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MiR-144-3p 靶向 SGK3 通过 Hippo 信号通路抑制 卵巢癌的生长和侵袭

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[摘要] 目的: 探讨miR-144-3p对卵巢癌生长和侵袭的作用及机制。方法: RT-qPCR检测正常卵巢上皮细胞和卵巢癌细胞miR-144-3p与SGK3 mRNA的表达, 双荧光素酶报告检测靶向关系, 将卵巢癌SKOV3细胞分为对照组(Control组)、模拟物对照组(mimic-NC组)、miR-144-3p高表达组(miR-144-3p mimic组)、SGK3高表达组(pc-SGK3组)、miR-144-3p和SGK3都高表达组(miR-144-3p+SGK3组), CCK-8法检测细胞增殖, 克隆形成实验检测细胞生长, 流式细胞术检测细胞周期和细胞凋亡, Transwell实验检测细胞侵袭, 蛋白质印迹分析检测p-MST, p-LATS, p-YAP, MST, LATS, YAP蛋白表达水平; 裸鼠后肢腹侧皮下注射SKOV3细胞悬液构建移植瘤, 每周检测移植瘤体积, 第30天颈椎脱位法处死裸鼠, 完整取出皮下肿瘤, 电子天平称重, 蛋白质印迹分析检测SGK3, p-MST, p-LATS, p-YAP, MST, LATS, YAP蛋白表达水平。结果: MiR-144-3p在卵巢癌细胞中低表达, 而SGK3高表达; miR-144-3p靶向负调控SGK3; miR-144-3p过表达能够明显减弱卵巢癌细胞增殖、减少每视野中卵巢癌细胞克隆数目, 减短细胞周期, 增加细胞凋亡率, 减少侵袭细胞数目、上调p-MST/MST, p-LATS/LATS, p-YAP/YAP蛋白表达($P<0.01$), 再加入SGK3高表达和Hippo信号通路抑制剂XMU-MP-1后均能逆转上述反应。结论: MiR-144-3p靶向SGK3通过Hippo信号通路抑制卵巢癌的生长和侵袭。

[关键词] miR-144-3p; SGK3; Hippo信号通路; 卵巢癌

MiR-144-3p inhibits the growth and invasion of ovarian cancer by targeting SGK3 via Hippo signaling pathway

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Abstract **Objective:** To investigate the effect and mechanism of miR-144-3p on the growth and invasion of ovarian cancer. **Methods:** The expression of miR-144-3p and SGK3 mRNA in normal ovarian epithelial cells and ovarian cancer cells was detected by RT-qPCR, and the targeting relationship was detected by dual luciferase assay. The ovarian

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cancer SKOV3 cells were divided into a control group, a mimic-NC group, a miR-144-3p mimic group, a pc-SGK3 group, and a miR-144-3p+ SGK3 group. Cell proliferation was detected by CCK-8 method, cell growth was detected by colony formation assay, cell cycle and apoptosis were detected by flow, cell invasion was detected by transwell assay, and the expression levels of p-MST, p-LATS, p-YAP, MST, LATS and YAP were detected by Western blotting. The transplanted tumor was constructed by subcutaneous injection of SKOV3 cell suspension into the hind limb of nude mice. The volume of the transplanted tumor was detected weekly. On the 30th day, the nude mice were sacrificed by cervical dislocation. The subcutaneous tumor was completely removed and the electronic balance was weighed. The expression levels of SGK3, p-MST, p-LATS, p-YAP, MST, LATS, and YAP proteins were detected by Western blotting. **Results:** MiR-144-3p was down-regulated in ovarian cancer cells, while SGK3 was highly expressed; miR-144-3p was targeted to negatively regulate SGK3; miR-144-3p overexpression significantly reduced ovarian cancer cell proliferation, reduced the number of ovarian cancer cell clones per field of vision, shortened the cell cycle, increased the rate of apoptosis, reduced the number of invading cells, and up-regulated p-MST/MST, p-LATS/LATS, and p-YAP/YAP protein expression ($P<0.01$), the addition of SGK3 high expression and Hippo signaling pathway inhibitor XMU-MP-1 reversed the above reaction. **Conclusion:** MiR-144-3p inhibits the growth and invasion of ovarian cancer by targeting SGK3 via Hippo signaling pathway.

Keywords miR-144-3p; SGK3; Hippo signaling pathway; ovarian cancer

卵巢癌是指生长在卵巢上的恶性肿瘤, 是女性中最常见和最致命的妇科恶性肿瘤之一^[1]。由于缺乏明显的症状和早期生物标志物, 大约75%的卵巢癌患者被诊断为晚期或转移期, 5年生存率仅为25%~30%^[2]。此外, 高复发率和耐药导致这些患者预后更差, 病死率更高^[3]。因此, 迫切需要寻找卵巢癌的特异性有效早期检测标志物。现已发现miRNA在各种人类癌症中均存在异常表达, 其作为致癌基因或肿瘤抑制因子调节着癌症的发展, miRNA已经成为癌症的新标志, 并成为癌症诊断和治疗的新希望^[4]。已有研究^[5]发现: 卵巢癌组织中miR-144-3p的表达水平显著低于正常卵巢组织和卵巢良性肿瘤组织, 与卵巢癌患者的肿瘤直径、病理分期、淋巴结转移情况及分化程度均有关, 可在一定程度上反映卵巢癌患者的病情严重程度。因此, miR-144-3p有望成为卵巢癌诊断和治疗的新靶点。但miR-144-3p对卵巢癌的作用机制尚不清楚, 本文对其进行深入探讨, 以期对卵巢癌的诊断和治疗提供新的参考数据。

1 材料与方法

1.1 材料

胎牛血清(货号: 16000-044)、青霉素和链霉素双抗溶液(货号: 15140122)购自上海索尔生物科技有限公司; DMEM培养基(货号: SH3002.01B)购

自上海善然生物科技有限公司; mimic-NC, miR-144-3p mimic, pc-SGK3质粒及各种引物购自上海生工生物工程股份有限公司; lipofectamine 2000转染试剂(货号: 11668-027)购自上海恪敏生物科技有限公司; SYBR-Green PCR试剂盒(货号: 4309155)购自赛默飞世尔科技公司; 双荧光素酶报告基因检测试剂盒(货号: GN201-01)购自北京原平皓生物技术有限公司; CCK-8试剂盒(货号: CT-K-5)购自上海炎熙生物科技有限公司; RIPA裂解缓冲液(货号: SBJ-0999)购自南京海克尔生物科技有限公司; BCA试剂盒(货号: BC201)购自上海易色医疗科技有限公司; 兔来源的血清和糖皮质激素调节蛋白激酶3(serum and glucocorticoid-regulated protein kinase 3, SGK3)、巯基丙酮酸硫转移酶(thiothiopyruvate transferase, MST)、p-MST、大肿瘤抑制因子(large tumor suppressor, LATS)、p-LATS、Yes相关蛋白(Yes-associated protein, YAP)、p-YAP单克隆一抗, 辣根过氧化物酶偶联的山羊抗兔二抗(货号: ab126108, ab85377, ab79199, ab70561, ab111344, ab205270, ab76252)购自上海艾博抗生物科技有限公司。

1.2 方法

1.2.1 细胞及培养

人卵巢癌细胞系(3AO, A2780, OVCAR3, SKOV3)和正常人卵巢表面上皮(HOSEpic)细胞购

自美国典型培养物保藏中心。用含有100 U/mL青霉素和100 $\mu\text{g}/\text{mL}$ 链霉素的体积分数为10%的牛血清DMEM培养基中,在37 $^{\circ}\text{C}$,5% CO_2 培养箱培养。

1.2.2 细胞转染

取对数期的卵巢癌SKOV3细胞,接种于6孔板(1×10^6 /孔)。当达到80%融合,根据Lipofectamine 2000说明书将100 nmol/L的miR-144-3p mimic质粒、miR-NG质粒、pc-SGK3质粒分别或联合转染进入SKOV3细胞。

1.2.3 RT-qPCR 检测 miR-144-3p 与 SGK3 mRNA 的表达

采用QIAzol裂解试剂提取总RNA,采用cDNA反转录试剂盒合成cDNA。RT-qPCR采用SYBR-Green PCR试剂盒说明书操作进行。U6作为内参,使用 $2^{-\Delta\Delta\text{Ct}}$ 方法计算。引物序列:miR-144-3p正向引物5'-CCCTACAGTATAGATGATG-3',反向引物5'-TGCAGGGTCCGAGGT-3';SGK3正向引物5'-CCGCTCGAGACCATGGCCCTGAAGATTC-3',反向引物5'-CGCGGATCCAAAATAAGTCTTCTG-3';U6正向引物5'-CTCGCTTCGGCAGCACACA-3',U6反向引物5'-AACGCTTCACGAATTTGCGT-3'。

1.2.4 双荧光素酶报告检测靶向关系

收集生长至对数期的SKOV3细胞,铺于96孔板,每孔约 4×10^3 个细胞,24 h后,分别转染mimic-NC+SGK3 WT, mimic-NC+SGK3 MUT, miR-144-3p mimic + SGK3 WT, miR-144-3p mimic + SGK3 MUT,根据双荧光素酶报告基因试剂盒说明进行测定,用萤火虫荧光素酶活性和肾荧光素酶活性比值表示荧光素酶的相对活性。

1.2.5 CCK-8 法检测细胞增殖

将细胞以每孔 2×10^3 的密度接种在96孔板中培养0,12,24,36或48 h。孵育后,每孔加入10 μL CCK-8溶液,孵育1 h,使用酶标仪在450 nm处检测每个样品的OD值,实验重复3次。

1.2.6 克隆形成实验检测细胞生长

将细胞以 5×10^3 每孔接种到6孔板中,37 $^{\circ}\text{C}$ 培养。培养基每2天更新1次。10 d后,用PBS洗涤细胞两次,并在4%多聚甲醛中固定30 min。用PBS缓冲液洗涤2次后,用1%结晶紫染色缓冲液将细胞染色30 min,用数码相机拍照。

1.2.7 流式细胞术检测细胞周期

收集各组3细胞并用70%乙醇固定30 min,然后用冰冷的PBS洗涤2次。将细胞离心分离,并在冰上用含RNase的PBS(稀释比例为1:100)重悬,然后用碘化丙锭染色,最后使用流式细胞仪FACS方法分析细胞周期进展。

1.2.8 流式细胞术检测细胞凋亡

培养48 h后,离心收集细胞,按 1×10^6 个/mL的细胞浓度重悬。细胞悬液中加入5 μL Annexin-V-FITC和5 μL PI,在黑暗的房间里孵化15 min后用流式细胞仪分析细胞凋亡情况。细胞凋亡率=早期细胞凋亡率+晚期细胞凋亡率。

1.2.9 Transwell 实验检测细胞侵袭

在Transwell小室上室接种SKOV3细胞悬浮液和不含血清的培养基,终浓度为 2.5×10^5 个/mL,并在Transwell小室下室加入含血清和丝裂霉素C的培养基,培养48 h后,取出Transwell小室,用结晶紫染色,并在显微镜下观察分析。

1.2.10 蛋白质印迹分析检测 SGK3, p-MST, p-LATS, p-YAP, MST, LATS, YAP 蛋白表达水平

在裂解缓冲液中提取总蛋白质,并用BCA测定试剂盒测量蛋白质浓度。将10 μg 蛋白样品用10% SDS-PAGE分离后转移至PVDF膜上。用5%脱脂奶粉封闭膜,然后加入单克隆一抗(SGK3 1:500, p-MST 1:500, p-LATS 1:500, p-YAP 1:10 000, MST 1:100, LATS 1:5 000, YAP 1:1 000)在4 $^{\circ}\text{C}$ 封闭过夜,接着加入对应辣根过氧化物酶偶联的二抗室温孵育1 h,然后再次用TBST洗涤3次。使用ECL系统检测结合的抗体。

1.2.11 移植瘤实验

将裸鼠随机分为control组和miR-144-3p mimic组,每组16只;在两组裸鼠后肢腹侧皮下分别注射0.2 mL的 1×10^7 个/mL SKOV3细胞和转染miR-144-3p mimic的SKOV3细胞悬液;接着在SPF条件下正常饮食饲养。每周检测移植瘤体积,第30天颈椎脱位法处死裸鼠,完整取出皮下肿瘤,电子天平称重,蛋白质印迹分析检测SGK3, p-MST, p-LATS, p-YAP, MST, LATS, YAP蛋白表达水平。

1.3 统计学处理

采用SPSS 21.0统计软件进行数据分析。图形使用GraphPad Prism 6.0构建的。测量数据以均数 \pm 标准差($\bar{x} \pm s$)表示,多组比较使用单因素方差分析,两组比较使用 t 检验, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 MiR-144-3p 在卵巢癌细胞中低表达,而 SGK3 高表达

通过RT-qPCR检测人卵巢癌细胞系(3AO, A2780, OVCAR3, SKOV3)和正常人卵巢表面上

皮(HOSEpiC)细胞中miR-144-3p与SGK3 mRNA的表达可知, 与正常人卵巢表面上皮HOSEpiC细胞相比, 人卵巢癌细胞系3AO, A2780, OVCAR3, SKOV3中miR-144-3p mRNA均明显下调, SGK3 mRNA均明显上调($P<0.01$, 图1), 选择人卵巢癌细胞系SKOV3做后续实验。

2.2 MiR-144-3p 靶向负调控 SGK3

根据TargetScan数据库的预测, miR-144-3p与SGK3 3'-UTR区存在结合位点(图2A)。为进一步验证miR-144-3p直接作用于SGK3, 荧光素酶报告基因实验显示: miR-144-3p高表达明显抑制了含有野生型SGK3质粒的荧光素酶活性, 但对突变型SGK3质粒的荧光素酶活性无影响($P<0.01$, 图2B)。为进一步验证miR-144-3p高表达对SGK3的作用, 采用RT-qPCR检测SGK3 mRNA的表达, 蛋白质印迹法检测SGK3蛋白的表达。与mimic-NC组细胞相比, miR-144-3p mimic组细胞中SGK3 mRNA和蛋白表达均明显下调($P<0.01$, 图2C, 2D)。因此, miR-144-3p直接靶向负调控SGK3。

2.3 MiR-144-3p 靶向 SGK3 抑制卵巢癌的生长

与control组相比, mimic-NC组卵巢癌细胞生长情况并无明显变化, miR-144-3p mimic组卵巢癌细胞增殖明显减弱、细胞克隆数目明显减少、 G_0/G_1 期明显增长、细胞凋亡率明显增加($P<0.01$), pc-SGK3组卵巢癌细胞增殖明显增强、细胞克隆数目明显增多、 G_0/G_1 期明显减短、细胞凋亡率明显

减少($P<0.01$); 与miR-144-3p mimic组相比, miR-144-3p+SGK3组卵巢癌细胞增殖明显减弱、细胞克隆数目明显增多、 G_0/G_1 期明显减短、细胞凋亡率明显减少($P<0.01$, 图3, 图4)。

2.4 MiR-144-3p 靶向 SGK3 抑制卵巢癌的侵袭

通过Transwell实验检测卵巢癌细胞侵袭情况(图5), 与control组相比, mimic-NC组每视野中卵巢癌细胞侵袭数目无明显变化, miR-144-3p mimic组每视野中卵巢癌细胞侵袭数目明显减少($P<0.01$), pc-SGK3组每视野中卵巢癌细胞侵袭数目明显增多($P<0.01$); 与miR-144-3p mimic组相比, miR-144-3p+SGK3组每视野中卵巢癌细胞侵袭数目明显减少($P<0.01$)。

2.5 MiR-144-3p 靶向 SGK3 激活 Hippo 信号通路

蛋白质印迹分析检测Hippo信号通路蛋白p-MST/MST, p-LATS/LATS, p-YAP/YAP蛋白表达水平(图6), 结果显示: 与control组相比, mimic-NC组卵巢癌细胞p-MST/MST, p-LATS/LATS, p-YAP/YAP蛋白表达无明显变化, miR-144-3p mimic组卵巢癌细胞p-MST/MST, p-LATS/LATS, p-YAP/YAP蛋白表达明显上调($P<0.01$), pc-SGK3组卵巢癌细胞p-MST/MST、p-LATS/LATS, p-YAP/YAP蛋白表达明显下调($P<0.01$); 与miR-144-3p mimic组相比, miR-144-3p+SGK3组卵巢癌细胞p-MST/MST, p-LATS/LATS, p-YAP/YAP蛋白表达明显下调($P<0.01$)。

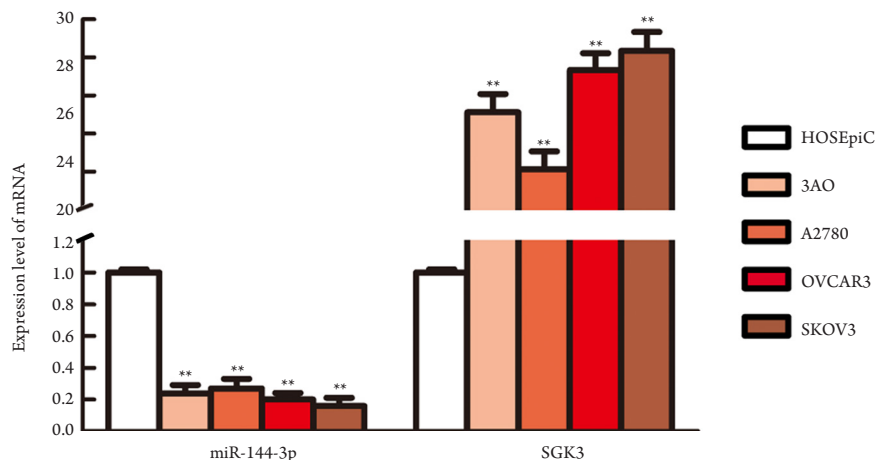


图1 RT-qPCR检测miR-144-3p与SGK3 mRNA的表达

Figure 1 Expression of miR-144-3p and SGK3 mRNA was detected by RT-qPCR

与正常卵巢细胞HOSEpiC相比, $**P<0.01$, $n=9$ 。

Compared with ovarian cell HOSEpiC cells, $**P<0.01$, $n=9$.

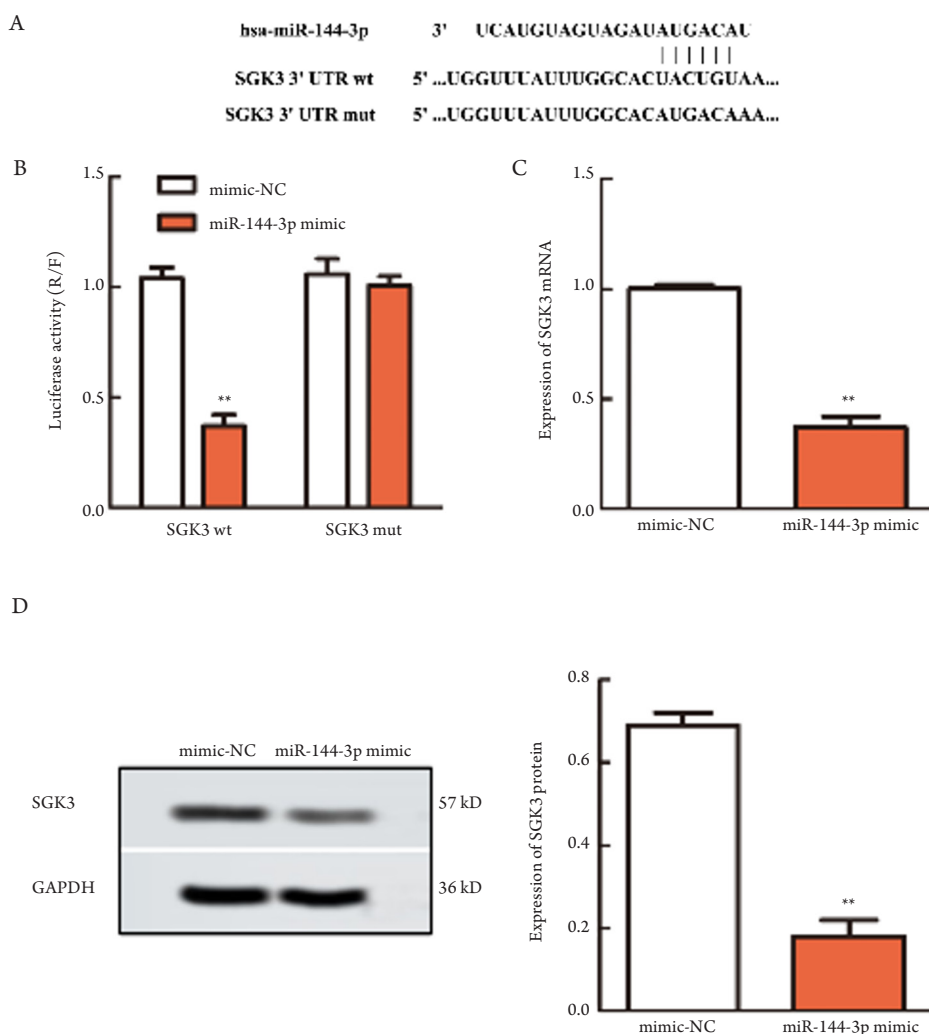


图2 MiR-144-3p直接靶向负调控SGK3

Figure 2 MiR-144-3p directly targets negative regulation of SGK3

(A) TargetScan数据库预测miR-144-3p与SGK3 3'-UTR区存在结合位点; (B) 双荧光素酶报告基因实验检测miR-144-3p与SGK3靶向关系; (C) RT-qPCR检测SGK3 mRNA的表达; (D) 蛋白质印迹法检测SGK3蛋白的表达。与mimic-NC组相比, ** $P < 0.01$ 。 $n = 9$ 。

(A) Binding site of miR-144-3p and SGK3 3'-UTR region is predicted by TargetScan database; (B) Relationship between miR-144-3p and SGK3 is detected by dual luciferase reporter gene assay; (C) Expression of SGK3 mRNA was detected by RT-qPCR; (D) Expression of SGK3 protein was detected by Western blotting. ** $P < 0.01$ versus the mimic-NC group. $n = 9$.

2.6 MiR-144-3p 通过激活 Hippo 信号通路抑制卵巢癌细胞生长和侵袭

为研究Hippo信号通路对卵巢癌细胞生长和侵袭的作用, 用100 nmol/L的Hippo信号通路抑制剂XMU-MP-1处理卵巢癌细胞。与control组相比, XMU-MP-1组卵巢癌细胞增殖情况明显增强, 每视野克隆细胞数目增加, G_0/G_1 期明显缩短, 细胞凋亡率明显减少以及侵袭细胞数目均增多($P < 0.01$, 图7); 与miR-144-3p mimic组相比, XMU-MP-1+miR-144-3p组卵巢癌细胞增殖情况明显增强, 每视野克隆细胞数目减少, G_0/G_1 期明显

缩短, 细胞凋亡率明显减少和侵袭细胞数目均增多($P < 0.01$)。

2.7 MiR-144-3p 抑制卵巢癌 SKOV3 移植瘤的发展

采用卵巢癌移植瘤实验研究miR-144-3p对卵巢癌发生发展的影响, 结果表明: 与control组相比, miR-144-3p mimic组大鼠体内移植瘤的重量和体积明显减小($P < 0.01$; 图8A, 8B, 8C), miR-144-3p mimic组大鼠体内移植瘤SGK3蛋白表达明显下调($P < 0.01$); p-MST/MST, p-LATS/LATS, p-YAP/YAP蛋白表达明显上调($P < 0.01$, 图8D)。

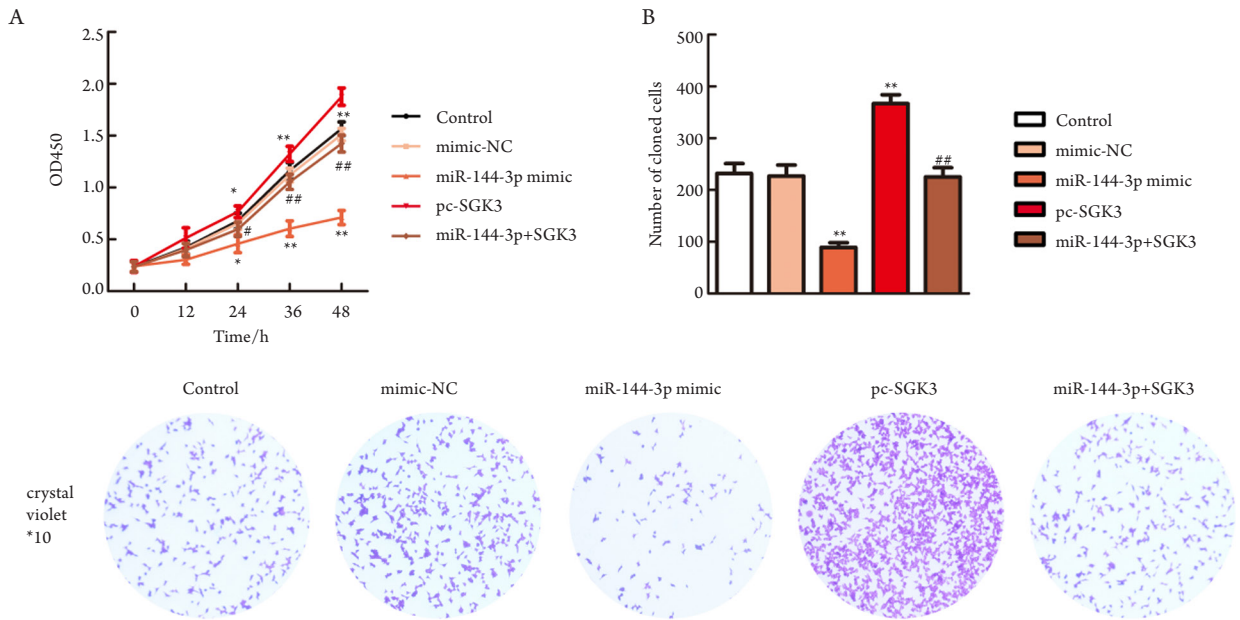


图3 MiR-144-3p靶向SGK3抑制卵巢癌的生长增殖

Figure 3 MiR-144-3p inhibits the growth and proliferation of ovarian cancer by targeting SGK3

(A)通过CCK-8法检测卵巢癌细胞增殖情况；(B)通过克隆形成实验检测卵巢癌细胞生长情况。与control组相比，** $P < 0.01$ ；与miR-144-3p mimic组相比，** $P < 0.01$ 。n=9。

(A) Proliferation of ovarian cancer cells was detected by CCK-8 method; (B) Growth of ovarian cancer cells was detected by colony formation assay. ** $P < 0.01$ vs the control group; ** $P < 0.01$ vs the miR-144-3p mimic group. n=9.

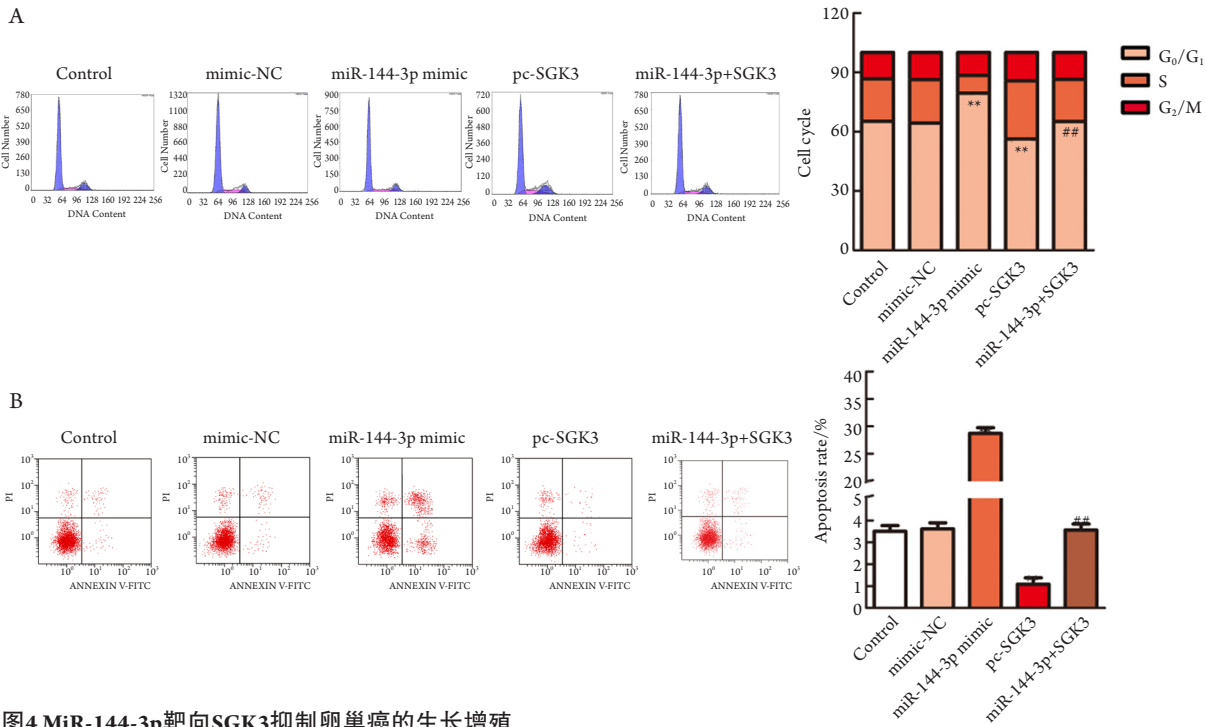


图4 MiR-144-3p靶向SGK3抑制卵巢癌的生长增殖

Figure 4 MiR-144-3p inhibits the growth and proliferation of ovarian cancer by targeting SGK3

(A)通过流式检测卵巢癌细胞周期；(B)通过流式检测卵巢癌细胞凋亡。与control组相比，** $P < 0.01$ ；与miR-144-3p mimic组相比，** $P < 0.01$ 。n=9。

(A) Ovarian cancer cell cycle is detected by flow cytometry; (B) Ovarian cancer cell apoptosis is detected by flow cytometry. ** $P < 0.01$ vs the control group, ** $P < 0.01$ vs the miR-144-3p mimic group. n=9.

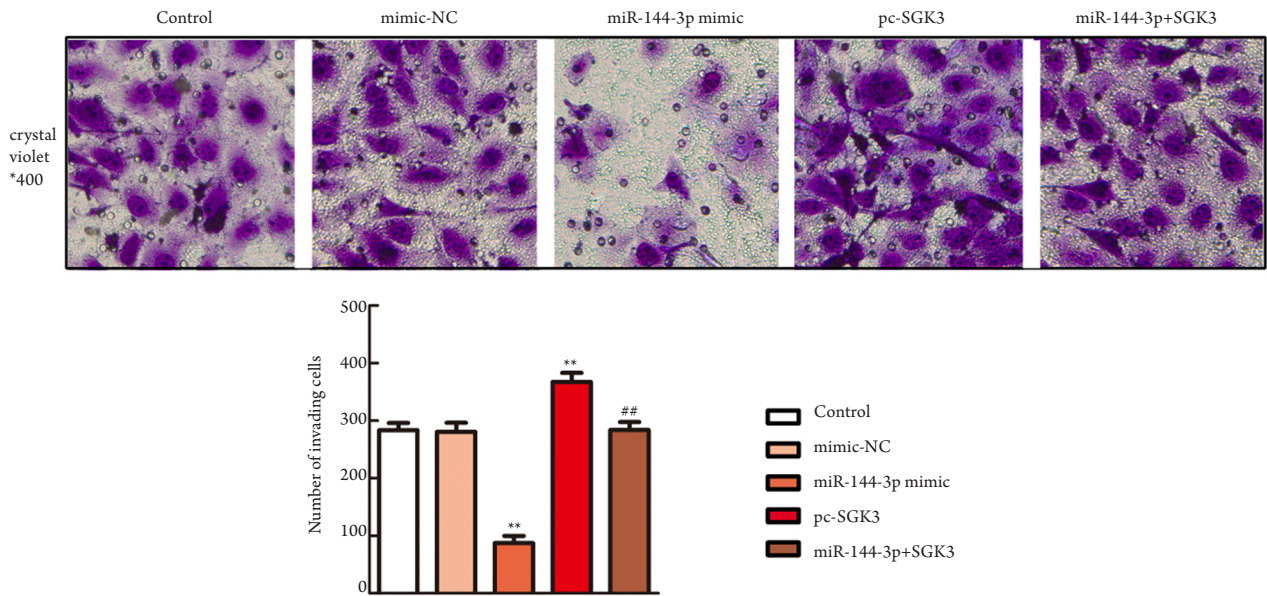


图5 Transwell实验检测各组卵巢癌细胞侵袭($\times 400$)

Figure 5 Invasive ability of each group of ovarian cancer cells was detected by Transwell experiment ($\times 400$)

与control组相比, $**P < 0.01$; 与miR-144-3p mimic组相比, $##P < 0.01$; $n = 9$ 。

$**P < 0.01$ versus the control group; $##P < 0.01$ versus the miR-144-3p mimic group; $n = 9$ 。

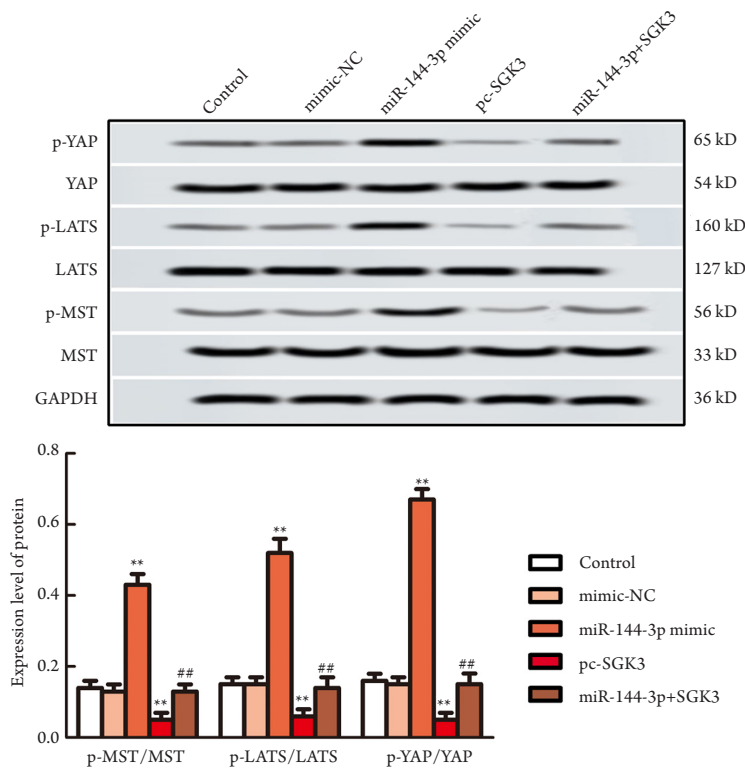


图6 蛋白质印迹分析检测Hippo信号通路蛋白p-MST/MST, p-LATS/LATS, p-YAP/YAP蛋白表达水平

Figure 6 Expression levels of p-MST/MST, p-LATS/LATS, p-YAP/YAP proteins were detected by Western blotting

与control组相比, $**P < 0.01$; 与miR-144-3p mimic组相比, $##P < 0.01$; $n = 9$ 。

$**P < 0.01$ vs the control group; $##P < 0.01$ vs the miR-144-3p mimic group; $n = 9$ 。

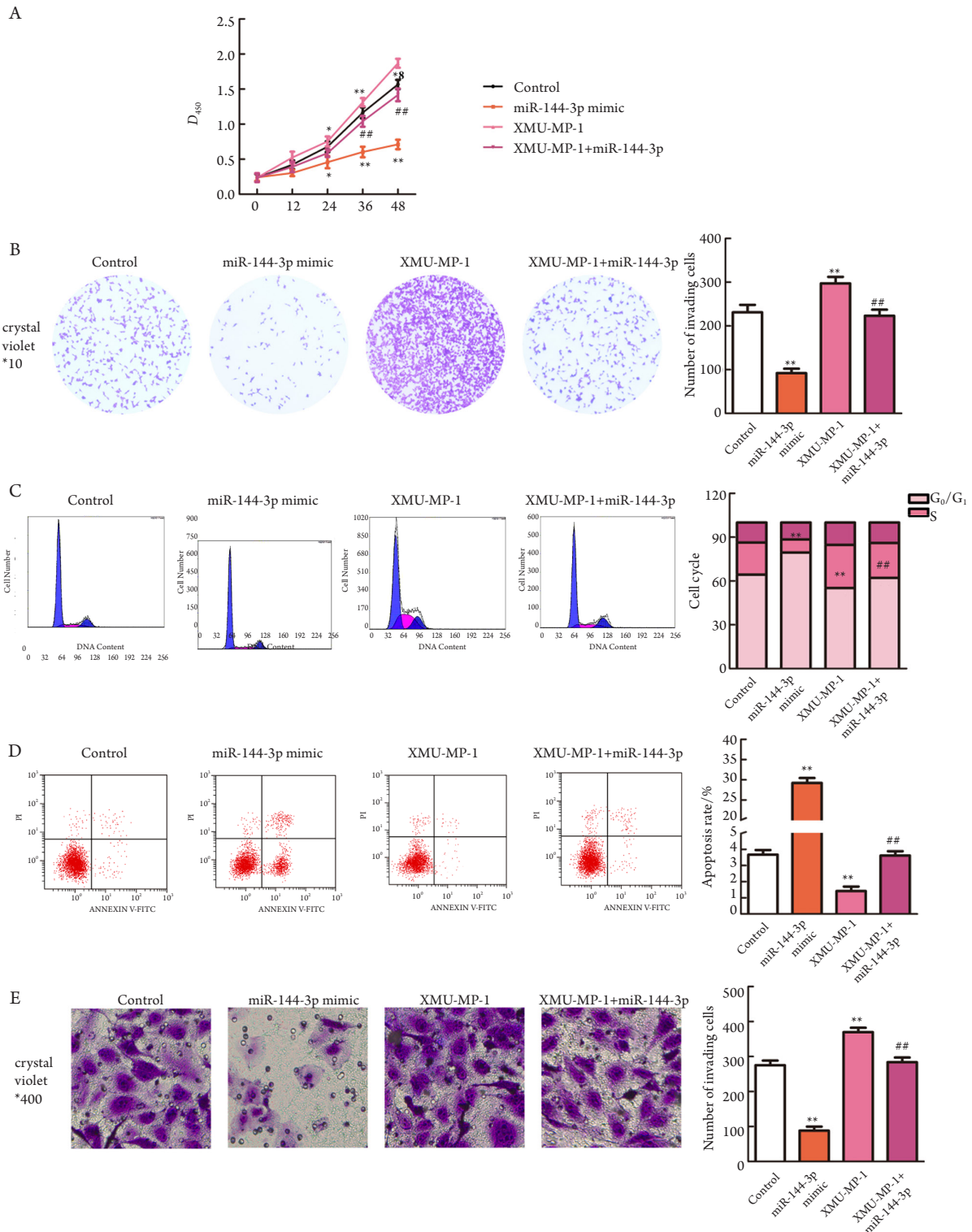


图7 MiR-144-3p通过激活Hippo信号通路抑制卵巢癌细胞生长和侵袭

Figure 7 MiR-144-3p inhibits ovarian cancer cell growth and invasion by activating Hippo signaling pathway

(A)通过CCK-8法检测卵巢癌细胞增殖情况；(B)通过克隆形成实验检测卵巢癌细胞生长情况；(C)通过流式检测细胞周期；(D)通过流式检测细胞凋亡；(E)Transwell实验检测各组卵巢癌细胞侵袭($\times 400$)。与control组相比，** $P < 0.01$ ；与miR-144-3p mimic组相比，** $P < 0.01$ 。 $n = 9$ 。

(A) Proliferation of ovarian cancer cells was detected by CCK-8 method; (B) Growth of ovarian cancer cells was detected by colony formation assay; (C) Cell cycle detection by flow cytometry; (D) Apoptosis detection by flow cytometry; (E) Invasive ability of each group of ovarian cancer cells was detected by Transwell experiment. ** $P < 0.01$ vs the control group; ** $P < 0.01$ vs the miR-144-3p mimic group. $n = 9$.

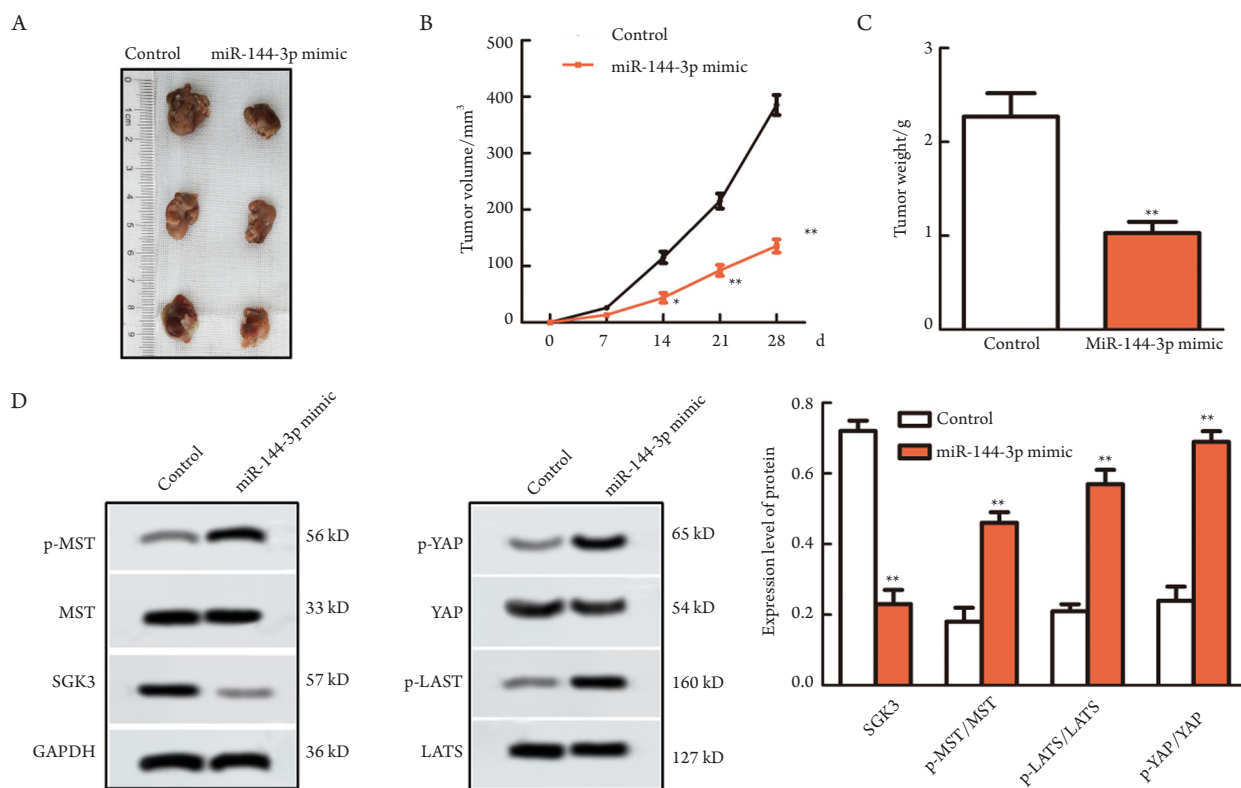


图8 MiR-144-3p抑制卵巢癌SKOV3移植瘤的发展

Figure 8 MiR-144-3p inhibits the development of ovarian cancer SKOV3 xenografts

(A)移植瘤照片;(B)SKOV3移植瘤体积;(C)SKOV3移植瘤重量;(D)蛋白质印迹分析检测SGK3, p-MST/MST, p-LATS/LATS, p-YAP/YAP蛋白表达水平。与control组相比, ** $P < 0.01$; $n = 9$ 。

(A) Photo of tumor; (B) Volume of SKOV3 xenografts; (C) Weight of SKOV3 xenografts; (D) Expression levels of SGK3, p-MST/MST, p-LATS/LATS, p-YAP/YAP proteins detected by Western blotting. ** $P < 0.01$ vs the control group; $n = 9$.

3 讨论

MiRNA对细胞增殖、侵袭、凋亡和分化等多种生命活动具有调节作用,在包括癌症在内的多种疾病的发生过程中起关键作用。已有证据^[6]表明在肿瘤发展和转移过程中存在大量miRNA的异常表达。因此,揭示miRNA在癌症中的潜在作用和机制可以促进对肿瘤分子生物学的了解,并提供新的治疗方法和诊断方法。MiR-144-3p作为肿瘤的抑制因子已在多种癌症类型中得到证实,如Zhao等^[7]报道miR-144-3p靶向c-Met抑制多发性骨髓瘤细胞增殖,诱导细胞凋亡;Liu等^[8]报道miR-144-3p靶向AP-1转录因子亚单位抑制胰腺癌细胞PANC-1的增殖、迁移和侵袭;Li等^[9]报道miR-144-3p通过靶向PBX3抑制胃癌上皮细胞向间质转化。相反,miR-144-3p在某些癌症中作为致癌因子也有报道,如Liu等^[10]报道miR-144-3p通过靶向配对框

基因8促进甲状腺乳头状癌的生长和转移。本研究发现:miR-144-3p在卵巢癌细胞中表达明显降低,这与前人研究结果^[5]相符。此外,本研究还发现:miR-144-3p表达的恢复能够明显抑制卵巢癌细胞的生长增殖和侵袭。

为更好地了解miR-144-3p对卵巢癌的作用机制,使用TargetScan数据库预测miR-144-3p靶基因,发现miR-144-3p与SGK3 3'-UTR区存在结合位点,并通过双荧光素酶报告实验验证了该靶向关系。Zhang等^[5]研究发现卵巢癌组织中SGK3表达水平显著高于正常卵巢组织和卵巢良性肿瘤组织,这与本研究结果SGK3在卵巢癌细胞中表达明显降低一致。此外,本研究还发现:miR-144-3p靶向负调控SGK3。SGK3又称细胞因子独立生存激酶,由8q12.2号染色体编码,是磷脂酰肌醇3的下游介质,因其含有一个N端PX域,在SGK家族具有独特地位^[11-12]。SGK是一种的丝/苏氨酸蛋白激酶,与

其他蛋白激酶显著不同的是, SGK的转录、活性和在细胞内定位受到不同因素的调节, 是多种胞内信号途径的交汇点, 参与了细胞存活与凋亡、离子通道调节等过程, 与高血压、糖尿病性肾病等疾病密切相关^[13]。已有研究^[14]报道: SGK3在乳腺癌、卵巢癌和肝细胞癌等多种癌症中作为致癌因子存在。本研究发现: SGK3过表达能够促进卵巢癌细胞的生长、增殖和侵袭, miR-144-3p过表达通过靶向抑制SGK3可以抑制卵巢癌细胞的生长增殖和侵袭。

本研究还发现: miR-144-3p靶向SGK3激活Hippo信号通路。Hippo信号通路在哺乳动物进化上是保守的, 其通过调节细胞增殖和存活来控制器官大小和肿瘤发生^[15]。在哺乳动物中, Hippo信号转导途径的核心是激酶级联磷酸化^[16]。Hippo信号通路上游膜蛋白受体作为胞外生长抑制信号的感受器, 一旦感受到胞外生长抑制信号, 就会激活一系列激酶级联磷酸化反应, 最终磷酸化下游效应因子YAP和TAZ^[17]。而细胞骨架蛋白会与磷酸化后的YAP和TAZ结合, 使它滞留在细胞质内, 降低其细胞核活性, 从而实现对细胞生长的调控^[18]。已有研究^[19]发现卵巢癌中Hippo信号通路被抑制, 鞘氨醇-1-磷酸盐通过抑制Hippo信号促进卵巢癌细胞的增殖。本研究发现: 添加Hippo信号通路抑制剂XMU-MP-1明显促进卵巢癌细胞的生长、增殖和侵袭, miR-144-3p过表达通过激活Hippo信号通路来抑制卵巢癌细胞的生长、增殖和侵袭。

综上所述, miR-144-3p过表达靶向抑制SGK3通过激活Hippo信号通路来抑制卵巢癌细胞的生长、增殖和侵袭, miR-144-3p作为一种卵巢癌中的肿瘤抑制因子, 有望成为卵巢癌治疗和诊断的靶基因, 其对药物耐药性的作用及机制还有待进一步研究。

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