circGFRA1靶向miR-642a-5p调控类风湿关节炎滑膜成纤维细胞增殖、迁移和侵袭的机制研究

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摘要 该研究探讨了circGFRA1对类风湿关节炎(RA)滑膜成纤维细胞(SFs)增殖、迁移和侵袭的影响及其可能机制。收集了39例RA患者的滑膜组织和39例膝关节创伤且无其他关节异常病史患者的滑膜组织(正常滑膜组织), qRT-PCR法检测组织中circGFRA1和miR-642a-5p表达情况。体外分离培养RASFs, 分别转染circGFRA1小干扰RNA、miR-642a-5p模拟物, 或共转染circGFRA1小干扰RNA与miR-642a-5p抑制剂后, CCK-8法、划痕实验、Transwell小室分别检测细胞增殖、迁移和侵袭; 蛋白质印迹法检测细胞中Ki-67、E-cadherin和N-cadherin蛋白表达; 双荧光素酶报告基因实验验证circGFRA1和miR-642a-5p的调控关系。结果显示, RA患者滑膜组织中circGFRA1表达水平较正常滑膜组织显著升高(P<0.05), miR-642a-5p较正常滑膜组织显著降低(P<0.05); 下调circGFRA1或上调miR-642a-5p后, RASFs细胞D值、划痕愈合率、侵袭数及细胞中Ki-67、N-cadherin蛋白表达均呈显著下降趋势(P<0.05), E-cadherin蛋白表达水平显著升高(P<0.05); circGFRA1在RASFs中靶向负调控miR-642a-5p; 下调miR-642a-5p逆转了下调circGFRA1对RASFs增殖、迁移和侵袭的影响。综上, circGFRA1可能通过靶向下调miR-642a-5p促进类风湿性关节炎细胞增殖、迁移和侵袭。

关键词 类风湿关节炎; circGFRA1; miR-642a-5p; 细胞增殖; 迁移; 侵袭

Study on the Mechanism of circGFRA1 Targeting miR-642a-5p to Regulate the Proliferation, Migration and Invasion of Rheumatoid Arthritis Synovial Fibroblasts

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Abstract This study investigated the effect of circGFRA1 on the proliferation, migration and invasion of RA (rheumatoid arthritis) SFs (synovial fibroblasts) and its possible mechanism. The synovial tissues of 39 RA patients and 39 patients who have knee joint trauma and have no other joint abnormalities were collected. The expression of circGFRA1 and miR-642a-5p in the tissues were detected by qRT-PCR. RASFs were isolated and cultured *in vitro*. After RASFs were transfected with circGFRA1 small interfering RNA or miR-642a-5p mimic, or co-transfected with circGFRA1 small interfering RNA and miR-642a-5p inhibitor, CCK-8 method, scratch test, and Transwell chamber detected cell proliferation, migration and invasion. And the expression of Ki-67, E-cadherin and N-cadherin protein in the cells was detected by Western blot. The dual luciferase reporter gene experiment

收稿日期: 2022-02-16 接受日期: 2022-04-06

甘肃省科技计划项目(批准号: 21JR7RA014)资助的课题

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Received: February 16, 2022 Accepted: April 6, 2022

This work was supported by the Gansu Provincial Science and Technology Plan Project (Grant No.21JR7RA014)

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verified the regulatory relationship between circGFRA1 and miR-642a-5p. The results showed that the expression of circGFRA1 in the synovial tissues of RA patients was significantly higher than that in normal synovial tissues (P<0.05), but the expression of miR-642a-5p was significantly lower than that in normal synovial tissues (P<0.05). After down-regulating circGFRA1 or up-regulating miR-642a-5p, the D value, scratch healing rate, invasion number of RASFs, the protein expression of Ki-67 and N-cadherin in cells were all decreased (P<0.05), but the protein expression of E-cadherin was increased (P<0.05). circGFRA1 could target and negatively regulate miR-642a-5p in RASFs. Down-regulating miR-642a-5p reversed the effect of down-regulating circGFRA1 on the proliferation, migration and invasion of RASFs. Taken together, circGFRA1 may promote the proliferation, migration and invasion of rheumatoid arthritis cells by targeting and negatively regulating miR-642a-5p.

Keywords rheumatoid arthritis; circGFRA1; miR-642a-5p; cell proliferation; migration; invasion

类风湿关节炎(rheumatoid arthritis, RA)是一种自身免疫疾病, 以关节滑膜异常增生和炎症反应为主要病理特征。滑膜成纤维细胞(synovial fibroblasts, SFs)是滑膜增生的主要效应细胞[1], 抑制其过度增殖对RA的治疗尤为重要。circGFRA1是一种环状RNA(circRNA), 其在卵巢癌、胶质瘤及肝细胞癌中均表达上调, 降低circGFRA1表达水平对以上肿瘤细胞的繁殖、转移及侵袭具有显著的抑制作用, 从而可以阻碍肿瘤进一步恶化[2-4]。RASFs具有肿瘤细胞特性, 易增殖和侵袭[5]。因此, 推测circGFRA1也可能参与调控RASFs的增殖和侵袭。StarBase靶基因在线软件预测显示, circGFRA1可能靶向调控miR-642a-5p。miR-642a-5p在结肠癌和前列腺癌中表达下调, 上调miR-642a-5p可分别靶向抑制COL1A1、WT1的表达, 阻碍结肠癌和前列腺癌细胞的恶性行为[6-7]。然而, miR-642a-5p对RASFs生物学行为的影响还未知。本研究主要探究了circGFRA1和miR-642a-5p对RASFs增殖、迁移及侵袭的影响, 并观察了circGFRA1能否靶向miR-642a-5p参与调控RASFs增殖、迁移及侵袭, 以期了解circGFRA1/miR-642a-5p轴在RA发生发展中的作用, 为RA的治疗提供新靶点。

1 资料与方法

1.1 组织样本

以39例于本院(第一作者所在单位, 下同)行过膝关节滑膜切除术的RA患者为研究对象, 女21例, 男18例, 平均年龄为(46.38±8.25)岁。纳入标准: 无其他关节异常疾病; 无其他全身性疾病。另以同时期39例膝关节创伤且无其他关节异常病史的患者为对照组, 女25例, 男14例, 平均年龄为(46.38±8.25)岁。将获取的两组患者的滑膜组织置于液氮中保存。研究符合《赫尔辛基宣言》原则, 并经本院伦理委员会批准(批准号: 20190087)和患者知情同意。

1.2 细胞和试剂

PCR实验相关试剂盒购自大连宝生物工程有限公司; 胎牛血清购自浙江天杭生物科技有限公司; DMEM培养液、CCK-8试剂盒、BCA蛋白检测试剂盒及双荧光素酶活性检测试剂盒购自北京索莱宝科技有限公司; Lipofectamine™ 2000试剂盒购自美国Invitrogen公司; Ki-67、E-cadherin和N-cadherin和GAPDH抗体购自Abcam公司; 引物序列、circGFRA1小干扰RNA(si-circGFRA1)及阴性对照序列(si-NC)、miR-642a-5p模拟物(mimcs)及抑制剂(anti-miR-642a-5p)、模拟对照序列(miR-NC)及抑制剂阴性对照序列(anti-miR-NC)、野生型(WT)及突变型(MUT)circGFRA1荧光素酶报告基因载体均购自上海生工生物工程有限公司。

1.3 方法

1.3.1 qRT-PCR法检测circGFRA1和miR-642a-5p表达 于液氮保护下充分研磨组织样本, 利用RNA提取试剂盒获得组织中总RNA。将获取的RNA利用逆转录试剂盒逆转录为cDNA, 然后进行PCR反应。引物序列见表1。$2^{-\Delta\Delta Ct}$法计算circGFRA1相对*GADPH*、miR-642a-5p相对*U6*的表达量。

1.3.2 分离培养RASFs 参照文献[8]方法分离培养RASFs。将获取的RA患者的滑膜组织用手术剪碎, 大小约为1 mm×1 mm×1 mm, 加I型胶原酶进行消化处理, 消化温度为37 °C, 时间为4 h。然后将其过200目细胞滤网, 并转移至15 mL离心管中, 1 000 r/min离心5 min, 去除上清液, 加适量PBS溶液, 混合均匀。1 000 r/min离心5 min, 去除上清液, 加含

表1 引物序列

Table 1 Primer sequences

基因 Gene	上游 Upstream	下游 Downstream
circGFRA1	5′-GTC GTG CTG AGG CGC GAT C-3′	5′-CGT GAT GCC TGA CCT AGC CG-3′
GAPDH	5′-AGA AGG CTG GGG CTC ATT TG-3′	5′-AGG GGC CAT CCA CAG TCT TC-3′
miR-642a-5p	5′-GCG GTC CCT CTC CAA ATG T-3′	5′-AGT GCA GGG TCC GAG GTA TT-3′
U6	5′-CTC GCT TCG GCA GCA CA-3′	5′-AAC GCT TCA CGA ATT TGC GT-3′

10%胎牛血清的DMEM培养液, 转移至25 cm^2培养瓶中培养。待细胞融合至85%左右时, 胰蛋白酶消化, 传代培养, 取3~6代细胞用于以下实验。

1.3.3 细胞转染 于6孔板中接种2.5 mL RASFs(5.0×10^4个/mL), 培养24 h, 弃培养基。将Lipofectamine™ 2000试剂分别与si-circGFRA1(si-circGFRA1组)、si-NC(si-NC组)、miR-642a-5p mimics(miR-642a-5p组)、miR-NC(miR-NC组)、anti-miR-642a-5p(miR-642a-5p组)、anti-miR-NC(anti-miR-NC组)、si-circGFRA1、anti-miR-642a-5p(si-circGFRA1+anti-miR-642a-5p组)、si-circGFRA1和anti-miR-NC(si-circGFRA1+anti-miR-NC组)相结合并混匀, 分别加至6孔板中, 每孔加入100 μL试剂。37 °C连续孵育12 h后, 将培养液丢弃, 并加入完全培养液, 再培养24 h, qRT-PCR法检测细胞中circGFRA1或miR-642a-5p表达情况验证转染效果。

1.3.4 CCK-8法检测细胞增殖 于96孔板中分别接种0.2 mL si-circGFRA1组、si-NC组、miR-642a-5p组、miR-NC组、si-circGFRA1+anti-miR-642a-5p组和si-circGFRA1+anti-miR-NC组细胞悬液(5.0×10^4个/mL), 培养24 h后, 加10 μL CCK-8, 37 °C孵育2 h, 用酶标仪检测各孔光密度(*D*)值。

1.3.5 划痕实验检测细胞迁移 于6孔板中分别接种2.5 mL si-circGFRA1组、si-NC组、miR-642a-5p组、miR-NC组、si-circGFRA1+anti-miR-642a-5p组和si-circGFRA1+anti-miR-NC组细胞悬液(5.0×10^4个/mL), 37 °C连续孵育4 h, 弃掉培养液。在细胞接种的6孔板底部采用200 μL的移液器枪头划两条相互平行的细线, 位于划痕处的细胞除外, 测量两条细线之间的距离, 并将其记为$d_{0\,h}$。在加入完全培养液后连续培养24 h, 按照上述方法再次进行测量, 并记为$d_{24\,h}$。划痕愈合率(%)=$(d_{0\,h}-d_{24\,h})/d_{0\,h}\times100\%$。

1.3.6 Transwell检测细胞侵袭 Matrigel基质胶放置在4 °C冰箱中, 融化后铺于Transwell小室的上室, 自然晾干。然后分别取100 μL si-circGFRA1组、si-NC组、miR-642a-5p组、miR-NC组、si-circGFRA1+anti-miR-642a-5p组和si-circGFRA1+anti-miR-NC组细胞悬液(5.0×10^4个/mL)加至上室中, 另取500 μL完全培养液加至下室。培养24 h后, 弃培养液, 先用多聚甲醛将细胞固定20 min, 然后置于结晶紫染色液中染色15 min。用PBS清洗后, 显微镜观察, 计数。

1.3.7 蛋白质印迹法检测细胞中Ki-67、E-cadherin和N-cadherin蛋白表达情况 于6孔板中分别接种2.5 mL si-circGFRA1组、si-NC组、miR-642a-5p组、miR-NC组、si-circGFRA1+anti-miR-642a-5p组和si-circGFRA1+anti-miR-NC组细胞悬液(5.0×10^4个/mL), 培养24 h后, 将RIPA裂解液(400 μL)加入到收集到的细胞中并对细胞总蛋白进行提取。采用随机提取细胞BCA的实验方法进行测定各组细胞胶原蛋白质的细胞浓度, 行SDS-PAGE电泳反应(40 μg蛋白)后转至PVDF膜, 用5%脱脂牛奶于37 °C封闭2 h。于4 °C冰箱中分别用稀释度均为1:1 000的Ki-67、E-cadherin、N-cadherin、GAPDH一抗孵育过夜, 洗膜后, 在置于稀释度为1:2 000的山羊抗兔二抗中室温孵育1 h。1 h后向细胞内滴加ECL显影, 应用ImageJ软件分析各分组细胞的总条带胶原蛋白细胞灰度的统计数值及Ki-67、E-cadherin、N-cadherin相对GAPDH的表达量。

1.3.8 双荧光素酶报告基因实验 于6孔板中接种2.5 mL RASFs(5.0×10^4个/mL), 培养24 h, 弃培养基。将Lipofectamine™ 2000试剂分别与WT-circGFRA1、miR-642a-5p mimics、WT-circGFRA1、miR-NC、MUT-circGFRA1、miR-642a-5p mimics、MUT-circGFRA1和miR-NC相结合并混匀, 分别加至6孔板中, 每孔加入100 μL试剂。连续孵育12 h后, 将上层清液及培养液丢弃, 并加入完全培养液于37 °C继续孵育。培养24 h后, 弃培养液, 将细胞行裂解处理, 3 500 r/min离心10 min。取20 μL上清液, 加100 μL 1× 萤火虫或海

肾荧光素酶反应工作液, 对萤火虫和海肾的荧光强度进行检测。以萤火虫/海肾荧光强度的数值表示细胞荧光素酶活性。

1.4 统计学分析

SPSS.22.0软件进行统计学分析。计量资料以均数±标准差($\bar{x}\pm s$)表示。RA患者滑膜组织与正常滑膜组织中circGFRA1和miR-642a-5p表达的比较以及si-NC组、si-circGFRA1组、miR-NC组、miR-642a-5p组、si-circGFRA1+anti-miR-NC组、si-circGFRA1+anti-miR-642a-5p组各检测指标的比较均用独立样本t检验。以P<0.05表示差异有统计学意义。

2 结果

2.1 circGFRA1和miR-642a-5p在RA患者中的表达情况

RA患者滑膜组织中circGFRA1的表达量为(4.85±0.44), 较正常滑膜组织中circGFRA1的表达量(1.00±0.09)显著升高(t=53.535, P<0.05, 图1); RA患者滑膜组织中miR-642a-5p的表达量为(0.38±0.04), 较正常滑膜组织中miR-642a-5p的表达量(1.00±0.07)显著降低(t=48.025, P<0.05, 图1)。

2.2 下调circGFRA1对RASFs增殖的影响

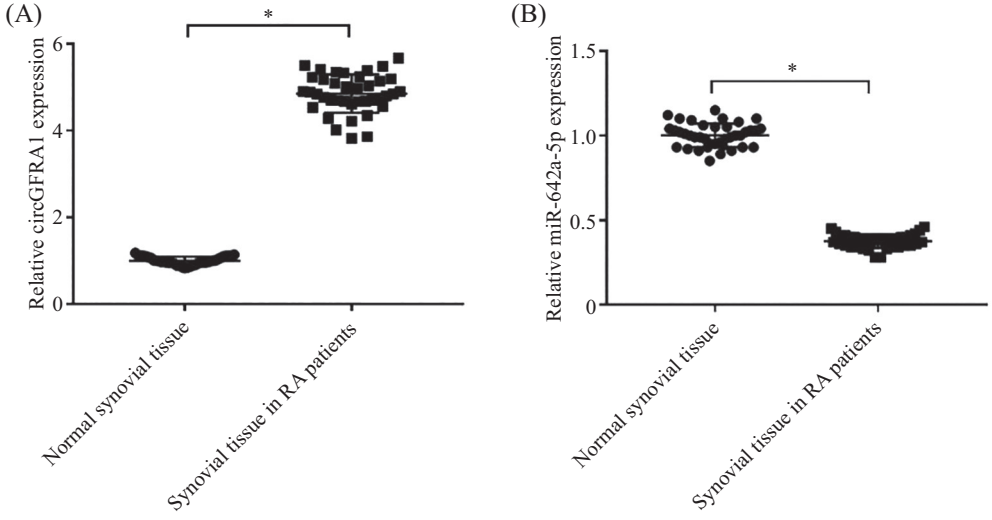
si-NC组和si-circGFRA1组RASFs中circGFRA1的表达量分别为(1.00±0.00)、(0.27±0.03), si-circGFRA1组circGFRA1的表达量较si-NC组显著下调(t=73.000, P<0.05, 图2和表2)。si-circGFRA1组细胞D值及细胞中Ki-67蛋白表达较si-NC组均显著降低(P<0.05, 图2和表2)。

2.3 下调circGFRA1对RASFs迁移和侵袭的影响

相比于si-NC组, si-circGFRA1组细胞划痕愈合率、侵袭数及细胞中N-cadherin蛋白表达量明显降低(P<0.05, 图3和表3), 同时E-cadherin蛋白表达量显著升高(P<0.05, 图3和表3)。

2.4 circGFRA1靶向调控miR-642a-5p的表达

StarBase软件结果显示circGFRA1与miR-642a-5p的结合位点(图4)。与WT-circGFRA1和miR-NC的细胞荧光素酶活性(1.04±0.06)相比, 共转染WT-circGFRA1与miR-642a-5p mimics的RASFs荧光素酶活性为(0.52±0.05), 明显降低(t=19.974, P<0.05, 表4); 共转染MUT-circGFRA1与miR-642a-5p mimics的细胞荧光素酶活性为(1.03±0.06), 较共转染MUT-circGFRA1与miR-NC的细胞荧光素酶活性(1.01±0.05)无显著差异(t=0.768, P=0.454, 表4), 说明circGFRA1可靶向结合miR-642a-5p。同时, si-NC组和si-circGFRA1组RASFs中miR-642a-5p的表达量分别为(1.00±0.00)、(2.88±0.24), si-circGFRA1组miR-642a-5p的表达量较si-NC组显著升高(t=23.500, P<0.05, 表4), 说明下调circGFRA1促进miR-642a-5p的表达。

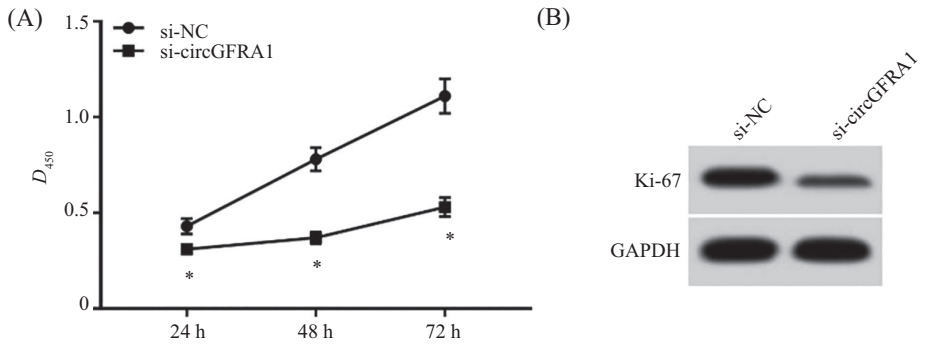


A: circGFRA1在RA患者滑膜组织中的表达; B: miR-642a-5p在RA患者滑膜组织中的表达。*P<0.05, 与正常滑膜组织比较。

A: the expression of circGFRA1 in the synovial tissue of RA patients; B: the expression of miR-642a-5p in the synovial tissue of RA patients. *P<0.05 compared with normal synovial tissue.

图1 circGFRA1和miR-642a-5p在RA患者滑膜组织中的表达

Fig.1 The expression of circGFRA1 and miR-642a-5p in synovial tissue of RA patients



A: 下调circGFRA1对RASFs增殖的影响; B: 下调circGFRA1对RASFs中Ki-67蛋白表达的影响。*P<0.05, 与si-NC组比较。

A: the effect of down-regulating circGFRA1 on the proliferation of RASFs; B: the effect of down-regulating circGFRA1 on the expression of Ki-67 protein in RASFs. *P<0.05 compared with si-NC group.

图2　下调circGFRA1对RASFs增殖的影响

Fig.2　The effect of down-regulating circGFRA1 on the proliferation of RASFs

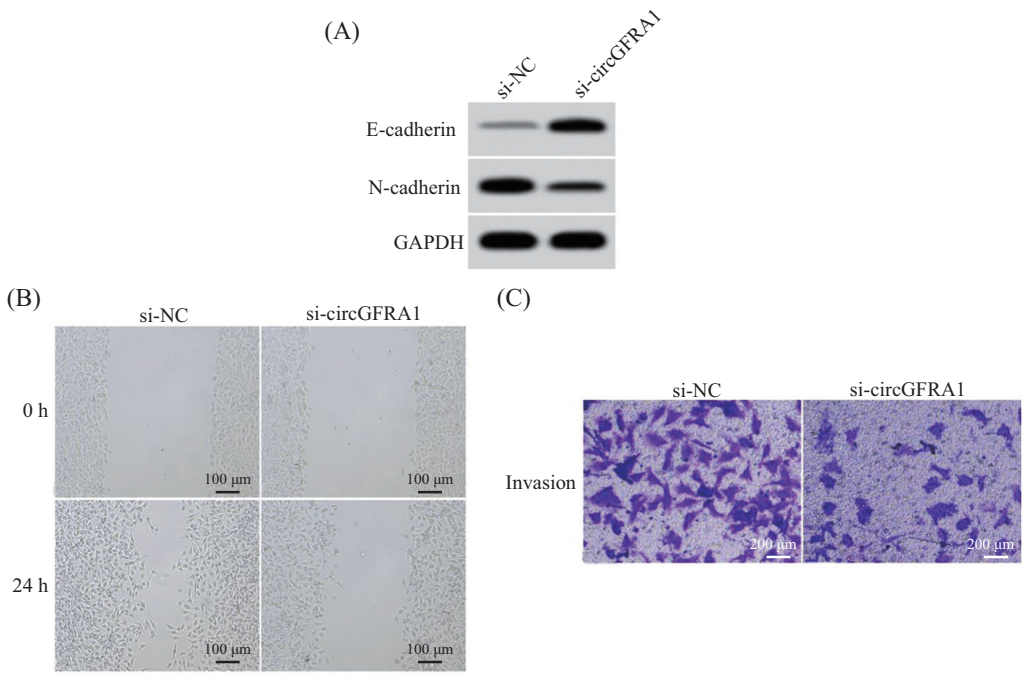
表2　下调circGFRA1对RASFs增殖的影响

Table 2　The effect of down-regulating circGFRA1 on the proliferation of RASFs

分组 Group	D_{450}			Ki-67蛋白 Ki-67 protein
	24 h	48 h	72 h	
si-NC	0.43±0.04	0.78±0.06	1.11±0.09	0.64±0.05
si-circGFRA1	0.31±0.03*	0.37±0.03*	0.53±0.05*	0.23±0.02*
t	7.200	18.336	16.900	22.841
P	0.000	0.000	0.000	0.000

*P<0.05, 与si-NC组比较。

*P<0.05 compared with si-NC group.



A: 下调circGFRA1对RASFs中E-cadherin和N-cadherin蛋白表达的影响; B: 下调circGFRA1对RASFs迁移的影响; C: 下调circGFRA1对RASFs侵袭的影响。

A: the effect of down-regulating circGFRA1 on the protein expression of E-cadherin and N-cadherin in RASFs; B: the effect of down-regulating circGFRA1 on the migration of RASFs; C: the effect of down-regulating circGFRA1 on the invasion of RASFs.

图3　下调circGFRA1对RASFs迁移和侵袭的影响

Fig.3　The effect of down-regulating circGFRA1 on the migration and invasion of RASFs

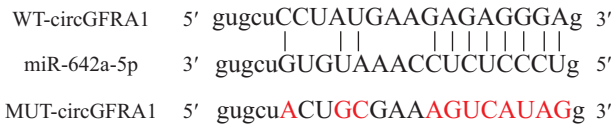
表3 下调circGFRA1对RASFs迁移和侵袭的影响

Table 3 The effect of down-regulating circGFRA1 on the migration and invasion of RASFs

分组 Group	划痕愈合率% Scratch healing rate /%	侵袭细胞数 Number of invasive cells	E-cadherin蛋白 E-cadherin protein	N-cadherin蛋白 N-cadherin protein
si-NC	64.08±5.64	105.06±11.08	0.16±0.02	0.75±0.05
si-circGFRA1	24.42±2.26*	47.65±4.24*	0.57±0.04*	0.29±0.03*
t	19.582	14.518	27.504	23.667
P	0.000	0.000	0.000	0.000

*P<0.05, 与si-NC组比较。

*P<0.05 compared with si-NC group.



红色字母为突变序列。

The red letters are mutation sequences.

图4 circGFRA1与miR-642a-5p互补的核苷酸序列

Fig.4 Complementary nucleotide sequence between circGFRA1 and miR-642a-5p

表4 各组荧光素酶活性检测结果

Table 4 Luciferase activity detection results in each group

分组 Group	野生型-circGFRA1 WT-circGFRA1	突变型-circGFRA1 MUT-circGFRA1
miR-NC	1.04±0.06	1.01±0.05
miR-642a-5p	0.52±0.05*	1.03±0.06
t	19.974	0.768
P	0.000	0.454

*P<0.05, 与miR-NC组比较。

*P<0.05 compared with miR-NC group.

2.5 上调miR-642a-5p对RASFs增殖、迁移和侵袭的影响

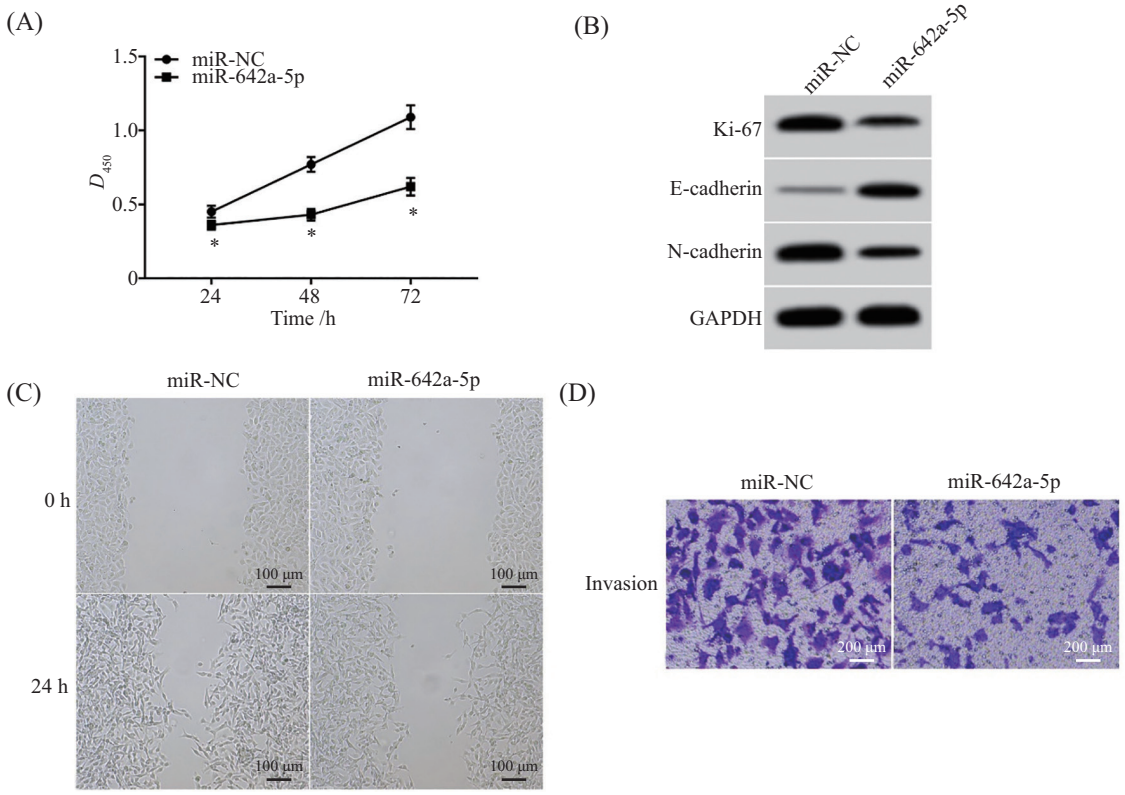
miR-NC组和miR-642a-5p组RASFs中miR-642a-5p的表达量分别为(1.00±0.00)、(3.23±0.27), miR-642a-5p组miR-642a-5p的表达量较miR-NC组显著上调(t=24.778, P<0.05, 图5和表5)。miR-642a-5p组细胞D值、划痕愈合率、侵袭数及细胞中Ki-67、N-cadherin蛋白表达较miR-NC组均显著降低(P<0.05), 而E-cadherin蛋白表达较miR-NC组显著升高(P<0.05, 图5和表5)。

2.6 下调miR-642a-5p逆转了下调circGFRA1对RASFs增殖、迁移和侵袭的作用

si-circGFRA1+anti-miR-NC组和si-circGFRA1+anti-miR-642a-5p组RASFs中miR-642a-5p的表达量分别为(1.00±0.00)、(0.36±0.03), si-circGFRA1+anti-miR-642a-5p组miR-642a-5p的表达量较si-circGFRA1+anti-miR-NC组显著下调(t=64.000, P<0.05, 图6和表6)。si-circGFRA1+anti-miR-642a-5p组细胞D值、划痕愈合率、侵袭数及细胞中Ki-67、N-cadherin蛋白表达量较si-circGFRA1+anti-miR-NC组均显著升高(P<0.05, 图6和表6), 而E-cadherin蛋白表达较si-circGFRA1+anti-miR-NC组显著降低(P<0.05, 图6和表6)。

3 讨论

目前, 临床RA的治疗多采用糖皮质激素、非甾体类抗炎药等, 但长期使用副作用较大[9]。因此, 需要寻找用于治疗RA的新方法。滑膜成纤维细胞的过度增殖和侵袭是RA滑膜增生的主要原因, 探究影响滑膜成纤维细胞过度增殖和侵袭的分子机制可为RA的治疗提供靶点。circRNA和微小RNA(miRNA)作为不同类型的非编码RNA, circRNA能够通过miRNA分子海绵作用, 从而对miRNA靶基因的表达



A: 上调miR-642a-5p对RASFs增殖的影响; B: 上调miR-642a-5p对RASFs中Ki-67、E-cadherin和N-cadherin蛋白表达的影响; C: 上调miR-642a-5p对RASFs迁移的影响; D: 上调miR-642a-5p对RASFs侵袭的影响。*P<0.05, 与miR-NC组比较。

A: the effect of up-regulating miR-642a-5p on the proliferation of RASFs; B: the effect of up-regulating miR-642a-5p on the protein expression of Ki-67, E-cadherin and N-cadherin in RASFs; C: the effect of up-regulating miR-642a-5p on the migration of RASFs; D: the effect of up-regulating miR-642a-5p on the invasion of RASFs. *P<0.05 compared with miR-NC group.

图5 上调miR-642a-5p对RASFs增殖、迁移和侵袭的影响

Fig.5 The effect of up-regulating miR-642a-5p on the proliferation, migration and invasion of RASFs

表5 上调miR-642a-5p对RASFs增殖、迁移和侵袭的影响

Table 5 The effect of up-regulating miR-642a-5p on the proliferation, migration and invasion of RASFs

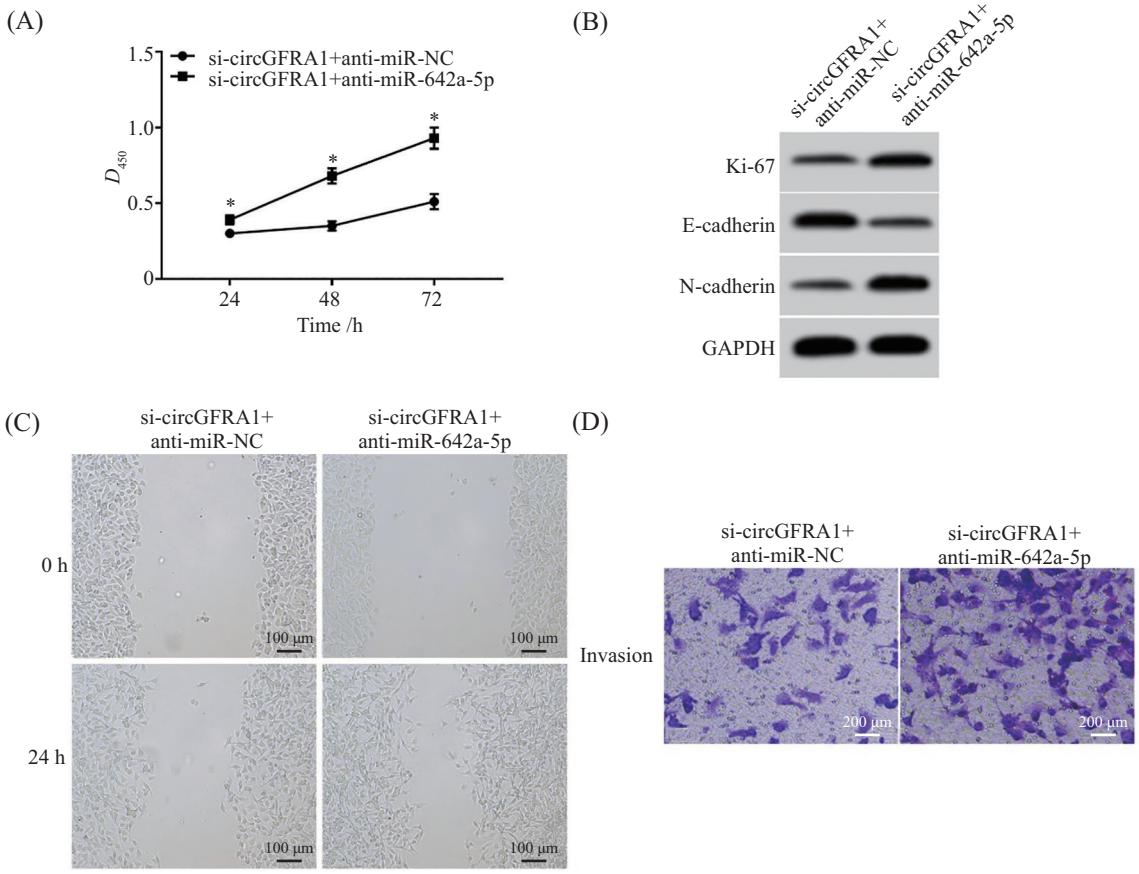
分组 Groups	D_{450}			划痕愈合率/% Cell migration rate /%	侵袭细胞数 Number of invasive cells	Ki-67蛋白 Ki-67 protein	E-cadherin蛋白 E-cadherin protein	N-cadherin蛋白 N-cadherin protein
	24 h	48 h	72 h					
miR-NC	0.45±0.04	0.77±0.05	1.09±0.08	66.02±5.83	108.71±11.67	0.65±0.05	0.14±0.02	0.77±0.05
miR-642a-5p	0.36±0.03	0.43±0.04*	0.62±0.06*	31.21±2.99*	53.24±5.05*	0.30±0.03*	0.51±0.05*	0.34±0.03*
t	5.400	15.930	14.100	15.939	13.087	18.007	20.612	22.123
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

*P<0.05, 与miR-NC组比较。

*P<0.05 compared with miR-NC group.

进行调节及控制。既往研究显示, circRNA/miRNA/靶基因这一调控通路对RASFs的增殖、迁移及侵袭具有重要影响, 为RA的治疗提供了潜在分子靶点。例如, circPTTG1IP、circ0088036和circAFF2均是RA患者滑膜组织中表达上调的circRNA, 且分别通过靶向miR-671-5p/TLR4轴、miR-140-3p/SIRT1、miR-650/CNP轴促进RASFs增殖、迁移和侵袭及炎症反应, 其表达有利于延缓RA的发展进程[10-12]。

作为一种circRNA, 还未见circGFRA1影响RA发生发展的相关报道。本研究主要观察了circGFRA1在RA患者滑膜组织中的表达及其对RASFs增殖、迁移和侵袭的影响, 结果显示, circGFRA1在RA患者滑膜组织中的表达明显高于正常滑膜组织, 下调circGFRA1显著抑制了RASFs增殖、迁移及侵袭,



A: 下调miR-642a-5p逆转了下调circGFRA1对RASFs增殖的影响; B: 下调miR-642a-5p逆转了下调circGFRA1对RASFs中Ki-67、E-cadherin和N-cadherin蛋白表达影响; C: 下调miR-642a-5p逆转了下调circGFRA1对RASFs迁移的影响; D: 下调miR-642a-5p逆转了下调circGFRA1对RASFs侵袭的影响。*P<0.05, 与si-circGFRA1+anti-miR-NC组比较。

A: down-regulating miR-642a-5p reversed the effect of down-regulating circGFRA1 on the proliferation of RASFs; B: down-regulating miR-642a-5p reversed the effect of down-regulating circGFRA1 on the protein expression of Ki-67, E-cadherin and N-cadherin in RASFs; C: down-regulating miR-642a-5p reversed the effect of down-regulating circGFRA1 on the migration of RASFs; D: down-regulating miR-642a-5p reversed the effect of down-regulating circGFRA1 on the invasion of RASFs RASFs. *P<0.05 compared with si-circGFRA1+anti-miR-NC group.

图6 下调miR-642a-5p逆转了下调circGFRA1对RASFs增殖、迁移和侵袭的作用

Fig.6 Down-regulating miR-642a-5p reversed the effect of down-regulating circGFRA1 on the proliferation, migration and invasion of RASFs

表6 下调miR-642a-5p逆转了下调circGFRA1对RASFs增殖、迁移和侵袭的作用

Table 6 Down-regulation of miR-642a-5p reversed the effects of down-regulation of circGFRA1 on the proliferation, migration and invasion of RASFs

分组 Groups	miR-642a-5p	D_{450} 24 h	D_{450} 48 h	D_{450} 72 h	划痕愈合率/% Cell migration rate /%	侵袭细胞数 Number of invasive cells	Ki-67蛋白 Ki-67 protein	E-cadherin蛋白 E-cadherin protein	N-cadherin蛋白 N-cadherin protein
si-circGFRA1+anti-miR-NC	1.00±0.00	0.30±0.03	0.35±0.03	0.51±0.05	22.54±2.67	45.53±4.15	0.22±0.02	0.58±0.04	0.27±0.03
si-circGFRA1+anti-miR-642a-5p	0.36±0.03*	0.39±0.03*	0.68±0.05*	0.93±0.07*	56.35±5.07*	90.58±7.39*	0.55±0.04*	0.28±0.03*	0.62±0.06*
t	64.000	6.364	16.978	14.647	17.701	15.946	22.137	18.000	15.652
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

*P<0.05, 与si-circGFRA1+anti-miR-NC组比较。

*P<0.05 compared with si-circGFRA1+anti-miR-NC group.

提示circGFRA1对RA的发生发展起促进作用, 下调其表达可能起到治疗RASFs的作用, 但尚需在体内进行验证。Ki-67是细胞增殖的标志性蛋白[13]。上皮间质转化是细胞失去上皮极性而获得间质细胞特性的过程, 在此过程中, E-cadherin等上皮标志物表达减少, 而N-cadherin等间质标志物表达增加[14]。RASFs在发生上皮间质转化后, 细胞间黏附作用降低, 更易于迁移和侵袭。本研究结果显示, 下调circGFRA1降低了RASFs中Ki-67和N-cadherin蛋白表达, 而促进了E-cadherin蛋白表达, 进一步提示下调circGFRA1抑制了RASFs增殖、迁移及侵袭。

此外, 本研究证实circGFRA1在RASFs中可靶向负调控miR-642a-5p。miR-642a-5p参与多种疾病的发展进程。ZHENG等[15]研究显示, miR-361-5p可通过靶向抑制TLR4的表达降低三阴性乳腺癌细胞对紫杉醇的耐药性, miR-361-5p可作为改善三阴性乳腺癌紫杉醇耐药性的分子靶点。本研究探究了miR-642a-5p对RASFs增殖、迁移及侵袭的影响, 结果显示, miR-642a-5p在RA患者患者滑膜组织中呈低表达, 上调miR-642a-5p显著削弱了RASFs的增殖、迁移及侵袭性, 这提示miR-642a-5p可能是抑制RA发生发展的有利因素。同时, 恢复实验结果显示, 下调miR-642a-5p逆转了下调circGFRA1对RASFs增殖、迁移及侵袭的抑制作用, 进一步提示circGFRA1可能通过靶向负调控miR-642a-5p来影响RASFs的增殖、迁移及侵袭, 但其具体调控的miR-642a-5p的靶基因还有待进一步探究。

综上, circGFRA1在RA患者滑膜组织中表达量升高, 而miR-642a-5p表达量降低; 下调circGFRA1有效削弱了RASFs的增殖、迁移及侵袭性, 这可能与下调circGFRA1促进了细胞中miR-642a-5p的表达有关。circGFRA1/miR-642a-5p轴可能为RA的治疗提供了新靶点。

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