研究论文

LncRNA OSER1-AS1通过调控miR-433-3p影响宫颈癌细胞增殖、迁移及侵袭

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摘要 该研究旨在探讨lncRNA OSER1-AS1对宫颈癌细胞增殖、迁移、侵袭的影响及其对miR-433-3p的调控作用。利用Western blot和qRT-PCR方法检测宫颈癌细胞中OSER1-AS1、miR-433-3p的表达水平, 发现宫颈癌细胞中OSER1-AS1的表达水平显著升高(P<0.05), 而miR-433-3p的表达水平显著降低(P<0.05); 将si-NC、si-OSER1-AS1、miR-NC、miR-433-3p、si-OSER1-AS1+anti-miR-NC、si-OSER1-AS1+anti-miR-433-3p转染至SiHa细胞, 进一步应用CCK-8法与平板克隆实验检测细胞增殖能力, 发现转染si-OSER1-AS1或miR-433-3p mimics后, 细胞活力显著降低(P<0.05), 克隆形成数、迁移及侵袭细胞数显著减少(P<0.05); 而转染si-OSER1-AS1+anti-miR-433-3p后, 细胞活力显著升高(P<0.05), 且克隆形成数、迁移及侵袭细胞数显著增多(P<0.05); 最后用双荧光素酶报告实验检测OSER1-AS1、miR-433-3p的靶向关系, 发现OSER1-AS1能够竞争性地结合miR-433-3p。结果说明, OSER1-AS1可负向调控miR-433-3p的表达进而调控细胞增殖、迁移及侵袭。提示干扰OSER1-AS1表达可通过上调miR-433-3p的表达从而抑制宫颈癌细胞的增殖、迁移及侵袭。

关键词 OSER1-AS1; miR-433-3p; 宫颈癌; 增殖; 迁移; 侵袭

LncRNA OSER1-AS1 Affects the Proliferation, Migration and Invasion of Cervical Cancer Cells by Regulating miR-433-3p

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Abstract This study is aimed to explore the effect of lncRNA OSER1-AS1 on the proliferation, migration and invasion of cervical cancer cells and its regulation on miR-433-3p. Western blot and qRT-PCR were used to detect the expression levels of OSER1-AS1 and miR-433-3p in cervical cancer cells, finding that the expression level of OSER1-AS1 in cervical cancer cells was significantly increased (P<0.05), while the expression level of miR-433-3p was significantly reduced (P<0.05). si-NC, si-OSER1-AS1, miR-NC, miR-433-3p, si-OSER1-AS1+anti-miR-NC, and si-OSER1-AS1+anti-miR-433-3p were transfected into SiHa cells. Furthermore, CCK-8 method and plate cloning experiment were used to detect cell proliferation ability, finding that transfection of si-OSER1-AS1 or miR-433-3p mimics significantly reduced cell viability (P<0.05), and the number of clone formation, migration

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and invasion cells were significantly reduced (P<0.05). However, when transfected with si-OSER1-AS1+anti-miR-433-3p, cell viability was significantly increased (P<0.05), and the number of clone formation, migration and invasion cells were significantly increased (P<0.05). Finally, the dual luciferase reporter experiment was used to detect the targeting relationship of OSER1-AS1 and miR-433-3p, finding that OSER1-AS1 could competitively bind to miR-433-3p. The results indicated that OSER1-AS1 could negatively regulate the expression of miR-433-3p and mediate cell proliferation, migration and invasion. It was suggested that interference with OSER1-AS1 expression could inhibit the proliferation, migration and invasion of cervical cancer cells by up-regulating the expression of miR-433-3p.

Keywords OSER1-AS1; miR-433-3p; cervical cancer; proliferation; migration; invasion

宫颈癌是威胁女性生命安全的恶性肿瘤之一, 随着现代生活节奏的加快, 宫颈癌发病率逐年上升且呈年轻化趋势, 研究表明基因异常表达是导致肿瘤发生的根本原因, 因而从基因水平探究宫颈癌的发病机制对宫颈癌诊断及治疗均具有重要意义[1]。长链非编码RNA(long non-coding RNA, lncRNA)是内源性非编码RNA分子, 其可调控基因表达从而参与宫颈癌等肿瘤的发生及发展过程, lncRNA可竞争性结合微小RNA(microRNA, miRNA), 并可调控miRNA表达, 而miRNA可通过调控下游靶基因表达从而参与宫颈癌发生过程[2-4]。LncRNA OSER1-AS1(OSER1 antisense RNA 1)在肝细胞癌中表达水平升高, 并可通过竞争性结合miR-372-3p从而促进肝细胞癌发生[5]。但OSER1-AS1在宫颈癌中的表达及其可能的作用机制尚未可知。生物信息学分析显示, miR-433-3p可能是OSER1-AS1的靶标, 研究表明, miR-433在宫颈癌中表达水平降低, lncRNA-ATB(activated by transforming growth factor beta)可通过负向调控miR-433的表达从而促进宫颈癌细胞迁移及侵袭[6]。因此, 本研究主要探究OSER1-AS1对宫颈癌细胞增殖、迁移及侵袭的影响, 探讨其对miR-433-3p的靶向调控作用, 为宫颈癌靶向治疗提供潜在靶点。

1 材料与方法

1.1 材料与试剂

人正常宫颈细胞Ect1/E6E7与人宫颈癌细胞系HeLa、SiHa购自美国ATCC细胞库; DMEM培养基购自美国Gibco公司; 胎牛血清购自美国Hyclone公司; Lipofectamine® 3000购自美国Invitrogen公司; si-NC、si-OSER1-AS1、miR-NC、miR-433-3p mimics、anti-miR-NC、anti-miR-433-3p购自广州锐博生物科技有限公司; pcDNA3.1购自上海索宝生物科技有限公司; Trizol试剂、反转录与荧光定量检测试剂盒均购自美国Thermo Fisher公司; CCK-8检测试剂盒购自南京固与生物有限公司; Transwell小室购自美国Corning公司; Matrigel基质胶购自美国BD公司; 兔抗人MMP-2、MMP-9抗体购自美国Santa Cruz公司; 辣根过氧化物酶(horseradish peroxidase, HRP)标记的山羊抗兔二抗购自美国Abcam公司。

1.2 方法

1.2.1 *实验分组* Ect1/E6E7、HeLa、SiHa细胞培养于含10%胎牛血清、100 U/mL青霉素与100 μg/mL链霉素的DMEM培养基中, 于培养箱内培养, 待细胞生长至80%融合度时, 使用0.25%胰蛋白酶消化, 细胞进行传代培养。取对数生长期SiHa细胞接种于96孔板(1×10^4个/孔), 待细胞汇合率达到大约80%时, 用Lipofectamine® 3000转染试剂将si-NC(小干扰RNA对照)、si-OSER1-AS1(si-OSER1-AS1#1、si-OSER1-AS1#2和si-OSER1-AS1#3)、miR-NC(miRNA模拟物对照)、miR-433-3p mimics(miR-433-3p模拟物)分别转染至SiHa细胞, 分别记作si-NC组、si-OSER1-AS1组、miR-NC组、miR-433-3p mimics组; 将si-OSER1-AS1与miR-433-3p抑制剂及si-OSER1-AS1与miR-433-3p抑制剂阴性对照共转染至SiHa细胞, 分别记作si-OSER1-AS1+anti-miR-NC组、si-OSER1-AS1+anti-miR-433-3p组。各组转染6 h后, 将培养基更换为含有10%胎牛血清的培养基继续培养48 h。

1.2.2 *qRT-PCR检测细胞中OSER1-AS1、miR-433-3p、MMP-2及MMP-9的表达水平* 取Ect1/E6E7、HeLa、SiHa细胞与转染后的各组SiHa细胞, 采用Trizol法提取细胞总RNA。利用Nanodrop2000c超微量分光光度计检测RNA浓度与纯度, RNA的D_{260}/D_{280}

比值应处于1.8~2.0之间。参照反转录试剂盒说明书将总RNA反转录合成cDNA。OSER1-AS1正向引物5′-TTG AGC TCG TGA GTG ACA GT-3′, 反向引物5′-CAT GCA ACC CTG TTC AAG CT-3′; miR-433-3p正向引物5′-AGA AGT ACG GTG AGC CTG TC-3′, 反向引物5′-AGT CTC ACT CTG TCA CCC A-3′; *MMP-2*正向引物5′-GAT AAC CTG GAT GCC GTC GTG-3′, 反向引物5′-GGT GTG CAG CGA TGA AGA TGA TA-3′; *MMP-9*正向引物5′-CCA TGC ACT GGG CTT AGA TCA-3′, 反向引物5′-GGC CTT GGG TCA GGC TTA GA-3′; *U6*正向引物5′-GCT TCG GCA GCA CAT ATA CT-3′, 反向引物5′-GT GCA GGG TCC GAG GTA TTC-3′; *GAPDH*正向引物5′-GGA GCG AGA TCC CTC CAA AAT-3′, 反向引物5′-GGC TGT TGT CAT ACT TCT CAT GG-3′; *β-actin*正向引物5′-CAT CCG TAA AGA CCT CCC CAA C-3′, 反向引物5′-ATG GAG CCA CCG ATC CAC A-3′。引物由上海生工生物工程股份有限公司合成。以cDNA为模板进行qRT-PCR反应, 反应条件: 95 °C预变性2 min; 95 °C变性30 s, 60 °C退火30 s, 72 °C延伸30 s, 共40个循环。OSER1-AS1以*GAPDH*为内参, miR-433-3p以*U6*为内参, *MMP-2*及*MMP-9*以*β-actin*为内参, 采用$2^{-\Delta\Delta Ct}$法计算OSER1-AS1、miR-433-3p、*MMP-2*及*MMP-9*的相对表达量。

1.2.3 CCK-8法检测细胞增殖 取各组SiHa细胞接种于96孔板(1×10^4个/孔), 继续培养24 h, 每孔分别加入10 μL CCK-8溶液, 置于培养箱(37 °C、体积分数为5% CO_2)中继续培养2 h, 利用酶标仪检测各孔在450 nm处的光密度(*D*)值。

1.2.4 平板克隆形成实验 取各组SiHa细胞接种于6孔板(1×10^3个/孔), 置于培养箱内继续培养, 直至出现肉眼可见的克隆, 终止培养, 加入4%多聚甲醛固定15 min, 分别加入结晶紫染液染色5 min, PBS洗涤, 观察克隆形成数。

1.2.5 Transwell实验检测细胞迁移与侵袭 细胞迁移及侵袭实验: 取各组SiHa细胞接种于Transwell小室的上室(3×10^4个/孔), 下室加入含有胎牛血清的培养液(600 μL/孔), 置于培养箱中继续培养24 h, 取出小室, PBS清洗, 分别加入4%多聚甲醛固定15 min, 用0.1%结晶紫染液染色10 min, 置于显微镜下观察迁移及侵袭细胞数。细胞侵袭实验前还需使用培养液稀释Matrigel基质胶, 将Matrigel基质胶稀释液加入上室(40 μL/孔), 室温孵育5 h。

1.2.6 双荧光素酶报告基因检测OSER1-AS1与miR-433-3p的靶向关系 starBase数据库预测显示, OSER1-AS1与miR-433-3p核苷酸序列存在结合位点(序列为5′-AGG CCG AGG UGG GUG GAU CAU GAG-3′), 将含有的结合位点与突变位点克隆至荧光素酶报告基因载体psi-CHECK, 分别构建野生型载体WT-OSER1-AS1与突变型载体MUT-OSER1-AS1, 用Lipofectamine® 3000转染试剂分别将miR-NC(miRNA模拟物对照)、miR-433-3p mimics(miR-433-3p模拟物)与WT-OSER1-AS1、MUT-OSER1-AS1共转染至SiHa细胞, 置于培养箱继续培养24 h, 弃去培养基, 采用胰消化酶消化, 收集细胞, 检测各组细胞的相对荧光素酶活性, 操作步骤严格按照双荧光素酶活性检测试剂盒说明书进行。

1.2.7 蛋白免疫印迹(Western blot)检测MMP-2、MMP-9蛋白表达 取各组SiHa细胞, 加入蛋白裂解液提取细胞总蛋白, 测定蛋白浓度, 将蛋白高温变性, 用SDS-PAGE分离蛋白, 将分离的蛋白凝胶转移至PVDF膜, 采用5%脱脂奶粉室温封闭2 h, 分别加入一抗稀释液(1:10 000), 4 °C孵育过夜, TBST洗涤, 分别加入二抗稀释液(1:2 000), 室温孵育1 h, TBST洗涤, 滴加ECL, 暗室内曝光显影, 应用Image J软件分析各条带灰度值。

1.3 统计学处理

采用SPSS 21.0统计学软件分析数据, 计量资料以$\bar{x}\pm s$表示且均符合正态分布, 两组间比较采用独立样本*t*检验, 多组间比较采用单因素方差分析, 多组间两两比较采用SNK-*q*检验, 以$P<0.05$表示差异具有统计学意义。

2 结果

2.1 宫颈癌细胞OSER1-AS1显著高表达, 而miR-433-3p表达水平显著降低

与Ect1/E6E7细胞比较, 人宫颈癌细胞系HeLa、SiHa中OSER1-AS1的表达水平显著升高($P<0.05$), 而miR-433-3p的表达水平显著降低($P<0.05$), 其中OSER1-AS1在宫颈癌细胞SiHa中的表达水平相对较高, 因而选用宫颈癌细胞SiHa进行后续研究(表1)。

2.2 干扰OSER1-AS1表达明显抑制SiHa细胞增殖

与si-NC组比较, si-OSER1-AS1组OSER1-AS1

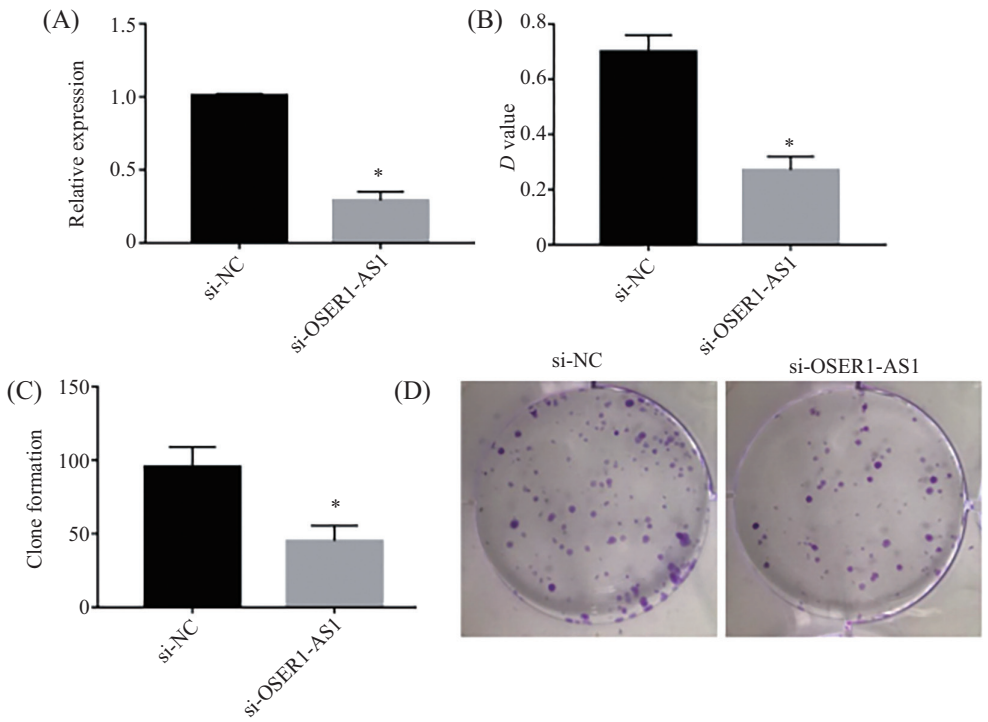
表1 宫颈癌细胞与正常宫颈细胞中OSER1-AS1、miR-433-3p的表达量

Table 1 The expression levels of OSER1-AS1 and miR-433-3p in cervical cancer cells and normal cervical cells

Groups 组别	OSER1-AS1	miR-433-3p
Ect1/E6E7	1.01±0.01	1.00±0.02
HeLa	1.63±0.27*	0.69±0.13*
SiHa	3.13±0.34*	0.28±0.06*
F	170.093	168.502
P	0	0

*P<0.05, 与Ect1/E6E7细胞比较。

*P<0.05 compared with Ect1/E6E7 cells.



A: 采用qRT-PCR检测OSER1-AS1的表达量; B: 采用CCK-8法检测细胞*D*值; C、D: 采用平板克隆形成实验检测克隆形成数。*P<0.05, 与si-NC组比较。si-NC组: SiHa细胞中转染si-OSER1-AS1的阴性对照si-NC; si-OSER1-AS1组: SiHa细胞中转染si-OSER1-AS1。

A: qRT-PCR was used to detect the expression of OSER1-AS1; B: CCK-8 method was used to detect the *D* value of cell; C,D: the plate clone formation experiment was used to detect the number of clone formation. *P<0.05 compared with the si-NC group. si-NC group: the negative control si-NC for si-OSER1-AS1 was transfected into SiHa cells; si-OSER1-AS1 group: si-OSER1-AS1 was transfected into SiHa cells.

图1 干扰OSER1-AS1表达对SiHa细胞增殖能力的影响

Fig.1 The effect of interference with OSER1-AS1 on the proliferation of SiHa cells

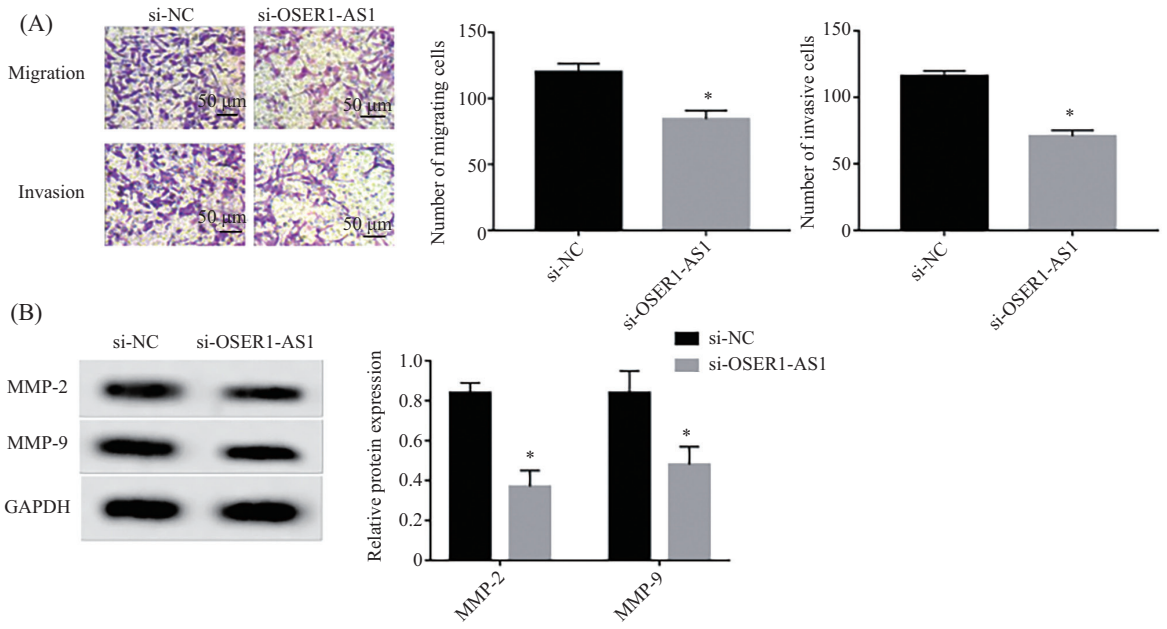
的表达水平降低, 提示转染效果良好。与si-NC组比较, si-OSER1-AS1组细胞活力显著降低(P<0.05), 克隆形成数显著减少(P<0.05)(图1)。

2.3 干扰OSER1-AS1表达明显抑制SiHa细胞迁移及侵袭

与si-NC组比较, si-OSER1-AS1组迁移及侵袭细胞数显著减少(P<0.05), MMP-2、MMP-9蛋白水平显著降低(P<0.05)(图2)。

2.4 miR-433-3p是OSER1-AS1的靶标, OSER1-AS1负向调控miR-433-3p

starBase数据库预测结果显示, OSER1-AS1与miR-433-3p存在结合位点(图3A)。双荧光素酶报告实验结果显示, miR-433-3p过表达可降低野生型载体WT-OSER1-AS1的荧光素酶活性(P<0.05), 而对突变型载体MUT-OSER1-AS1的荧光素酶活性无明显影响(P>0.05)(图3B)。与si-NC组比较, si-OSER1-

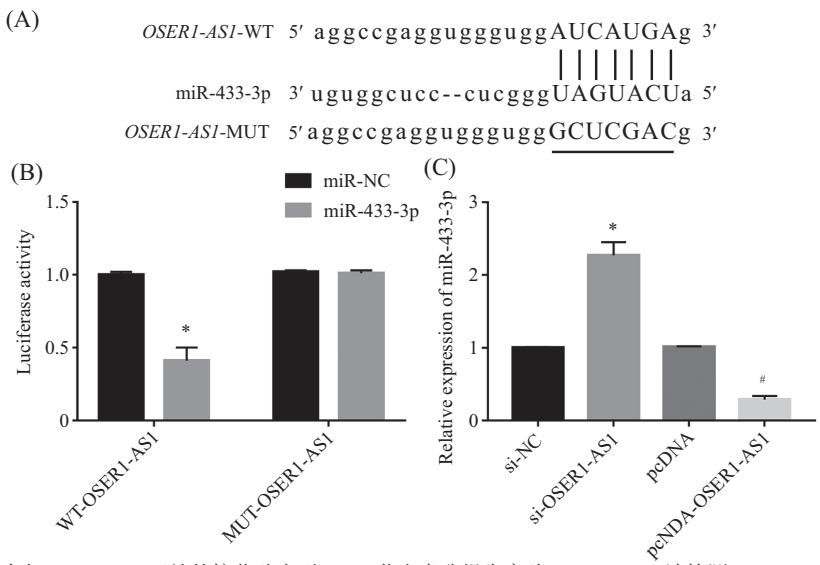


A: 采用Transwell小室实验检测细胞迁移及侵袭; B: Western blot法检测MMP-2、MMP-9蛋白表达。*P<0.05, 与si-NC组比较。si-NC组: SiHa细胞中转染si-OSER1-AS1的阴性对照si-NC; si-OSER1-AS1组: SiHa细胞中转染si-OSER1-AS1。

A: Transwell chamber experiment was used to detect cell migration and invasion; B: Western blot method was used to detect the protein expression of MMP-2 and MMP-9. *P<0.05 compared with the si-NC group. si-NC group: the negative control si-NC for si-OSER1-AS1 was transfected into SiHa cells; si-OSER1-AS1 group: si-OSER1-AS1 was transfected into SiHa cells.

图2 干扰OSER1-AS1表达对SiHa细胞迁移及侵袭能力的影响

Fig.2 The effect of interference with OSER1-AS1 on the migration and invasion ability of SiHa cells



A: OSER1-AS1的序列中含有与miR-433-3p互补的核苷酸序列; B: 双荧光素酶报告实验; C: qRT-PCR法检测OSER1-AS1调控miR-433-3p的表达。*P<0.05, 与si-NC组比较; #P<0.05, 与pcDNA组比较。WT-OSER1-AS1: 含有结合位点序列; MUT-OSER1-AS1: 利用基因突变技术将结合位点进行突变, 含有突变位点; si-NC组: SiHa细胞中转染si-NC; si-OSER1-AS1组: SiHa细胞中转染si-OSER1-AS1; pcDNA组: SiHa细胞中转染过表达的阴性对照; pcDNA-OSER1-AS1组: SiHa细胞中转染OSER1-AS1过表达序列。

A: the sequence of OSER1-AS1 contains a nucleotide sequence complementary to miR-433-3p; B: dual luciferase report experiment; C: qRT-PCR method was used to detect the expression of miR-433-3p regulated by OSER1-AS1. *P<0.05 compared with the si-NC group; #P<0.05 compared with the pcDNA group. WT-OSER1-AS1: contains the binding site sequence; MUT-OSER1-AS1: gene mutation technology was used to mutate the binding site and contains the mutation site. si-NC group: SiHa cells were transfected with si-NC; si-OSER1-AS1 group: SiHa cells were transfected with si-OSER1-AS1; pcDNA group: SiHa cells were transfected with overexpressed negative control; pcDNA-OSER1-AS1 group: OSER1-AS1 overexpression sequence was transfected into SiHa cells.

图3 OSER1-AS1调控miR-433-3p

Fig.3 OSER1-AS1 regulates miR-433-3p

AS1组miR-433-3p的表达水平显著升高(P<0.05); 与pcDNA组比较, pcDNA-OSER1-AS1组miR-433-3p的表达水平显著降低(P<0.05)(图3C)。

2.5 miR-433-3p过表达抑制SiHa细胞增殖、迁移及侵袭

与miR-NC组比较, miR-433-3p组miR-433-3p的表达水平降低, 提示转染效果良好。与miR-NC组比较, miR-433-3p组细胞活力显著降低(P<0.05), 克隆形成数显著减少(P<0.05), 迁移及侵袭细胞数显著减少(P<0.05), MMP-2、MMP-9蛋白及mRNA水平显著降低(P<0.05)(图4)。

2.6 抑制miR-433-3p表达可减弱干扰OSER1-AS1表达对SiHa细胞增殖、迁移及侵袭的抑制作用

与si-OSER1-AS1+anti-miR-NC组比较, si-OSER1-AS1+anti-miR-433-3p组miR-433-3p的表达水平降低, 提示转染效果良好。与si-OSER1-AS1+anti-miR-NC组比较, si-OSER1-AS1+anti-miR-433-3p组细胞活力显著升高(P<0.05), 克隆形成数显著增多(P<0.05), 迁移及侵袭细胞数显著增多(P<0.05), MMP-2、MMP-9蛋白及mRNA水平显著升高(P<0.05)(图5)。

3 讨论

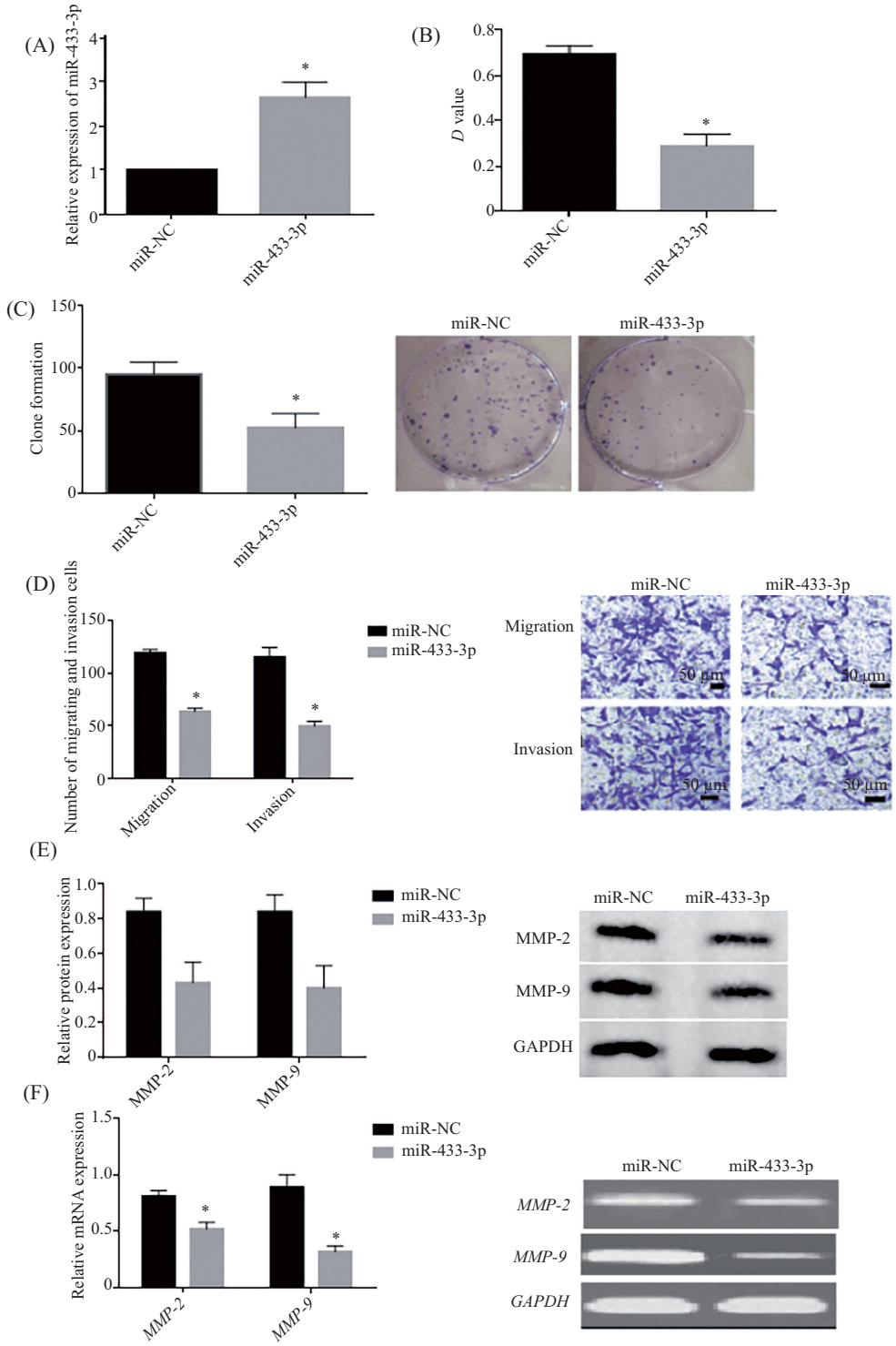
LncRNA与宫颈癌等肿瘤密切相关, 具有促进或抑制肿瘤细胞转移的作用, 还可作为肿瘤靶向治疗的潜在靶点[7-10]。但关于lncRNA OSER1-AS1在宫颈癌中的作用研究相对较少, 因而, 本研究主要探究OSER1-AS1在宫颈癌中的表达及其在宫颈癌中的生物学功能, 以期揭示OSER1-AS1在宫颈癌细胞增殖、迁移及侵袭等生物学过程中的作用机制。

在非小细胞肺癌中, OSER1-AS1表达水平降低, 并可抑制细胞增殖、迁移及侵袭[11]。本研究结果显示, 宫颈癌细胞中OSER1-AS1的表达水平升高, 这与上述研究结果不同, 原因可能是OSER1-AS1在不同类型肿瘤中表达状态不同, 提示OSER1-AS1在宫颈癌发生过程中可能发挥癌基因作用。本研究进一步分析显示, 干扰OSER1-AS1表达后宫颈癌细胞增殖能力明显减弱, 且迁移及侵袭细胞数减少, 提示干扰OSER1-AS1表达可抑制宫颈癌细胞增殖、迁移及侵袭。肿瘤细胞转移能力与基质金属蛋白酶类有关, 其中MMP-2、MMP-9属于基质金属蛋白酶成员, 其可降解细胞外基质沉积, 促进细胞转移[12]。本研究结果显示, 干扰OSER1-AS1表达后宫颈癌细胞中MMP-2、MMP-9蛋白及mRNA的表达水平降低, 进一步证实干扰MMP-2、MMP-9蛋白及mRNA可减弱宫颈癌细胞的迁移及侵袭能力。

为进一步探究OSER1-AS1在宫颈癌细胞增殖、迁移及侵袭等生物学过程中的作用机制, 本研究通过双荧光素酶报告实验证实, OSER1-AS1可靶向结合miR-433-3p, 并可负向调控miR-433-3p的活性。研究表明, miR-433-3p在食管鳞状细胞癌中表达水平降低, 并可通过靶向生长因子受体结合蛋白2(growth factor receptor-bound protein 2, GRB2)而抑制食管鳞状细胞癌细胞增殖及侵袭[13]。Linc01234通过调控miR-433/p21活化激酶4(p21-activated kinase 4, PAK4)轴促进口腔鳞状细胞癌的细胞增殖及转移[14]。沉默lncRNA LINC00460通过上调miR-433-3p表达而下调膜联蛋白A2(annexin A2, ANXA2)表达, 从而抑制结肠癌细胞迁移及侵袭[15]。LncRNA核内小RNA宿主基因14(small nucleolar RNA host gene 14, SNHG14)通过调控miR-433-3p/FBXO22(F-box protein 22)轴而促进骨肉瘤进展[16]。LncRNA PCGEM1通过靶向miR-433-3p而促进肾癌的进展[17]。综合上述研究报道证实, miR-433-3p在肿瘤发生及发展过程中发挥抑癌基因作用。本研究结果显示, 宫颈癌细胞中miR-433-3p的表达水平降低, 这与上述研究报道相似, 进一步分析显示, miR-433-3p过表达可明显降低宫颈癌细胞增殖、迁移及侵袭能力, 提示miR-433-3p在宫颈癌发生过程中发挥抑癌作用。同时本研究将si-OSER1-AS1与anti-miR-433-3p共转染至宫颈癌细胞, 结果显示细胞增殖、迁移及侵袭能力明显恢复, 提示抑制miR-433-3p表达可减弱干扰OSER1-AS1表达对SiHa细胞增殖、迁移及侵袭的抑制作用。

另外, 转染si-OSER1-AS1或miR-433-3p mimics及转染si-OSER1-AS1或anti-miR-433-3p, MMP-2和MMP-9蛋白表达与mRNA表达有同样的变化趋势, 提示MMP-2和MMP-9的3′UTR可能与miR-433-3p或OSER1-AS1存在潜在结合位点, 有必要进一步深入研究。

综上所述, OSER1-AS1在宫颈癌细胞中表达上调, miR-433-3p表达下调, 干扰OSER1-AS1表达或miR-433-3p过表达均可抑制宫颈癌细胞增殖、迁移及侵袭, 且其作用机制与OSER1-AS1靶向调控miR-433-3p的表达有关, OSER1-AS1、miR-433-3p可能

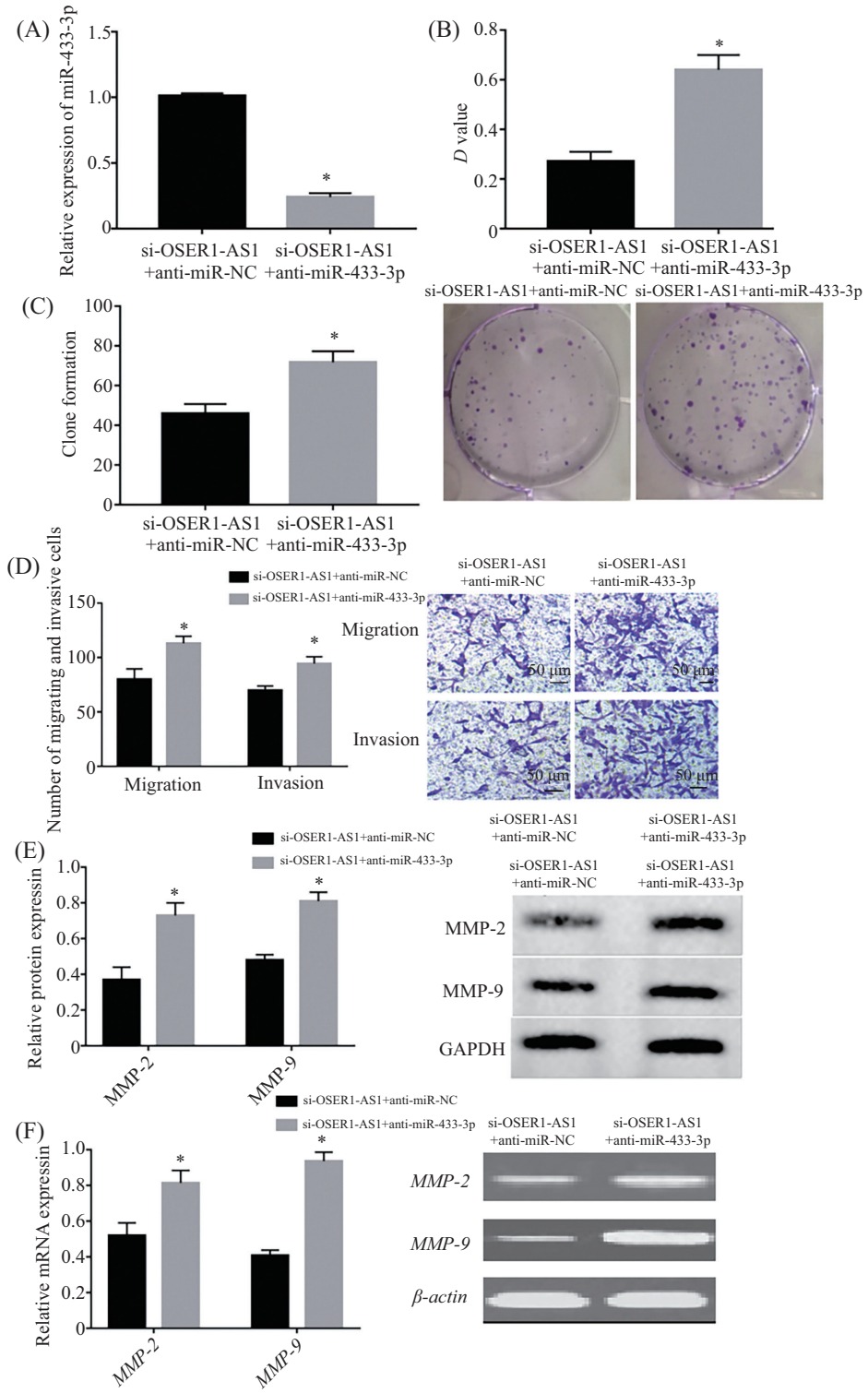


A: 采用qRT-PCR法检测miR-433-3p的相对表达量; B: 采用CCK-8法检测细胞增殖; C: 采用平板克隆形成实验检测克隆形成细胞数; D: 采用Transwell小室实验检测细胞迁移及侵袭; E: 采用Western blot法检测MMP-2、MMP-9蛋白相对表达量; F: 采用qRT-PCR法检测MMP-2、MMP-9 mRNA相对表达量。*P<0.05, 与miR-NC组比较。

A: qRT-PCR method was used to detect the relative expression of miR-433-3p; B: CCK-8 method was used to detect cell proliferation; C: the plate clone formation experiment was used to detect the number of clone forming cells; D: Transwell chamber experiment was used to detect cell migration and invasion; E: Western blot method was used to detect the relative expression of MMP-2 and MMP-9 proteins; F: qRT-PCR was used to detect the relative expression of *MMP-2* and *MMP-9* mRNAs. *P<0.05 compared with the miR-NC group.

图4 miR-433-3p过表达对SiHa细胞增殖、迁移及侵袭的影响

Fig.4 The effect of miR-433-3p overexpression on the proliferation, migration and invasion of SiHa cells



A: 采用qRT-PCR法检测miR-433-3p的相对表达量; B: 采用CCK-8法检测细胞增殖; C: 采用平板克隆形成实验检测克隆形成细胞数; D: 采用Transwell小室实验检测细胞迁移及侵袭; E: 采用Western blot法检测MMP-2、MMP-9蛋白相对表达量; F: 采用qRT-PCR 法检测*MMP-2*、*MMP-9* mRNAs相对表达量。*P<0.05, 与si-OSER1-AS1+anti-miR-NC组比较。

A: qRT-PCR method was used to detect the relative expression of miR-433-3p; B: CCK-8 method was used to detect cell proliferation; C: the plate clone formation experiment was used to detect the number of clone forming cells; D: Transwell chamber experiment was used to detect cell migration and invasion; E: Western blot method was used to detect the relative expression of MMP-2 and MMP-9 proteins; F: qRT-PCR was used to detect the relative expression of *MMP-2* and *MMP-9* mRNAs. *P<0.05 compared with the si-OSER1-AS1+anti-miR-NC group.

图5 抑制miR-433-3p表达可减弱干扰OSER1-AS1表达对SiHa细胞增殖、迁移及侵袭的抑制作用

Fig.5 Inhibition of miR-433-3p expression can attenuate the inhibitory effect of interfering OSER1-AS1 expression on the proliferation, migration and invasion of SiHa cells

是宫颈癌靶向治疗的潜在靶点。但OSER1-AS1、miR-433-3p的具体作用机制仍需进一步探究。

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