LINC01128在子宫内膜癌组织中的表达及其调节miR-367-3p/*KLF5*轴对细胞恶性进展的影响

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摘要 该文旨在探究LINC01128在子宫内膜癌(EC)组织中的表达情况及其调节miR-367-3p/Krüppel样因子5(*KLF5*)轴对细胞恶性进展的影响。qRT-PCR检测LINC01128、miR-367-3p和*KLF5* mRNA在EC组织、EC细胞系HEC-1A、人子宫内膜上皮细胞CP-H058中的表达水平。将体外培养的HEC-1A细胞随机分为: NC组(正常培养)、si-LINC01128组、si-NC组、miR-367-3p mimic组及miR-NC组。si-NC组、si-LINC01128组、miR-367-3p mimic组、miR-NC组分别转染si-NC、si-LINC01128、miR-367-3p mimic、miR-NC。CCK-8法、TUNEL染色分别检测HEC-1A细胞增殖、凋亡情况; Transwell、划痕实验分别检测HEC-1A细胞侵袭、迁移情况; 双荧光素酶报告基因实验验证LINC01128与miR-367-3p、miR-367-3p与*KLF5*的靶向关系; Western blot检测细胞中*KLF5*表达情况。与癌旁组织相比, EC组织中LINC01128和*KLF5* mRNA表达水平升高, miR-367-3p表达水平降低; 与CP-H058细胞相比, EC细胞HEC-1A中LINC01128和*KLF5* mRNA表达水平升高, miR-367-3p表达水平降低($P<0.05$)。NC组与si-NC组LINC01128、miR-367-3p、*KLF5* mRNA表达水平以及HEC-1A细胞增殖、凋亡、迁移、侵袭情况无显著差异($P>0.05$)。与si-NC组相比, si-LINC01128组LINC01128、*KLF5* mRNA表达水平以及D_{450}值、迁移率、侵袭数、Bcl-2表达水平均降低, miR-367-3p表达水平、凋亡率及Bax表达水平均升高($P<0.05$)。NC组与miR-NC组LINC01128、miR-367-3p、*KLF5* mRNA表达水平以及HEC-1A细胞增殖、凋亡、迁移及侵袭情况无显著差异($P>0.05$)。与miR-NC组对比, miR-367-3p mimic组LINC01128表达水平无显著差异($P>0.05$), *KLF5* mRNA表达水平、D_{450}值、迁移率、侵袭数及Bcl-2表达水平均降低, miR-367-3p表达水平、凋亡率及Bax表达水平均升高($P<0.05$)。双荧光素酶报告基因实验结果显示, miR-367-3p和LINC01128、*KLF5*均存在靶向调控关系。LINC01128在EC组织和细胞中呈高表达, 干扰LINC01128可通过调节miR-367-3p/*KLF5*轴抑制EC恶性进展。

关键词 子宫内膜癌; LINC01128; miR-367-3p; Krüppel样因子5; 恶性进展

Expression of LINC01128 in Endometrial Cancer Tissue and Its Effect on Cell Malignant Progression by Regulating miR-367-3p/*KLF5* Axis

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荆门市科技计划(批准号: 2020YDKY064)资助的课题

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Abstract This study aims to investigate the expression of LINC01128 in EC (endometrial cancer) tissue and its effect on cell malignant progression by regulating the miR-367-3p/*KLF5* (Krüppel like factor 5) axis. qRT-PCR was applied to detect the expression levels of LINC01128, miR-367-3p, and *KLF5* mRNA in EC tissue, cell line HEC-1A, human endometrial epithelial cells CP-H058. HEC-1A cells cultured *in vitro* were randomly separated into five groups: NC group (normal culture), si-LINC01128 group, si-NC group, miR-367-3p mimic group, and miR-NC group. And the si-NC group, si-LINC01128 group, miR-367-3p mimic group, and miR-NC group were transfected with si-NC, si-LINC01128, miR-367-3p mimic, and miR-NC, respectively. CCK-8 method and TUNEL staining were applied to detect the proliferation and apoptosis of HEC-1A cells, respectively. Transwell and scratch experiments were applied to detect the invasion and migration of HEC-1A cells, respectively. Dual luciferase reporter gene experiment was applied to verify the targeting relationship between LINC01128 and miR-367-3p, and between miR-367-3p and *KLF5*. Western blot was applied to detect the expression of KLF5 in cells. Compared with adjacent tissues, the expression of LINC01128 and *KLF5* mRNA in EC tissues was increased, while miR-367-3p expression was decreased; compared with CP-H058 cell, the expression of LINC01128 and *KLF5* mRNA in EC cell HEC-1A was increased, while miR-367-3p expression was reduced ($P<0.05$). There was no significant difference in the expression of LINC01128, miR-367-3p, *KLF5* mRNA, as well as HEC-1A cell proliferation, apoptosis, migration, and invasion between the NC group and the si-NC group ($P>0.05$). Compared with the si-NC group, the expression of LINC01128 and *KLF5* mRNA, D_{450} value, migration rate, invasion number, and Bcl-2 expression in the si-LINC01128 group were all reduced, while miR-367-3p expression, apoptosis rate, and Bax expression were all increased ($P<0.05$). There was no great difference in the expression of LINC01128, miR-367-3p, *KLF5* mRNA, as well as HEC-1A cell proliferation, apoptosis, migration, and invasion between the NC group and the miR-NC group ($P>0.05$). Compared with the miR-NC group, there was no great difference in LINC01128 expression in the miR-367-3p mimic group ($P>0.05$), the *KLF5* mRNA expression, D_{450} value, migration rate, invasion number, and Bcl-2 expression were all reduced, while miR-367-3p expression, apoptosis rate, and Bax expression were all increased ($P<0.05$). The results of dual luciferase reporter gene experiment showed that miR-367-3p had targeted regulatory relationship with LINC01128 and *KLF5*. LINC01128 is highly expressed in EC tissues and cells, and interference with LINC01128 can inhibit malignant progression of EC by regulating the miR-367-3p/*KLF5* axis.

Keywords endometrial cancer; LINC01128; miR-367-3p; Krüppel like factor 5; malignant progression

子宫内膜癌(endometrial cancer, EC)起源于子宫内膜, 是女性六大常见恶性肿瘤之一, 其发病率为女性生殖系统恶性肿瘤的20%~30%, 且在全球范围内每年约有76 000人死于EC[1]。癌症中心数据显示, EC发病率持续上升, 且呈年轻化趋势, 严重威胁女性生殖及生命健康[2]。对于早期EC患者, 手术切除和术后放化疗的利用可显著延长生存期, 5年生存率约为85%, 然而对于晚期转移或复发EC患者, 预后较差, 5年生存率较低[3]。因此亟需探索EC发生发展的分子机制, 以期为药物开发寻找新的治疗靶点。长度超200 nt的长链非编码RNA(long non-coding RNA, lncRNA)在人类基因组转录本中占比较大, 虽然其不具有编码蛋白质的功能, 但它在各种生物过程中发挥至关重要的作用[4]。据报道, lncRNA参与全身多种生理和病理过程, 特别是在致癌和癌症进展中发挥重要作用[5]。LINC01128是一种在癌症中新发现的lncRNA, HU等[6]研究显示, LINC01128通过靶向miR-383-5p/SFN轴参与宫颈癌的恶性进展, 但LINC01128对EC细胞的影响尚未可知。miRNA可参与调控多种生物过程, 在肿瘤发展过程中充当促癌或抑癌因子[7-8]。miR-367-3p是miRNA家族成员之一, 通过生物信息学网站预测发现miR-367-3p与LINC01128、Krüppel样因子5(Krüppel like factor 5, *KLF5*)存在靶向关系, 且miR-367-3p与多种肿瘤细胞恶性进展联系密切[9]。*KLF5*属于KLF家族, 可通过调控Wnt/β-catenin信号通路参与EC细胞的增殖和侵袭[10]。因此, 推测LINC01128可能通过调控miR-367-3p/*KLF5*轴影响EC恶性进展。本研究将探索LINC01128对EC恶性行为及其对miR-367-3p/*KLF5*轴的影响, 以期为EC诊断及治疗提供可能靶点。

1 材料与方法

1.1 临床资料

收集2021年8月至2023年11月在荆门市中医医院进行手术治疗的59例EC患者的癌组织和癌旁组织, 患者年龄为32~61(43.36±4.01)岁; 国际妇产科学联盟(International Federation of Gynecology and Obstetrics, FIGO) I~II期31例, III~IV期28例。59例EC患者均首次诊断为EC, 未行任何抗肿瘤治疗。本研究经荆门市中医医院伦理委员会审批通过(批准号: 2021-0025)。

1.2 材料与试剂

人EC细胞系HEC-1A购于优利科(上海)生命科学有限公司; 人子宫内膜上皮细胞CP-H058购于武汉普诺赛生命科技有限公司; si-LINC01128及其阴性对照si-NC购于苏州吉玛基因股份有限公司; miR-367-3p mimic及其阴性对照miR-NC购于上海吉玛制药技术有限公司; DMEM培养基购于美国Hyclone公司; 胎牛血清购于北京索莱宝科技有限公司; Trizol试剂购于美国Invivogen公司; CCK-8细胞增殖试剂盒购于汉恒生物工程有限公司; TUNEL试剂盒购于上海优宁维生物有限公司; Transwell小室、Matrigel胶购于美国Corning公司; Bax、Bcl-2一抗及二抗购于美国Abcam公司。

1.3 方法

1.3.1 细胞转染、分组 HEC-1A细胞用专用培养基培养, 隔日换液, 当细胞融合度超80%时传代, 选对数期、生长良好的细胞进行后续研究。将HEC-1A细胞分为NC组(正常培养)、si-LINC01128组、si-NC组、miR-367-3p mimic组及miR-NC组。根据转染试剂盒说明书, si-NC组、si-LINC01128组、miR-367-3p mimic组、miR-NC组分别转染si-NC、si-LINC01128、miR-367-3p mimic、miR-NC, 培养24 h(37 °C、5% CO_2、饱和湿度培养箱)后进行后续研究。

1.3.2 qRT-PCR检测LINC01128、miR-367-3p、*KLF5* mRNA表达情况 收集各组细胞、EC组织及癌旁组织, 利用Trizol裂解提取组织及细胞中总RNA, 检测RNA纯度、浓度。利用逆转录试剂盒合成cDNA并进行qRT-PCR定量检测。反应体系: 4 μL Master Mix PCR预混液, 上下游引物各0.5 μL, 2 μL cDNA模板, 双蒸水补至10 μL。扩增环境: 97 °C预变性8 min; 97 °C变性30 s, 60 °C退火20 s, 72 °C延伸10 s, 共40个循环。利用$2^{-\Delta\Delta Ct}$法计算、定量LINC01128、miR-367-3p、*KLF5* mRNA的相对表达量, 引物序列见表1。

1.3.3 CCK-8法、TUNEL染色分别检测细胞增殖、凋亡情况 CCK-8法: 将1.3.1各组HEC-1A细胞铺于96孔板(5×10^4个/孔)中, 48 h后终止培养并向各孔加入10 μL CCK-8试剂, 1 h后, 使用酶标仪对450 nm处的吸光度(*D*)值进行测定。

TUNEL染色: 将1.3.1各组HEC-1A细胞接种至24孔板细胞爬片(2×10^5个/mL)上, 48 h后取出爬片, 4%多聚甲醛在室温下固定15 min, TUNEL染色严格根据TUNEL试剂盒说明书进行, 随后加入磷酸盐缓冲液冲洗, 荧光显微镜下拍照, 记录TUNEL阳性核占每个视野的百分比。

1.3.4 Transwell检测细胞侵袭情况 Matrigel基质胶铺于Transwell小室上室, 无血清培养基重悬1.3.1各组HEC-1A细胞(1×10^5个/mL), 200 μL细胞悬液接种于Transwell小室上室, 下室加入600 μL DMEM, 37 °C孵育24 h后用磷酸盐缓冲液清洗2遍,

表1 引物序列

Table 1 Primer sequences

引物名称 Primers name	引物序列 Primer sequences
LINC01128	F: 5′-AAG GTG AGG TGA GAG GAC AGG AAG-3′
	R: 5′-CAA GGC AGG CAC TCA ACG GTA G-3′
KLF5	F: 5′-AGC TCA CCT GAG GAC TCA TA-3′
	R: 5′-GTG CGC AGT GCT CAG TTC T-3′
GAPDH	F: 5′-AGA AGG CTG GGG CTC ATT TG-3′
	R: 5′-AAC GCT TCA CGA ATT TGC GT-3′
miR-367-3p	F: 5′-AGT GCA GGG TCC GAG GTA TT-3′
	R: 5′-CGA CGA ATT GCA CTT TAG C-3′
U6	F: 5′-CTC GCT TCG GCA GCA CA-3′
	R: 5′-AAC GCT TCA CGA ATT TGC GT-3′

4%多聚甲醛室温固定30 min, 0.1%结晶紫室温下染色20 min, 光学显微镜记录拍照, 随机拍照5个视野, 利用ImageJ软件计数。

1.3.5 划痕实验检测细胞迁移情况 取1.3.1中各组对数期HEC-1A细胞制成单细胞悬液, 6孔板中接种HEC-1A细胞(5×10^5个/mL), 当细胞融合度超80%时, 用枪头在6孔板底部作划痕, 37 ℃、5% CO_2、饱和湿度培养箱中培养48 h, 光学显微镜拍摄细胞间距离。

1.3.6 双荧光素酶报告基因检测 分别构建野生型(LINC01128-WT、*KLF5*-WT)和突变型(LINC01128-MUT、*KLF5*-MUT)载体。将上述质粒再分别与miR-367-3p mimic及miR-NC共转染HEC-1A细胞, 48 h后测定荧光素酶活性。

1.3.7 Western blot检测Bax、Bcl-2蛋白表达情况 RIPA裂解法裂解各组细胞并抽提蛋白。BCA法定量蛋白浓度后, 利用10% SDS-PAGE电泳分离蛋白并将其转移到PVDF膜上。封闭液中室温封闭2 h后, 添加Bax、Bcl-2一抗(稀释比例1:1 000)于4 ℃孵育过夜, TBST洗膜后加入二抗(1:1 000), 室温下孵育1 h后添加曝光液上机曝光, 利用ImageJ软件对条带强度进行定量分析。

1.4 统计分析

利用SPSS 26.0和GraphPad Prism 9.0软件处理实验数据, 计量资料以均值±标准差($\bar{x}\pm s$)表示。多组间比较采用单因素方差分析, 进一步两两对比采用SNK-q检验; 两组间比较采用t检验, P<0.05表示差异具有显著性。

2 结果

2.1 LINC01128、miR-367-3p、*KLF5* mRNA在EC组织及细胞中的表达情况

与癌旁组织对比, EC组织中LINC01128、*KLF5* mRNA表达水平升高, miR-367-3p表达水平降低(P<0.05, 表2); HEC-1A细胞中LINC01128、*KLF5* mRNA表达水平均高于人子宫内膜上皮细胞CP-H058, miR-367-3p表达水平低于人子宫内膜上皮细胞CP-H058(P<0.05, 表3)。

2.2 沉默LINC01128对HEC-1A细胞LINC01128、miR-367-3p、*KLF5* mRNA表达以及细胞增殖、凋亡、迁移及侵袭的影响

与NC组对比, si-NC组LINC01128、*KLF5* mRNA表达水平, HEC-1A细胞增殖、凋亡、迁移、侵袭及Bax、Bcl-2蛋白表达水平均无显著差异(P>0.05)。与si-NC组对比, si-LINC01128组LINC01128、*KLF5* mRNA表达水平以及D_{450}值、迁移率、侵袭数、Bcl-2表达水平均降低, miR-367-3p表达水平、凋亡率及Bax表达水平均升高(P<0.05, 图1~图4、表4)。

2.3 上调miR-367-3p对HEC-1A细胞LINC01128、miR-367-3p、*KLF5* mRNA表达水平以及增殖、凋亡、迁移、侵袭的影响

与NC组对比, miR-NC组LINC01128、miR-367-3p、*KLF5* mRNA表达水平, HEC-1A细胞增殖、凋亡、迁移、侵袭及Bax、Bcl-2蛋白表达水平无显著差异(P>0.05)。与miR-NC组对比, miR-

表2 LINC01128、miR-367-3p、*KLF5* mRNA在EC及癌旁组织中的表达情况

Table 2 Expression of LINC01128, miR-367-3p and *KLF5* mRNA in EC and paracancer tissues

类别 Category	LINC01128	miR-367-3p	*KLF5* mRNA
Para-carcinoma tissue	1.01±0.13	1.02±0.12	1.04±0.14
EC tissue	2.14±0.23	0.30±0.05	2.51±0.26
t	32.853	42.542	38.237
P	<0.05	<0.05	<0.05

表3 不同细胞中LINC01128、miR-367-3p、*KLF5* mRNA表达水平对比

Table 3 Comparison of LINC01128, miR-367-3p and *KLF5* mRNA expression in different cells

类别 Category	LINC01128	miR-367-3p	*KLF5* mRNA
CP-H058 cell	1.01±0.12	0.99±0.10	1.04±0.13
HEC-1A cell	1.71±0.11	0.62±0.07	1.52±0.11
t	10.382	7.425	6.904
P	<0.05	<0.05	<0.05

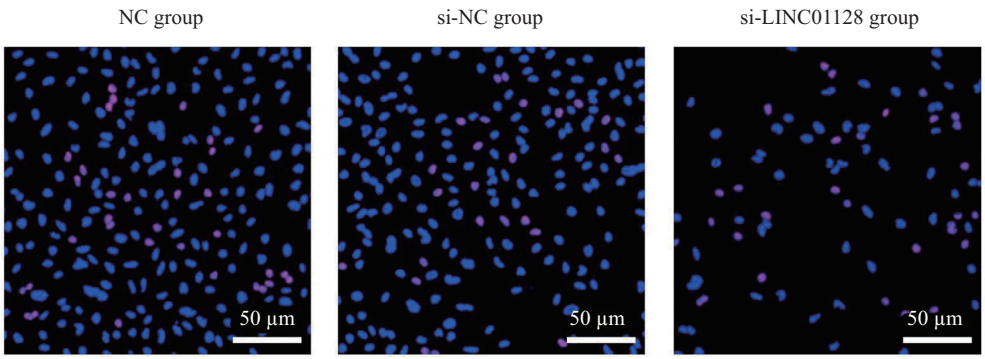


图1 TUNEL染色检测细胞凋亡情况

Fig.1 Cell apoptosis was detected by TUNEL staining

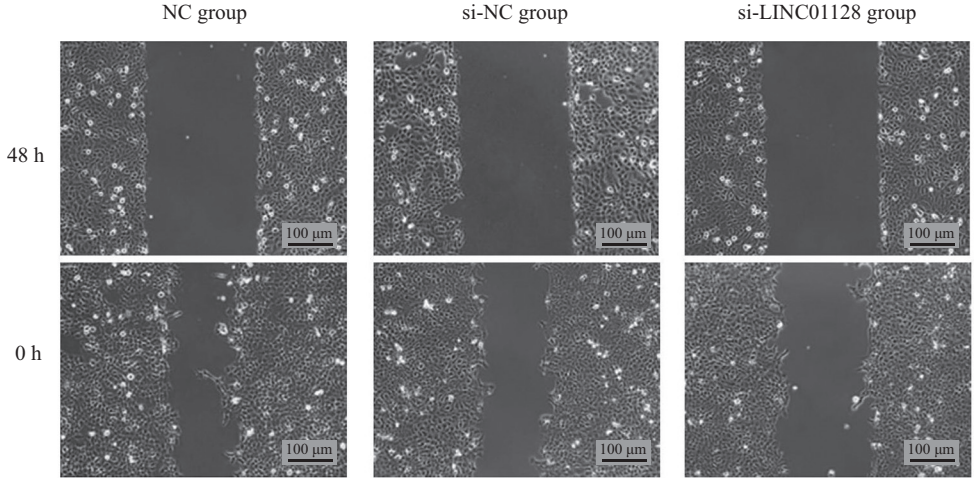


图2 划痕实验检测细胞迁移情况

Fig.2 Cell migration was detected by scratch test

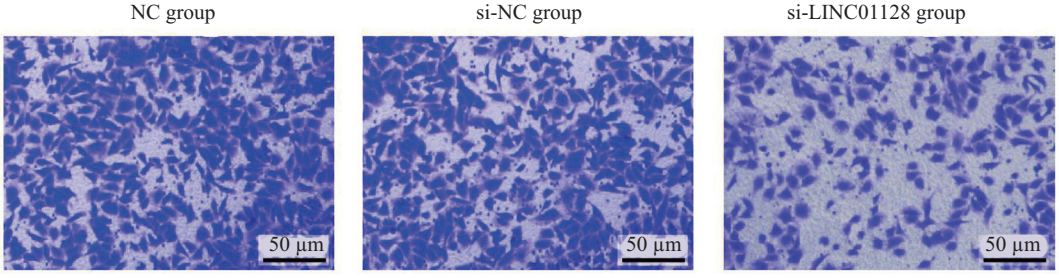


图3 Transwell检测细胞侵袭情况

Fig.3 Cell invasion was detected by Transwell

367-3p mimic组LINC01128表达水平无显著差异(P>0.05), *KLF5* mRNA表达水平、D_{450}值、迁移率、侵袭数、Bcl-2表达水平均降低, miR-367-3p表达水平、凋亡率、Bax表达水平均升高(P<0.05, 图5~图8、表5)。

2.4 miR-367-3p与LINC01128的靶向关系

miR-367-3p与LINC01128存在结合位点(图9A)。与miR-NC和LINC01128-WT共转染组(1.05±0.12)相比, miR-367-3p mimic和LINC01128-WT共转染组(0.40±0.05)荧光素酶活性降低(t=19.031, P<0.05); 与miR-NC和LINC01128-MUT共转染组(1.11±0.15)对比, miR-367-3p mimic与LINC01128-MUT共转染组(1.03±0.08)荧光素酶活性无显著差异(t=1.153, P>0.05)(图9B)。qRT-PCR结果显示, 与si-NC组(1.05±0.11)相比, si-LINC01128组miR-367-3p水平(1.88±0.15)显著升高(P<0.05)(表4), 提示LINC01128

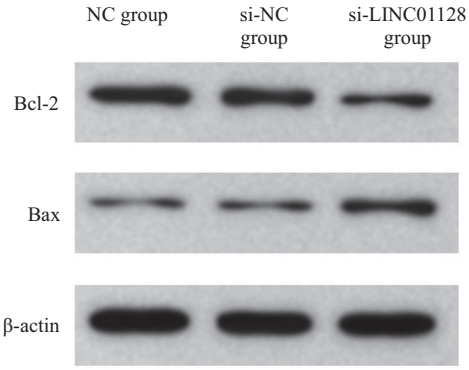


图4 Western blot检测Bax、Bcl-2蛋白表达情况

Fig.4 Western blot analysis of Bax and Bcl-2 protein expression

表4 沉默LINC01128对HEC-1A细胞中LINC01128、miR-367-3p、*KLF5* mRNA表达水平以及增殖、凋亡、迁移及侵袭的影响

Table 4 Effects of silencing LINC01128 on expression levels of LINC01128, miR-367-3p and *KLF5* mRNA, and proliferation, apoptosis, migration and invasion of HEC-1A cells

组别 Group	凋亡率/% Apoptosis rate /%	划痕愈合率/% Cratch healing rate /%	细胞侵袭数 Cell invasion number	D_{450}	LINC01128	miR-367-3p	*KLF5* mRNA	Bcl-2	Bax
NC group	13.11±1.08	48.92±4.17	186.31±16.74	0.85±0.08	1.04±0.11	1.07±0.12	1.05±0.11	0.93±0.12	0.25±0.03
si-NC group	11.62±1.04	47.13±4.01	182.42±16.43	0.83±0.07	1.01±0.10	1.05±0.11	1.01±0.11	0.90±0.12	0.22±0.02
si-LINC01128 group	39.11±2.57*#	26.53±2.41*#	101.36±11.04*#	0.53±0.04*#	0.30±0.04*#	1.88±0.15*#	0.42±0.05*#	0.43±0.04*#	0.52±0.05*#

*P<0.05, 与NC组对比; #P<0.05, 与si-NC组对比。

*P<0.05 compared with NC group; #P<0.05 compared with si-NC group.

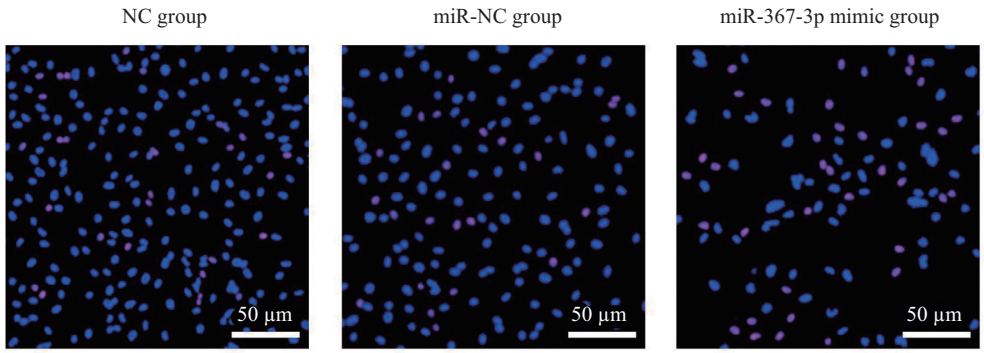


图5 TUNEL染色检测细胞凋亡情况

Fig.5 Cell apoptosis was detected by TUNEL staining

可靶向负调控miR-367-3p的表达。

2.5 miR-367-3p与*KLF5*的靶向关系

miR-367-3p与*KLF5*存在结合位点(图10A)。与miR-NC和*KLF5*-WT共转染组(1.03±0.12)相比, miR-367-3p mimic和*KLF5*-WT共转染组(0.40±0.05)荧光素酶活性降低(t=11.871, P<0.05); 与miR-NC和*KLF5*-MUT共转染组(1.08±0.11)对比, miR-367-3p mimic与*KLF5*-MUT共转染组(1.01±0.08)荧光素酶活性无显著差异(t=1.261, P>0.05)(图10B)。Western blot结果显示, 与miR-NC组(0.48±0.05)相比, miR-367-3p mimic组的*KLF5*蛋白表达水平(0.27±0.03)显著降低(P<0.05), 提示*KLF5*为miR-367-3p的靶基因, miR-367-3p可负调控*KLF5*的表达(图11)。

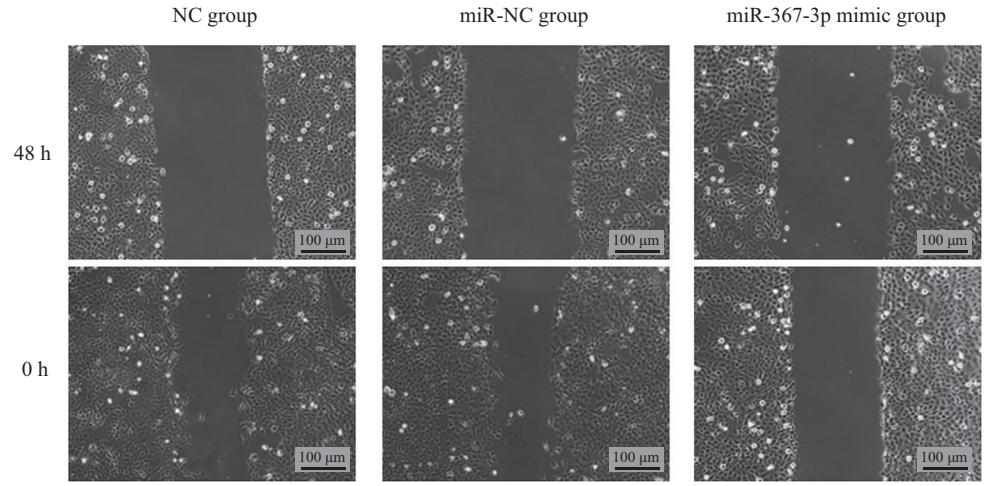


图6 划痕实验检测细胞迁移

Fig.6 Cell migration was detected by scratch test

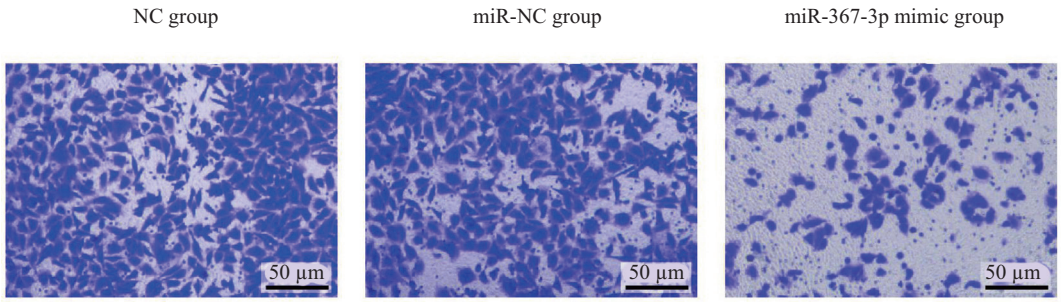


图7 Transwell检测细胞侵袭情况

Fig.7 Cell invasion was detected by Transwell

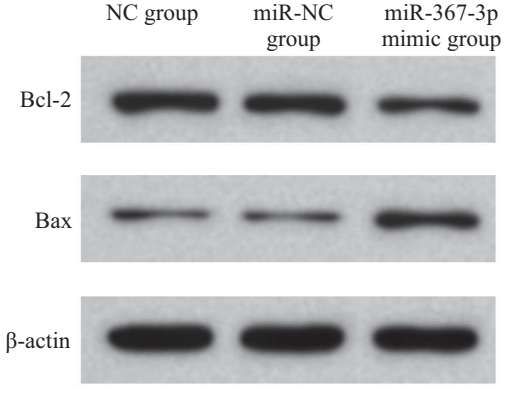


图8 Western blot检测Bax、Bcl-2蛋白表达情况

Fig.8 Western blot analysis of Bax and Bcl-2 protein expression

3 讨论

EC是一种依赖雌激素的女性生殖系统恶性肿瘤, 其发病率和死亡率逐年递增, 每年约有7.6万女性死于EC, 其现已成为妇科第二大恶性肿瘤[11]。随着医疗技术的不断发展, EC诊断技能及治疗策略均获得了突破性进展, 目前超70%的EC患者可在早期诊断, 通过手术及放化疗治疗预后良好, 然而对于晚期EC患者, 治疗选择有限, 生存质量及预后仍不理想[12-13]。因此积极探索EC的潜在发病机制, 探索新的治疗手段, 对改善患者预后具有重要临床意义。

LncRNA是一类功能性RNA分子, 长度超过200 nt, 是基因表达和染色单体修饰的重要调节因

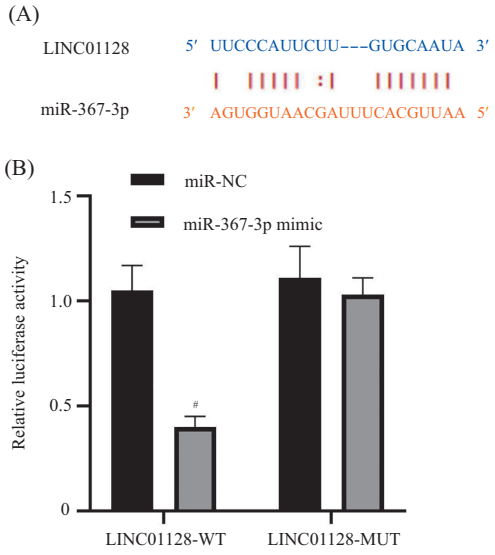
表5 上调miR-367-3p对HEC-1A细胞miR-367-3p、LINC01128、*KLF5* mRNA表达及以增殖、凋亡、迁移及侵袭的影响

Table 5 Effects of up-regulation of miR-367-3p on expression of miR-367-3p, LINC01128 and *KLF5* mRNA, and proliferation, apoptosis, migration and invasion of HEC-1A cells

组别 Group	凋亡率/% Apoptosis rate /%	划痕愈合率/% Cratch healing rate /%	细胞侵袭数 Cell invasion number	D_{450}	LINC01128	miR-367-3p	*KLF5* mRNA	Bcl-2	Bax
NC group	12.81±1.09	49.12±4.23	185.41±16.82	0.83±0.07	1.03±0.11	1.06±0.12	1.04±0.11	0.91±0.11	0.24±0.04
miR-NC group	11.27±1.05	48.55±4.10	183.50±16.48	0.81±0.06	1.07±0.12	1.04±0.14	1.03±0.12	0.88±0.10	0.21±0.03
miR-367-3p mimic group	38.64±2.43*&	19.87±2.52*&	73.56±9.14*&	0.47±0.05*&	1.01±0.11	2.24±0.19*&	0.43±0.05*&	0.41±0.04*&	0.50±0.05*&

*P<0.05, 与NC组对比; &P<0.05, 与miR-NC组对比。

*P<0.05 compared with NC group; &P<0.05 compared with miR-NC group.



A: miR-367-3p与LINC01128存在靶向结合位点; B: 双荧光素酶报告基因实验验证miR-367-3p与LINC01128的靶向关系; #P<0.05, 与miR-NC和LINC01128-WT共转染组对比。

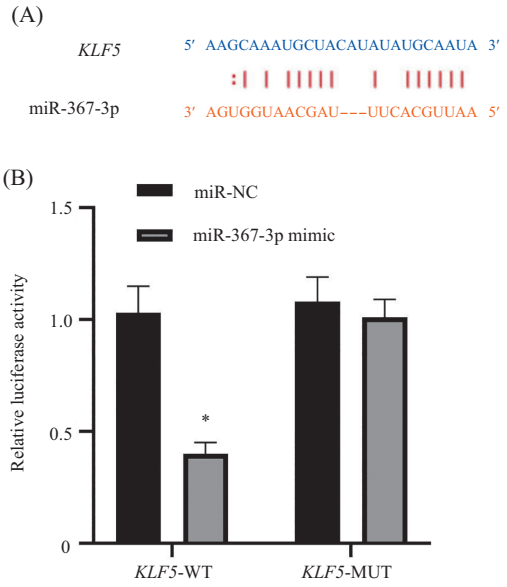
A: there was a targeted binding site between miR-367-3p and LINC01128; B: double luciferase reporter gene assay verified the targeting relationship between miR-367-3p and LINC01128; #P<0.05 compared with miR-NC and LINC01128-WT cotransfection group.

图9 miR-367-3p与LINC01128的靶向关系

Fig.9 Targeting relationship between miR-367-3p and LINC01128

子[14]。有研究显示, lncRNA参与多种生理、病理过程, 值得注意的是, lncRNA参与多种恶性肿瘤的发病机制和恶性进展, 可作为肿瘤诊断和预后标志物[15]。在EC中, 许多lncRNA表达异常并参与该病的发病机制。尽管现已发现众多lncRNA在EC中具有致癌或抗癌作用, 但仍有大量lncRNA可能有助于EC的进展, 且尚未深入探索[16]。LINC01128位于1号染色体上, 是一种新发现的lncRNA, 现已被证实在肿瘤进展中扮演重要角色。例如, LINC01128在神经胶质瘤组织和细胞株中表达上调, 其过表达可促进神经胶质瘤细胞增殖、迁移和侵袭[17]。本研究发现, LINC01128在EC组织中表达水平升高, 沉默LINC01128表达可使HEC-1A细胞增殖、迁移、侵袭能力及Bcl-2表达水平降低, 使细胞凋亡率及Bax表达水平升高, 提示LINC01128是EC的促癌因子, 沉默LINC01128可以抑制HEC-1A细胞恶性进展, 其可作为EC的靶向分子。

LncRNA通常与下游miRNA结合发挥作用, miRNA是内源性单链非编码RNA, 在细胞增殖、分化和凋亡中扮演重要角色, 现已被证实与多种恶性肿瘤的发生密切相关[18]。为进一步分析LINC01128抑制EC细胞恶性进展的机制, 本研究通过Starbase网



A: miR-367-3p与*KLF5*存在靶向结合位点; B: 双荧光素酶报告基因实验验证miR-367-3p与*KLF5*的靶向关系; *P<0.05, 与miR-NC和*KLF5*-WT共转染组对比。

A: there were targeted binding sites between miR-367-3p and *KLF5*; B: double luciferase reporter gene assay verified the targeting relationship between miR-367-3p and *KLF5*; *P<0.05 compared with miR-NC and *KLF5*-WT co-transfection group.

图10 miR-367-3p与*KLF5*的结合位点

Fig.10 Binding sites of miR-367-3p and *KLF5*

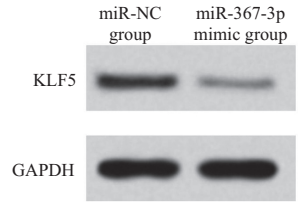


图11 Western blot检测KLF5蛋白表达情况

Fig.11 Western blot analysis of KLF5 protein expression

站预测发现, LINC01128与miR-367-3p存在结合位点。miR-367-3p具有促癌或抑癌功能, 如杨凌博等[19]研究显示, miR-367-3p在肾癌组织和细胞系中表达水平升高, 下调miR-367-3p表达可有效抑制肾癌细胞的增殖和转移。DU等[20]研究显示, miR-367-3p在前列腺癌组织和细胞系中表达水平降低, miR-367-3p过表达可抑制前列腺癌细胞的增殖、侵袭和转移。本研究发现miR-367-3p在EC组织和细胞系中表达水平降低, 上调miR-367-3p可抑制EC细胞的恶性生物学行为, 提示miR-367-3p在EC中发挥抑癌作用。为进一步探讨miR-367-3p调控EC细胞的机制, 本研究通过Starbase网站对miR-367-3p潜在靶基因进行预测发现, miR-367-3p与*KLF5*存在结合位点。*KLF5*在多种细胞中特异性表达, 在发育、代谢和细胞多能性中发挥重要作用[21]。此外, *KLF5*还可驱动多种肿瘤的进展和转移, 如WU等[21]研究显示, *KLF5*在胃癌组织中表达上调, 沉默*KLF5*增强了胃癌细胞自噬、凋亡。本研究结果发现, *KLF5*在过表达miR-367-3p的EC细胞中表达水平降低, 荧光素酶实验结果显示, miR-367-3p与*KLF5*之间为负性调控关系, 提示干扰LINC01128通过调节miR-367-3p/*KLF5*轴抑制EC恶性进展。

综上所述, LINC01128在EC组织和细胞中高表达, 干扰LINC01128可通过调节miR-367-3p/*KLF5*轴抑制EC恶性进展。然而LINC01128与EC患者临床病理特征的关系尚未明确, 且未进行动物实验进一步验证, 未来将深入探究完善, 为EC的靶向治疗提供新靶点。

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