·基础研究·

miR-96-5p 靶向叉头状转录因子 O1 对 Ishikawa 细胞的作用

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摘　要：【目的】探讨微小 RNA(miR)-96-5p 通过靶向叉头状转录因子 O1(*FOXO1*)基因对子宫内膜癌细胞增殖和侵袭的影响。【方法】qRT-PCR 检测正常组织和子宫内膜癌组织中 miR-96-5p 和 *FOXO1* mRNA 的表达，Western blot 法检测 FOXO1 蛋白表达。分析 miR-96-5p 及 FOXO1 表达与子宫内膜癌临床病理特征的关系。转染 pcDNA、pcDNA-FOXO1、inhibitor NC、miR-96-5p inhibitor、miR-96-5p mimic、miR-96-5p + FOXO1 到子宫内膜癌 Ishikawa 细胞，分别记为 pcDNA 组、FOXO1 组、inhibitor NC 组、miR-96-5p inhibitor 组、miR-96-5p 组、miR-96-5p + FOXO1 组，对照组不进行转染。取稳定转染的各组细胞，CCK-8 和 Transwell 小室检测细胞活力和侵袭能力，Western blot 检测细胞周期蛋白 D1(Cyclin D1)、活化多聚 ADP 核糖聚合酶(cleaved PARP)、p21 及波形蛋白的表达水平。TargetScan 网站预测 miR-96-5p 与 FOXO1 的靶向关系，双荧光素酶报告基因检测细胞荧光素酶活性。Ishikawa 细胞分为 mimic NC 组、miR-96-5p 组、inhibitor NC 组、miR-96-5p inhibitor 组，qRT-PCR 检测各转染组 miR-96-5p 和 *FOXO1* mRNA 的表达，Western blot 法检测 FOXO1 蛋白表达。【结果】子宫内膜癌组织中 miR-96-5p 的相对表达量较正常组织上调，相反，*FOXO1* mRNA 和蛋白相对表达量下调(P<0.01)。子宫内膜癌患者中 miR-96-5p 高表达与病理分级和临床分期呈正相关(P=0.034，P=0.010)，与年龄和是否绝经无明显相关性(P=0.370，P=0.166)；FOXO1 低表达与病理分级和临床分期呈负相关(P=0.023，P=0.007)，与年龄和是否绝经无明显相关性(P=0.344，P=0.144)。过表达 FOXO1 或抑制 miR-96-5p 表达后，Ishikawa 细胞活力明显降低(P<0.05)、侵袭细胞数减少(P<0.01)，cyclin D1、Vimentin 蛋白表达水平明显降低，而 cleaved PARP、p21 蛋白表达水平明显升高(P<0.01)。与对照组比较，miR-96-5p 组 Ishikawa 细胞活力明显升高(P<0.05)、侵袭细胞数增加(P<0.01)，Cyclin D1、Vimentin 蛋白表达水平明显升高，而 cleaved PARP、p21 蛋白表达水平明显降低(P<0.01)。与 miR-96-5p 组比较，过表达 FOXO1 可明显逆转 miR-96-5p 对 Ishikawa 细胞活力和侵袭的促进作用(P<0.05)。miR-96-5p 靶向并负调控 FOXO1 的表达 (P<0.05)。【结论】miR-96-5p 可通过靶向负调控 FOXO1 促进子宫内膜癌细胞的增殖和侵袭。

关键词：子宫内膜癌；miR-96-5p；FOXO1；细胞周期蛋白 D1；侵袭
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Effect of miR-96-5p on Ishikawa Cells by Targeting Forkhead Box Transcription Factor O1

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Abstract:【Objective】To study the effect of microRNA-96-5p (miR-96-5p) on the proliferation and invasion of endometrial cancer cells by targeting forkhead box transcription factor O1 (*FOXO1*) genes.【Methods】The expressions of miR-96-5p and *FOXO1* mRNA in normal and endometrial carcinoma tissues were detected by qRT-PCR, and FOXO1 protein expression was detected by Western blot. The relationship between miR-96-5p and FOXO1 expression and clinicopathological features of endometrial carcinoma was analyzed. pcDNA, pcDNA-FOXO1, inhibitor NC, miR-96-5p inhibitor, miR-96-5p mimic and miR-96-5p + FOXO1 were transfected into endometrial carcinoma Ishikawa cells, which were recorded as pcDNA group, FOXO1 group, inhibitor NC group, miR-96-5p inhibitor group, miR-96-5p group and miR-96-5p + FOXO1 group, respectively. Cells in stable transfection groups were taken, CCK-8 and Transwell chamber were employed to detect cell viability and invasion, and the expression levels of cyclin D1, cleaved PARP, p21 and Vimentin were measured by Western blot. TargetScan website was used to predict the targeting relationship between miR-96-5p and FOXO1, and dual luciferase reporter gene was used to detect cell luciferase activity. Ishikawa cells were divided into mimic NC group, miR-96-5p group, inhibitor NC group and miR-96-5p inhibitor group. The expressions of miR-96-5p and *FOXO1* mRNA were detected by qRT-PCR, and the protein expression of FOXO1 was detected by Western blot.【Results】The relative expression level of miR-96-5p in endometrial carcinoma tissues was up-regulated compared with that in normal tissues. On the contrary, the relative expression levels of *FOXO1* mRNA and protein were down-regulated (P<0.01). The high expression of miR-96-5p was positively correlated with pathological grade and clinical stage (P=0.034, P=0.010), but not significantly correlated with age and menopause in endometrial carcinoma patients (P=0.370, P=0.166). The low expression of FOXO1 was negatively correlated with pathological grade and clinical stage (P=0.023, P=0.007), but not significantly correlated with the age and menopause in endometrial cancer patients (P=0.344, P=0.144). After FOXO1 overexpression or inhibition of miR-96-5p expression, the viability of Ishikawa cells decreased significantly (P < 0.05), the number of invasive cells decreased (P < 0.01), the expression levels of cyclin D1 and Vimentin proteins decreased significantly, and the expression levels of cleaved PARP and p21 proteins increased significantly (P < 0.01). Compared with the control group, the viability of Ishikawa cells was significantly increased (P < 0.05), the number of invasive cells was increased (P < 0.01), the expression levels of cyclin D1 and Vimentin were significantly increased, and the expression levels of cleaved PARP and p21 were significantly decreased in miR-96-5p group (P < 0.01). Compared with the miR-96-5p group, overexpression of FOXO1 could significantly reverse the promotion of miR-96-5p on the viability and invasion of Ishikawa cells (P < 0.05). miR-96-5p targeted and negatively regulated FOXO1 expression (P < 0.05).【Conclusion】miR-96-5p promotes the proliferation and invasion of endometrial cancer cells by targeting and negatively regulating FOXO1.

Key words: endometrial cancer; miR-96-5p; forkhead box transcription factor O1; cyclin D1; invasion

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子宫内膜癌是女性生殖道最常见的恶性肿瘤，近年来其发病率呈上升趋势[1]。目前治疗子宫内膜癌的方法主要包括手术切除、放化疗法和激素疗法[2]。但是，由于晚期转移性或复发性子宫内膜癌患者失去了手术机会，治疗失败率较高，因此，需要研制新的治疗方法。目前，越来越多的分子靶向药物正在临床测试应用，且部分药物已显示出有效结果[3-4]。微小RNA(microRNA，miRNA)是可以调节一组靶基因并导致翻译抑制或mRNA降解的分子[5]。最近的研究表明，子宫内膜癌中大量miRNA失调并调节细胞的生长和转移，继而影响肿瘤的发生和发展[6]。有研究发现，miR-96-5p在结肠癌[7]、卵巢癌[8]和肺癌[9]中的表达均明显上调，并促进癌细胞的增殖和迁移；miR-96-5p在子宫内膜癌中表达上调，可能与患者不良预后相关[10]。而叉头状转录因子O1(forkhead box transcription factor O1，FOXO1)在各种癌症(包括子宫内膜癌)的进展中起抑癌作用[11]。因此，本文主要探讨了miR-96-5p是否通过调控FOXO1影响子宫内膜癌细胞的增殖和侵袭。

1 材料与方法

1.1 临床组织标本

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大学第一附属医院行子宫切除术的50例患者子宫内膜癌和癌旁正常组织样本，-80 ℃保存。子宫内膜癌均经病理学确诊，且术前未接受过任何其他治疗。本研究经河南中医药大学第一附属医院伦理委员会批准（批件号：HNZYYDXFSYY-2020-029），所有参与者均知情同意。

1.2　主要试剂及仪器

DMEM培养基、胎牛血清（fetal bovine serum，FBS）（美国Gibco公司）；Lipofectamine2000和Trizol试剂（美国Invitrogen公司）；cDNA反转录试剂盒和SYBR Green PCR Master Mix试剂盒（日本TaKaRa公司）；CCK-8试剂、RIPA裂解缓冲液、BCA试剂盒和ECL发光剂（上海碧云天生物技术有限公司）；兔抗FOXO1抗体（ab39670）、兔抗细胞周期蛋白D1（cyclin D1）抗体（ab16663）、兔抗活化多聚ADP核糖聚合酶（cleaved poly-ADP ribose polymerase，cleaved PARP）抗体（ab32064）、兔抗p21抗体（ab109520）、兔抗波形蛋白抗体（ab92547）、兔抗GAPDH（ab9485）（英国Abcam公司）；辣根过氧化物酶偶联的山羊抗兔二抗（#7074，美国CST公司）；双荧光素酶报告系统（美国Promega公司）。高速低温离心机（美国Beckman公司）；Varioskan LUX酶标仪（美国Thermo Fisher公司）；Promotor® 实时荧光定量PCR仪（杭州艾康生物技术有限公司）；Primo Star iLED显微镜（德国蔡司公司）；电泳仪，电转仪（北京六一生物科技有限公司）；Tanon 3500凝胶成像系统（上海天能公司）。

1.3　方法

1.3.1　*细胞培养及细胞转染*　子宫内膜癌Ishikawa细胞购自美国菌种保藏中心（American type culture collection，ATCC）。Ishikawa细胞在含有100 mL/L FBS的DMEM培养基（含100 U/mL青霉素和100 μg/mL链霉素）于37 ℃和50 mL/L CO_2的加湿培养箱中培养。当细胞达到80% 融合时，根据Lipofectamine2000试剂说明书进行转染。向Ishikawa细胞转染pcDNA、pcDNA-FOXO1、inhibitor NC、miR-96-5p inhibitor、mimic NC、miR-96-5p mimic以及共转染miR-96-5p mimic和pcDNA-FOXO1，分别记为pcDNA组、FOXO1组、inhibitor NC组、miR-96-5p inhibitor组、mimic NC组、miR-96-5p组和miR-96-5p+FOXO1组，对照组不进行转染。以上载体和基因序列均由上海吉玛基因公司设计和提供。

1.3.2　*qRT-PCR检测正常组织和子宫内膜癌组织中miR-96-5p和FOXO1 mRNA的表达*　采用Trizol试剂提取组织标本及转染细胞的总RNA，然后用分光光度计检测RNA纯度。根据反转录试剂盒说明，取10 ng RNA用于cDNA合成。反应程序：16 ℃ 30 min；42 ℃ 30 min，85 ℃ 5 min。使用SYBR Green PCR Master Mix试剂盒进行qRT-PCR反应，程序如下：95 ℃ 2 min；95 ℃ 15 s，60 ℃ 1 min，40个循环；以U6或GAPDH对照，采用$2^{-\Delta\Delta ct}$法计算miR-96-5p和FOXO1的表达。qRT-PCR反应特异性引物如下所示：FOXO1，正向5′-TGGACATGCTCAGCAGACATC-3′，反向5′-TTGGGTCAGGCGGTTCA-3′；GAPDH，正向5′-TATGATGATATCAAGAGGGTAGT-3′，反向5′-TGTATCCAAACTCATTGTCATAC-3′；miR-96-5p，正向5′-ACACTCCAGCTGGGTTTGGCACTAGCACATTT-3′，反向5′-CTCAACTGGTGTCGTGGA-3′；U6，正向5′-CTCGCTTCGGCAGCACA-3′，反向5′-AACGCTTCACGAATTTGCGT-3′。

1.3.3　*Western blot检测FOXO1、cyclin D1、cleaved-PARP、p21和Vimentin蛋白表达*　用RIPA裂解缓冲液裂解组织，12 000×*g*离心后收集上清液，BCA测定蛋白浓度。凝胶电泳分离等量的蛋白质并电转移到聚偏二氟乙烯（polyvinylidene fluoride，PVD）膜。50 g/L的脱脂牛奶封闭后，PVDF膜用抗GAPDH（1∶1 000）、FOXO1（1∶1 000）、cyclin D1（1∶1 000）、cleaved PARP（1∶1 000）、p21（1∶1 000）和波形蛋白（1∶1 000）的一抗4 ℃孵育过夜。然后用辣根过氧化物酶（horseradish peroxidase，HRP）偶联的山羊抗兔二抗（1∶3 000）在37 ℃下处理2 h，再用化学发光液观察蛋白条带。

1.3.4　*CCK-8法检测细胞活力*　将传至第3~5代的Ishikawa细胞接种于96孔板内，转染后各组连续培养24、48、72 h，每孔加入10 μL CCK-8试剂，37 ℃培养2 h后使用酶标仪在450 nm检测吸光度。

1.3.5　*Transwell小室检测细胞侵袭情况*　每个上室的膜用Matrigel（100 μg/cm^2）包被，然后37 ℃孵育过夜以胶凝。转染后Ishikawa细胞重悬于无血清培养基中，并以3×10^{4}细胞/孔密度接种到上层Transwell室中。下室加入500 μL含100 mL/L FBS的DMEM培养基作为化学诱导剂。温育24 h后，除去未侵袭的细胞，用5 g/L的结晶紫对侵袭的细胞进行染色，并于显微镜下计数。

1.3.6 双荧光素酶报告基因验证实验 Targetscan网站预测分析FOXO1 3′UTR上miR-96-5p的结合位点。将FOXO1 wt或FOXO1 mut质粒分别与mimic NC或miR-96-5p mimic使用Lipofectamine2000试剂共转染至Ishikawa细胞。培养48 h后,按照说明书操作测定细胞裂解液中的荧光素酶活性。

1.4 统计学处理

采用Graphpad 6.0软件进行数据统计和分析。结果表示为平均值±标准差($\bar{x}\pm s$),采用单因素方差分析数据,进一步两两比较采用LSD检验;两个分类变量的关联程度分析,先采用χ^2检验,再计算miR-96-5p表达或FOXO1表达与子宫内膜癌临床病理特征的关联系数ϕ或*Cramér's V*。P<0.05为差异有统计学意义。

2 结 果

2.1 miR-96-5p和FOXO1在子宫内膜癌组织中的表达

qRT-PCR和Western blot检测结果显示,各组*FOXO1* mRNA和蛋白表达水平差异有统计学意义(t=23.850, P<0.001; t=33.99, P<0.001;图1),各组miR-96-5p表达水平差异有统计学意义(t=12.550, P<0.001)。与正常组织比较,子宫内膜癌组织中*FOXO1* mRNA和蛋白表达量显著下调,miR-96-5p表达量显著上调(P<0.001)。

2.2 miR-96-5p和FOXO1表达与子宫内膜癌临床病理特征的关系

子宫内膜癌患者中miR-96-5p高表达与病理分级和临床分期呈正相关(*Cramér's V* =0.367, P=0.034; ϕ=0.362, P=0.010),即随着miR-96-5p表达升高,病理分级和临床分期等级越高,而与年龄和是否绝经无明显相关性(P=0.370, P=0.166);子宫内膜癌患者中FOXO1低表达与病理分级和临床分期呈负相关(*Cramér's V* =0.389, P=0.023; ϕ=0.384, P=0.007),即随着FOXO1表达降低,病理分级和临床分期等级越高,与年龄和是否绝经无明显相关性(P=0.344, P=0.144;表1)。

2.3 FOXO1过表达对子宫内膜癌Ishikawa细胞存活和侵袭的影响

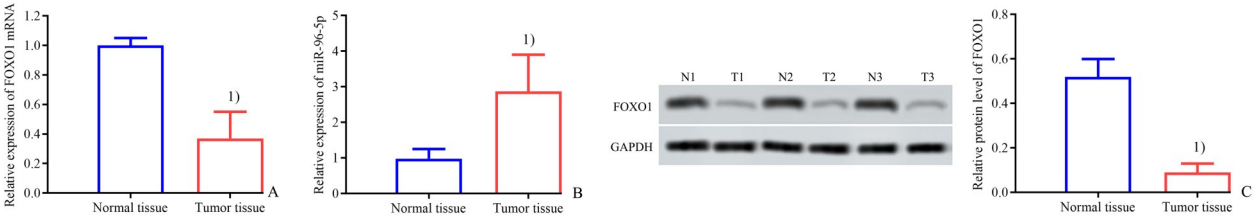
Western blot实验结果显示,各组FOXO1蛋白表达水平差异有统计学意义(F=123.400, P<0.001)。与对照组比较,pcDNA组FOXO1蛋白表

表1 miR-96-5p和FOXO1表达与子宫内膜癌临床病理特征的关系

Table 1 Relationship between miR-96-5p and FOXO1 expression and clinicopathological features of endometrial carcinoma

Clinicopathological features	miR-96-5p		χ^2	P	FOXO1		χ^2	P
	High	Low			High	Low		
Age/years			0.802	0.370			0.897	0.344
<45(n=17)	10	7			8	9		
≥45(n=33)	15	18			11	22		
Whether menopause			1.919	0.166			2.131	0.144
Pre-menopausal (n=28)	15	13			12	16		
Post-menopausal (n=22)	16	6			14	8		
Pathological grade			6.77	0.034			7.527	0.023
G_1(n=9)	5	4			4	5		
G_2(n=14)	8	6			5	9		
G_3(n=27)	24	3			2	25		
Clinical stage			6.551	0.010			7.390	0.007
Ⅰ+Ⅱ(n=17)	6	11			12	5		
Ⅲ(n=33)	24	9			10	23		

The expression of miR-96-5p and pathological grade *Cramér's V*=0.367; The expression of miR-96-5p and clinical stage ϕ=0.362; The expression of FOXO1 and pathological grade *Cramér's V*=0.389; The expression of FOXO1 and clinical stage ϕ=0.384.



A: *FOXO1* mRNA expression in tissues was detected by qRT-PCR; B: Expression of miR-96-5p in tissues was detected by qRT-PCR; C: Western blot analysis of FOXO1 protein expression in tissues; N: Normal; T: tumor. [1)] $P<0.001$ compared with normal tissue.

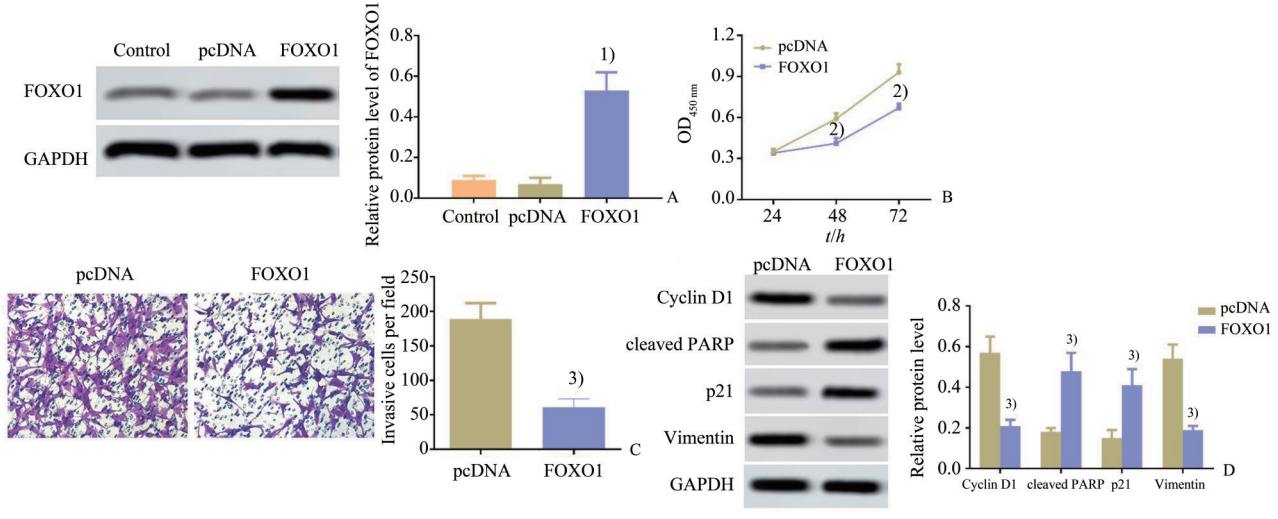
图1　FOXO 1蛋白在子宫内膜癌组织中的表达

Fig. 1　Expression of FOXO 1 protein in endometrial carcinoma

达量变化无统计学意义（$P>0.05$），FOXO1组Ishikawa细胞中FOXO1蛋白表达量明显上调（$P<0.01$；图2A）。CCK-8结果显示，各组吸光度值在总体差异上均具有统计学意义（$F=17.890$，$P<0.001$）。如图2B所示，与pcDNA组比较，FOXO1组48 h和72 h的细胞活力均明显降低（$P<0.05$）。Transwell实验结果表明，2组侵袭细胞数差异具有统计学意义（$t=8.546$，$P=0.001$）。Western blot结果显示，2组中各蛋白表达水平差异具有统计学意义（cyclin D1：$t=7.298$，$P=0.002$；cleaved PARP：$t=5.637$，$P=0.005$；p21：$t=5.040$，$P=0.007$；Vimentin：$t=8.328$，$P=0.001$）。与pcDNA组相比，FOXO1组侵袭细胞数目明显减少（$P<0.01$；图2C），Cyclin D1和波形蛋白的表达水平明显降低，cleaved PARP和p21蛋白表达水平明显升高（$P<0.01$；图2D）。

2.4　抑制miR-96-5p表达对子宫内膜癌Ishika-wa细胞存活和侵袭的影响

qRT-PCR结果显示，各组miR-96-5p表达水平差异有统计学意义（$F=137.600$，$P<0.001$）。与对照组比较，inhibitor NC组Ishikawa细胞中miR-96-5p表达量变化无统计学差异（$P>0.05$），miR-96-5p inhibitor组中miR-96-5p表达量明显下调（$P<0.05$；图3A）。CCK-8结果显示，各组吸光度值在总体差异上均具有统计学意义（$F=10.320$，$P=0.003$）。如图3B所示，与inhibitor NC组比较，miR-96-5p inhibitor组Ishikawa细胞48 h和72 h的细胞活力明显减弱（$P<0.05$）。Transwell实验结果表明，2组侵袭

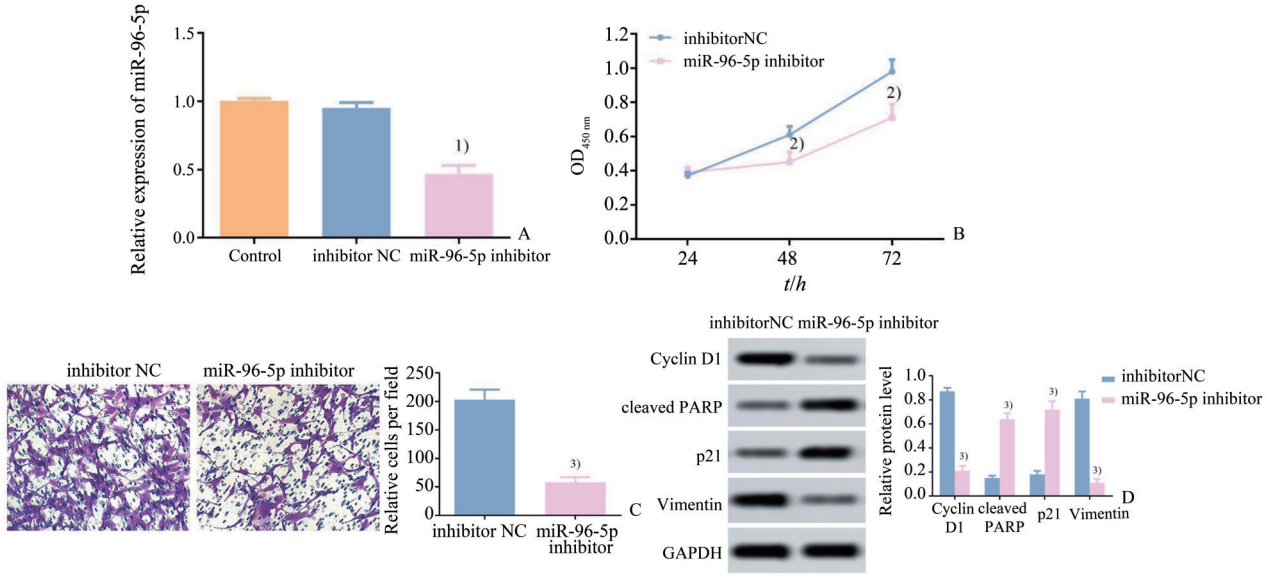


A: Western blot detection of FOXO1 protein levels in each group; B: CCK-8 detection of changes in cell viability in each group; C: Changes in the number of cells invaded in each group (Transwell, ×400); D: Western blot detection of the protein expression levels of Cyclin D1, cleaved PARP, p21 and Vimentin. [1)] $P<0.01$compared with the control group; [2)] $P<0.05$, [3)] $P<0.01$ compared with the pcDNA group.

图2　Western blot检测Ishikawa细胞FOXO1蛋白的表达情况

Fig. 2　Expression of FOXO1 protein of Ishikawa cell detected by Western blot

细胞数差异具有统计学意义(t=12.480,P=0.002)。Western blot结果显示,2组中各蛋白表达水平差异具有统计学意义(cyclin D1:t=22.860,P=0.001;cleaved PARP:t=15.760,P=0.003;p21:t=12.280,P=0.002;Vimentin:t=18.070,P=0.001)。与inhibitor NC组比较,miR-96-5p inhibitor组侵袭细胞数明显减少(P<0.01,图3C),cyclin D1和波形蛋白的表达水平明显降低,cleaved PARP和p21蛋白表达水平明显升高(P<0.01;图3D)。



A: The expression level of miR-96-5p in each group was detected by qRT-PCR; B: The change of cell viability in each group was detected by CCK-8 method; C: The change in the number of cells invaded in each group (Transwell, ×400); D: Western blot was used to detect the expression levels of Cyclin D1, cleaved PARP, p21 and Vimentin; [1] P<0.05 compared with the control group; [2] P<0.05, [3] P<0.01 compared with the inhibitor NC group.

图3 抑制miR-96-5p表达对Ishikawa细胞活力和侵袭的影响

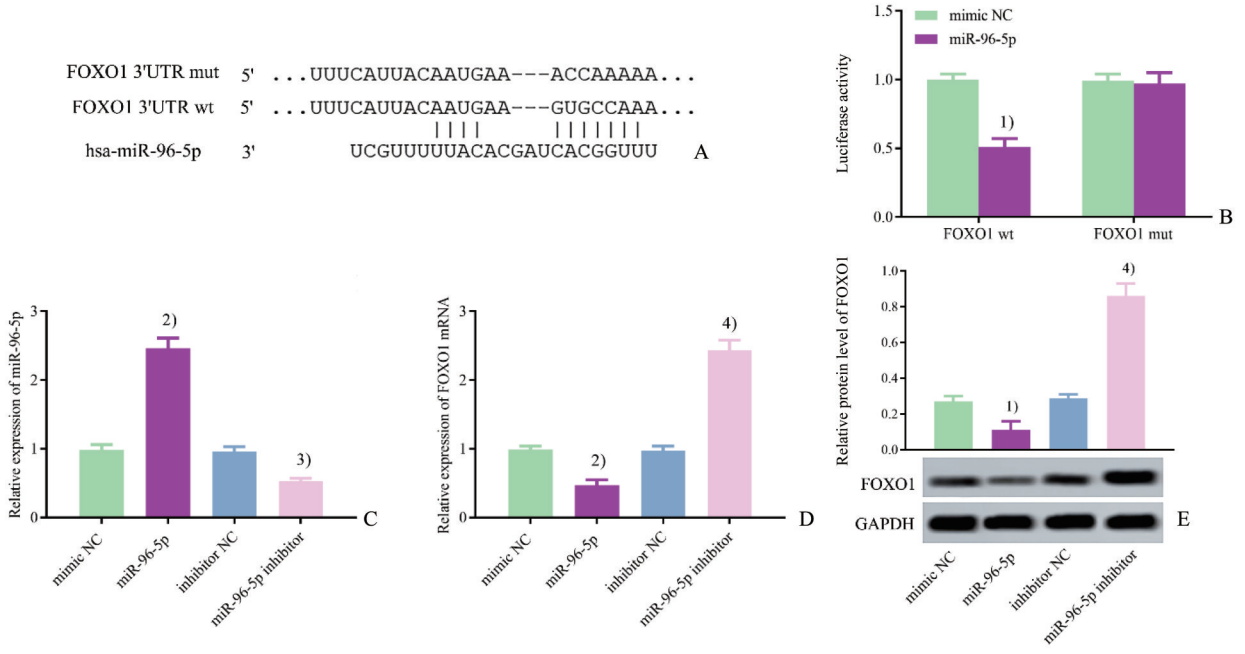
Fig. 3 Effects of inhibition of miR-96-5p expression on Ishikawa cell viability and invasion

2.5 miR-96-5p靶向负调控FOXO1的表达

Targetscan预测结果显示,FOXO1 3′UTR部分序列与miR-96-5p互补配对(图4A)。荧光素酶报告基因实验结果显示,各组总体差异有统计学意义(F=47.000,P<0.001)。如图4B所示,与mimic-NC+FOXO1 wt组比较,转染FOXO1 mut的细胞荧光素酶活性无明显变化,miR-96-5p+FOXO1 wt组细胞荧光素酶活性明显降低(P<0.01)。qRT-PCR和Western blot检测结果显示,各组FOXO1 mRNA和蛋白表达水平差异有统计学意义(F=236.000,P<0.001;F=148.70,P<0.001),各组miR-96-5p表达水平差异有统计学意义(F=241.600,P<0.001)。如图4C-E所示,与mimic NC组比较,miR-96-5p组miR-96-5p表达明显上调,*FOXO1* mRNA和蛋白表达明显下调(P<0.01);与inhibitor NC组比较,miR-96-5p inhibitor组miR-96-5p表达明显下调(P<0.05),*FOXO1* mRNA和蛋白表达明显上调(P<0.01)。

2.6 miR-96-5p靶向调控FOXO1促进Ishikawa细胞的活力和侵袭

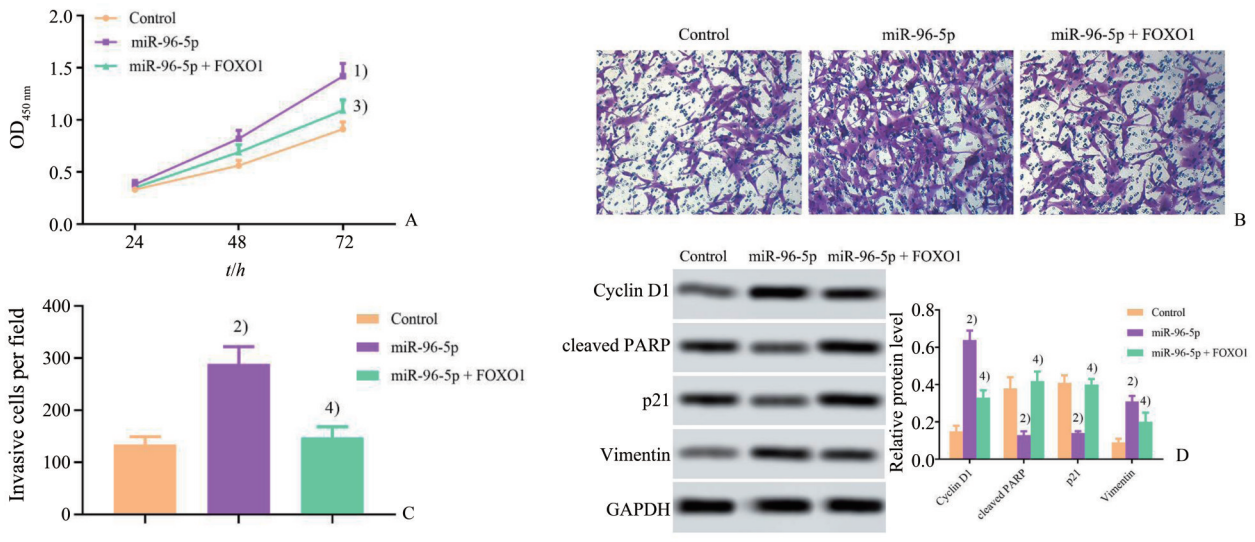
CCK-8结果显示,各组吸光度值在总体差异上均具有统计学意义(F=8.126,P<0.001)。与对照组比较,miR-96-5p组Ishikawa细胞活力明显升高,与miR-96-5p组比较,miR-96-5p+FOXO1组细胞活力明显降低(P<0.05;图5A)。Transwell实验结果表明,各组侵袭细胞数差异具有统计学意义(F=37.910,P<0.001)。Western blot结果显示,各组蛋白表达水平差异具有统计学意义(Cyclin D1:F=110.600,P<0.001;cleaved PARP:F=34.200,P<0.001;p21:F=81.120,P<0.001;Vimentin:F=28.660,P=0.001)。如图5B-D所示,与对照组比较,miR-96-5p组侵袭细胞数明显增多(P<0.01),cyclin D1和波形蛋白表达水平明显升高,cleaved PARP和p21蛋白水平明显降低(P<0.01),表明细胞凋亡被抑制;与miR-96-5p组比较,miR-96-5p+FOXO1组侵袭细胞数明显减少(P<0.01),cyclin D1



A: FOXO1 3′UTR contains a nucleotide sequence complementary to miR-96-5p; B. Dual-luciferase reporter assay; C: qRT-PCR detection of the expression level of miR-96-5p in each group; D: qRT- PCR detection of *FOXO1* mRNA expression in each group; E: Western blot detection of FOXO1 protein expression in each group. [1] $P<0.05$, [2] $P<0.01$ compared with the mimic NC group; [3] $P<0.05$, [4] $P<0.01$ compared with the inhibitor NC group.

图4　miR-96-5p靶向调控FOXO1的表达

Fig. 4　miR-96-5p targeted the expression of FOXO1



A: CCK-8 detection of cell viability in each group; B: Transwell detection of cell invasion ability (×400); C: Statistics of the number of invasive cells in each group; D: Western blot was used to detect the expression of Cyclin D1, cleaved PARP, p21 and Vimentin. [1] $P<0.05$, [2] $P<0.01$ compared with the control group; [3] $P<0.05$, [4] $P<0.01$ compared with the miR-96-5p group.

图5　miR-96-5p通过调控FOXO1表达对Ishikawa细胞活力和侵袭的影响

Fig. 5　Effects of miR-96-5p on Ishikawa cell viability and invasion by regulating FOXO1 expression

和波形蛋白表达水平明显降低，cleaved PARP和p21蛋白表达水平明显升高（$P<0.05$，$P<0.01$），表明细胞凋亡被诱导。

3　讨 论

由于高复发、高转移、预后差及5年生存率低，

子宫内膜癌的临床治疗已越来越受到关注[3]。叉头蛋白家族成员FOXO1转录因子是PI3K/Akt信号通路调控细胞存活的一个关键底物,在细胞的增殖、发育、凋亡和代谢的过程中发挥重要作用[12]。FOXO1参与了包括子宫内膜癌在内的等多种癌症的发生发展,与癌症的进展、侵袭与转移密切相关[13-14]。MiR-96-3p在多种肿瘤中发挥癌基因作用,Qin等[15]研究表明miR-96-5p可通过激活MEK/ERK信号传导促进乳腺癌的迁移。Wang等[16]研究表明miR-96-5p可通过直接靶向FOXF2促进口腔癌细胞的增殖和侵袭。

本研究结果表明子宫内膜癌组织中miR-96-5p明显上调,FOXO1明显下调,且在子宫内膜癌中miR-96-5p高表达或FOXO1低表达与肿瘤病理分级和临床分期具有相关性,与患者年龄和是否绝经无明显相关性。抑癌基因p21通过停滞细胞周期,阻断细胞分裂,有利于基因组的损伤后修复[17]。Cleaved PARP为Caspase家族凋亡基因启动子,蛋白表达增加时说明细胞凋亡增加[18]。波形蛋白是间质标志物,在癌症转移等过程中发挥了重要作用[19]。过表达FOXO1或抑制miR-96-5p表达均明显抑制Ishikawa细胞的活力、侵袭及Cyclin D1和波形蛋白表达,明显促进cleaved PARP和p21蛋白表达,从而诱导细胞凋亡。上述结果揭示miR-96-5p在子宫内膜癌中发挥促癌作用,而FOXO1起着抑癌作用。

一系列miRNA(miR-9、miR-27、miR-96、miR-153、miR-182、miR-183)明显降低了HEC-1B细胞中FOXO1的丰度,并促进子宫内膜的肿瘤发生[20]。此外,miR-135通过下调FOXO1明显刺激了Ishikawa细胞的增殖[21]。本研究结果显示,miR-96-5p直接靶向负调控FOXO1。miR-96-5p过表达对Ishikawa细胞活力和侵袭的促进作用可被过表达FOXO1所逆转。因此,miR-96-5p通过下调FOXO1的表达水平在子宫内膜癌中发挥促癌作用。

综上所述,miR-96-5p在子宫内膜癌中表达上调,FOXO1表达下调,并与子宫内膜癌的临床分期和病理分级有关。miR-96-5p通过靶向负调控FOXO1的表达促进子宫内膜癌细胞的存活和侵袭,表明miR-96-5p可能成为治疗子宫内膜癌的有效靶点。

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