRNA XIST组相比较; ▲P<0.05, 与si-LncRNA XIST+anti-NC组相比较。$\bar{x}\pm s$, n=6。

A: cleaved-Caspase-3 and Caspase-3 protein expression bands; B: comparison of cleaved-Caspase-3 and Caspase-3 protein expression; *P<0.05 compared with the Control group; #P<0.05 compared with the si-NC group; △P<0.05 compared with the si-LncRNA XIST group; ▲P<0.05 compared with the si-LncRNA XIST+anti-NC group. $\bar{x}\pm s$, n=6.

图11　沉默LncRNA XIST对HKF中cleaved-Caspase-3、Caspase-3蛋白表达的影响

Fig.11　The effect of silencing LncRNA XIST on the protein expressions of cleaved-Caspase-3 and Caspase-3 in HKF



A: miR-7-5p及*ADAM10* mRNA表达水平比较; B: ADAM10蛋白表达条带; C: ADAM10蛋白表达情况比较; **P*<0.05, 与Control组相比较; #*P*<0.05, 与mimic-NC组相比较; △*P*<0.05, 与miR-7-5p mimic组相比较; ▲*P*<0.05, 与miR-7-5p mimic+pcDNA-NC组相比较。$\bar{x}\pm s$, *n*=6。

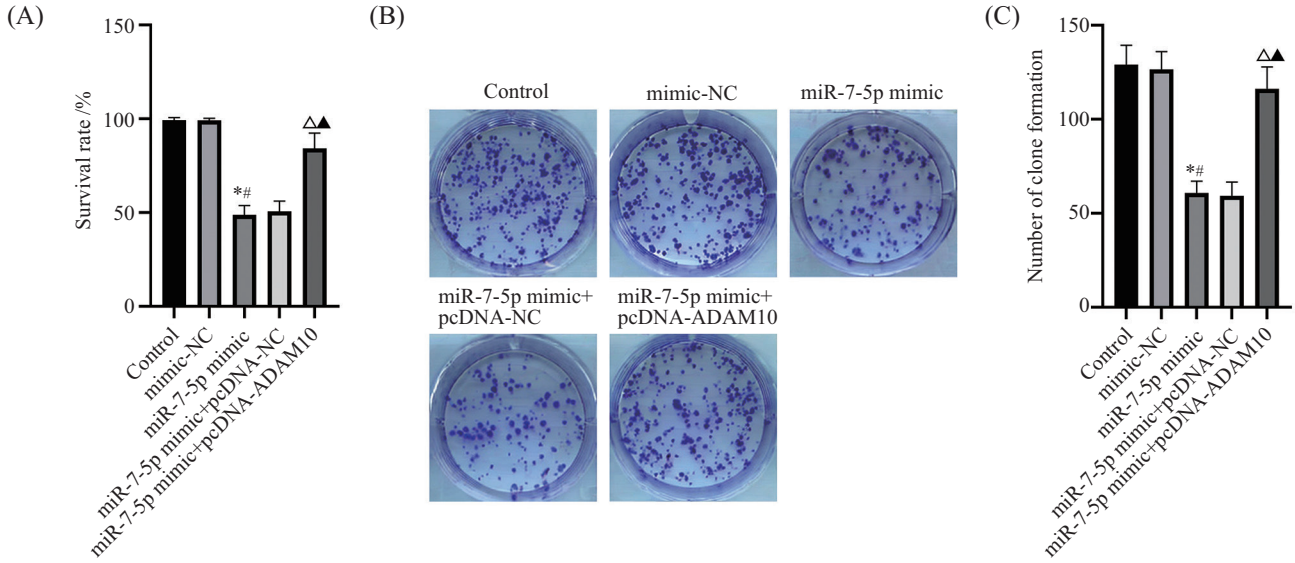
A: comparison of miR-7-5p and *ADAM10* mRNA expressions; B: ADAM10 protein expression bands; C: comparison of ADAM10 protein expression; **P*<0.05 compared with the Control group; #*P*<0.05 compared with the mimic-NC group; △*P*<0.05 compared with the miR-7-5p mimic group; ▲*P*<0.05 compared with the miR-7-5p mimic+pcDNA-NC group. $\bar{x}\pm s$, *n*=6.

图12 各组HKF中LncRNA XIST、miR-7-5p以及ADAM10 mRNA和蛋白的表达情况

Fig.12 Expression of LncRNA XIST, miR-7-5p and ADAM10 mRNA and protein in HKF of each group

等[19]研究结果一致, 其在瘢痕疙瘩组织中的表达与LncRNA XIST呈负相关。随后的双荧光素酶报告基因实验进一步验证了LncRNA XIST确实能够作为竞争性内源RNA, 靶向性地负向调控miR-7-5p的表达水平。更深入的实验结果显示, 沉默LncRNA XIST可上调miR-7-5p表达, 同时抑制HKF增殖, 促进其凋亡。然而, 当下调miR-7-5p的表达水平时, 沉默LncRNA XIST对HKF的增殖抑制作用会显著减弱。这揭示, 沉默LncRNA XIST可能通过上调miR-7-5p表达, 进而调控下游靶mRNA的表达, 从而对HKF增殖产生抑制作用。

此外, 在非小细胞肺癌研究中, miR-7-5p在癌组织及细胞系中的表达水平降低, 而ADAM10表达水平升高, 并且*ADAM10*是miR-7-5p的靶基因, 研究表明, 沉默circ-SFMBT2能够通过调控miR-7-5p/ADAM10分子轴来减弱非小细胞肺癌细胞增殖、克隆形成及侵袭能力, 并诱导其凋亡, 最终抑制移植瘤的生长[21]。本研究通过生物信息学分析及双荧光素酶报告实验, 进一步证实了*ADAM10*与miR-7-5p之间存在明确的靶向负相关关系。众多研究报道均指出, LncRNA可以通过靶向特定的miRNA, 进而调节ADAM10的表达水平, 最终影响细胞的增殖与凋亡过程[22-24]。本研究观察到, 沉默LncRNA XIST表达后, miR-7-5p表达水平升高, 而ADAM10表达水平相应降低, 进而抑制HKF的增殖, 并促进其凋亡的发生。进一步的研究发现, 当抑制miR-7-5p的表达时, ADAM10的表达水平随之上调, 同时逆转沉默LncRNA XIST对HKF增殖及凋亡的影响。同时,

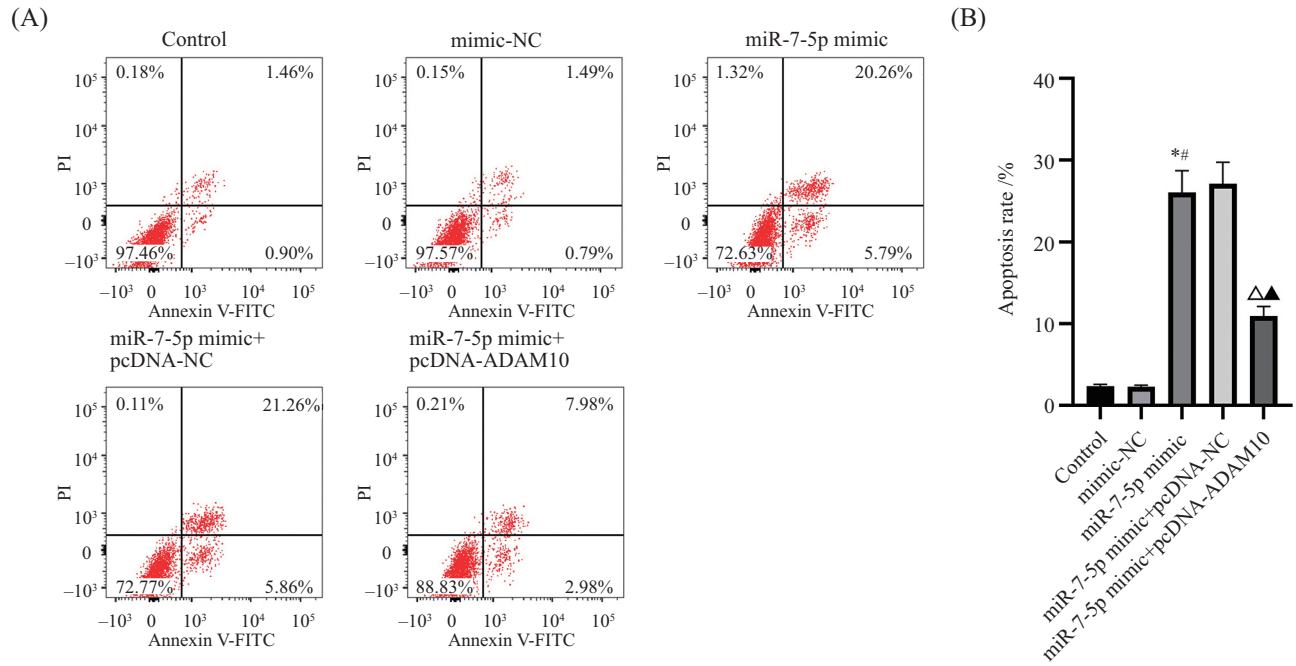


A: 细胞存活率比较; B: 细胞克隆结果; C: 克隆形成数比较; *P<0.05, 与Control组相比较; #P<0.05, 与mimic-NC组相比较; △P<0.05, 与miR-7-5p mimic组相比较; ▲P<0.05, 与miR-7-5p mimic+pcDNA-NC组相比较。$\bar{x}\pm s$, n=6。

A: comparison of cell survival rates; B: cell cloning results; C: comparison of the number of clone formations; *P<0.05 compared with the Control group; #P<0.05 compared with the mimic-NC group; △P<0.05 compared with the miR-7-5p mimic group; ▲P<0.05 compared with the miR-7-5p mimic+pcDNA-NC group. $\bar{x}\pm s$, n=6.

图13　沉默LncRNA XIST对HKF增殖的影响

Fig.13　The effect of silencing LncRNA XIST on the proliferation of HKF

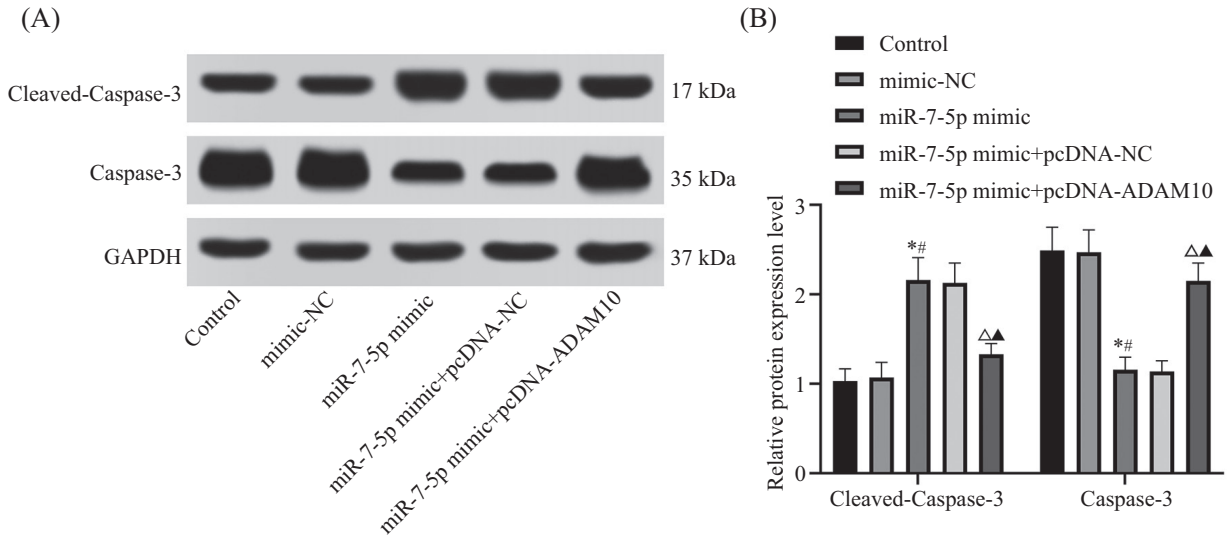


A: 流式细胞仪检测细胞凋亡; B: 细胞凋亡率比较; *P<0.05, 与Control组相比较; #P<0.05, 与mimic-NC组相比较; △P<0.05, 与miR-7-5p mimic组相比较; ▲P<0.05, 与miR-7-5p mimic+pcDNA-NC组相比较。$\bar{x}\pm s$, n=6。

A: flow cytometry was used to detect cell apoptosis; B: comparison of cell apoptosis rates; *P<0.05 compared with the Control group; #P<0.05 compared with the mimic-NC group; △P<0.05 compared with the miR-7-5p mimic group; ▲P<0.05 compared with the miR-7-5p mimic+pcDNA-NC group. $\bar{x}\pm s$, n=6.

图14　过表达miR-7-5p对HKF凋亡的影响

Fig.14　The effect of overexpression of miR-7-5p on the apoptosis of HKF



A: cleaved-Caspase-3、Caspase-3蛋白表达条带; B: cleaved-Caspase-3、Caspase-3蛋白表达比较; *P<0.05, 与Control组相比较; $^{\#}P$<0.05, 与mimic-NC组相比较; $^{\triangle}P$<0.05, 与miR-7-5p mimic组相比较; $^{\blacktriangle}P$<0.05, 与miR-7-5p mimic+pcDNA-NC组相比较。$\bar{x}\pm s$, n=6。

A: cleaved-Caspase-3 and Caspase-3 protein expression bands; B: comparison of cleaved-Caspase-3 and Caspase-3 protein expression; *P<0.05 compared with the Control group; $^{\#}P$<0.05 compared with the mimic-NC group; $^{\triangle}P$<0.05 compared with the miR-7-5p mimic group; $^{\blacktriangle}P$<0.05 compared with the miR-7-5p mimic+pcDNA-NC group. $\bar{x}\pm s$, n=6.

图15 过表达miR-7-5p对HKF中cleaved-Caspase-3、Caspase-3蛋白表达的影响

Fig.15 The effect of overexpression of miR-7-5p on the protein expressions of cleaved-Caspase-3 and Caspase-3 in HKF

当上调ADAM10表达时, 过表达miR-7-5p对HKF的增殖抑制作用会显著减弱。这些研究结果显示, 沉默LncRNA XIST可能通过靶向调控miR-7-5p, 进而下调ADAM10表达, 从而影响HKF增殖、凋亡等生物学行为, 其具体机制可能是LncRNA XIST通过竞争性结合miR-7-5p, 解除miR-7-5p对ADAM10的抑制, 则会促进HKF增殖, 抑制其凋亡。据报道, 沉默ADAM17可能会抑制EGFR/ERK通路的活性, 从而抑制细胞外基质在瘢痕疙瘩成纤维细胞中的沉积, 进一步抑制细胞增殖[9]。ADAM17敲除可降低TGF-β1/Smad3通路激活介导的心肌成纤维细胞向肌成纤维细胞转化水平, 减少糖尿病诱导的心功能障碍和抑制心脏胶原沉积[25]。下调ADAM17表达能够抑制实验性胰腺炎小鼠的炎性细胞浸润及胰腺组织纤维化, 这与IL-6反式信号通路/STAT3轴的活性减弱或者轴的相关蛋白的表达下调有关[26]。基于以上描述, ADAM17在胶原沉积和炎症方面的下游通路较多, 但LncRNA XIST/miR-7-5p/ADAM10轴是否能够通过上述通路发挥促进HKF增殖、抑制其凋亡的作用还不清楚, 需进一步研究证实。

本研究目前仅在细胞水平验证了LncRNA XIST/miR-7-5p/ADAM10调控轴的生物学功能, 若将其作为瘢痕疙瘩的临床生物标志物, 仍需通过大量动物模型实验及临床样本数据进一步验证。未来研究需持续深化机制探索, 为临床转化提供更充分的理论支撑。此外, 本研究因聚焦机制分析未设置阳性对照, 后续将补充该组实验以明确LncRNA XIST的生物学效应。值得关注的是, 当前研究局限于瘢痕疙瘩领域, 后续拟拓展至肝纤维化、肺纤维化等其他纤维化疾病, 通过跨疾病比较分析提升研究的普适性与学术影响力。

综上所述, 沉默LncRNA XIST可能通过上调miR-7-5p, 进而下调ADAM10的表达, 从而抑制HKF增殖, 促进其凋亡。基于上述机制, 可从多维度探索干预策略与药物靶点: 直接利用反义寡核苷酸(antisense oligonucleotide, ASO)或siRNA沉默LncRNA XIST; 开发miR-7-5p模拟物, 借助脂质体或纳米颗粒载体提高递送效率, 强化对ADAM10的抑制; 针对ADAM10, 设计靶向其催化位点的小分子抑制剂或采用RNA干扰技术阻断其功能。此外, LncRNA XIST、miR-7-5p、ADAM10的多靶点联合干预有望产生协同增效作用。不过, 这些策略仍需通过基础研究与临床试验验证其安全性和有效性, 以推动临床转化。

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