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·基础研究·

hsa_circ_0011946靶向miR-767-3p调控宫颈癌细胞增殖、迁移和侵袭

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[摘 要] **目的** 探讨hsa_circ_0011946靶向miR-767-3p在宫颈癌进展中的作用。**方法** RT-qPCR分析hsa_circ_0011946、miR-767-3p在宫颈癌组织、癌旁正常组织、人宫颈上皮永生化细胞(H8)及宫颈癌细胞(SiHa、HeLa、Caski)中的表达水平。分别用si-NC、si-hsa_circ_0011946、miR-NC、miR-767-3p、pcDNA、pcDNA-hsa_circ_0011946、anti-miR-NC+si-hsa_circ_0011946、anti-miR-767-3p+si-hsa_circ_0011946转染SiHa细胞,并设为si-NC组、si-hsa_circ_0011946组、miR-NC组、miR-767-3p组、pcDNA组、pcDNA-hsa_circ_0011946组、anti-miR-NC+si-hsa_circ_0011946组、anti-miR-767-3p+si-hsa_circ_0011946组,未转染的细胞作为NC组。采用RT-qPCR检测各组SiHa细胞中hsa_circ_0011946和miR-767-3p的表达;CCK-8法检测细胞活力;平板克隆形成实验检测细胞克隆数;Transwell实验检测细胞迁移和侵袭数;Western blot检测MMP-2、MMP-9蛋白表达。circular RNA interactome预测miR-767-3p与hsa_circ_0011946的结合位点,双荧光素酶报告基因实验分析hsa_circ_0011946和miR-767-3p的靶向关系。**结果** 与癌旁正常组织比较,宫颈癌组织中hsa_circ_0011946表达水平显著升高($P<0.05$),miR-767-3p表达水平显著降低($P<0.05$)。与H8细胞比较,SiHa、HeLa、Caski细胞中hsa_circ_0011946表达水平显著升高($P<0.05$),miR-767-3p表达水平显著降低($P<0.05$)。与NC组比较,si-hsa_circ_0011946组SiHa细胞hsa_circ_0011946表达水平、吸光度(A)值、克隆数、迁移数、侵袭数及MMP-2、MMP-9蛋白表达水平显著降低/减少($P<0.05$)。与miR-NC组比较,miR-767-3p组SiHa细胞miR-767-3p表达水平显著升高($P<0.05$),A值、克隆数、迁移数、侵袭数及MMP-2、MMP-2蛋白表达水平显著降低/减少($P<0.05$)。与pcDNA组比较,pcDNA-hsa_circ_0011946组SiHa细胞miR-767-3p表达水平显著降低($P<0.05$),hsa_circ_0011946表达水平显著升高($P<0.05$)。共转染miR-767-3p mimics和WT-hsa_circ_0011946的SiHa细胞相对荧光素酶活性低于共转染miR-NC和WT-hsa_circ_0011946($P<0.05$)。hsa_circ_0011946与miR-767-3p直接特异性结合。与anti-miR-NC+si-hsa_circ_0011946组比较,anti-miR-767-3p+si-hsa_circ_0011946组SiHa细胞miR-767-3p表达水平显著降低($P<0.05$),A值、克隆数、迁移数、侵袭数及MMP-2、MMP-9蛋白表达水平显著升高/增加($P<0.05$)。**结论** 干扰hsa_circ_0011946通过靶向上调miR-767-3p抑制宫颈癌细胞的增殖、迁移和侵袭。

[关键词] hsa_circ_0011946;miR-767-3p;宫颈癌;增殖;迁移;侵袭

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hsa_circ_0011946 regulates the proliferation, migration and invasion of cervical cancer cells via targeting miR-767-3p

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Abstract: Objective To investigate the role of hsa_circ_0011946 targeting miR-767-3p in the progression of cervical cancer. **Methods** The expression levels of hsa_circ_0011946 and miR-767-3p in cervical cancer tissues, adjacent tissues, immortalized human cervical epithelial cells (H8) and cervical cancer cells (SiHa, HeLa, and Cashi) were determined by RT-qPCR. SiHa cells were transfected with si-NC, si-hsa_circ_0011946, miR-NC, miR-767-3p, pcDNA, pcDNA-hsa_circ_0011946, anti-miR-NC+si-hsa_circ_0011946, anti-miR-767-3p+si-hsa_circ_0011946, respectively, as the si-NC group, the si-hsa_circ_0011946 group, the miR-NC group, the miR-767-3p group, the pcDNA group, the pcDNA-hsa_circ_0011946 group, the anti-miR-NC+si-hsa_circ_0011946 group, and the anti-miR-767-3p+si-hsa_circ_0011946 group, and the untransfected cells were used as the NC group. The expression of hsa_circ_0011946 and miR-767-3p of SiHa cells in each group was detected by RT-qPCR, the cell viability was detected by CCK-8, the number of cell clone was detected by plate clone formation assay, the cell migration and invasion numbers were detected by Transwell assay, and the protein expression of MMP-2 and MMP-9 was detected by Western blot. The binding site of miR-767-3p to hsa_circ_0011946 was predicted by circular RNA interactome, and the targeted relationship between hsa_circ_0011946 and miR-767-3p was analyzed by dual luciferase reporter assay. **Results** Compared with the adjacent tissues, the expression level of hsa_circ_0011946 in the cervical cancer tissues was significantly increased ($P<0.05$), while the expression level of miR-767-3p was significantly decreased ($P<0.05$). Compared with H8 cells, the expression levels of hsa_circ_0011946 in SiHa, HeLa and Caski cells were significantly increased ($P<0.05$), while the expression levels of miR-767-3p were significantly decreased ($P<0.05$). Compared with the NC group, the expression level of hsa_circ_0011946, absorbance (A) value, number of clone, number of migration, number of invasion and the protein expression levels of MMP-2 and MMP-9 were significantly decreased in SiHa cells in the miR-767-3p group ($P<0.05$). Compared with the miR-NC group, the expression level of miR-767-3p in SiHa cells in the miR-767-3p group was significantly increased ($P<0.05$), and the cell A value, number of clone, number of migration, number of invasion and the protein expression levels of MMP-2 and MMP-9 were significantly decreased ($P<0.05$). Compared with the pcDNA group, the expression level of miR-767-3p in SiHa cells in the pcDNA-hsa_circ_0011946 group was significantly decreased ($P<0.05$), and the expression level of hsa_circ_0011946 was significantly increased ($P<0.05$). The relative luciferase activity of SiHa cells co-transfected with miR-767-3p mimics and WT-hsa_circ_0011946 was lower than that of co-transfected with miR-NC and WT-hsa_circ_0011946 ($P<0.05$). hsa_circ_0011946 bound to miR-767-3p directly and specifically. Compared with the anti-miR-NC+si-hsa_circ_0011946 group, the expression level of miR-767-3p in SiHa cells in the anti-miR-767-3p+si-hsa_circ_0011946 group was significantly decreased ($P<0.05$), and the cell A value, number of clone, number of migration, number of invasion and protein expression levels of MMP-2 and MMP-9 were significantly increased ($P<0.05$). **Conclusion** Interfering hsa_circ_0011946 can inhibit the proliferation, migration and invasion of cervical cancer cells through targeted up-regulation of miR-767-3p.

Keywords: hsa_circ_0011946; miR-767-3p; cervical cancer; proliferation; migration; invasion

宫颈癌是女性最严重的恶性癌症之一,具有较高的浸润性和病死率[1]。因此,阐明宫颈癌进展的机制,寻找新的、有效的治疗靶点至关重要。环状RNA(circular RNA,circRNA)是具有共价闭合环的RNA分子,其有竞争内源性RNA活性,即circRNA可与微小RNA(microRNA,miRNA)识别元件结合发挥miRNA抑制作用,从而提高靶mRNA的表达[2]。circRNA表达改变与宫颈癌进展密切相关,再加上其发育阶段特异性表达模式,circRNA被认为是宫颈癌治疗的理想靶点[3-4]。研究显示,hsa_circ_0011946在乳腺癌中的表达异常上调,敲减hsa_circ_0011946可明显降低乳腺癌细胞迁移和侵袭能力[5]。miRNA是重要的转录后调控因子,其表达失调参与肿瘤细胞多个恶性进程[6-7]。miR-767-3p可诱导胶质瘤细胞甲基转移酶(O6-methylguanine-DNA methyltransferase,MGMT)mRNA降解,增强其对替莫唑胺的敏感性[8]。肺腺癌中miR-767-3p表达下调,过表达miR-767-3p可抑制肺腺癌细胞的增殖、迁移和侵袭[9]。靶基因预测显示,miR-767-3p是hsa_circ_0011946的潜在靶点。但hsa_circ_0011946、miR-767-3p在宫颈癌中的表达和功能,以及hsa_circ_0011946是否通过靶向miR-767-3p调控宫颈癌进展目前尚不清楚。因此,本研究旨在揭示hsa_circ_0011946靶向miR-767-3p在宫颈癌进展中的作用,以期为宫颈癌的治疗提供有效靶点。

1 材料与方法

1.1 材料

1.1.1 组织来源 收集2017年5月至2019年5月我院收治的43例宫颈癌患者的癌组织和癌旁正常组织。患者年龄45~73岁,中位年龄57岁,均未接受放疗、化疗、免疫治疗等抗肿瘤治疗。组织标本离体后保存在-80 ℃冰箱备用。本研究经我院医学伦理委员会审核批准(XTYYKY2017006),患者及家属均对本研究知情同意。

1.1.2 细胞和试剂 人宫颈上皮永生化细胞H8(批号:QS-H283)购自旗赛生物科技(武汉)有限公司;宫颈癌SiHa细胞(批号:CL-0210)、HeLa细胞(批号:CL-0101)、Caski细胞(批号:CL-0048)及MEM培养基(批号:PM150410P)、青链霉素双抗(批号:PB180120)、胎牛血清(批号:164210)购自武汉普诺赛生命科技公司;PrimeScript逆转录试剂盒(批号:RR037A)、SYBR Premix Ex Taq试剂盒(批号:DRR041A)购自大连TaKaRa公司;miScript逆转录试剂盒(批号:218161)、miScript SYBR Green PCR试剂盒(批号:218073)购自德国Qiagen公司;si-hsa_circ_0011946、si-NC、miR-767-3p、pcDNA、pcDNA-hsa_circ_0011946、anti-miR-NC、anti-miR-767-3p、miR-NC、重组荧光素酶报告质粒购自上海生工生物公司;CCK-8、NP-40缓冲液购自上海碧云天生物公司;MMP-2兔多抗(批号:AF0234)、MMP-9兔多抗(批号:AF5234)、β-actin兔单抗(批号:AF5003)、辣根过氧化物酶标记山羊抗兔IgG(批号:A0208)购自上海碧云天生物公司。

1.2 RT-qPCR检测hsa_circ_0011946、miR-767-3p表达

使用TRIzol试剂从H8细胞、SiHa细胞、HeLa细胞、Caski细胞及癌组织和癌旁正常组织中提取总RNA,分别用PrimeScript逆转录试剂盒、miScript逆转录试剂盒合成circRNA、miRNA的cDNA,分别用SYBR Premix Ex Taq试剂盒、miScript SYBR Green PCR试剂盒对上述cDNA进行RT-qPCR。PCR反应条件:95 ℃预变性5 min;95 ℃变性30 s,55 ℃退火30 s,72 ℃延伸30 s,循环40次。U6为miR-767-3p的内参,β-actin为hsa_circ_0011946的内参,采用$2^{-\Delta\Delta Ct}$法分析hsa_circ_0011946、miR-767-3p的表达水平。miR-767-3p上游引物5′-TCCATTTGTTTTGATGATGGACT-3′,下游引物5′-CTCAACTGGTGTCGTGGAGTC-3′;U6上游引物5′-CTCGCTTCGGCAGCACAT-3′,下游引物5′-AACGCTTCACGAATTTGCGT-3′;hsa_circ_0011946上游引物5′-GCTGGTGTTCCTTGACTGGA-3′,下游引物5′-CACTGTAGCAAACCAGCATTTCT-3′;β-actin上游引物5′-GTCCACCGCAAATGCTTCTA-3′,下游引物5′-TGCTGTCACCTTCACCGTTC-3′。

1.3 细胞培养和分组

SiHa细胞接种含1%青链霉素双抗、10%胎牛血清的MEM培养基,并在37 ℃、含5%CO_2的培养箱中孵育。用胰蛋白酶消化80%融合的SiHa细胞,1∶3传代。取第3代对数期SiHa细胞以2×10⁴个/孔接种至96孔板,用Lipofectamine 2000将si-NC、si-hsa_circ_0011946、miR-NC、miR-767-3p、pcDNA、pcDNA-hsa_circ_0011946、anti-miR-NC+si-hsa_circ_0011946、anti-miR-767-3p+si-hsa_circ_0011946分别转染50%融合的SiHa细胞,分别作为si-NC组、si-hsa_circ_0011946组、miR-NC组、miR-767-3p组、pcDNA组、pcDNA-hsa_circ_0011946组、anti-miR-NC+si-hsa_circ_0011946组、anti-miR-767-3p+si-hsa_circ_0011946组。未转染的SiHa细胞作为NC组。转染48 h后,收集SiHa细胞,RT-qPCR检测hsa_circ_0011946、miR-767-3p的表达以验证转染效果,随后进行后续实验。

1.4 CCK-8法检测细胞活力

将转染48 h后的SiHa细胞以5×10³个/孔接种至96孔板,细胞贴壁后每孔添加10 μL CCK-8试剂,37 ℃孵育2 h。酶标仪在450 nm波长处测定吸光度(absorbance,A)值以表示细胞活力。

1.5 平板克隆形成实验检测细胞克隆数

将转染48 h后的SiHa细胞以5×10²个/孔接种至6孔板,培养约12 d至出现肉眼可见细胞克隆。4%多聚甲醛固定1 h,然后用0.1%结晶紫溶液染色。显微镜下计数大于50个细胞的克隆数。

1.6 Transwell法检测细胞迁移和侵袭数

将转染48 h后的SiHa细胞重悬于无血清培养基中,调整细胞密度为1×10⁶/mL。Transwell上室添加100 μL细胞悬液,下室添加600 μL完全培养基,然后在培养箱中孵育24 h。用棉签除去膜上表面的细胞,膜下表面的细胞为迁移细胞。用4%多聚甲醛固定迁移细胞,0.1%结晶紫溶液染色后,显微镜下对迁移细胞进行拍照。选取5个随机视野细胞数的均值表示各组细胞迁移数。侵袭数测定时先用0.5 mg/mL的基质胶包被Transwell上室膜,其余步骤同迁移测定。

1.7 Western blot检测MMP-2、MMP-9蛋白表达

用NP-40缓冲液提取蛋白并测定浓度。将50 μg蛋白样品在100 V下用10%十二烷基硫酸钠聚丙烯

酰胺凝胶电泳分离,并转移到硝酸纤维素膜(200 mA,80 min)。膜封闭后,除去封闭液,添加MMP-2(1∶1 000)、MMP-9(1∶1 500)、β-actin(1∶1 500)一抗,4 ℃摇床孵育2 h,然后与辣根过氧化物酶标记二抗(山羊抗兔IgG,1∶2 000)在37 ℃下孵育1 h。用化学发光试剂盒进行显色反应。以Image J软件测得的目的条带和β-actin灰度值的比值表示蛋白表达水平。

1.8 双荧光素酶报告基因实验

circular RNA interactome预测miR-767-3p与hsa_circ_0011946的结合位点。将含miR-767-3p结合位点的hsa_circ_0011946野生序列或不含预测位点的突变序列分别克隆到pmirGLO质粒,构建WT-hsa_circ_0011946和MUT-hsa_circ_0011946重组荧光素酶报告质粒。用Lipofectamine 2000将WT-hsa_circ_0011946或MUT-hsa_circ_0011946分别与miR-767-3p mimics、miR-NC共转染至SiHa细胞,用双荧光素酶报告基因系统测定转染48 h后的相对荧光素酶活性。

1.9 统计学方法

采用SPSS 22.0软件进行统计学分析,呈正态分布的数据以均数±标准差($\bar{x}\pm s$)表示,2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,进一步两两比较采用*LSD-t*检验。P<0.05为差异有统计学意义。

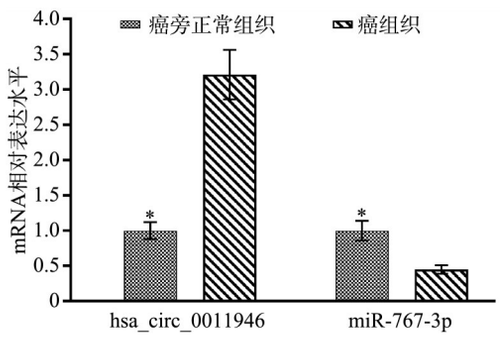
2 结果

2.1 宫颈癌组织及癌旁正常组织中hsa_circ_0011946和miR-767-3p表达

宫颈癌组织中hsa_circ_0011946表达水平显著高于癌旁正常组织(P<0.05),miR-767-3p表达水平显著低于癌旁正常组织(P<0.05),见图1。

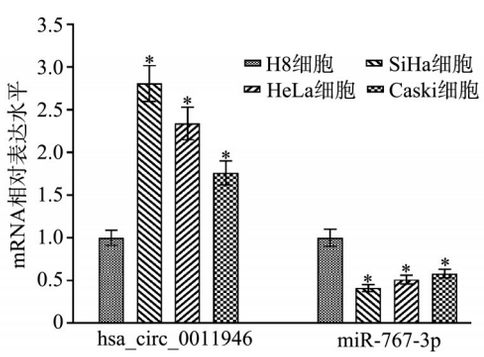
2.2 不同细胞系中hsa_circ_0011946和miR-767-3p的表达

与H8细胞比较,宫颈癌细胞系(SiHa、HeLa、Caski)中hsa_circ_0011946表达水平显著升高(P<0.05),miR-767-3p表达水平显著降低(P<0.05),见图2。选择表达差异较大的SiHa细胞进行后续实验。



*:与癌组织比较,P<0.05

图1 宫颈癌组织及癌旁正常组织中hsa_circ_0011946和miR-767-3p表达

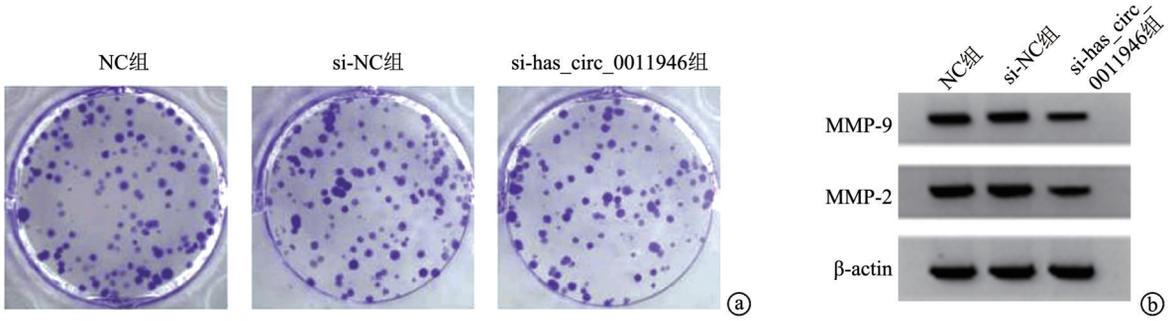


*:与H8细胞比较,P<0.05

图2 宫颈癌细胞系中hsa_circ_0011946和miR-767-3p的表达情况

2.3 干扰hsa_circ_0011946表达抑制宫颈癌SiHa细胞增殖、迁移和侵袭

与NC组比较,si-hsa_circ_0011946组SiHa细胞hsa_circ_0011946表达水平、A值、克隆数、迁移数、侵袭数及MMP-2、MMP-9蛋白表达水平显著降低/减少(P<0.05),见图3、表1。



a:细胞克隆形成实验;b:Western blot检测MMP-2、MMP-9蛋白表达

图3 干扰hsa_circ_0011946表达对宫颈癌SiHa细胞克隆及蛋白表达的影响

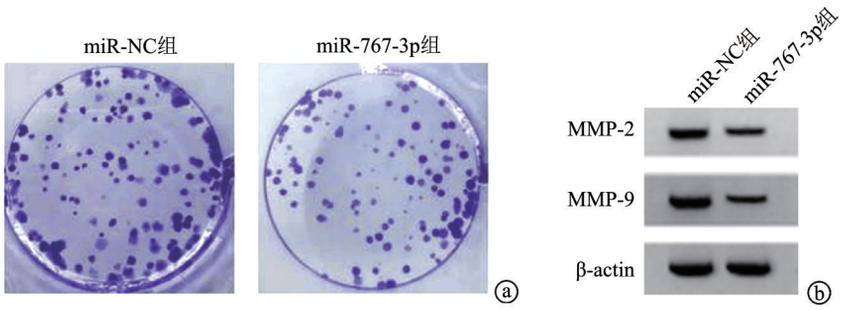
2.4 过表达miR-767-3p抑制宫颈癌SiHa细胞增殖、迁移和侵袭

与miR-NC组比较，miR-767-3p组SiHa细胞miR-767-3p表达水平显著升高（$P<0.05$），细胞A值、克隆数、迁移数、侵袭数及MMP-2、MMP-9蛋白表达水平显著降低/减少（$P<0.05$），见图4、表2。

表1 干扰hsa_circ_0011946表达抑制宫颈癌SiHa细胞增殖、迁移和侵袭（$\bar{x}\pm s$）

组别	hsa_circ_0011946	MMP-2	MMP-9	A值	细胞克隆数（个）	细胞迁移数（个）	细胞侵袭数（个）
NC组	1.00±0.10	0.93±0.07	0.75±0.06	1.10±0.09	123.14±9.03	234.49±18.17	171.32±13.67
si-NC组	1.02±0.09	0.91±0.09	0.73±0.07	1.11±0.10	121.32±9.16	231.06±19.03	168.43±9.72
si-hsa_circ_0011946组	0.45±0.04*	0.42±0.04*	0.37±0.03*	0.53±0.05*	63.41±5.02*	108.37±9.35*	71.09±6.19*
F	143.406	154.295	131.362	144.469	163.446	178.744	274.923
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

*：与NC组比较，$P<0.05$



a：细胞克隆形成实验；b：Western blot检测MMP-2、MMP-9蛋白表达

图4 过表达miR-767-3p对宫颈癌SiHa细胞克隆及蛋白表达的影响

表2 过表达miR-767-3p抑制SiHa宫颈癌细胞增殖、迁移和侵袭（$\bar{x}\pm s$）

组别	miR-767-3p	MMP-2	MMP-9	A值	细胞克隆数（个）	细胞迁移数（个）	细胞侵袭数（个）
miR-NC组	1.00±0.10	0.92±0.09	0.75±0.08	1.11±0.08	124.35±11.02	228.49±17.54	164.32±12.51
miR-767-3p组	2.51±0.22	0.48±0.04	0.36±0.03	0.57±0.05	57.16±4.36	117.68±9.54	79.24±7.13
t	18.745	13.403	13.694	17.013	17.008	16.649	17.726
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

2.5 hsa_circ_0011946靶向调控miR-767-3p的表达

circular RNA interactome预测到miR-767-3p和hsa_circ_0011946存在特异性结合序列（图5）。与miR-NC组比较，miR-767-3p组WT-hsa_circ_0011946的SiHa细胞相对荧光素酶活性降低（$P<0.05$）；而MUT-hsa_circ_0011946的SiHa细胞相对荧光素酶活性差异无统计学意义（$P<0.05$），见图6。pcDNA-hsa_circ_0011946组SiHa细胞hsa_circ_0011946表达水平显著高于pcDNA组（$P<0.05$），miR-767-3p表达水平显著低于pcDNA组（$P<0.05$）；si-hsa_circ_0011946组SiHa细胞hsa_circ_0011946表达水平显著低于si-NC组（$P<0.05$），miR-767-3p表达水平显著高于si-NC组（$P<0.05$），见图7。

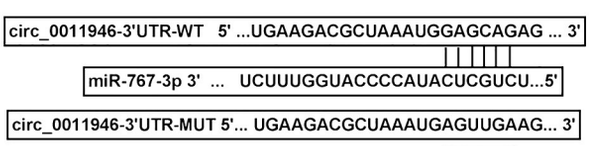
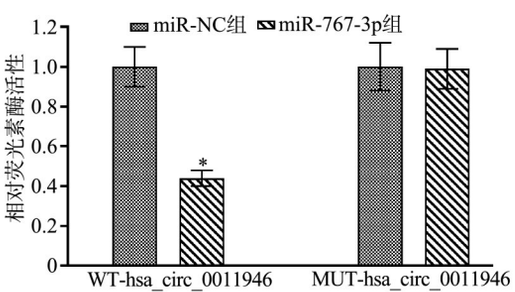
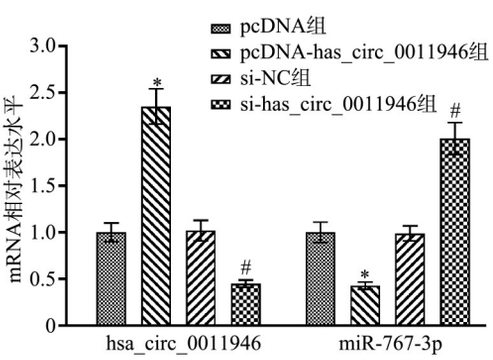


图5 miR-767-3p与hsa_circ_0011946的结合位点



*：与miR-NC组比较，$P<0.05$

图6 双荧光素酶报告基因检测实验

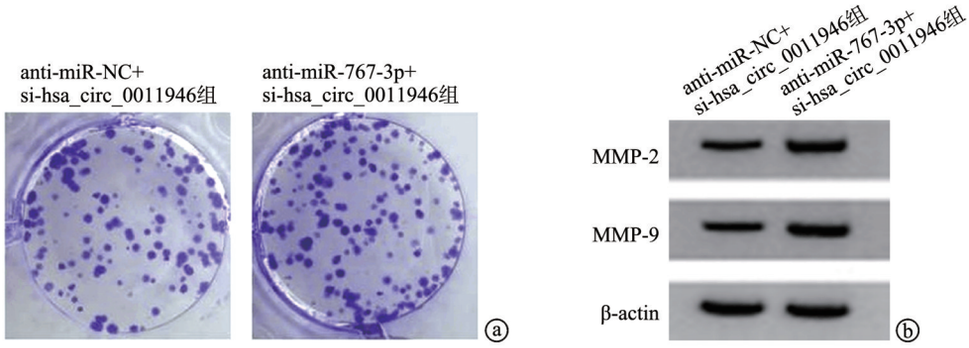


*：与pcDNA组比较，$P<0.05$；#：与si-NC组比较，$P<0.05$

图7 RT-qPCR检测hsa_circ_0011946、miR-767-3p的表达

2.6 抑制miR-767-3p表达逆转干扰hsa_circ_0011946表达对SiHa细胞增殖、迁移和侵袭的影响

与anti-miR-NC+si-hsa_circ_0011946组比较，anti-miR-767-3p+si-hsa_circ_0011946组SiHa细胞miR-767-3p表达水平显著降低（$P<0.05$），细胞A值、克隆数、迁移数、侵袭数及MMP-2、MMP-9蛋白表达水平显著升高/增加（$P<0.05$），见图8、表3。



a：细胞克隆形成实验；b：Western blot检测MMP-2、MMP-9蛋白表达

图8 抑制miR-767-3p表达逆转干扰hsa_circ_0011946对SiHa细胞克隆及蛋白表达的影响

表3 抑制miR-767-3p表达逆转干扰hsa_circ_0011946表达对SiHa细胞增殖、迁移和侵袭的影响（$\bar{x}\pm s$）

组别	miR-767-3p	MMP-2	MMP-9	A值	细胞克隆数（个）	细胞迁移数（个）	细胞侵袭数（个）
anti-miR-NC+si-hsa_circ_0011946组	1.00±0.09	0.41±0.04	0.35±0.03	0.54±0.05	66.31±4.19	112.34±10.31	74.38±7.02
anti-miR-767-3p+si-hsa_circ_0011946组	0.42±0.03	0.95±0.09	0.78±0.07	1.15±0.10	123.89±9.76	271.46±24.18	185.23±15.41
t	18.341	16.449	16.939	16.583	16.263	18.160	19.638
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

3 讨论

circRNA已被证实参与调控多种生物过程，在宫颈癌发生发展中发挥关键作用。如宫颈癌临床标本中circ_0000388表达上调，且与FIGO分期、淋巴结转移和浸润深度相关，circ_0000388高表达可显著增强宫颈癌细胞的增殖和转移能力[10]。circRNA8924通过抑制miR-518d-5p/519-5p活性促进宫颈癌细胞恶性生物学行为[11]。过表达circEYA1可降低宫颈癌细胞活力，促进细胞凋亡，抑制异种移植瘤生长[12]。circMTO1通过调控miR-6893促进宫颈癌的发生和化疗耐药性[13]。本研究发现，hsa_circ_0011946在宫颈癌组织及细胞中表达显著升高，表明hsa_circ_0011946可能参与宫颈癌的进展。hsa_circ_0011946已被证实参与肝细胞癌的进展，其表达与肝细胞癌的淋巴转移相关，敲除hsa_circ_0011946可抑制肝细胞癌细胞的迁移和侵袭，提示hsa_circ_0011946在肝细胞癌中发挥致癌作用[14]。本研究结果显示，干扰hsa_circ_0011946表达可抑制SiHa细胞增殖、迁移和侵袭，这与既往研究[5,14]报道的hsa_circ_0011946在其他肿瘤细胞中的作用相吻合。MMP-2、MMP-9是降解基底膜、细胞外基质的主要蛋白酶，为细胞迁移和侵袭提供有利条件[15-16]。研究证实，沉默circ-0000212可通过抑制MMP-2和MMP-9蛋白表达降低宫颈癌细胞的侵袭和迁移能力[17]。本研究干扰hsa_circ_0011946表达后，MMP-2和MMP-9蛋白表达显著降低，进一步证实干扰hsa_circ_0011946表达对宫颈癌细胞迁移和侵袭的抑制作用。

研究表明，circRNA在宫颈癌中的功能与调控miRNA表达有关，如circ_0000515可通过靶向miR-326抑制宫颈癌细胞凋亡和自噬，诱导细胞增殖和侵袭[18]。本研究中双荧光素酶检测和RT-qPCR证实，miR-767-3p是hsa_circ_0011946的特异性靶点。miR-767-3p已被报道在非小细胞肺癌中表达下调，敲减circ_0018818通过上调miR-767-3p表达抑制非小细胞肺癌细胞的增殖和侵袭，并阻碍上皮-间质转化过程[19]。此外，miR-767-3p还介导敲减hsa_circ_0000673对肝癌细胞恶性行为的抑制作用[20]。本研究发现，宫颈癌组织和细胞中miR-767-3p表达水平显著下降，过表达miR-767-3p可下调MMP-2、MMP-9蛋白表达，抑制SiHa细胞增殖、迁移和侵袭。hsa_circ_0011946对miR-767-3p具有负调控作用，且过表达miR-767-3p与干扰hsa_circ_0000673的抗肿瘤作用一致，故本研究推测宫颈癌中可能存在hsa_circ_0011946/miR-767-3p调控途径。为进一步证实干扰hsa_circ_0011946的作用依赖于miR-767-3p表达上调，本研究将si-hsa_circ_

0011946和anti-miR-767-3p共转染至SiHa细胞，结果显示，抑制miR-767-3p表达可显著削弱干扰hsa_circ_0011946对SiHa细胞的抗增殖、抗迁移和抗侵袭作用，进一步证实了hsa_circ_0011946靶向miR-767-3p促进SiHa细胞的恶性生物学行为。

综上，宫颈癌中hsa_circ_0011946呈高表达，miR-767-3p呈低表达。干扰hsa_circ_0011946通过靶向上调miR-767-3p抑制宫颈癌细胞增殖、迁移和侵袭。因此，hsa_circ_0011946/miR-767-3p途径可能是抑制宫颈癌进展的潜在有效靶点。

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