

长链非编码 RNA LINC00973 对上皮性卵巢癌迁移、侵袭和远端转移的促进作用及其分子机制

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[摘 要] **目的:** 探讨长链非编码 RNA (lncRNA) LINC00973 对上皮性卵巢癌迁移、侵袭和远端转移的影响, 并阐明其分子机制。**方法:** 将人卵巢癌 SKOV3 和 OVCAR3 细胞分为 EF1a-FH 空载体对照组、LINC00973 OE 组、U6-shRNA 空载体对照组 (SHV 组) 和 LINC00973 KD 组。分别转染插入无义序列的 pLent-EF1a-FH-CMV-copGFP-P2A-Puro、LINC00973 过表达、插入无义序列的 pLent-U6-shRNA-CMV-copGFP-P2A-Puro 和 LINC00973 小发夹 RNA (shRNA) 慢病毒, 经嘌呤霉素筛选得到稳定转染的 SKOV3 和 OVCAR3 细胞。采用实时荧光定量 PCR (RT-qPCR) 法检测各组细胞中目的基因 mRNA 表达水平, 细胞划痕实验检测各组细胞迁移率, Transwell 小室实验检测各组细胞穿膜细胞数。小鼠腹腔注射 SKOV3 野生型 (WT 组)、LINC00973 OE (LINC00973 OE 组) 和 LINC00973 KD (LINC00973 KD 组) 细胞, 构建上皮性卵巢癌腹腔种植转移模型, 采用 HE 染色观察各组小鼠结肠和肝脏组织形态表现, RNA 测序 (RNA-seq) 分析 SHV 和 LINC00973 KD 组 SKOV3 细胞差异基因, RT-qPCR 法检测正常卵巢上皮细胞 IOSE-80 和上皮性卵巢癌 SKOV3、A2780 及 OVCAR3 细胞中 LINC00973 mRNA 表达水平, 各组细胞中 LINC00973、波形蛋白 (Vimentin), 蜗牛家族转录因子 1 (Snail)、Twist 家族碱性螺旋-环-螺旋转录因子 1 (Twist)、锌指 E 盒结合蛋白 1 (ZEB1)、锌指 E 盒结合蛋白 2 (ZEB2), 趋化因子 CXCL8 和基质金属蛋白酶 (MMP) 16 mRNA 表达水平及各组小鼠肝脏和结肠组织中 LINC00973、Vimentin 和 Twist mRNA 表达水平。**结果:** 与正常卵巢上皮 IOSE-80 细胞比较, 上皮性卵巢癌 SKOV3、OVCAR3 和 A2780 细胞中 LINC00973 mRNA 表达水平均明显升高 ($P < 0.01$), SKOV3 和 OVCAR3 细胞中 LINC00973 mRNA 表达水平最高, 因此选择该 2 种细胞进行后续实验。SKOV3 和 OVCAR3 细胞中, 与对照组比较, LINC00973 OE 组细胞中 LINC00973 mRNA 表达水平升高 ($P < 0.01$); 与 SHV 组比较, LINC00973 KD 组细胞中 LINC00973 mRNA 表达水平降低 ($P < 0.05$ 或 $P < 0.01$), 表明 SKOV3 和 OVCAR3 LINC00973 过表达及敲低细胞系构建成功。在 SKOV3 细胞中, 与对照组比较, LINC00973 OE 组细胞中 Vimentin 和 Twist mRNA 表达水平升高 ($P < 0.05$ 或 $P < 0.01$), Snail mRNA 表达水平差异无统计学意义 ($P > 0.05$); 与 SHV 组比较, LINC00973 KD 组细胞中 Vimentin、Snail 和 Twist mRNA 表达水平降低 ($P < 0.01$)。在 OVCAR3 细胞中, 与对照组比较, LINC00973 OE 组细胞中 Vimentin、Snail 和 Twist mRNA 表达水平升高 ($P < 0.01$); 与 SHV 组比较, LINC00973 KD 组细胞中 Vimentin、Snail 和 Twist mRNA 表达水平降低 ($P < 0.01$)。细胞划痕实验, 在 SKOV3 和 OVCAR3 细胞中, 与对照组比较, LINC00973 OE 组细胞划痕愈合率均明显升高 ($P < 0.01$); 与 SHV 组比较, LINC00973 KD

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组细胞划痕愈合率均明显降低 ($P<0.01$)。Transwell小室实验,在SKOV3和OVCAR3细胞中,与对照组比较,LINC00973 OE组穿膜细胞数均明显增加 ($P<0.01$);与SHV组比较,LINC00973 KD组穿膜细胞数均明显减少 ($P<0.01$)。与WT组比较,LINC00973 OE组小鼠腹腔形成的结节数增多,肝脏表面粗糙,有多处结节形成,肠系膜和结肠表面也有结节形成,结肠组织中LINC00973、Vimentin和Twist mRNA表达水平均升高 ($P<0.01$);与WT组比较,LINC00973 KD组小鼠腹腔无结节形成,肝脏表面光滑、无结节,且肝脏组织中LINC00973、Vimentin和Twist mRNA表达水平降低 ($P<0.01$),肠系膜和结肠表面也无结节形成。HE染色,与WT组比较,LINC00973 OE组小鼠肝脏和结肠出现多处病变,表现为细胞大小不一、形状不规则、细胞边界不清,细胞核分裂增多,细胞质出现深浅不一的红色;LINC00973 KD组小鼠肝脏和结肠组织细胞排列整齐、形状规则,细胞核和细胞质分布均匀。RNA-seq测序,与SHV组比较,LINC00973 KD组未富集到与肿瘤转移相关的关键信号通路,而与肿瘤转移相关的基因CXCL8、MMP16、ZEB1和ZEB2转录水平降低。RT-qPCR法,与对照组比较,LINC00973 OE组细胞中ZEB1、ZEB2、CXCL8和MMP16 mRNA表达水平明显升高 ($P<0.01$);与SHV组比较,LINC00973 KD组细胞中ZEB1、ZEB2、CXCL8和MMP16的mRNA表达水平明显降低 ($P<0.01$)。结论:LINC00973可以上调转移相关因子Vimentin、Snail、Twist、ZEB1、ZEB2、CXCL8和MMP16表达,促进上皮性卵巢癌的迁移、侵袭和远端转移。

【关键词】 卵巢肿瘤;转移;LINC00973;实时荧光定量PCR;基质金属蛋白酶

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Long non-coding RNA LINC00973 promotes migration, invasion and distal metastasis of epithelial ovarian cancer and its molecular mechanism

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ABSTRACT Objective: To discuss the effect of long non-coding RNA (lncRNA) LINC00973 on the migration, invasion, and distant metastasis of epithelial ovarian cancer, and to clarify its molecular mechanism. **Methods:** The human ovarian cancer SKOV3 and OVCAR3 cells were divided into EF1a-FH empty vector control group (control group), LINC00973 overexpression group (LINC00973 OE group), U6-shRNA empty vector control group (SHV group), and LINC00973 knockdown group (LINC00973 KD group), and were transfected with lentivirus containing nonsense sequence (pLent-EF1a-FH-CMV-copGFP-P2A-Puro), LINC00973 overexpression, nonsense sequence (pLent-U6-shRNA-CMV-copGFP-P2A-Puro) and LINC00973 shRNA, respectively, followed by puromycin screening to obtain stably transfected SKOV3 and OVCAR3 cells. Real-time fluorescence quantitative PCR (RT-qPCR) method was used to detect the mRNA expression levels of target genes in the cells in various groups; wound healing assay was used to detect the migration rate of the cells in various groups; Transwell chamber assay was used to detect the number of transmembrane cells in various groups; The mice were divided into control group (WT group), LINC00973 OE group, and LINC00973 KD group, with 4 mice in each group. SKOV3 wild-type cells, LINC00973 OE cells, and LINC00973 KD cells were intraperitoneally injected into the mice in various groups, respectively, to establish the epithelial ovarian cancer intraperitoneal implantation and metastasis model; HE staining was used to observe the morphology of the colon and liver

tissues of the mice in various groups; RNA-sequencing (RNA-seq) was used to analyze the differentially expressed genes between SHV and LINC00973 KD groups in the SKOV3 cell line; RT-qPCR method was used to detect the mRNA expression levels of LINC00973 in the normal ovarian epithelial cells IOSE-80 and epithelial ovarian cancer cells SKOV3, A2780 and OVCAR3, the mRNA expression levels of LINC00973, Vimentin, Snail family transcriptional repressor 1 (Snail), Twist family basic helix-loop-helix transcription factor 1 (Twist), zinc finger E-box binding homeobox 1 (ZEB1), zinc finger E-box binding homeobox 2 (ZEB2), CXCL8 and matrix metalloproteinase (MMP) 16 in the cells in various groups, and the mRNA expression levels of LINC00973, Vimentin and Twist in liver and colon tissues of the mice in various groups. **Results:** Compared with normal ovarian epithelial cells IOSE-80, the expression level of LINC00973 mRNA in the epithelial ovarian cancer cells SKOV3, OVCAR3 and A2780 was significantly increased ($P < 0.01$), with the highest expression level of LINC00973 in SKOV3 and OVCAR3 cells, which were therefore selected for subsequent experiments. In SKOV3 and OVCAR3 cells, compared with control group, the expression level of LINC00973 mRNA in the cells in LINC00973 OE group was increased ($P < 0.01$); compared with SHV group, the expression level of LINC00973 mRNA in the cells in LINC00973 KD group was decreased ($P < 0.05$ or $P < 0.01$), indicating successful construction of LINC00973 overexpression and knockdown cell lines. In SKOV3 cells, compared with control group, the mRNA expression levels of Vimentin and Twist in LINC00973 OE group were increased ($P < 0.05$ or $P < 0.01$), while no significant difference was observed in Snail mRNA expression level ($P > 0.05$); compared with SHV group, the mRNA expression levels of Vimentin, Snail and Twist in LINC00973 KD group were decreased ($P < 0.01$). In OVCAR3 cells, compared with control group, the mRNA expression levels of Vimentin, Snail and Twist in LINC00973 OE group were increased ($P < 0.01$); compared with SHV group, the expression levels of Vimentin, Snail, and mRNA Twist in LINC00973 KD group were decreased ($P < 0.01$). The wound healing assay results showed that compared with control group, the wound healing rates of the SKOV3 and OVCAR3 cells in LINC00973 OE group were significantly increased ($P < 0.01$); compared with SHV group, the wound healing rates of the cells in LINC00973 KD group were significantly decreased ($P < 0.01$). The Transwell chamber assay results showed that compared with control group, the numbers of transmembrane cells of the SKOV3 and OVCAR3 cells in LINC00973 OE group were significantly increased ($P < 0.01$); compared with SHV group, the numbers of transmembrane cells in LINC00973 KD group were significantly decreased ($P < 0.01$). Compared with WT group, the number of peritoneal nodules in LINC00973 OE group was increased, with rough liver surface and multiple nodules formed on mesentery and colon surface, and the expression levels of LINC00973, Vimentin, and Twist mRNA in colon tissue were increased ($P < 0.01$); compared with WT group, no nodules were formed in the peritoneal cavity of LINC00973 KD group, with smooth liver surface, no nodules in liver tissue, and decreased expression levels of LINC00973, Vimentin, and Twist mRNA, and no nodules were observed on mesentery and colon surface. The HE staining results showed that compared with WT group, the multiple lesions were observed in liver and colon tissues in LINC00973 OE group, manifested as uneven cell size, irregular shape, unclear cell boundaries, increased nuclear division, and uneven red staining in cytoplasm, while in LINC00973 KD group, the cells in liver and colon tissues were arranged neatly with regular shape, and uniform distribution of nuclei and cytoplasm. The RNA-seq results showed that compared with SHV group, no key signaling pathways related to tumor metastasis were enriched in LINC00973 KD group, and the transcription levels of metastasis-related genes CXCL8, MMP16, ZEB1, and ZEB2 were decreased. The RT-qPCR results showed that compared with control group, the expression levels of ZEB1, ZEB2, CXCL8, and MMP16 mRNA in the cells in LINC00973 OE group were significantly increased ($P < 0.01$); compared with SHV group, the expression levels of ZEB1, ZEB2, CXCL8, and MMP16 mRNA in the cells in LINC00973 KD group were

significantly decreased ($P < 0.01$). **Conclusion:** LINC00973 can up-regulate the expression of metastasis-related factors Vimentin, Snail, Twist, ZEB1, ZEB2, CXCL8, and MMP16, and promote the migration, invasion, and distant metastasis of epithelial ovarian cancer.

KEYWORDS Ovarian neoplasm; Neoplasm metastasis; LINC00973; Real-time fluorescence quantitative PCR; Matrix metalloproteinases

卵巢癌是女性生殖系统三大恶性肿瘤之一,上皮性卵巢癌是最常见的亚型,其起病隐匿,被发现时往往已经是晚期,且预后差,死亡率高,是全球女性癌症死亡的主要原因^[1]。卵巢癌极易发生转移,其中最常见的方式是盆腔种植转移。尽管多数卵巢癌患者最初对标准化治疗方案有效,但80%的患者最终会复发和转移^[2],而肿瘤转移是导致患者预后差的重要原因。因此,阐明卵巢癌转移的分子机制、寻找新的治疗靶点和改善卵巢癌转移现状十分重要。

长链非编码RNA (long non coding RNA, lncRNA) 是长度大于200个核苷酸的RNA分子,不编码蛋白质,可编码少数多肽^[3-4], lncRNA与肿瘤的发生发展密切相关,可在转录、转录后和翻译水平调控基因表达^[5]。越来越多的lncRNA表达失调被证实与卵巢癌细胞的迁移和侵袭有关,如lncRNA KCNQ1重叠转录物1 (KCNQ1 opposite strand transcript 1, KCNQ1OT1) 通过促进真核翻译起始因子2B亚基5 (eukaryotic translation initiation factor 2B subunit 5, EIF2B5) 启动子的甲基化促进卵巢癌的转移^[6], lncRNA RUNX1基因内含子转录本1 (RUNX1 intronic transcript 1, RUNX1-IT1) 通过激活核因子 κ B (nuclear factor kappa B, NF- κ B) 信号通路促进了卵巢癌进展^[7], 癌症相关长链非编码RNA 15 (cancer-associated long non-coding RNA 15, CASC15) 通过竞争性结合一些微小RNA (microRNA, miRNA), 如miR-23b-3p和miR-24-3p协同上调SMAD家族成员3 (SMAD family member 3, Smad3) 的表达促进卵巢癌的上皮-间质转化 (epithelial-mesenchymal transition, EMT) 和转移^[8], 因此靶向LncRNA有抑制卵巢癌转移的潜能。LINC00973表达升高与许多肿瘤的进展和不良预后显著相关,其可促进结直肠癌的耐药和增殖,抑制结直肠癌细胞的凋亡^[9-10],而在透明细胞肾细胞癌和非小细胞肺癌中,LINC00973可促进肿瘤细胞的免疫逃逸^[11-12]。然而LINC00973在卵巢癌中的作用尚未见相关报道,探讨LINC00973在卵巢癌中迁移侵袭中的作

用,并阐明其作用机制。

1 材料与方法

1.1 细胞、主要试剂和仪器 人正常卵巢上皮IOSE-80细胞和人上皮性卵巢癌A2780细胞购于宁波明舟生物科技有限公司,人上皮性卵巢癌SKOV3和OVCAR3细胞、Mc coy'5A培养基及胰岛素生长因子购于武汉普诺赛生命科技有限公司,胎牛血清和RPMI 1640培养基购于美国BI公司,LINC00973慢病毒包装由维真生物科技有限公司完成,PCR所需引物由生工生物工程(上海)股份有限公司合成,总RNA提取试剂盒购于北京全式金生物技术有限公司,逆转录试剂盒购于美国赛默飞世尔科技有限公司,Transwell小室购于广州洁特生物过滤股份有限公司,2 $\times$ FastStart Universal SYBR Green Master染料(E301)购于近岸蛋白质科技有限公司,HE染色试剂盒购于北京索莱宝科技有限公司。CO₂细胞培养箱和生物安全柜购于新加坡Esco公司,全自动细胞计数仪购于上海睿钰生物科技有限公司,倒置荧光显微镜(型号:Olympus IX 53)购于日本Olympus公司,微量紫外分光光度计(型号:BioPhotometer D30)和高速离心机(型号:eppendorf 5427R)购于德国Eppendorf公司。

1.2 细胞培养 IOSE-80细胞培养在含10%胎牛血清和1%青-链霉素的RPMI 1640培养基中,SKOV3细胞培养在含10%胎牛血清和1%青-链霉素的Mc coy's 5A培养基中,OVCAR3细胞培养在含20%胎牛血清、10 mg $\cdot$ L⁻¹重组人胰岛素溶液(10 g $\cdot$ L⁻¹)和1%青-链霉素的RPMI 1640培养基中,并置于37 $^{\circ}$ C恒温、5% CO₂培养箱中培养。

1.3 细胞转染和分组 将处于对数生长期的SKOV3和OVCAR3细胞以适当密度铺至6孔细胞培养板上,过夜培养后细胞密度达到60%~70%时,设EF1a-FH空载体对照组、LINC00973过表达组(LINC00973 overexpression, LINC00973 OE组)、U6-shRNA空载体对照组(SHV组)和LINC00973敲低组(LINC00973 knockdown,

LINC00973 KD组)。按照病毒辅助转染试剂：完全培养基=1：1 000的比例先加入助转染试剂，对照组加入插入无义序列的 pLent-EF1a-FH-CMV-copGFP-P2A-Puro 病毒，LINC00973 OE组加入LINC00973过表达慢病毒，SHV组加入插入无义序列的 pLent-U6-shRNA-CMV-copGFP-P2A-Puro慢病毒，LINC00973 KD组加入LINC00973短发夹RNA（short hairpin RNA，shRNA）慢病毒，转染48 h后，加入嘌呤霉素（1 g·L⁻¹）筛选稳转细胞株，待各组细胞稳定生长后，RT-qPCR法验证过表达和敲低LINC00973的转染效果。

1.4 RNA提取和RT-qPCR法验证 按照试剂盒说明书方法提取总RNA，分光光度计检测RNA的浓度和纯度，采用逆转录试剂盒合成cDNA，以GAPDH为内参基因，对相关基因进行RT-qPCR法检测。反应体系：2×SYBR Super mix plus 10 μL，模板cDNA 200 μg，上下游引物各1 μL，ddH₂O补齐至20 μL。反应条件：反应温度梯度95℃、4 min，95℃、15 s，54℃、20 s，72℃、20 s，共40个循环。采用2^{-△△C_t}法计算各组细胞中目的基因mRNA表达水平。

1.5 细胞划痕实验检测各组细胞划痕愈合率 将处于对数生长期的SKOV3或OVCAR3细胞接种至12孔细胞培养板上，每孔2×10⁵个细胞，每组设置

3个复孔，待细胞生长到80%的密度时，采用200 μL无菌吸管尖刮擦细胞单层。磷酸盐缓冲液（phosphated buffer saline，PBS）清洗去除细胞碎片后换无血清培养基，倒置荧光显微镜下拍摄0 h划痕距离（L0），培养箱中孵育48 h，再次拍摄相同位置的划痕距离（L1），计算细胞划痕愈合率。细胞划痕愈合率=（L1-L0）/L0×100%。

1.6 Transwell小室实验检测各组细胞的穿膜细胞数 Transwell小室置于24孔细胞培养板中，上室中加入200 μL含8 000个细胞的无血清培养基，下室加入800 μL含20%胎牛血清的培养基，细胞培养箱中培养72 h后，PBS缓冲液清洗Transwell小室，结晶紫染色5 min，PBS缓冲液洗掉结晶紫浮色，用棉棒擦除上室中未穿膜的细胞后，各组随机选取3个视野于倒置显微镜下拍照，统计各组穿膜细胞数。

1.7 动物实验 小鼠卵巢癌腹腔移植瘤转移模型：12只6~8周龄雌性BALB/C-nu裸鼠随机分为对照（wild type，WT）组、LINC00973 KD组和LINC00973 OE组，每组4只，每组每只小鼠右下腹腹腔分别注射5×10⁶个SKOV3 WT、LINC00973 KD和LINC00973 OE细胞。3周后处死小鼠，解剖观察小鼠腹腔内肿瘤结节形成情况并留取小鼠结肠和肝脏组织置于4%多聚甲醛中用于后续实验。

1.8 HE染色观察各组小鼠结肠和肝脏组织形态表现 小鼠结肠和肝脏组织固定后，常规石蜡包埋，切片厚度4 μm，二甲苯中脱蜡2次，每次5 min。系列乙醇（100%、95%、85%和75%）复水，每梯度3 min，蒸馏水浸泡2 min，苏木素染液染色5 min，蒸馏水洗去浮色。分化液分化30 s，自来水浸洗2次，每次3~5 min，置于伊红染色1 min，倾去多余染色液后快速脱水，75%乙醇、85%乙醇、95%乙醇和100%乙醇各浸洗2~3 s，100%乙醇浸洗1 min，二甲苯透明2次，每次1 min，最后中性树胶封固，自然晾干，镜下观察不同组织切片的形态表现并拍照。

1.9 RNA测序（RNA sequencing，RNA-seq）测序 将3对SKOV3 LINC00973 KD和对照组细胞样本进行RNA-seq测序（武汉华大基因科技服务有限公司完成），通过云分析（武汉华大基因科技服务有限公司完成）平台数据分析LINC00973 KD组与肿瘤转移相关的关键信号通路。

表1 引物序列
Tab. 1 Sequences of primers

Gene	Primer(5'-3')
LINC00973	F: CAAGGACAATTATTCCTCAC R: GACAAAGCCAAGGATTCAGT
Vimentin	F: CGCCAACCTACATCGACAAGGTGC R: CTGGTCCACCTGCCGGCGCAG
Snail	F: TCCACGAGGTGTGACTAACT R: CCGACAAGTGACAGCCATTA
Twist	F: CTAGAGACTCTGGAGCTGGATA R: TCTGGAATCACTGTCCACG
ZEB1	F: AAGTGGCGGTAGATGGTA R: TGTTGTATGGGTGAAGCA
ZEB2	F: TCTGCGACATAAATACGA R: GAGTGAAGCCTTGAGTGC
MMP16	F: TGTGATGGACCAACAGAC R: ATACAAGAGGCATAAGG
CXCL8	F: CTAGTTTGATACTCCCAGTC R: AAGTTTCAACCAGCAAGA
GAPDH	F: CTCCTCCTGTTTCGACAGTCAGC R: CCAATACGACCAATCCGTT

1.10 RT-qPCR 法检测各组细胞中目的基因 mRNA 表达水平 RT-qPCR 法检测正常卵巢上皮 IOSE-80 细胞和上皮性卵巢癌 SKOV3、A2780 及 OVCAR3 细胞中 LINC00973 mRNA 表达水平, 各组细胞中 LINC00973 和波形蛋白 (Vimentin), 促 EMT 转录因子蜗牛家族转录因子 1 (snail family transcriptional repressor 1, Snail)、Twist 家族碱性螺旋-环-螺旋转录因子 1 (twist family BHLH transcription factor 1, Twist)、锌指 E-盒结合同源框蛋白 1 (zinc finger E-box-binding homeobox 1, ZEB1) 和锌指 E-盒结合同源框蛋白 2 (zinc finger E-box-binding homeobox 2, ZEB2), 趋化因子 CXCL8 和基质金属蛋白酶 (matrix metalloproteinases, MMP) 16 mRNA 表达水平以及各组小鼠肝脏和结肠组织中 LINC00973、Vimentin 及促 EMT 转录因子 Twist mRNA 表达水平。

1.11 统计学分析 采用 GraphPad Prism 8.0.1 统计软件进行统计学分析。IOSE-80、SKOV3、A2780、OVCAR3 细胞和转染的各组细胞中 LINC00973 mRNA 表达水平, 各组细胞划痕愈合率, 各组穿膜细胞数, 各组细胞中 Vimentin、Snail、Twist、ZEB1、ZEB2、MMP16 和 CXCL8 以及各组小鼠结肠及肝脏组织中 LINC00973、Vimentin 和 Twist mRNA 表达水平均符合正态分布, 以 $\bar{x} \pm s$ 表示, 多组间样本均数比较采用单因素方差分析或多因素方差分析, 组间均数两两比较采用 LSD-*t* 检验。以 $P < 0.05$ 为差异有统计学意义。

2 结 果

2.1 各种细胞中 LINC00973 mRNA 表达水平 与 IOSE-80 细胞比较, SKOV3、OVCAR3 和 A2780 细胞中 LINC00973 mRNA 表达水平均明显升高 ($P < 0.01$), 其中 SKOV3 和 OVCAR3 细胞中 LINC00973 mRNA 表达水平最高 (表 2), 因此选择该 2 种细胞进行后续实验。

2.2 各组 SKOV3 和 OVCAR3 细胞中 LINC00973 mRNA 表达水平 在 SKOV3 和 OVCAR3 细胞中, 与对照组比较, LINC00973 OE 组细胞中 LINC00973 mRNA 表达水平升高 ($P < 0.01$); 与 SHV 组比较, LINC00973 KD 组细胞中 LINC00973 mRNA 表达水平降低 ($P < 0.05$ 或 $P < 0.01$), 表明 SKOV3 和 OVCAR3 LINC00973 过表达和敲低细胞系构建成功。见表 3 和 4。

表 2 各种细胞中 LINC00973 mRNA 表达水平

Tab. 2 Expression levels of LINC00973 in different kinds of cells ($n=3, \bar{x} \pm s$)

Group	Expression level of LINC00973
IOSE-80	1.000±0.019
SKOV3	211.916±15.037*
OVCAR3	82.331±0.051*
A2780	69.105±1.297*
F	22.460
P	<0.01

* $P < 0.01$ vs IOSE-80.

表 3 SKOV3 细胞中 LINC00973 mRNA 表达水平

Tab. 3 Expression levels of LINC00973 mRNA in SKOV3 cells ($n=3, \bar{x} \pm s$)

Group	LINC00973
Control	1.001±0.049
LINC00973 OE	448.815±24.601*
SHV	1.001±0.047
LINC00973 KD	0.351±0.024 [△]

* $P < 0.01$ vs control group; [△] $P < 0.01$ vs SHV group.

表 4 OVCAR3 细胞中 LINC00973 mRNA 表达水平

Tab. 4 Expression levels of LINC00973 mRNA in OVCAR3 cells ($n=3, \bar{x} \pm s$)

Group	LINC00973
Control	1.003±0.074
LINC00973 OE	206.375±25.735*
SHV	1.001±0.039
LINC00973 KD	0.696±0.060 [△]

* $P < 0.01$ vs control group; [△] $P < 0.05$ vs SHV group.

2.3 各组细胞中 Vimentin、Snail 和 Twist mRNA 表达水平 在 SKOV3 细胞中, 与对照组比较, LINC00973 OE 组细胞中 Vimentin 和 Twist mRNA 表达水平升高 ($P < 0.05$ 或 $P < 0.01$), Snail mRNA 表达水平差异无统计学意义 ($P > 0.05$); 与 SHV 组比较, LINC00973 KD 组细胞中 Vimentin、Snail 和 Twist mRNA 表达水平降低 ($P < 0.01$)。在 OVCAR3 细胞中, 与对照组比较, LINC00973 OE 组细胞中 Vimentin、Snail 和 Twist mRNA 表达水平升高 ($P < 0.01$); 与 SHV 组比较, LINC00973 KD 组细胞中 Vimentin、Snail 和 Twist mRNA 表达水平降低 ($P < 0.01$)。见图 1。

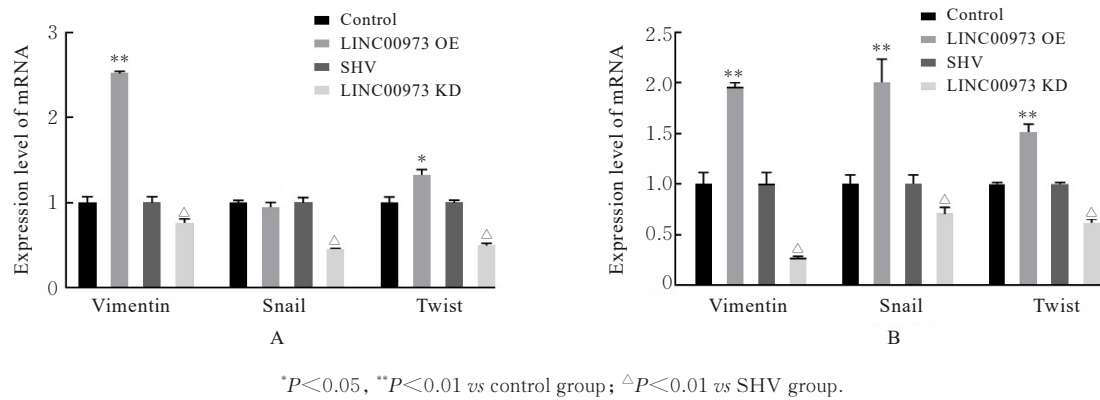


图1 SKOV3(A)和OVCAR3(B)细胞中Vimentin、Snail及Twist mRNA表达水平

Fig. 1 Expression levels of Vimentin, Snail, and Twist mRNA in cells SKOV3(A) and OVCAR3(B) cells

2.4 各组细胞划痕愈合率 在 SKOV3 和 OVCAR3 细胞中，与对照组比较，LINC00973 OE 组细胞划痕愈合率均明显升高 ($P < 0.01$)；与 SHV 组比较，LINC00973 KD 组细胞划痕愈合率均明显降低 ($P < 0.01$)。见图 2 和 3。

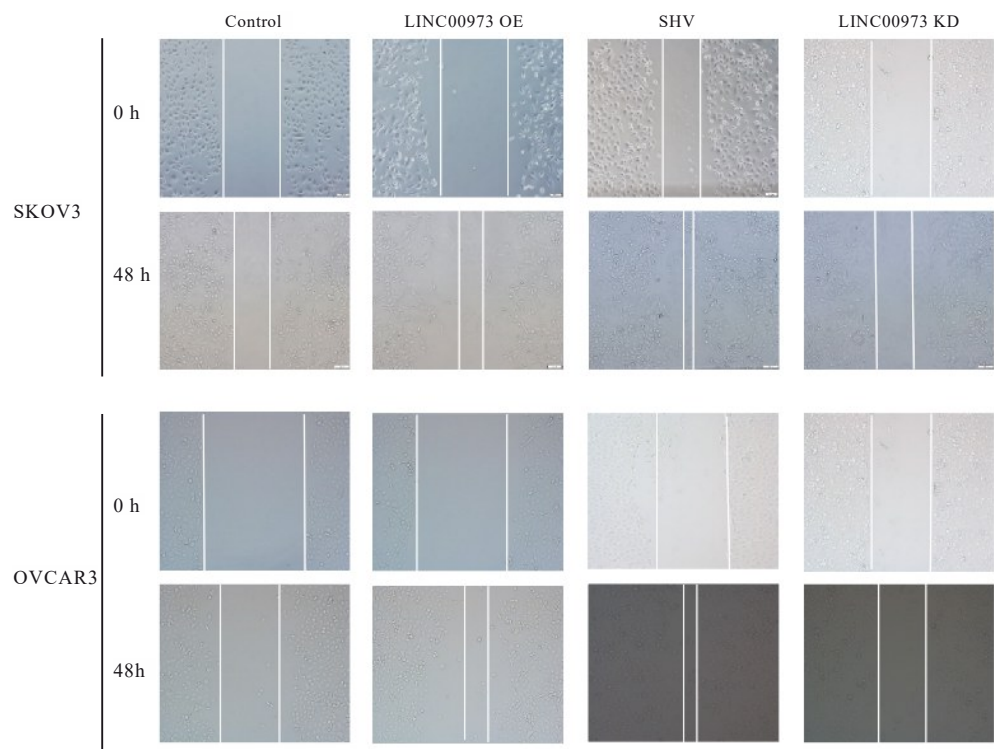
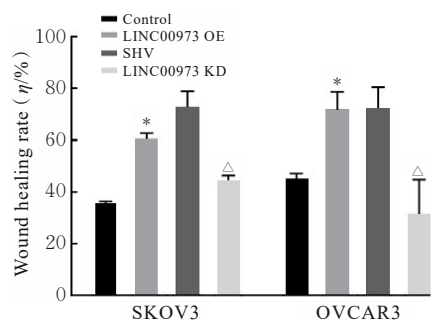


图2 各组细胞划痕愈合情况(×400)

Fig. 2 Wound healing of cells in various groups (×400)

2.5 各组细胞中穿膜细胞数 在 SKOV3 和 OVCAR3 细胞中，与对照组比较，LINC00973 OE 组穿膜细胞数均明显增加 ($P < 0.01$)；与 SHV 组比较，LINC00973 KD 组穿膜细胞数均明显减少 ($P < 0.01$)。见图 4 和 5。

2.6 各组细胞体内转移情况 与 WT 组比较，LINC00973 OE 组小鼠腹腔形成的结节数增多，肝脏表面粗糙，有多处结节形成，肠系膜和结肠表面也有结节形成，结肠组织中 LINC00973、Vimentin 和 Twist mRNA 表达水平均升高 ($P < 0.01$)；与 WT 组比较，LINC00973 KD 组小鼠腹腔无结节形成，肝脏表面光滑、无结节，且肝脏组织中



* $P < 0.01$ vs control group; $\Delta P < 0.01$ vs SHV group.

图3 各组细胞划痕愈合率

Fig. 3 Wound healing rates of cells in various groups

LINC00973、Vimentin和Twist mRNA表达水平降低 ($P < 0.01$), 肠系膜和结肠表面也无结节形成。

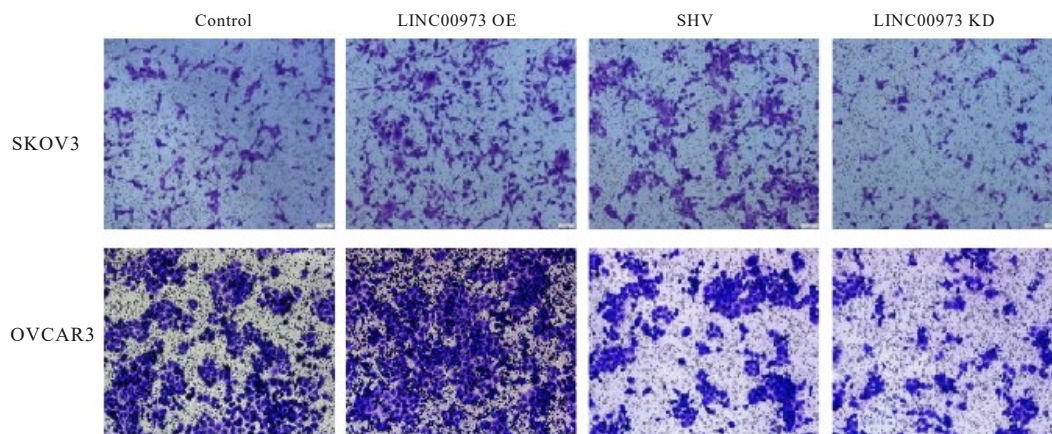
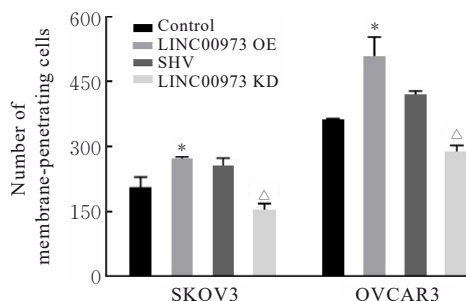


图4 各组细胞的穿膜情况(结晶紫, $\times 400$)

Fig.4 Membrane-penetrating in cells in various groups (Crystal violet, $\times 400$)



* $P < 0.01$ vs control group; $\Delta P < 0.01$ vs SHV group.

图5 各组细胞中穿膜细胞数

Fig. 5 Numbers of membrane-penetrating cells in various groups

2.8 各组细胞中ZEB1、ZEB2、CXCL8和MMP16 mRNA表达水平 在SKOV3和OVCAR3细胞中, 与对照组比较, LINC00973 OE组细胞中ZEB1、

各组小鼠肝脏和结肠组织切片的HE染色检测结果显示: 与WT组比较, LINC00973 OE组小鼠肝脏和结肠出现多处病变, 表现为细胞大小不一且形状不规则, 细胞边界不清, 细胞核分裂增多, 细胞质出现深浅不一的红色; LINC00973 KD组小鼠肝脏和结肠组织细胞排列整齐、形状规则, 细胞核和细胞质分布均匀。见图6~8。

2.7 RNA-seq 测序 将3对SKOV3 LINC00973 KD和对照组SHV细胞样本进行RNA-seq测序和云分析, 结果显示: 与SHV组比较, LINC00973 KD组未富集到与肿瘤转移相关的关键信号通路, 而与肿瘤转移相关的基因CXCL8、MMP16、ZEB1和ZEB2转录水平降低。见图9。

ZEB2、CXCL8和MMP16 mRNA表达水平明显升高 ($P < 0.01$); 与SHV组比较, LINC00973 KD组细胞中ZEB1、ZEB2、CXCL8和MMP16 mRNA表达水平明显降低 ($P < 0.01$)。见图10。

3 讨论

卵巢癌极具侵袭性, 易转移, 预后差^[13], 现有的治疗方案无法有效阻止卵巢癌转移, 因此迫切需要寻找新的作用靶点, 抑制卵巢癌的转移, 改善患者预后。研究^[14-17]显示: lncRNA可影响多种肿瘤的侵袭转移过程, 如lncRNA CEBPA基因差异转录本(CEBPA divergent transcript, CEBPA-DT)可促进肝癌的转移, LINC02273通过表观遗传方式激活癌基因前梯度蛋白2同源物(anterior gradient 2 homolog, AGR2)转录, 从而驱动乳腺

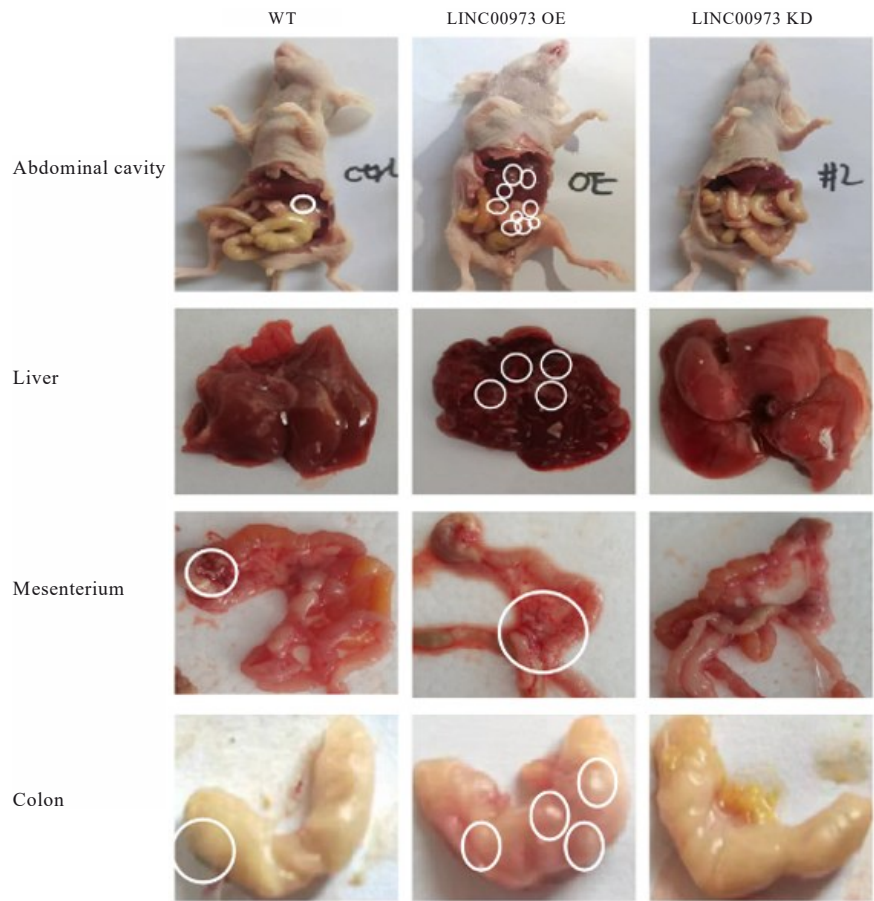
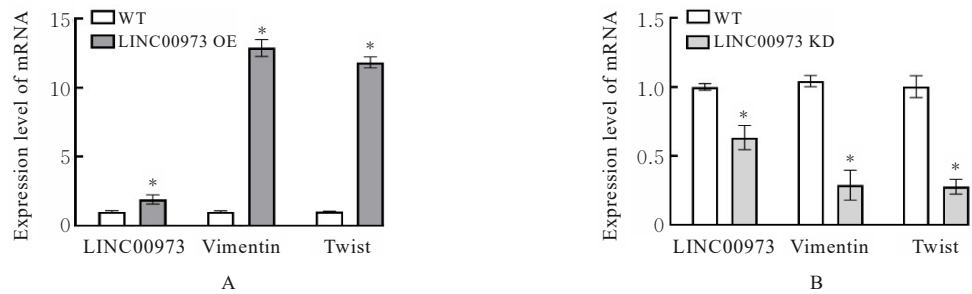


图 6 各组小鼠腹腔形成结节情况和肝脏表面形态表现

Fig. 6 Formation of peritoneal nodules and morphology of liver surface of mice in various groups



* $P < 0.01$ vs WT group.

图 7 各组小鼠结肠(A)和肝脏(B)组织中 LINC00973、Vimentin 及 Twist mRNA 表达水平

Fig. 7 Expression levels of LINC00973, Vimentin, and Twist mRNA in colon(A) and liver(B) tissues of mice in various groups

癌转移；lncRNA MIR503 宿主基因（MIR503 host gene, MIR503HG）可抑制非小细胞肺癌的 EMT 和转移；LINC00982 被证实抑制结直肠癌的转移。在卵巢癌的转移过程中，lncRNA 也发挥了重要的调控作用，如 lncRNA HAS2-AS1、LINC00629 和 lncRNA MEG3 等均参与了卵巢癌细胞的迁移和侵

袭过程^[18-20]，本研究结果显示：LINC00973 在卵巢癌细胞中的转录水平明显升高，并可明显促进卵巢癌细胞的迁移、侵袭和在腹腔中的远端转移，因此 LINC00973 有望成为抑制卵巢癌进展的理想靶点。为了进一步探讨 LINC00973 调控上皮性卵巢癌细胞迁移、侵袭和远端转移的分子机制，本课题

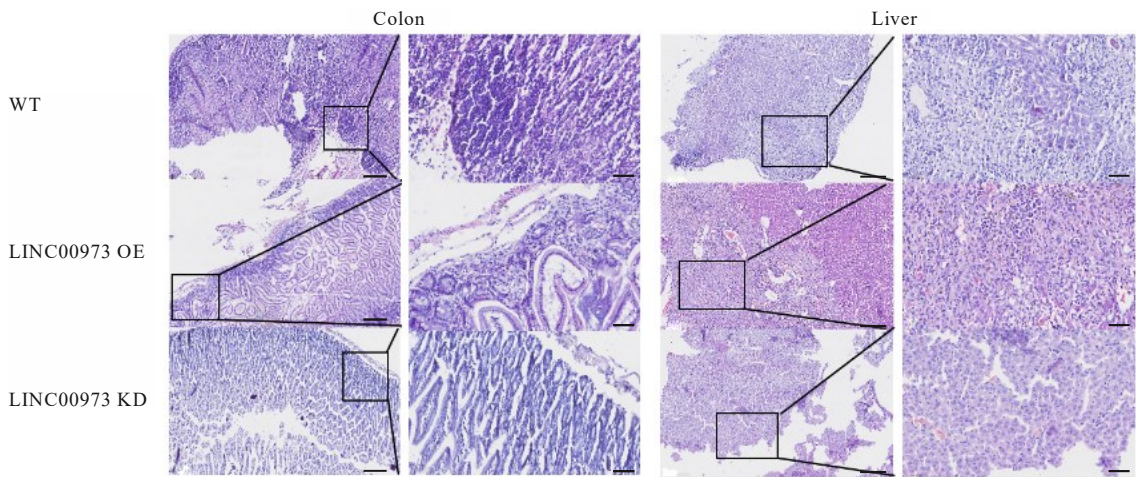
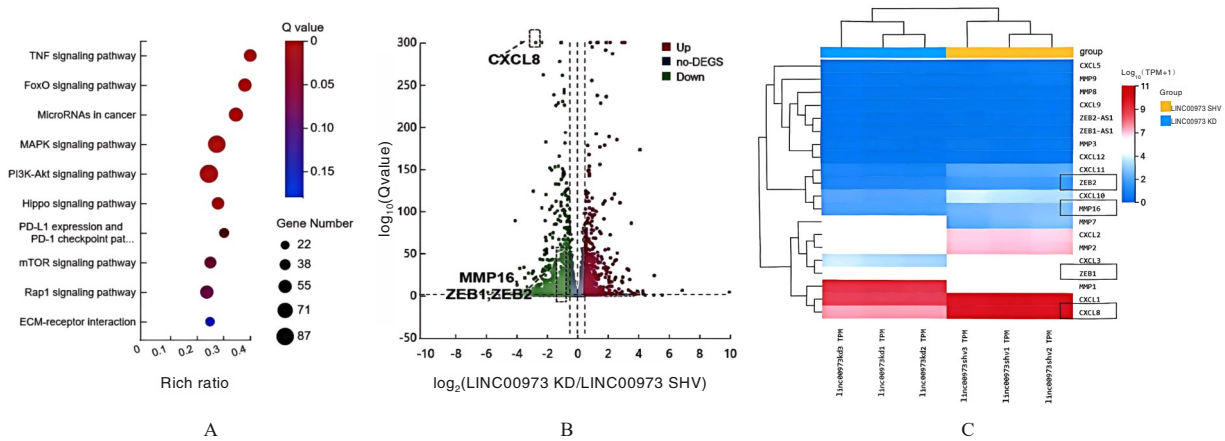


图8 HE染色观察各组小鼠肝脏和结肠组织形态表现(Bar=200 μm)

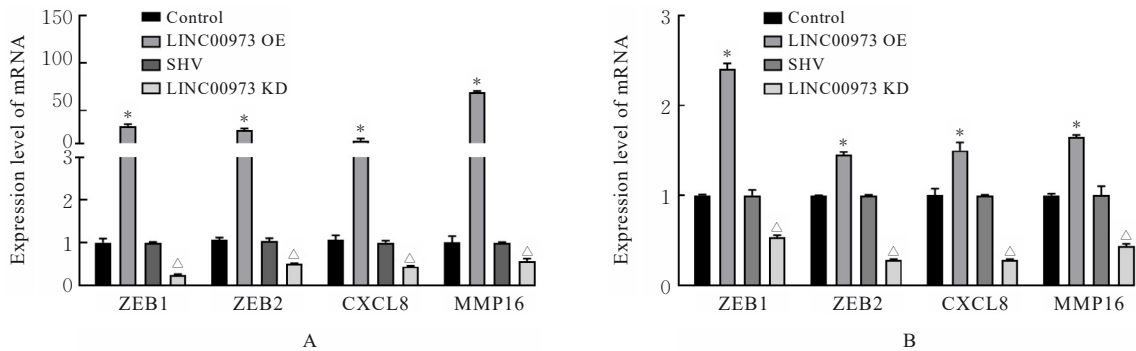
Fig. 8 Morphology of liver and colon tissues of mice in various groups detected by HE staining (Bar=200 μm)



A: KEGG analysis of signaling cascade stimulated in LINC00973 KD group compared with SHV group in SKOV3 cells; B: Volcano plot of genes upregulated and down regulated in LINC00973 KD group compared with SHV group in SKOV3 cells; C: Heat map of oncogenic pathways in LINC00973 KD group compared with SHV group in SKOV3 cells.

图9 LINC00973调控卵巢癌细胞迁移、侵袭和远端转移的分子机制

Fig. 9 Molecular mechanism of migration, invasion, and distant metastasis of ovarian cancer cells regulated by LINC00973



A: SKOV3 cells; B: OVCAR3 cells. * $P < 0.01$ vs control group; $\triangle P < 0.01$ vs SHV group.

图10 各组细胞中ZEB1、ZEB2、CXCL8和MMP16 mRNA表达水平

Fig. 10 Expression levels of ZEB1, ZEB2, CXCL8, and MMP16 mRNA in cells in various groups

组对 SKOV3 LINC00973 低表达和对照组进行转录组测序, 通过对测序结果分析和验证, 寻找到 LINC00973 下游与癌症转移相关的作用靶点为 ZEB1/ZEB2、MMP16 和 CXCL8, 其中 ZEB1/ZEB2 是调控 EMT 的关键转录因子, 可以下调 E-钙黏蛋白和其他编码细胞黏附和基底膜蛋白的基因来调节 EMT^[21-22], EMT 在肿瘤细胞迁移和侵袭的早期阶段发挥重要作用^[23]。LINC01296/miR-141-3p/ZEB1-ZEB2 组成的调控通路可促进非小细胞肺癌和结直肠癌的转移进程^[24]; ZEB2 通过调节肿瘤干细胞的侵袭性和成瘤性促进高级别浆液性卵巢癌的腹膜转移^[25]; MMP16 能降解 I 型纤维胶原, 促进黑色素瘤的转移^[26], 还可作为一些 miRNA 的靶基因, 介导胶质瘤和肾癌等肿瘤的迁移及侵袭^[27-29]; C-X-C 基序趋化因子配体 (C-X-C motif chemokine ligand, CXCL) 8 是 CXC 家族的一种典型趋化因子, 由肿瘤细胞、内皮细胞和淋巴细胞分泌, CXCL8-CXCR1/2 轴可激活磷脂酰肌醇 3 激酶-蛋白激酶 B (phosphatidylinositol 3-kinase-protein kinase B, PI3K-AKT)、磷脂酶 C (phospholipase C, PLC) 及 Janus 激酶-信号转导和转录激活因子 (Janus kinase-signal transducer and activator of transcription, JAK-STAT) 等信号通路, 调节肿瘤的增殖、周期、分化、侵袭转移、凋亡、EMT 和血管生成^[30-31], 并且 CXCL8 能促进卵巢癌和胃癌等肿瘤的腹膜转移^[32]。本研究中 RT-qPCR 法检测结果表明: LINC00973 可以正向调控 ZEB1/ZEB2、MMP16 和 CXCL8 mRNA 表达, 进而促进上皮性卵巢癌的迁移、侵袭和远端转移。

竞争性的结合 microRNA (competing endogenous RNAs, ceRNAs) 调节基因表达是 lncRNA 最常见的作用机制。研究^[33]显示: miR-200b 靶向 ZEB2 促进了胃癌的迁移和侵袭, miR-200 靶向 ZEB1/ZEB2 促进了非小细胞肺癌的迁移及侵袭, ZEB1/ZEB2 是 miR-200 家族的靶基因, 而 miR-200 家族是卵巢癌 EMT 主要的调控因子^[34], LINC00973 是否通过竞争性结合 miR-200 家族调控 ZEB1/ZEB2 的表达有待进一步验证。MMP16 作为 miR-146b-5p 的靶基因抑制胶质瘤的迁移和侵袭^[29], CXCL8 作为 miR-182 靶基因促进了肺癌的骨转移^[35]。LINC00973 是否以 ceRNAs 机制调控 ZEB1/ZEB2、MMP16 和 CXCL8 表达, 还需进一步探讨。

综上所述, LINC00973 通过上调 ZEB1/ZEB2、MMP16 和 CXCL8 的表达促进上皮性卵巢癌细胞的迁移、侵袭及远端转移, LINC00973 是抑制卵巢癌转移的潜在靶点。

利益冲突声明:

所有作者声明不存在利益冲突。

作者贡献声明:

夏云秀参与实验设计和论文撰写, 张硕参与细胞表型实验和论文校对, 张恒海参与数据整理和分析, 王飞参与实验指导, 董洪亮和杜静参与论文审阅。

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