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LncRNA DLEU2调节miR-30a-5p/SOX4信号通路对卵巢癌细胞恶性生物学行为的影响

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摘要 该研究旨在探讨LncRNA DLEU2调节miR-30a-5p/SOX4信号通路对卵巢癌细胞恶性生物学行为的影响。对卵巢癌细胞(SK-OV-3、OVCAR-8、A2780、OV90)进行表型筛选, 选择SK-OV-3并将其分为control组、si-NC组、si-DLEU2组、mimic NC组、miR-30a-5p mimic组、si-DLEU2+inhibitor NC组、si-DLEU2+miR-30a-5p inhibitor组。qRT-PCR检测LncRNA DLEU2、miR-30a-5p、*SOX4* mRNA表达水平; CCK-8法检测细胞增殖情况; 划痕实验检测细胞迁移情况; Transwell检测细胞侵袭情况; Hoechst 33258染色检测细胞凋亡情况; Western blot法检测SOX4蛋白表达水平; 双荧光素酶报告基因实验检测LncRNA DLEU2与miR-30a-5p、miR-30a-5p与*SOX4*的相互作用。结果显示, SK-OV-3与人正常卵巢细胞相比, miR-30a-5p表达水平降低, LncRNA DLEU2、*SOX4* mRNA表达水平升高(P<0.05); 与control组以及si-NC组比较, si-DLEU2组LncRNA DLEU2表达水平、SOX4表达水平及细胞增殖、迁移和侵袭能力降低, miR-30a-5p表达水平和细胞凋亡率升高(P<0.05)。转染miR-30a-5p mimic对SK-OV-3细胞的影响与转染si-DLEU2相同; 与si-DLEU2+inhibitor NC组比较, si-DLEU2+miR-30a-5p inhibitor组细胞SOX4表达水平升高, 增殖、迁移和侵袭能力提高, miR-30a-5p水平、细胞凋亡率降低(P<0.05); 双荧光素酶活性检测表明, LncRNA DLEU2与miR-30a-5p之间、miR-30a-5p与*SOX4*之间存在靶向关系。总结得出, 干扰LncRNA DLEU2可能通过上调miR-30a-5p表达, 抑制SOX4表达, 抑制卵巢癌细胞恶性生物学行为。

关键词 LncRNA DLEU2; miR-30a-5p; SOX4; 卵巢癌细胞; 恶性生物学行为

The Effect of LncRNA DLEU2 on the Malignant Biological Behavior of Ovarian Cancer Cells by Regulating the miR-30a-5p/SOX4 Signaling Pathway

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Abstract This study aimed to investigate the effect of LncRNA DLEU2 on the malignant biological behavior of ovarian cancer cells by regulating the miR-30a-5p/SOX4 signaling pathway. Phenotypic screening of ovarian cancer cells (SK-OV-3, OVCAR-8, A2780, OV90), ovarian cancer cells SK-OV-3 were selected and assigned into control group, si-NC group, si-DLEU2 group, mimic NC group, miR-30a-5p mimic group, si-

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DLEU2+inhibitor NC group, and si-DLEU2+miR-30a-5p inhibitor group. qRT-PCR was applied to detect the mRNA expression levels of LncRNA DLEU2, miR-30a-5p, and *SOX4*. CCK-8 method was applied to detect cell proliferation. Scratch experiment was applied to detect cell migration. Transwell method was applied to detect cell invasion. Hoechst 33258 staining was applied to detect cell apoptosis. Western blot was applied to detect the expression level of SOX4 protein. The dual luciferase reporter gene assay was applied to detect the interaction between LncRNA DLEU2 and miR-30a-5p, and between miR-30a-5p and *SOX4*. The results showed that compared with normal human ovarian cells, the expression of LncRNA DLEU2 and *SOX4* mRNA in SK-OV-3 increased, while the expression of miR-30a-5p decreased (P<0.05). Compared with the control group and si-NC group, the expression of LncRNA DLEU2, expression of SOX4, the abilities of cell proliferation, migration, and invasion were lower in SK-OV-3 cells in the si-DLEU2 group, while the expression of miR-30a-5p and apoptosis rate were higher (P<0.05). The effect of transfecting miR-30a-5p mimic on SK-OV-3 cells was the same as transfecting si-DLEU2. Compared with the si-DLEU2+inhibitor NC group, the abilities of proliferation, migration, and invasion of SK-OV-3 cells in si-DLEU2+miR-30a-5p inhibitor group were higher, the expression of SOX4 was higher, and miR-30a-5p expression and apoptosis rate were lower (P<0.05). The dual luciferase reporter gene experiment showed that LncRNA DLEU2 had a targeted relationship with miR-30a-5p, and miR-30a-5p had a targeted relationship with *SOX4*. In conclusion, interference with LncRNA DLEU2 may upregulate miR-30a-5p expression, inhibit SOX4 expression, and suppress the malignant biological behavior of ovarian cancer cells.

Keywords LncRNA DLEU2; miR-30a-5p; SOX4; ovarian cancer cells; malignant biological behavior

卵巢癌是全球女性最致命的妇科恶性肿瘤之一, 5年生存率约为46%, 在女性常见癌症中排名第七, 死亡率在癌症中排名第八[1]。由于临床表现不典型, 早期缺乏特异性和敏感性好的诊断生物标志物, 70%~90%的患者确诊时已经错过最佳治疗时机[2]。据2018年*The Lancet*报道, 中国卵巢癌患者的平均5年生存率10年来基本保持不变[3]。因此, 探究卵巢癌的发病机制、建立有效诊断方法以及寻找新的治疗靶点至关重要[4]。在多种癌症中, DLEU2起着促调控作用, 例如, DLEU2不仅可促进宫颈癌进展, 还可促进结直肠癌细胞增殖和侵袭[5]。DLEU2在口腔癌细胞中上调, 其高表达显著缩短患者的无病生存期, 抑制DLEU2表达可促进miR-30a-5p表达, 降低p38 MAPK磷酸化水平, 抑制细胞增殖、迁移和侵袭[6]。在非小细胞肺癌中, lncRNA DLEU2通过与miR-30a-5p结合, 抑制miR-30a-5p表达, 促进肿瘤发生和侵袭[7]。有研究表明, miR-30a-5p过表达可显著增强卵巢癌细胞增殖、迁移和侵袭能力, 而miR-30a-5p抑制则表现出相反趋势[8]。LINC00926过表达可通过抑制miR-30a-5p表达以促进Sry相关高迁移率-box4(SOX4)表达, 表明miR-30a-5p与SOX4具有靶向关系[9]。SOX4是SOX转录因子家族的成员, 其在多种癌症中被诱导表达。有研究表明, FEZF1-AS1通过靶向miR-130a-5p及其下游SOX4表达来促进卵巢癌细胞的转移和增殖[10]。通过Starbase分析发现, LncRNA DLEU2与miR-30a-5p、miR-30a-5p与*SOX4*的序列存在互补结合区域。因此, 本研究通过干扰LncRNA DLEU2表达和过表达miR-30a-5p, 探究LncRNA DLEU2对卵巢癌细胞的影响, 及miR-30a-5p和SOX4在其中的作用。

1 材料与方法

1.1 细胞来源

人卵巢癌细胞(SK-OV-3细胞系)购自武汉普诺赛生命科技有限公司; 人正常卵巢上皮细胞(IOSE80)、人卵巢癌细胞(OVCAR-8细胞系、A2780细胞系、OV90细胞系)购自思泰默(上海)生物科技有限公司。

1.2 实验试剂

si-DLEU2、si-NC、miR-30a-5p mimic、mimic NC、miR-30a-5p inhibitor、inhibitor NC购自广州市锐博生物科技有限公司; TRIzol试剂、逆转录试剂盒购自南京诺唯赞生物科技股份有限公司; SYBR® Green I核酸凝胶染液购自Sigma-Aldrich公司; 增强型CCK-8细胞活力检测试剂盒、Hoechst 33258染色液购自武汉尚恩生物技术有限公司; Lipofectamine

2000转染试剂购自ThermoFisher Scientific公司; Matrigel、细胞总蛋白提取试剂盒、Transwell小室、SDS-PAGE购自武汉三鹰生物技术有限公司; BCA试剂盒、DAB试剂、PVDF膜、增强型ECL化学发光试剂盒购自上海碧云天生物技术有限公司; SOX4、GAPDH兔单克隆抗体(货号: ab316850、ab9485)、HRP标记的山羊抗兔IgG抗体(货号: ab6721)购自英国Abcam公司。

1.3 方法

1.3.1 细胞培养与分组转染 使用RPMI-1640培养基培养细胞, 培养基中补充有10%胎牛血清。所有细胞均保存在37 °C、5% CO_2的培养箱中。所有实验均使用无支原体细胞进行。将SK-OV-3细胞分为七组, control组不转染质粒、si-NC组转染si-NC质粒、si-DLEU2组转染si-DLEU2质粒、mimic NC组转染mimic NC质粒、miR-30a-5p mimic组转染miR-30a-5p mimic质粒、si-DLEU2+inhibitor NC组共转染si-DLEU2与inhibitor NC质粒、si-DLEU2+miR-30a-5p inhibitor组共转染si-DLEU2与miR-30a-5p inhibitor质粒, 使用0.3 mg/mL质粒, 所有转染均使用Lipofectamine 2000进行。12 h后, 将培养上清液替换为含有10%胎牛血清(fetal bovine serum, FBS)的新鲜培养基, 进行各指标检测。

1.3.2 qRT-qPCR检测LncRNA DLEU2、miR-30a-5p和*SOX4* mRNA表达水平 使用TRIzol试剂提取细胞总RNA。根据制造商的手册, 使用逆转录试剂盒合成cDNA。qRT-PCR反应使用SYBR® Green I核酸凝胶染液进行。PCR反应在以下条件下进行: 95 °C预变性5 min; 95 °C变性10 s、55 °C退火20 s、72 °C延伸20 s, 40个循环。miR-30a-5p以*U6*为内参, 其余RNA以*GAPDH*为内参, 通过$2^{-\Delta\Delta Ct}$法计算基因相对表达量。使用的所有引物序列置于表1中。

1.3.3 CCK-8检测细胞增殖情况 将转染的细胞接种到96孔板中, 密度为每孔6×10^4个细胞。在24、48、72 h测定细胞的增殖情况。随后, 向每个孔中加入10 μL CCK-8溶液, 于37 °C下再孵育2 h, 然后在微孔板读数仪上读取450 nm处的吸光度(*D*)值。

1.3.4 划痕实验检测细胞迁移情况 将5×10^4个细胞接种于6孔板, 用小号枪头垂直划痕, 加入含有10% FBS的DMEM培养基。培养24 h后观察并记录结果。划痕愈合率=[(0 h时的划痕面积–24 h时的划痕面积)/0 h的划痕面积]×100%。

1.3.5 Transwell检测细胞侵袭情况 使用带有Matrigel的Transwell小室分析细胞侵袭情况。将5×10^5个细胞接种于200 μL无血清培养基中的上室(小室用Matrigel包被, Matrigel在DMEM中1:6稀释)。将下室加入0.6 mL DMEM, 在37 °C、5% CO_2的条件下孵育48 h后, 除去小室上表面的细胞。侵入下表面的细胞于4 °C下在4%多聚甲醛中固定30 min, 洗涤后室温下用结晶紫染色15 min, 然后在显微镜下拍照。

1.3.6 Hoechst 33258染色检测细胞凋亡情况 使用Hoechst 33258染色通过形态学观察评估凋亡细胞。各组细胞弃去培养基后, PBS洗涤3次, 在室温下用4%多聚甲醛固定细胞20 min, Triton X-100室温透化细胞10 min, 然后用Hoechst 33258染色液室温染色30 min。用荧光显微镜观察细胞, 细胞凋亡率=(凋亡细胞数/细胞总数)×100%。

1.3.7 Western blot检测SOX4蛋白表达情况 用磷酸盐缓冲液清洗细胞, 然后用细胞总蛋白提取试剂盒提取总蛋白, BCA试剂盒定量。通过SDS-PAGE分离蛋白质裂解物, 将蛋白质转移到PVDF膜, 随后在4 °C下与SOX4、GAPDH(稀释度均为1:1 000)一抗孵育过夜, 用TBST洗涤膜3次, 然后将膜与二抗(稀释度为1:5 000)在室温下孵育1 h。使用增强化学发光试剂盒显影, 使用成像系统进行定量, GAPDH作为内参对照。

表1 LncRNA DLEU2、miR-30a-5p、*SOX4*及内参引物序列

Table 1 LncRNA DLEU2, miR-30a-5p, *SOX4* and internal reference primer sequences

引物名称 Primer name	上游引物(5′→3′) Upstream primer (5′→3′)	下游引物(5′→3′) Downstream primer (5′→3′)
LncRNA DLEU2	TTG CTT TCC CAG AAA AGG TG	AAT TGC ACT TAG GCC ACA C
miR-30a-5p	AAA GTG GAA TTT GTA GAG A	CAG GTA CAG ACG GAT ATC TTG C
SOX4	ACA GTT TTG TGC CCT CA	GGG GTC GAT GCT GTG TTT TG
U6	GGT CGG GCA GGA AAG AGG GC	GCT AAT CTT CTC TGT ATC GTT CC
GAPDH	GCA AGA GCA CAA GAG GAA GA	ACT GTG AGG AGG GGA GAT TC

1.3.8 LncRNA DLEU2与miR-30a-5p、miR-30a-5p与*SOX4*的相互作用 SK-OV-3细胞接种于6孔板中培养, 生长旺盛时, 将LncRNA DLEU2野生型(WT)、突变型(MUT)质粒各自和mimic NC质粒、miR-30a-5p mimic质粒, 共转染SK-OV-3细胞; SOX4 WT、SOX4 MUT质粒各自和mimic NC质粒、miR-30a-5p mimic质粒, 共转染SK-OV-3细胞, 48 h后, 进行双荧光素酶检测实验测试荧光素酶活性。

1.4 统计学分析

实验数据用($\bar{x}\pm s$)表示, 采用Graphpad prism 7.0软件进行统计分析, 多组比较采用单因素方差分析。P<0.05表示差异有统计学意义。

2 结果

2.1 各细胞系中LncRNA DLEU2、miR-30a-5p、*SOX4* mRNA表达水平比较

与IOSE80细胞比较, SK-OV-3、OVCAR-8、A2780、OV90细胞中LncRNA DLEU2和*SOX4*的mRNA表达水平升高, miR-30a-5p表达水平降低(P<0.05), SK-OV-3细胞的LncRNA DLEU2和*SOX4*的mRNA表达水平最高, miR-30a-5p表达水平最低, 后续实验以SK-OV-3为研究细胞(表2)。

2.2 各组SK-OV-3中LncRNA DLEU2、miR-30a-5p和*SOX4*的mRNA表达水平

与control组、si-NC组比较, si-DLEU2组细胞中miR-30a-5p的mRNA水平升高, LncRNA DLEU2和*SOX4*的mRNA水平降低(P<0.05); 与mimic NC组比较, miR-30a-5p mimic组细胞中miR-30a-5p水平升高, *SOX4*水平降低(P<0.05); 与si-DLEU2+inhibitor NC组比较, si-DLEU2+miR-30a-5p inhibitor组细胞中miR-30a-5p水平降低, *SOX4*水平升高(P<0.05)(表3)。

2.3 各组SK-OV-3细胞增殖比较

与control组、si-NC组比较, si-DLEU2组细胞的D值降低(P<0.05); 与control组、mimic NC组比较,

表2 各细胞系LncRNA DLEU2、miR-30a-5p、*SOX4* mRNA表达比较

Table 2 Comparison of LncRNA DLEU2, miR-30a-5p, *SOX4* mRNA expression in various cell lines

细胞系 Cell lines	LncRNA DLEU2	miR-30a-5p	*SOX4*
IOSE80	1.04±0.11	0.99±0.11	1.05±0.11
SK-OV-3	2.96±0.31[a]	0.32±0.04[a]	3.04±0.31[a]
OVCAR-8	2.64±0.28[a]	0.47±0.05[a]	2.45±0.25[a]
A2780	2.69±0.28[a]	0.39±0.04[a]	2.21±0.26[a]
OV90	2.34±0.25[a]	0.55±0.06[a]	2.67±0.28[a]

$\bar{x}\pm s$, n=6; [a]P<0.05, 与IOSE80细胞相比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with IOSE80 cells.

表3 SK-OV-3中LncRNA DLEU2、miR-30a-5p、*SOX4*表达比较

Table 3 Comparison of LncRNA DLEU2, miR-30a-5p, *SOX4* expression in SK-OV-3

分组 Groups	LncRNA DLEU2	miR-30a-5p	*SOX4*
Control group	1.03±0.11	1.08±0.12	1.06±0.11
si-NC group	1.05±0.11	1.06±0.11	0.98±0.11
si-DLEU2 group	0.45±0.06[ab]	2.53±0.27[ab]	0.46±0.05[ab]
mimic NC group	1.03±0.11	1.04±0.11	1.05±0.11
miR-30a-5p mimic group	1.08±0.12	2.48±0.25[ad]	0.44±0.05[ad]
si-DLEU2+inhibitor NC group	0.44±0.05[ab]	2.54±0.27[ab]	0.45±0.05[ab]
si-DLEU2+miR-30a-5p inhibitor group	0.43±0.05	1.02±0.12[ce]	0.92±0.11[ce]
F	74.904	98.897	67.166
P	0.000	0.000	0.000

$\bar{x}\pm s$, n=6; [a]P<0.05, 与control组相比; [b]P<0.05, 与si-NC组相比; [c]P<0.05, 与si-DLEU2组相比; [d]P<0.05, 与mimic NC组相比; [e]P<0.05, 与si-DLEU2+inhibitor NC组相比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with control group; [b]P<0.05 compared with si-NC group; [c]P<0.05 compared with si-DLEU2 group; [d]P<0.05 compared with mimic NC group; [e]P<0.05 compared with si-DLEU2+inhibitor NC group.

miR-30a-5p mimic组细胞的*D*值降低(*P*<0.05); 与si-DLEU2+inhibitor NC组比较, si-DLEU2+miR-30a-5p inhibitor组细胞的*D*值升高(*P*<0.05)(表4)。

2.4 各组SK-OV-3细胞迁移比较

与control组、si-NC组比较, si-DLEU2组SK-OV-3细胞的划痕愈合率下降(*P*<0.05); 与mimic NC组比较, miR-30a-5p mimic组细胞的划痕愈合率下降(*P*<0.05); 与si-DLEU2+inhibitor NC组比较, si-DLEU2+miR-30a-5p inhibitor组细胞的划痕愈合率提高(*P*<0.05)(图1和表5)。

2.5 各组SK-OV-3细胞侵袭比较

与control组和si-NC组比较, si-DLEU2组SK-OV-3细胞的侵入细胞数减少(*P*<0.05); 与control组和mimic NC组比较, miR-30a-5p mimic组SK-OV-3细胞的侵入细胞数减少(*P*<0.05); 与si-DLEU2组、si-DLEU2+inhibitor NC组比较, si-DLEU2+miR-30a-5p inhibitor组SK-OV-3细胞的侵入细胞数增加(*P*<0.05)(图2和表6)。

2.6 各组SK-OV-3细胞凋亡比较

与control组和si-NC组细胞比较, si-DLEU2组细胞凋亡率上升(*P*<0.05); 与mimic NC组比较, miR-30a-5p mimic组细胞凋亡率上升(*P*<0.05); 与si-DLEU2+inhibitor NC组比较, si-DLEU2+miR-30a-5p inhibitor组细胞凋亡率下降(*P*<0.05)(图3和表7)。

2.7 各组SK-OV-3细胞中SOX4蛋白表达

与control组和si-NC组比较, si-DLEU2组细胞SOX4蛋白表达水平减少(*P*<0.05); 与mimic NC组比较, miR-30a-5p mimic组细胞SOX4蛋白表达水平减少(*P*<0.05)。与si-DLEU2+inhibitor NC组相比, si-DLEU2+miR-30a-5p inhibitor组细胞SOX4蛋白表达水平增多(*P*<0.05)(图4和表8)。

表4 各组SK-OV-3细胞增殖比较

Table 4 Comparison of SK-OV-3 cells proliferation in each group

分组 Groups	D_{450}		
	24 h	48 h	72 h
Control group	1.21±0.13	1.57±0.17	1.95±0.21
si-NC group	1.18±0.13	1.54±0.16	1.94±0.20
si-DLEU2 group	0.86±0.10^{ab}	1.18±0.12^{ab}	1.56±0.17^{ab}
mimic NC group	1.20±0.14	1.55±0.16	1.96±0.21
miR-30a-5p mimic group	0.84±0.09^{ad}	1.15±0.12^{ad}	1.53±0.16^{ad}
si-DLEU2+inhibitor NC group	0.85±0.09^{ab}	1.17±0.12^{ab}	1.51±0.16^{ab}
si-DLEU2+miR-30a-5p inhibitor group	1.09±0.12^{ce}	1.48±0.15^{ce}	1.87±0.19^{ce}
F	13.757	11.405	7.905
P	0.000	0.000	0.000

$\bar{x}\pm s$, n=6; ^{a}P<0.05, 与control组相比; ^{b}P<0.05, 与si-NC组相比; ^{c}P<0.05, 与si-DLEU2组相比; ^{d}P<0.05, 与mimic NC组相比; ^{e}P<0.05, 与si-DLEU2+inhibitor NC组相比。

$\bar{x}\pm s$, n=6; ^{a}P<0.05 compared with control group; ^{b}P<0.05 compared with si-NC group; ^{c}P<0.05 compared with si-DLEU2 group; ^{d}P<0.05 compared with mimic NC group; ^{e}P<0.05 compared with si-DLEU2+inhibitor NC group.

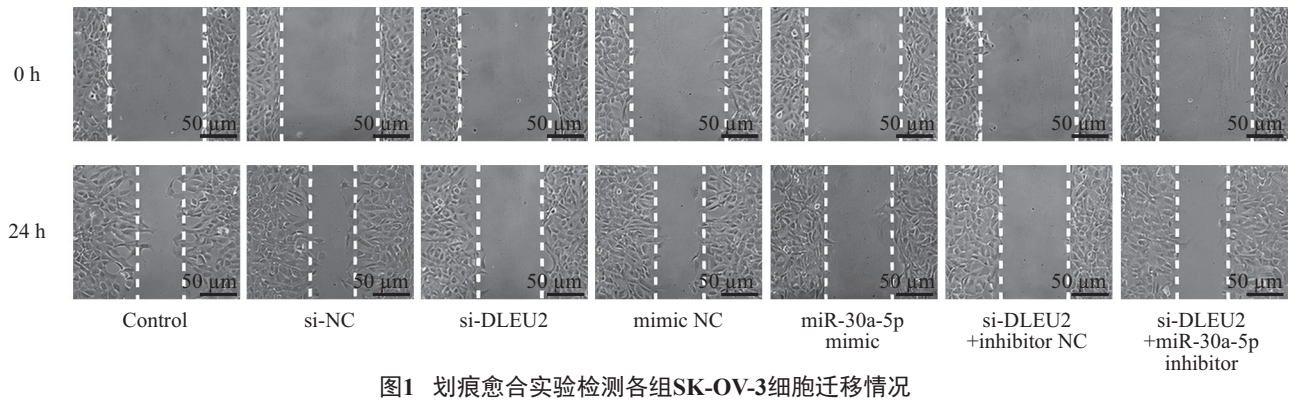


图1 划痕愈合实验检测各组SK-OV-3细胞迁移情况

Fig.1 Migration of SK-OV-3 cells in each group detected by scratch healing experiment

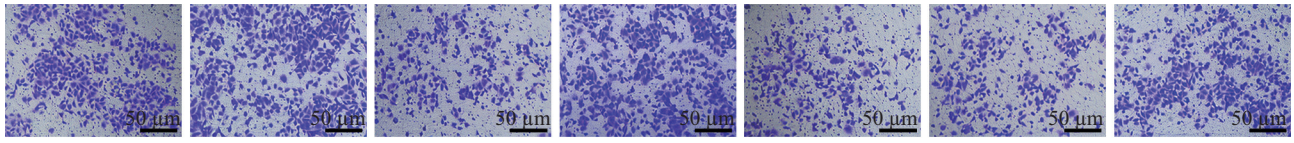
表5 各组SK-OV-3细胞迁移比较

Table 5 Comparison of SK-OV-3 cell migration in each group

分组 Groups	划痕愈合率/% Scratch healing rate /%
Control group	48.36±4.92
si-NC group	49.14±4.97
si-DLEU2 group	27.89±2.82[ab]
mimic NC group	48.52±4.87
miR-30a-5p mimic group	28.58±2.91[ad]
si-DLEU2+inhibitor NC group	27.68±2.79[ab]
si-DLEU2+miR-30a-5p inhibitor group	44.53±4.52[ce]
F	40.079
P	0.000

$\bar{x}\pm s$, n=6; [a]P<0.05, 与control组相比; [b]P<0.05, 与si-NC组相比; [c]P<0.05, 与si-DLEU2组相比; [d]P<0.05, 与mimic NC组相比; [e]P<0.05, 与si-DLEU2+inhibitor NC组相比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with control group; [b]P<0.05 compared with si-NC group; [c]P<0.05 compared with si-DLEU2 group; [d]P<0.05 compared with mimic NC group; [e]P<0.05 compared with si-DLEU2+inhibitor NC group.



Control | si-NC | si-DLEU2 | mimic NC | miR-30a-5p mimic | si-DLEU2 +inhibitor NC | si-DLEU2 +miR-30a-5p inhibitor

图2 Transwell小室检测各组SK-OV-3细胞侵袭情况

Fig.2 Transwell chamber detection of SK-OV-3 cell invasion in each group

表6 各组SK-OV-3细胞侵袭比较

Table 6 Comparison of SK-OV-3 cell invasion in each group

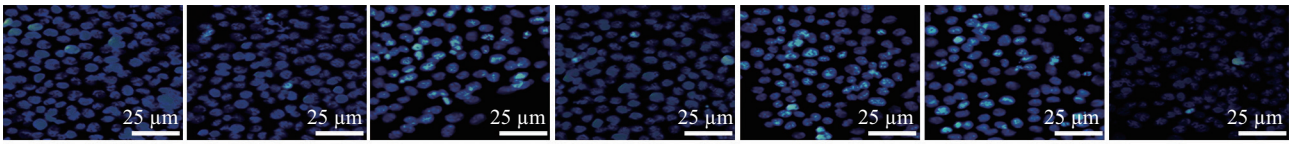
分组 Groups	侵入细胞数 Number of invading cells
Control group	96.49±9.82
si-NC group	95.78±9.76
si-DLEU2 group	58.26±4.93[ab]
mimic NC group	97.18±9.92
miR-30a-5p mimic group	56.78±4.74[ad]
si-DLEU2+inhibitor NC group	57.22±4.85[ab]
si-DLEU2+miR-30a-5p inhibitor group	89.25±8.97[ce]
F	38.437
P	0.000

$\bar{x}\pm s$, n=6; [a]P<0.05, 与control组相比; [b]P<0.05, 与si-NC组相比; [c]P<0.05, 与si-DLEU2组相比; [d]P<0.05, 与mimic NC组相比; [e]P<0.05, 与si-DLEU2+inhibitor NC组相比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with control group; [b]P<0.05 compared with si-NC group; [c]P<0.05 compared with si-DLEU2 group; [d]P<0.05 compared with mimic NC group; [e]P<0.05 compared with si-DLEU2+inhibitor NC group.

2.8 LncRNA DLEU2与miR-30a-5p、miR-30a-5p与*SOX4*相互关系

如图5和图6所示, Starbase分析显示LncRNA DLEU2与miR-30a-5p、miR-30a-5p与*SOX4*的序列存在互补结合部分。双荧光素酶检测得出, 与LncRNA DLEU2 MUT和mimic NC共转染比较, 共转LncRNA DLEU2 WT和miR-30a-5p mimic的荧光素酶活性明显降低(P<0.05)(表9); 与SOX4 MUT和mimic NC共



Control | si-NC | si-DLEU2 | mimic NC | miR-30a-5p mimic | si-DLEU2 +inhibitor NC | si-DLEU2 +miR-30a-5p inhibitor

图3 Hoechst 33258染色检测各组SK-OV-3细胞凋亡情况

Fig.3 Hoechst 33258 staining to detect apoptosis of SK-OV-3 cells in each group

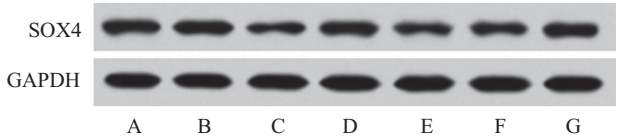
表7 各组SK-OV-3细胞凋亡比较

Table 7 Comparison of SK-OV-3 cell apoptosis in each group

分组 Groups	细胞凋亡率/% Cell apoptosis rate /%
Control group	4.97±0.52
si-NC group	5.14±0.57
si-DLEU2 group	23.37±2.41^{ab}
mimic NC group	4.12±0.43
miR-30a-5p mimic group	22.73±2.32^{ad}
si-DLEU2+inhibitor NC group	21.98±2.26^{ab}
si-DLEU2+miR-30a-5p inhibitor group	7.56±0.78^{ce}
F	204.766
P	0.000

$\bar{x}\pm s$, n=6; ^{a}P<0.05, 与control组相比; ^{b}P<0.05, 与si-NC组相比; ^{c}P<0.05, 与si-DLEU2组相比; ^{d}P<0.05, 与mimic NC组相比; ^{e}P<0.05, 与si-DLEU2+inhibitor NC组相比。

$\bar{x}\pm s$, n=6; ^{a}P<0.05 compared with control group; ^{b}P<0.05 compared with si-NC group; ^{c}P<0.05 compared with si-DLEU2 group; ^{d}P<0.05 compared with mimic NC group; ^{e}P<0.05 compared with si-DLEU2+inhibitor NC group.



A: control组; B: si-NC组; C: si-DLEU2组; D: mimic NC组; E: miR-30a-5p mimic组; F: si-DLEU2+inhibitor NC组; G: si-DLEU2+miR-30a-5p inhibitor组。

A: control group; B: si-NC group; C: si-DLEU2 group; D: mimic NC group; E: miR-30a-5p mimic group; F: si-DLEU2+inhibitor NC group; G: si-DLEU2+miR-30a-5p inhibitor group.

图4 Western blot检测各组SK-OV-3细胞SOX4和GAPDH的蛋白表达情况

Fig.4 Western blot detection of protein expression of SOX4 and GAPDH in SK-OV-3 cells in each group

转相比, SOX4 MUT和miR-30a-5p mimic共转的荧光素酶活性明显降低(P<0.05)(表10)。

3 讨论

卵巢癌是主要的妇科恶性肿瘤之一, 也是全球女性癌症相关死亡的主要原因。由于缺乏有效的早期肿瘤诊断方法, 卵巢癌的死亡率很高, 预后也很差[11]。化疗被广泛用于治疗卵巢癌患者, 但耐药性很常见, 治疗效果并不理想[12]。此外, 卵巢癌的复发率极高, 这也是导致该疾病死亡率高的原因之一[13]。

哺乳动物基因组中约有1.2%用于编码蛋白质, 大部分基因组对应于转录调控元件和非编码RNA。根据大小、序列和功能, ncRNA分为不同的亚类, 其中最著名的是LncRNA和miRNA。LncRNA长度超过200个核苷酸, 可以发挥竞争性内源性RNA(ceRNA)的作用, 通过与miRNA竞争性结合来调节靶基因的表达, 与肿瘤进展密切相关[14]。LncRNA DLEU2的宿主基因位于13号染色体上, 由DLEU2基因编码, 经多聚腺苷酸化和裂解, 与任何其他非编码RNA无同源性[15]。DLEU2可作为ceRNA通过调节miR-496/PRKACB表达来加速人类急性髓系白血病进程, 其还在胃癌组织中高表达[16]。在宫

表8 各组SK-OV-3细胞中SOX4蛋白表达水平比较

Table 8 Comparison of SOX4 protein expression levels in SK-OV-3 cells in each group

分组 Groups	SOX4
Control group	0.99±0.11
si-NC group	1.05±0.11
si-DLEU2 group	0.72±0.08[ab]
mimic NC group	1.03±0.11
miR-30a-5p mimic group	0.69±0.07[ad]
si-DLEU2+inhibitor NC group	0.71±0.08[ab]
si-DLEU2+miR-30a-5p inhibitor group	0.96±0.11[ce]
F	16.995
P	0.000

$\bar{x}\pm s$, n=6; [a]P<0.05, 与control组相比; [b]P<0.05, 与si-NC组相比; [c]P<0.05, 与si-DLEU2组相比; [d]P<0.05, 与mimic NC组相比; [e]P<0.05, 与si-DLEU2+inhibitor NC组相比。

$\bar{x}\pm s$, n=6; [a]P<0.05 compared with control group; [b]P<0.05 compared with si-NC group; [c]P<0.05 compared with si-DLEU2 group; [d]P<0.05 compared with mimic NC group; [e]P<0.05 compared with si-DLEU2+inhibitor NC group.

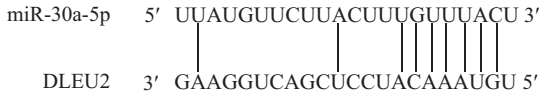


图5 LncRNA DLEU2与miR-30a-5p靶向结合位点预测

Fig.5 Prediction of target binding sites of LncRNA DLEU2 and miR-30a-5p

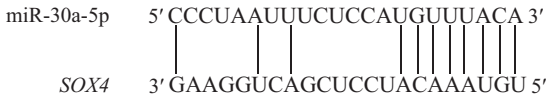


图6 miR-30a-5p与*SOX4*靶向结合位点预测

Fig.6 Prediction of target binding sites of miR-30a-5p and *SOX4*

表9 LncRNA DLEU2与miR-30a-5p双荧光素酶活性检测结果

Table 9 Results of dual luciferase activity detection of LncRNADLEU2 and miR-30a-5p

分组 Groups	LncRNA DLEU2 WT	LncRNA DLEU2 MUT
mimic NC	1.05±0.11	1.04±0.11
miR-30a-5p mimic	0.46±0.05	1.06±0.12
t	11.961	0.301
P	0.000	0.770

$\bar{x}\pm s$, n=6.

表10 miR-30a-5p与SOX4双荧光素酶活性检测结果

Table 10 Results of dual luciferase activity detection of miR-30a-5p and SOX4

分组 Groups	SOX4 WT	SOX4 MUT
mimic NC	1.02±0.11	1.01±0.12
miR-30a-5p mimic	0.47±0.05	1.07±0.11
t	11.150	0.903
P	0.000	0.883

$\bar{x}\pm s$, n=6.

颈癌组织中, DLEU2表达上调。敲低DLEU2可抑制宫颈癌细胞的增殖, 在体外诱导细胞凋亡和细胞周期停滞在G_2/M期, 在体内抑制肿瘤生长[17]。miR-30-5p属于miR-30家族, 通过调节肿瘤增殖和转移来抑制肿瘤发展, 在癌细胞中通常处于低水平, 是一种抑癌基因[18]。生物信息学分析已经预测, miR-30a-5p是与上皮性卵巢癌相关的枢纽基因[19]。miR-30a-5p在卵巢癌组织和顺铂耐药细胞系中显著低表达, miR-30a-5p过表达可抑制卵巢癌细胞恶性行为和顺铂耐药性[20]。因此, 本研究对LncRNA DLEU2和miR-30a-5p进行探究, 发现干扰LncRNA DLEU2表达后, SK-OV-3细胞的miR-30a-5p表达水平升高, 细胞凋亡率提高, 增殖、迁移和侵袭能力明显降低; 过表达miR-30a-5p对SK-OV-3细胞的作用与干扰LncRNA DLEU2表达相同; 干扰LncRNA DLEU2同时抑制miR-30a-5p表达可以逆转LncRNA DLEU2表达降低对SK-OV-3细胞的影响; 双荧光素酶活性结果表明, LncRNA DLEU2与miR-30a-5p具有靶向关系; 表明干扰LncRNA DLEU2可通过靶向提高miR-30a-5p表达, 抑制卵巢癌细胞增殖、迁移、侵袭, 促进细胞凋亡。

SOX4是一种必需的发育转录因子, 可调节干细胞特性、分化、祖细胞发育以及多种发育途径, 包括PI3K、Wnt和TGFβ信号转导[21]。SOX4在大多数恶性肿瘤中经常过表达, 在非小细胞肺癌中, miR-30a-5p抑制可增强*SOX4*表达, 促进非小细胞肺癌患者的免疫逃避[22]。在子宫内膜癌中, 提高SOX4表达可以促进细胞增殖[23]。SOX4是miR-2053在卵巢癌中的靶点, 卵巢癌标本中SOX4上调, 其表达与miR-2053水平呈负相关, 过表达SOX4会加强miR-2053抑制剂引起的卵巢癌细胞生物学行为变化[24]。因此, 本研究通过双荧光素酶报告基因实验进一步探究miR-30a-5p与*SOX4*的相互作用, 发现miR-30a-5p与*SOX4*存在靶向关系; 综合qRT-PCR和WB实验结果发现, miR-30a-5p过表达抑制SOX4的mRNA和蛋白表达, 降低SK-OV-3细胞增殖、迁移和侵袭能力, 提高细胞凋亡率; miR-30a-5p表达被抑制时, SOX4的mRNA和蛋白表达水平升高, 细胞增殖、迁移和侵袭能力提高, 细胞凋亡率降低; 这表明miR-30a-5p可能通过抑制*SOX4*表达来抑制卵巢癌细胞增殖、迁移和侵袭, 并促进细胞凋亡。

综上所述, LncRNA DLEU2在卵巢癌细胞中高表达, 可能通过调节miR-30a-5p/SOX4信号调控卵巢癌细胞的增殖、凋亡、迁移和侵袭。本研究为挖掘卵巢癌生物诊断标志物提供了新的思路, 并为优化卵巢癌治疗提供了候选靶点, 不足之处在于未进行体内实验, 后续需要进一步研究。

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