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circ-PHC3靶向miR-628-5p对宫颈癌细胞增殖及凋亡的影响

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摘要 该文旨在探讨环状RNA PHC3(circ-PHC3)靶向miR-628-5p对宫颈癌细胞增殖及凋亡的影响。将宫颈癌细胞SiHa分为干扰对照组、干扰PHC3组、过表达对照组、过表达miR-628-5p组、干扰PHC3+抑制对照组、干扰PHC3+抑制miR-628-5p组, 并转染相应质粒。利用qRT-PCR检测circ-PHC3和miR-628-5p表达水平, CCK-8法检测细胞增殖, 流式检测细胞凋亡, Transwell测定细胞迁移, Western blot检测B细胞淋巴瘤-2(Bcl-2)和Bcl-2相关X蛋白(BAX)表达情况, 双荧光素酶报告基因分析circ-PHC3与miR-628-5p的靶向关系。与人正常宫颈上皮细胞HUCEC比较, 宫颈癌细胞系HeLa、C33A、SiHa、ms751中circ-PHC3表达水平升高, miR-628-5p表达水平降低(P<0.05); 与干扰对照组比较, 干扰PHC3组SiHa细胞miR-628-5p水平升高, 增殖和迁移能力、Bcl-2表达水平降低, 凋亡率、BAX表达水平升高(P<0.05); 与过表达对照组比较, 过表达miR-628-5p组SiHa细胞增殖和迁移能力、Bcl-2表达水平降低, 凋亡率、BAX表达水平升高(P<0.05); 与干扰PHC3+抑制对照组相比, 干扰PHC3+抑制miR-628-5p组SiHa细胞miR-628-5p水平降低, 增殖和迁移能力、Bcl-2表达水平升高, 凋亡率、BAX表达水平降低(P<0.05); 报告基因分析证明circ-PHC3与miR-628-5p之间存在靶向关系(P<0.05)。circ-PHC3通过竞争性结合下调miR-628-5p表达促进宫颈癌细胞增殖, 并抑制宫颈癌细胞凋亡。

关键词 circ-PHC3; miR-628-5p; 宫颈癌细胞; 增殖及凋亡

Impacts of circ-PHC3 on the Proliferation and Apoptosis of Cervical Cancer Cells by Targeting miR-628-5p

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Abstract This article aims to investigate the effect of miR-628-5p targeted by circ-PHC3 (circular RNA PHC3) on the proliferation and apoptosis of cervical cancer cells. Cervical cancer cells SiHa were grouped into interference control group, interference PHC3 group, overexpression control group, overexpression miR-628-5p group, interference PHC3+inhibition control group, interference PHC3+inhibition miR-628-5p group, and transfected with corresponding plasmids. qRT-PCR was performed to measure the circ-PHC3 and miR-628-5p. CCK-8 method was used to measure cell proliferation. Flow cytometry was performed to detect cell apoptosis. Transwell method was performed to measure cell migration. Western blot was used to detect the Bcl-2 (B-cell lymphoma-2) and BAX (Bcl-2 associated X protein). Dual luciferase reporter gene was performed to explore the targeting relationship between circ-PHC3 and miR-628-5p. Compared with normal human cervical epithelial cells HUCEC, the

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circ-PHC3 increased and the miR-628-5p decreased in cervical cancer cell lines HeLa, C33A, SiHa, and ms751 (P<0.05). Compared with the interference control group, the miR-628-5p in SiHa cells in the interference PHC3 group increased, the proliferation and migration abilities, and Bcl-2 expression level decreased, and the apoptosis rate and BAX expression level increased (P<0.05). Compared with the overexpression control group, the proliferation and migration abilities, and Bcl-2 expression level in the miR-628-5p overexpression group decreased, and the apoptosis rate and BAX expression level increased (P<0.05). Compared with the interference PHC3+inhibition control group, the miR-628-5p in SiHa cells in the interference PHC3+inhibition miR-628-5p group decreased, the proliferation and migration abilities, and Bcl-2 expression level increased, and the apoptosis rate and BAX expression level decreased (P<0.05). Gene analysis reported that there was a targeted relationship between circ-PHC3 and miR-628-5p (P<0.05). Circ-PHC3 promotes proliferation of cervical cancer cells and inhibits apoptosis of cervical cancer cells by competitively binding and downregulating miR-628-5p.

Keywords circ-PHC3; miR-628-5p; cervical cancer cells; proliferation and apoptosis

宫颈癌与人乳头瘤病毒(human papillomavirus, HPV)相关, 是全球女性中第四大最常见的恶性肿瘤, 由于其病因明确, 因此可以预防和控制[1]。宫颈癌筛查方法已从细胞学方法演变为高度敏感的基于HPV的分子方法[2]。在复发性和转移性宫颈癌治疗中, 化疗联合免疫治疗或靶向治疗已经有所突破, 但仍然存在局限性[3]。环状RNA(circular RNA, circRNA)通过与微小RNA(microRNA, miRNA)及癌基因相关mRNA的相互调节参与宫颈癌的发生和发展, 多种circRNA在宫颈癌中表达异常, 与宫颈癌患者的临床病理特征有关[4-5]。在宫颈癌HeLa细胞中, 缺氧可增加多同源同系物3(polyhomologous homologues 3, PHC3) mRNA和circRNA表达水平, 表明*PHC3* mRNA和circ-PHC3在肿瘤起始中的协同作用[6]。在与遗传和表观遗传改变相关的癌症中, miRNA水平可以上调或下调[7]。在高危HPV的DNA整合位点发现许多miRNA位点, 表明高危HPV可能调节宿主细胞中的miRNA表达, miRNA在宫颈癌细胞的增殖、转移、凋亡、上皮–间充质转化和血管生成中发挥重要作用[8]。已有研究表明, miR-628-5p通过靶向血管内皮生长因子(vascular endothelial growth factor, VEGF)抑制宫颈癌细胞增殖并促进细胞凋亡[9]。使用starbase网站分析发现, circ-PHC3与miR-628-5p具有互补结合序列。在人卵巢癌细胞系和组织中circ-PHC3显著上调, circ-PHC3通过调节miR-497-5p/SRY-box转录因子9通路促进卵巢癌进展[10]。由于卵巢癌与宫颈癌同属于女性常见癌症, 因此, 本研究通过检测宫颈癌细胞系中circ-PHC3表达情况, 探究circ-PHC3靶向miR-628-5p对宫颈癌细胞增殖及凋亡的影响。

1 材料与方法

1.1 细胞来源

人宫颈癌细胞系HeLa(货号: STM-CL-5112)、C33A(货号: STM-CL-5088)、SiHa(货号: STM-CL-5047)、ms751(货号: STM-CL-5237)购自思泰默(上海)生物科技有限公司; 人正常宫颈上皮细胞HUCEC(YS1079C)购自上海雅吉生物科技有限公司。HeLa、C33A、SiHa、ms751所用培养基为含NEAA+10% FBS+1% P/S的MEM生长培养基; HUCEC采用角质细胞完全培养基培养, 培养箱条件设置为气相: 95%空气+5%二氧化碳。温度: 37 °C。

1.2 主要试剂

si-circ-PHC3、si-NC、mimic NC、inhibitor NC、miR-628-5p mimic、miR-628-5p inhibitor质粒购自广州锐博生物技术有限公司; 角质细胞完全培养基(货号: C120JV)购自上海源培生物科技股份有限公司; 蛋白质定量试剂盒(货号: KTD3001)、双荧光素酶报告基因检测试剂盒(货号: KTA8010)、增强型ECL发光液(货号: BMU101)购自亚科因生物技术有限公司; Lipofectamine 3000(货号: L3000015)购自美国ThermoFisher Scientific公司; miRNA反转录试剂盒(货号: ME0018)、miRNA荧光定量PCR试剂盒(货号: ME0020)购自北京百奥莱博科技有限公司; Annexin V-FITC/PI细胞凋亡检测试剂盒(货号: MA0220)购自大连美仑生物技术有限公司; Hieff UNICON® Universal Blue qPCR SYBR Green Master Mix(货号: 11184ES25)、Hifair® III 1st Strand

cDNA Synthesis Kit(货号: 11139ES60)购自翌圣生物科技(上海)股份有限公司; RIPA裂解液(货号: PC102)购自上海雅酶生物科技有限公司; B细胞淋巴瘤-2(Bcl-2)兔单抗(货号: ab32124)、Bcl-2相关X蛋白(BAX)兔单抗(货号: ab32503)、GAPDH兔单抗(货号: ab9485)、HRP标记的羊抗兔IgG抗体(货号: ab6721)购自英国Abcam公司。

1.3 方法

1.3.1 qRT-PCR检测各细胞系中circ-PHC3和miR-628-5p的表达情况 使用TRIzol试剂从各细胞系中提取总RNA。使用miRNA反转录试剂盒和Hifair® III 1st Strand cDNA Synthesis Kit合成和扩增cDNA, 并使用miRNA荧光定量PCR试剂盒和Hieff UNICON® Universal Blue qPCR SYBR Green Master Mix进行qPCR。以*GAPDH*和*U6*作为内参, 通过$2^{-\Delta\Delta Ct}$法计算基因相对表达水平。所用引物由百力格生物科技(上海)股份有限公司合成, 序列如下: miR-628-5p正向5′-GCC GAG ATG CTG ACA TAT TTA C-3′, 反向5′-CTC AAC TGG TGT CGT GGA-3′; *U6*正向5′-TCA TCA GAA ACA GTG GAG GT-3′, 反向5′-CAT CCT TAC ACA GGA GCC AT-3′; circ-PHC3正向5′-GGC TGC TGT ACA GTC-3′, 反向5′-GTG AGG TGG TGG TGG TG-3′; *GAPDH*正向5′-AAT GGG CAG CCG TTA GGA AA-3′, 反向5′-TGA AGG GGT CAT TGA TGG CA-3′。

1.3.2 细胞转染与分组 将SiHa细胞培养至80%~90%融合时, 将其分为对照组(不转染质粒)、干扰对照组(转染si-NC)、干扰PHC3组(转染si-circ-PHC3)、过表达对照组(转染mimic NC)、过表达miR-628-5p组(转染miR-628-5p mimic)、干扰PHC3+抑制对照组(si-circ-PHC3和inhibitor NC共转染)、干扰PHC3+抑制miR-628-5p组(si-circ-PHC3与miR-628-5p inhibitor共转染), 使用Lipofectamine 3000进行质粒转染, 转染48 h后, 使用qRT-PCR检测circ-PHC3和miR-628-5p表达情况, 以判断转染是否成功, 并进行后续相关实验。

1.3.3 CCK-8法检测细胞增殖情况 将分组转染的SiHa细胞铺在96孔板(2×10^4/孔)中, 并于每组培养24、48和72 h时, 添加10 μL CCK-8试剂培养1 h, 使用酶标仪在450 nm波长处测量吸光度(*D*)值。

1.3.4 流式细胞术检测细胞凋亡 转染后48 h, 收集SiHa细胞, 采用预冷的PBS冲洗细胞。利用AnnexinV-FITC/PI细胞凋亡检测试剂盒进行AnnexinV/PI双染色后, 用流式细胞术分析细胞凋亡情况。

1.3.5 Transwell迁移测定 将200 μL无血清培养基(5×10^4个处理细胞)转移至上室, 并将600 μL完全培养基加入下室。37 °C孵育24 h后, 在倒置光学显微镜下获得图像, 统计随机5个视野的迁移细胞数。

1.3.6 Western blot分析 使用RIPA裂解液从转染48 h后的细胞中提取总蛋白。使用BCA法测定蛋白质浓度。在4 °C下12 000 r/min离心15 min后, 用10% SDS-PAGE分离总蛋白, 并将其转移到聚偏二氟乙烯膜上, 在室温下用5%脱脂牛奶封闭1 h, 然后与Bcl-2、BAX、GAPDH一抗(1:1 000)在4 °C下孵育24 h, 将HRP偶联的二抗(1:5 000)与膜在室温下孵育1 h, 使用ECL发光液显色蛋白质条带, 并进行分析和定量, 以GAPDH为内参蛋白。

1.3.7 双荧光素酶报告基因分析 将circ-PHC3野生型(wild type, WT)、突变型(mutant, MUT)质粒分别与mimic NC、miR-628-5p mimic质粒共转染SiHa细胞, 48 h后, 用200 μL裂解液室温裂解细胞10 min, 加入荧光素酶检测试剂, 化学发光仪进行报告基因分析。

1.4 统计学分析

计量资料用($\bar{x}\pm s$)表示, 采用Graphpad Prism 7.0软件进行统计分析, 两组间比较采用*t*检验, 多组间比较采用单因素方差分析和Tukey's多重检验。$P<0.05$表示差异有统计学意义。

2 结果

2.1 各细胞系中circ-PHC3和miR-628-5p的表达水平

与人正常宫颈上皮细胞HUCEC比较, 宫颈癌HeLa、C33A、SiHa、ms751细胞系中circ-PHC3表达水平升高, miR-628-5p表达水平降低($P<0.05$); SiHa细胞差异最显著, 后续选择SiHa细胞进行实验, 见表1。

2.2 干扰PHC3对各组SiHa细胞circ-PHC3和miR-628-5p表达水平的影响

与干扰对照组比较, 干扰PHC3组SiHa细胞中circ-PHC3表达水平降低, miR-628-5p表达水平升高($P<0.05$); 与过表达对照组比较, 过表达miR-628-5p组SiHa细胞中miR-628-5p表达水平升高($P<0.05$); 与干扰PHC3+抑制对照组比较, 干扰PHC3+抑制miR-628-5p组SiHa细胞中miR-628-5p表达水平降低($P<0.05$), 见表2。

表1 各细胞系中circ-PHC3和miR-628-5p的表达水平比较

Table 1 Comparison of expression levels of circ-PHC3 and miR-628-5p in various cell lines

细胞 Cell	circ-PHC3	miR-628-5p
HUCEC	1.05±0.11	1.07±0.12
HeLa	1.96±0.24*	0.58±0.08*
C33A	2.43±0.27*	0.37±0.06*
ms751	2.93±0.32*	0.29±0.05*
SiHa	3.56±0.41*	0.24±0.04*

*P<0.05, 与HUCEC细胞相比。n=6。

*P<0.05 compared with HUCEC cells. n=6.

表2 SiHa细胞中circ-PHC3、miR-628-5p表达比较

Table 2 Comparison of circ-PHC3 and miR-628-5p expression in SiHa cells

分组 Groups	circ-PHC3	miR-628-5p
Interference control group	1.03±0.11	1.06±0.11
Interference PHC3 group	0.33±0.05*	2.85±0.34*
Overexpression control group	1.04±0.11	1.08±0.12
Overexpression miR-628-5p group	0.98±0.12	3.24±0.39$^{\#}$
Interference PHC3+inhibition control group	0.35±0.06	2.79±0.31
Interference PHC3+inhibition miR-628-5p group	0.34±0.05	1.04±0.13$^{\&}$
F	105.114	97.872
P	0.000	0.000

*P<0.05, 与干扰对照组相比; $^{\#}P$<0.05, 与过表达对照组相比; $^{\&}P$<0.05, 与干扰PHC3+抑制对照组相比。n=6。

*P<0.05 compared with the interference control group; $^{\#}P$<0.05 compared with the overexpression control group; $^{\&}P$<0.05 compared with the interference PHC3+inhibition control group. n=6.

2.3 干扰PHC3对各组SiHa细胞增殖的影响

与干扰对照组比较, 干扰PHC3组SiHa细胞D_{450}值降低(P<0.05); 与过表达对照组比较, 过表达miR-628-5p组SiHa细胞D_{450}值降低(P<0.05); 与干扰PHC3+抑制对照组比较, 干扰PHC3+抑制miR-628-5p组SiHa细胞D_{450}值升高(P<0.05), 见表3。

2.4 干扰PHC3对各组SiHa细胞凋亡的影响

与干扰对照组比较, 干扰PHC3组SiHa细胞凋亡率升高(P<0.05); 与过表达对照组比较, 过表达miR-628-5p组SiHa细胞凋亡率升高(P<0.05); 与干扰PHC3+抑制对照组比较, 干扰PHC3+抑制miR-628-5p组SiHa细胞凋亡率降低(P<0.05), 见图1与表4。

2.5 干扰PHC3对各组SiHa细胞迁移的影响

与干扰对照组比较, 干扰PHC3组SiHa迁移细胞数减少(P<0.05); 与过表达对照组比较, 过表达miR-628-5p组SiHa迁移细胞数减少(P<0.05); 与干扰PHC3+抑制对照组比较, 干扰PHC3+抑制miR-628-5p组SiHa迁移细胞数增加(P<0.05), 见图2与表5。

2.6 干扰PHC3对各组SiHa细胞中Bcl-2和BAX蛋白表达的影响

与干扰对照组比较, 干扰PHC3组SiHa细胞中Bcl-2表达水平降低, BAX表达水平升高(P<0.05); 与过表达对照组比较, 过表达miR-628-5p组SiHa细胞中Bcl-2表达水平降低, BAX表达水平升高(P<0.05); 与干扰PHC3+抑制对照组比较, 干扰PHC3+抑制miR-628-5p组SiHa细胞中Bcl-2表达水平升高, BAX表达水平降低(P<0.05), 见图3和表6。

2.7 circ-PHC3与miR-628-5p靶向关系

Starbase分析显示circ-PHC3与miR-628-5p能够互补结合, 见图4。报告基因分析得出, 与circ-PHC3 WT和mimic NC质粒共转染比较, circ-PHC3 WT和miR-628-5p mimic质粒共转染SiHa细胞后, 荧光素酶活性显著降低(P<0.05), 见表7。

3 讨论

超过90%的宫颈癌病例与高危HPV感染有关, 其中HPV-16和HPV-18最为危险[11]。高危HPV的致

表3 各组SiHa细胞增殖比较

Table 3 Comparison of SiHa cell proliferation in each group

分组 Groups	24 h D_{450}值 24 h D_{450} value	48 h D_{450}值 48 h D_{450} value	72 h D_{450}值 72 h D_{450} value
Interference control group	0.42±0.05	0.75±0.09	1.47±0.18
Interference PHC3 group	0.21±0.03*	0.38±0.06*	0.89±0.12*
Overexpression control group	0.43±0.06	0.74±0.08	1.49±0.19
Overexpression miR-628-5p group	0.19±0.04$^{\#}$	0.37±0.05$^{\#}$	0.84±0.11$^{\#}$
Interference PHC3+inhibition control group	0.20±0.03	0.39±0.06	0.91±0.14
Interference PHC3+inhibition miR-628-5p group	0.44±0.06$^{\&}$	0.76±0.09$^{\&}$	1.48±0.17$^{\&}$
F	43.832	45.864	27.235
P	0.000	0.000	0.000

*P<0.05, 与干扰对照组相比; $^{\#}P$<0.05, 与过表达对照组相比; $^{\&}P$<0.05, 与干扰PHC3+抑制对照组相比。n=6。

*P<0.05 compared with the interference control group; $^{\#}P$<0.05 compared with the overexpression control group; $^{\&}P$<0.05 compared with the interference PHC3+inhibition control group. n=6.

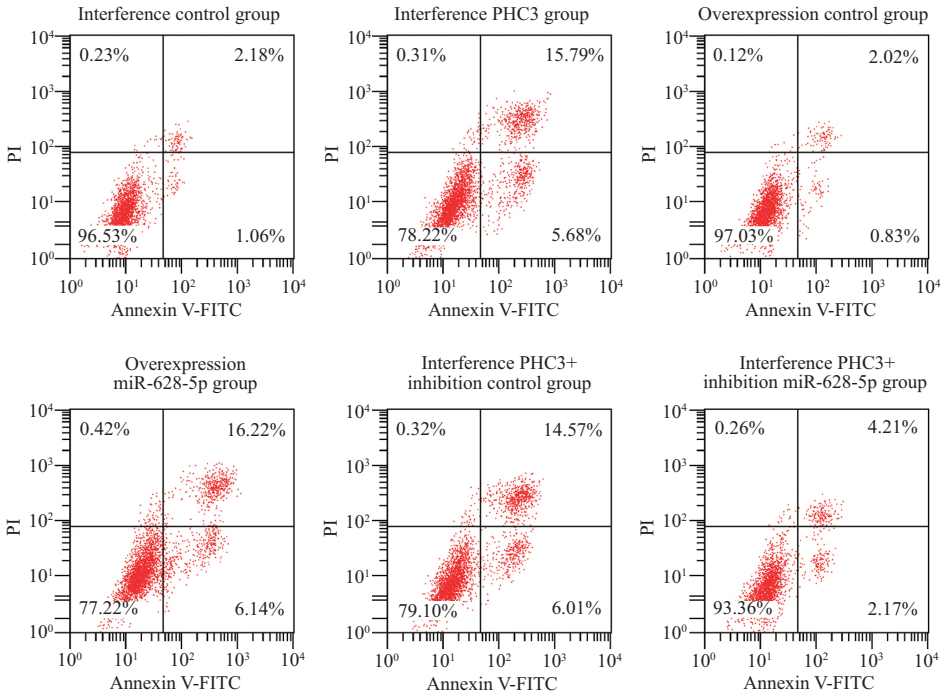


图1 流式细胞术分析各组SiHa细胞凋亡率

Fig.1 Analysis of apoptosis rate of SiHa cells in each group by flow cytometry

癌机制为其基因组整合到宿主基因组后, 破坏*E2*基因, 导致E6和E7癌蛋白过表达, 引发细胞转化。随着宿主染色体异常、点突变和基因甲基化模式改变等遗传变化不断积累, 宫颈上皮内瘤变可进展为宫颈癌[12]。

PHC3属于PcG蛋白家族; PcG蛋白是一类通过调控表观遗传以沉默基因表达的关键转录抑制因子, 在软组织发育、干细胞多能性维持、衰老及癌症等进程中发挥重要作用[13]。PcG复合物通过核心蛋白与PHC等辅因子的动态互作, 实现对靶基因激活或抑制的双重调控[14]。研究表明, 萝卜硫素通过调节音猬因子(sonic hedgehog, SHH)信号通路和PHC3抑制肺癌干细胞的自我更新[15]。本研究发现, 与人正常宫颈上皮细胞HUCEC比较, 宫颈癌细胞系HeLa、C33A、SiHa、ms751中circ-PHC3表达水平升高, 与前人研究[6]结果一致, 提示circ-PHC3可能作为宫颈癌的生物标志物。而干扰circ-PHC3能够抑制SiHa细胞增殖和迁移, 并促进SiHa细胞凋亡, 提示circ-PHC3可能作为宫颈癌的治疗靶点。

研究表明, circRNA可以与miRNA竞争性结

表4 各组SiHa细胞凋亡率比较

Table 4 Comparison of apoptosis rate of SiHa cells in each group

分组 Groups	凋亡率/% Apoptosis rate /%
Interference control group	3.24±0.54
Interference PHC3 group	21.47±2.56*
Overexpression control group	2.85±0.47
Overexpression miR-628-5p group	22.36±2.85#
Interference PHC3+inhibition control group	20.58±2.65
Interference PHC3+inhibition miR-628-5p group	6.38±0.92&
F	143.236
P	0.000

*P<0.05, 与干扰对照组相比; #P<0.05, 与过表达对照组相比; &P<0.05, 与干扰PHC3+抑制对照组相比。n=6。

*P<0.05 compared with the interference control group; #P<0.05 compared with the overexpression control group; &P<0.05 compared with the interference PHC3+inhibition control group. n=6.

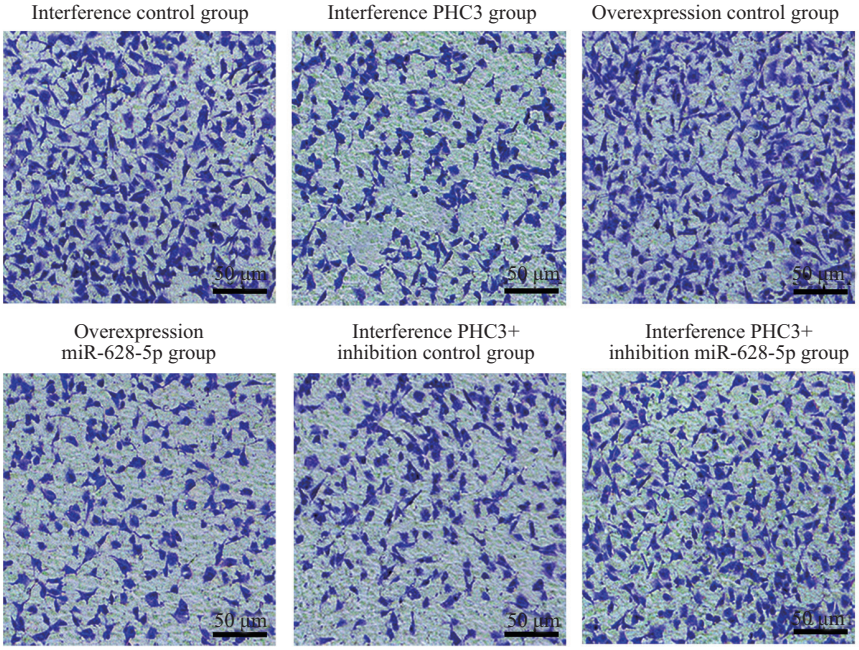


图2 各组SiHa迁移细胞数

Fig.2 Number of SiHa migrated cells in each group

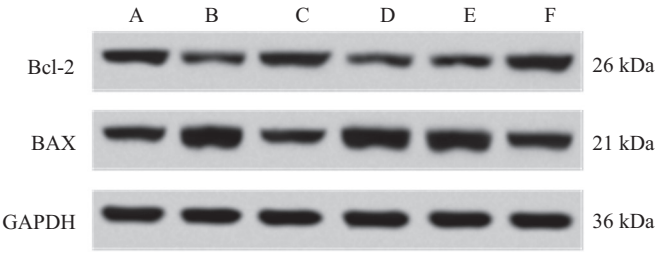
表5 各组SiHa迁移细胞数比较

Table 5 Comparison of the number of SiHa migrated cells in each group

分组 Groups	迁移细胞数 Number of migrated cells
Interference control group	135.38±17.63
Interference PHC3 group	83.94±12.48*
Overexpression control group	132.67±18.17
Overexpression miR-628-5p group	78.93±11.29#
Interference PHC3+inhibition control group	87.49±10.41
Interference PHC3+inhibition miR-628-5p group	130.26±18.43&
F	19.405
P	0.000

*P<0.05, 与干扰对照组相比; #P<0.05, 与过表达对照组相比; &P<0.05, 与干扰PHC3+抑制对照组相比。n=6。

*P<0.05 compared with the interference control group; #P<0.05 compared with the overexpression control group; &P<0.05 compared with the interference PHC3+inhibition control group. n=6.



A: 干扰对照组; B: 干扰PHC3组; C: 过表达对照组; D: 过表达miR-628-5p组; E: 干扰PHC3+抑制对照组; F: 干扰PHC3+抑制miR-628-5p组。
A: interference control group; B: interference PHC3 group; C: overexpression control group; D: overexpression miR-628-5p group; E: interference PHC3+inhibition control group; F: interference PHC3+inhibition miR-628-5p group.

图3 各组SiHa细胞中Bcl-2和BAX的蛋白表达条带

Fig.3 Protein expression bands of Bcl-2 and BAX in SiHa cells of each group

表6 各组SiHa细胞中Bcl-2和BAX蛋白表达水平比较

Table 6 Comparison of Bcl-2 and BAX protein expression levels in SiHa cells of each group

分组 Groups	Bcl-2	BAX
Interference control group	1.06±0.12	1.04±0.11
Interference PHC3 group	0.46±0.07*	2.32±0.27*
Overexpression control group	1.04±0.12	1.05±0.13
Overexpression miR-628-5p group	0.42±0.06$^{\#}$	2.46±0.29$^{\#}$
Interference PHC3+inhibition control group	0.49±0.08	2.21±0.28
Interference PHC3+inhibition miR-628-5p group	0.98±0.13$^{\&}$	1.13±0.16$^{\&}$
F	58.608	59.712
P	0.000	0.000

*P<0.05, 与干扰对照组相比; $^{\#}P$<0.05, 与过表达对照组相比; $^{\&}P$<0.05, 与干扰PHC3+抑制对照组相比。n=6。
*P<0.05 compared with the interference control group; $^{\#}P$<0.05 compared with the overexpression control group; $^{\&}P$<0.05 compared with the interference PHC3+inhibition control group. n=6.

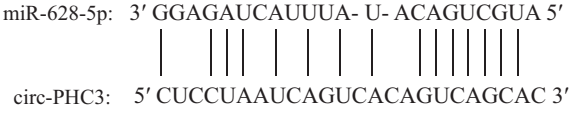


图4 circ-PHC3与miR-628-5p靶向结合位点预测

Fig.4 Prediction of target binding sites of circ-PHC3 and miR-628-5p

表7 双荧光素酶活性检测结果

Table 7 Double luciferase activity test results

分组 Groups	circ-PHC3 WT	circ-PHC3 MUT
mimic NC	1.04±0.11	1.03±0.11
miR-628-5p mimic	0.51±0.06	1.05±0.12
t	10.361	0.301
P	0.000	0.770

n=6.

合或与RNA结合蛋白结合, 通过竞争性内源性RNA(competing endogenous RNA, ceRNA)调节网络干扰宫颈癌成纤维细胞增殖, 影响癌症患者的预后[16]。长链非编码RNA天冬氨酰-tRNA合成酶反义RNA1通过miR-628-5p/锯齿状经典Notch配体1轴以激活Notch通路促进宫颈癌细胞增殖[17]。在宫颈癌ME-180细胞中, 柚皮苷上调miR-628-5p, 抑制其细胞增殖和周期进展, 并促进细胞凋亡[18]。另外, miR-628-5p在乳腺癌中差异表达, lncRNA-WFDC21P可通过与miR-628-5p竞争性结合上调SMAD家族成员3

基因表达, 从而促进三阴性乳腺癌增殖和迁移[19-20]。与上述研究一致的是, 本实验发现miR-628-5p在宫颈癌细胞中表达水平均降低, 过表达miR-628-5p后SiHa细胞的增殖和迁移被抑制, 凋亡率提高, 与干扰circ-PHC3结果类似。另外, 报告基因分析结果显示circ-PHC3与miR-628-5p具有靶向调节关系, 且干扰circ-PHC3后SiHa细胞中miR-628-5p水平升高。因此, 本研究在干扰circ-PHC3同时进一步抑制miR-628-5p表达, 结果发现, SiHa细胞的增殖和迁移能力得到恢复, 凋亡率也明显降低; 提示干扰circ-PHC3可能通过上调miR-628-5p抑制宫颈癌SiHa细胞增殖、迁移, 并促进细胞凋亡。既往研究显示, miR-93-3p[21]、miR-455-5p[22]、miR-497-5p[10]均为circ-PHC3的下游靶点, 但circ-PHC3是否通过上述靶点在宫颈癌细胞进展中发挥作用以及其与本文miR-628-5p的关系仍需进一步阐明。

综上所述, circ-PHC3通过竞争性结合下调miR-628-5p表达促进宫颈癌细胞增殖, 并抑制细胞凋亡。本研究证明circ-PHC3作为宫颈癌的治疗靶点具有潜在应用价值, 但不足之处在于仅使用一种宫颈癌细胞系进行研究且未进行体内实验验证, 后续研究需要对此进行补充。另外, 本研究目前只证实了circ-PHC3对miR-628-5p的靶向调控作用, 根据之前的研究, miR-628-5p的下游靶点或通路众多, 如Notch通路[17]、SMAD家族成员3[20]等, 未来将着重探讨miR-628-5p的下游分子机制。

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