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·基础研究·

circDNMT1调节miR-377-3p/PUM1轴对三阴性乳腺癌细胞增殖、凋亡和上皮-间质转化的影响

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[摘 要] **目的** 探讨circDNMT1调节miR-377-3p/PUM1轴对三阴性乳腺癌(TNBC)细胞增殖、凋亡和上皮-间质转化(EMT)的影响。**方法** 收集儋州市人民医院2018年至2021年收治的24例TNBC患者的TNBC组织和其邻近正常组织,采用qRT-PCR、Western blot法分别检测组织及小鼠正常乳腺上皮细胞系HC11和TNBC细胞系4T1、Eph41424、JC中circDNMT1表达、miR-377-3p表达、PUM1蛋白表达。将4T1细胞分为4T1组(未转染)、si-NC组(转染si-NC)、si-DNMT1组(转染si-DNMT1)、si-DNMT1+anti-NC组(同时转染si-DNMT1和anti-NC)、si-DNMT1+anti-miR-377-3p组(同时转染si-DNMT1和anti-miR-377-3p),qRT-PCR检测各组4T1细胞中circDNMT1、miR-377-3p表达;CCK-8检测各组4T1细胞增殖情况;流式细胞术检测各组4T1细胞凋亡情况;Western blot检测各组4T1细胞PUM1、EMT相关蛋白及凋亡相关蛋白表达;TargetScan网站预测miR-377-3p与circDNMT1、PUM1的结合位点,双荧光素酶报告验证miR-377-3p与circDNMT1、PUM1的靶向关系。BALB/c小鼠接种4T1细胞后随机分为空白对照组(注射等量生理盐水)、阴性对照组(尾静脉注射si-NC)、DNMT1沉默组(尾静脉注射si-DNMT1)、联合对照组(尾静脉注射si-DNMT1和anti-NC)、联合沉默组(尾静脉注射si-DNMT1和anti-miR-377-3p),记录小鼠肿瘤质量,采用苏木精-伊红染色观察肿瘤形态变化。**结果** 在TNBC组织和细胞中circDNMT1、PUM1上调,miR-377-3p下调,且在4T1细胞中表达差异最显著,因此本研究选择4T1细胞进行后续实验。与4T1组、si-NC组比较,si-DNMT1组4T1细胞中miR-377-3p表达、4T1细胞凋亡率及Bax、cleaved caspase-3、E-cadherin蛋白表达水平显著升高($P<0.05$),circDNMT1水平、PUM1蛋白表达水平、24 h和48 h OD值、Bcl-2蛋白表达水平、N-cadherin蛋白表达水平、Vimentin蛋白表达水平均显著减少/降低($P<0.05$);与si-DNMT1组、si-DNMT1+anti-NC组比较,si-DNMT1+anti-miR-377-3p组4T1细胞中miR-377-3p表达、4T1细胞凋亡率、Bax蛋白表达水平、cleaved caspase-3蛋白表达水平、E-cadherin蛋白表达水平显著降低($P<0.05$),PUM1蛋白表达水平、24 h和48 h OD值、Bcl-2蛋白表达水平、N-cadherin蛋白表达水平、Vimentin蛋白表达水平均显著增加/升高($P<0.05$)。与miR-NC+WT-circDNMT1组比较,miR-377-3p mimic+WT-circDNMT1组细胞荧光素酶活性显著降低($P<0.05$);与miR-NC+WT-PUM1组比较,miR-377-3p mimic+WT-PUM1组细胞荧光素酶活性显著降低($P<0.05$)。空白对照组、阴性对照组小鼠肿瘤细胞排列密集,边界清晰;DNMT1沉默组、联合对照组肿瘤细胞排列疏松,胞核固缩,细胞碎片增多;联合沉默组小鼠肿瘤细胞排列比较密集,边界趋于清晰。与空白对照组、阴性对照组比较,DNMT1沉默组小鼠肿瘤质量显著减少($P<0.05$);与DNMT1沉默组、联合对照组比较,联合沉默组小鼠肿瘤质量显著增加($P<0.05$)。**结论** 沉默circDNMT1可能通过上调miR-377-3p来抑制PUM1表达,进而抑制TNBC细胞增殖和EMT,促进细胞凋亡。

[关键词] circDNMT1;miR-377-3p;三阴性乳腺癌;细胞增殖;细胞凋亡;上皮-间质转化
[中图分类号] R737.9 [文献标志码] A [收稿日期] 2022-12-26

Effect of circDNMT1 regulating miR-377-3p/PUM1 axis on proliferation, apoptosis and epithelial-mesenchymal transition of triple-negative breast cancer cells

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Abstract: Objective To investigate the effects of circDNMT1 on the proliferation, apoptosis and epithelial-mesenchymal transition (EMT) of triple-negative breast cancer (TNBC) cells by regulating miR-377-3p/PUM1 axis. **Methods** The TNBC tissues and adjacent normal tissues of 24 patients with TNBC treated in Danzhou People's Hospital from 2018 to 2021 were collected. qRT-PCR and Western blot were used to detect the expression of circDNMT1, miR-377-3p, and PUM1 protein in tissue and mouse normal breast epithelial cell line HC11 and TNBC cell lines 4T1, Eph41424, and JC. 4T1 cells were divided into the 4T1 group (untransfected), the si-NC group (transfected with si-NC), the si-DNMT1 group (transfected with si-DNMT1), the si-DNMT1+anti-NC group (simultaneously transfected with si-DNMT1 and anti-NC), and the si-DNMT1+anti-miR-377-3p group (simultaneously transfected with si-DNMT1 and anti-miR-377-3p). qRT-PCR was used to detect the

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expression of circDNMT1 and miR-377-3p of 4T1 cells in each group; CCK-8 was used to detect the proliferation of 4T1 cells in each group; flow cytometry was used to detect the apoptosis of 4T1 cells in each group; Western blot was used to detect the expression of PUM1, EMT-related proteins and apoptosis-related proteins of 4T1 cells in each group; TargetScan website was used to predict the binding sites of miR-377-3p with circDNMT1 and PUM1; dual luciferase report was used to verify the targeting relationships of miR-377-3p with circDNMT1 and PUM1. After inoculation with 4T1 cells, BALB/c mice were randomly divided into the blank control group (injected with equal amount of normal saline), the negative control group (injected with si-NC via tail vein), the DNMT1 silencing group (injected with si-DNMT1 via tail vein), the combined control group (injected with si-DNMT1 and anti-NC via tail vein), and the combined silencing group (injected with si-DNMT1 and anti-miR-377-3p via tail vein), the tumor massess of mice were recorded and the morphological changes of tumors were observed by hematoxylin-eosin staining. **Results** circDNMT1 and PUM1 were up-regulated in TNBC tissues and cells, and miR-377-3p was down-regulated. The expression difference was most obvious in 4T1 cells, so 4T1 cells were selected for subsequent experiments. Compared with the 4T1 group and the si-NC group, the expression of miR-377-3p, the apoptosis rate of 4T1 cells, the expression levels of Bax, cleaved caspase-3 and E-cadherin protein of 4T1 cells in the si-DNMT1 group were significantly increased (P<0.05), the circ-DNMT1 level, the expression level of PUM1 protein, OD values at 24 hours and 48 hours, the expression level of Bcl-2, N-cadherin, Vimentin protein were significantly decreased (P<0.05). Compared with the si-DNMT1 group and the si-DNMT1+anti-NC group, the expression of miR-377-3p, the apoptosis rate of 4T1 cells, the expression levels of Bax, cleaved caspase-3 and E-cadherin proteins of 4T1 cells in the si-DNMT1+anti-miR-377-3p group were significantly decreased (P<0.05), the expression level of PUM1 protein, OD values at 24 hours and 48 hours, the expression levels of Bcl-2, N-cadherin, Vimentin proteins were significantly increased (P<0.05). Compared with the miR-NC+WT-circDNMT1 group, the cell luciferase activity in the miR-377-3p mimic+WT-circDNMT1 group was significantly decreased (P<0.05); compared with the miR-NC+WT-PUM1 group, the cell luciferase activity in the miR-377-3p mimic+WT-PUM1 group was significantly decreased (P<0.05). The tumor cells in the blank control group and the negative control group were densely arranged with clear boundary; the tumor cells in the DNMT1 silencing group and the combined control group were loosely arranged, the nuclei were pyknotic, and the cell fragments were increased; the tumor cells in the combined silencing group were densely arranged and the boundaries tended to be clear. Compared with the blank control group and the negative control group, the tumor mass of mice in the DNMT1 silencing group was significantly decreased (P<0.05). Compared with the DNMT1 silencing group and the combined control group, the tumor mass of mice in the combined silencing group was significantly increased (P<0.05). **Conclusion** Silencing circDNMT1 may inhibit the expression of PUM1 by up-regulating miR-377-3p, thereby inhibiting the proliferation and EMT of TNBC cells, and promoting cell apoptosis.

Keywords: circDNMT1; miR-377-3p; triple-negative breast cancer; cell proliferation; cell apoptosis; epithelial-mesenchymal transition

乳腺癌是女性最常见的恶性肿瘤之一，近年来其发病率持续上升。三阴性乳腺癌(triple-negative breast cancer,TNBC)约占乳腺癌的15%，复发率、转移率和病死率最高，且缺乏有效的靶向治疗方案[1]。TNBC的典型特征是缺乏雌激素受体、孕激素受体及人表皮生长因子受体2，因其无有效治疗靶点，治疗有一定局限，且预后较差[2]。因此，寻找新的TNBC预后标志物和分子治疗靶点十分重要。circRNAs是一类新型的非编码RNA，其特征是缺乏3′poly(A)尾巴的特殊环状结构[3]。有研究发现，circRNAs在许多癌症中是关键调节因子，如下调circDDX17可促进结直肠癌细胞增殖、迁移和侵袭，并抑制其凋亡[4]。敲低circDNMT1可抑制乳腺癌细胞集落形成、迁移、侵袭和肿瘤生长，促进细胞凋亡[5]。还有研究发现，circRNA/miRNA/mRNA轴参与TNBC的发展[6]。下调miR-377-3p会促进TNBC的进展[7]，而下调PUM1会抑制TNBC的进展[8]。但目前circDNMT1是否可通过调节miR-377-3p/PUM1轴影响TNBC的恶性生物学行为尚不清楚。因此，本研究旨在探究circDNMT1对TNBC细胞增殖、凋亡、上皮-间质转化(epithelial-mesenchymal transition,EMT)的影响及其作用机制，以期为该病的临床诊治提供参考。

1 材料与方法

1.1 主要材料

收集儋州市人民医院2018年至2021年收治的24例TNBC患者的TNBC组织(TNBC组)及其邻近正常组织(NOR组)。本研究经儋州市人民医院医学伦理委员会批准(2018-10099)，符合《赫尔辛基宣言》，所有患者均签署对手术的知情同意书。

TNBC细胞系4T1、Eph41424、JC，正常乳腺上皮细胞系HC11均购自上海酶研生物科技有限公司；si-NC、si-DNMT1、anti-NC、anti-miR-377-3p购自上海生工生物工程股份有限公司；CCK-8试剂盒购自上海吉至生化科技有限公司；PUM1、E-cadherin、N-cadherin、Vimentin、Bax、cleaved caspase-3、Bcl-2抗体均购自英国Abcam公司。30只BALB/c小鼠[体质量(20±2)g]购自广州锐格

生物科技有限公司[生产许可证号:SCXK(粤)2021-0059]。

1.2 qRT-PCR检测miR-377-3p、circDNMT1表达

使用TRIzol试剂提取组织和细胞的总RNA,然后反转录成cDNA。根据qRT-qPCR试剂盒配制反应体系,然后扩增。反应条件为:95 ℃预变性4 min,95 ℃ 45 s,60 ℃ 30 s,共40个循环。以U6或GAPDH为内参基因,采用$2^{-\Delta\Delta Ct}$法计算miR-377-3p、circDNMT1的相对表达量。引物序列见表1。

1.3 4T1细胞转染及分组

将4T1细胞添加至含10% FBS和1%青霉素-链霉素的DMEM培养基中,待4T1细胞培养至75%汇合时,用Lipofectamine 2000试剂盒进行转染,并分为4T1组(未处理的4T1细胞)、si-NC组(转染si-NC)、si-DNMT1组(转染si-DNMT1)、si-DNMT1+anti-NC组(同时转染si-DNMT1和anti-NC)、si-DNMT1+anti-miR-377-3p组(同时转染si-DNMT1和anti-miR-377-3p)。在细胞培养箱中培养48 h后用于后续检测。

1.4 CCK-8检测4T1细胞增殖水平

将各组4T1细胞按5×10^3/孔的密度接种至96孔板上,然后在细胞培养0、24、48 h时向每孔加入10 μL CCK-8试剂,置于细胞培养箱孵育2 h后,用酶标仪检测各组4T1细胞在450 nm波长处的光密度(optical density,OD)值。

1.5 流式细胞术检测4T1细胞凋亡水平

将各组4T1细胞加入胰酶消化成细胞悬液,用PBS清洗后调整4T1细胞浓度为5×10^5/mL。取100 μL细胞悬液与10 μL Annexin V-FITC和10 μL PI混合,置于4 ℃冰箱孵育15 min,PBS清洗2次,随后加入480 μL结合缓冲液重悬4T1细胞,上机检测细胞凋亡率。细胞凋亡率(%)=(细胞凋亡数目/细胞总数)×100%。

1.6 双荧光素酶报告验证miR-377-3p与PUM1、circDNMT1的关系

TargetScan网站预测miR-377-3p与circDNMT1、PUM1是否存在结合位点。将含miR-377-3p结合位点的野生型circDNMT1/PUM1序列和不含miR-377-3p结合位点的突变型circDNMT1/PUM1序列克隆到pmirGLO载体中,分别命名为WT-circDNMT1、MUT-circDNMT1、WT-PUM1、MUT-PUM1,然后将上述序列与mimic-NC、miR-377-3p mimic共转染到4T1细胞中,分别记为miR-NC+WT-circDNMT1组、miR-377-3p mimic+WT-circDNMT1组、miR-NC+MUT-circDNMT1组、miR-377-3p mimic+MUT-circDNMT1组和miR-NC+WT-PUM1组、miR-377-3p mimic+WT-PUM1组、miR-NC+MUT-PUM1组、miR-377-3p mimic+MUT-PUM1组。培养48 h后使用双荧光素酶检测系统检测各组4T1细胞的荧光素酶活性。

1.7 Western blot检测EMT及凋亡相关蛋白表达

将TNBC组织和邻近正常组织及各组4T1细胞经RIPA裂解缓冲液裂解后提取总蛋白,经凝胶电泳(120 V,蓝色染料到达胶的底端附近时停止电泳)分离蛋白后转移到PVDF膜(200 mA,1 kD约1 min)上,脱脂牛奶封闭,随后加入PUM1(1∶2 000)、E-cadherin(1∶1 000)、N-cadherin(1∶1 000)、Vimentin(1∶1 000)、Bax(1∶2 000)、cleaved caspase-3(1∶1 000)、Bcl-2(1∶2 000)一抗和GAPDH抗体(1∶2 000),4 ℃下放置过夜后,TBST清洗3次,然后加入辣根过氧化物酶标记的IgG二抗(1∶5 000),在37 ℃下孵育1 h后加入显色剂,用Image J软件量化目的蛋白的灰度值。

1.8 荷瘤小鼠模型验证circDNMT1对4T1移植瘤的影响

将4T1细胞接种至6周龄小鼠腹部脂肪垫(4×10^5/只),4 d后观察成瘤情况。将小鼠随机分为空白对照组(注射等量生理盐水)、阴性对照组(尾静脉注射si-NC)、DNMT1沉默组(尾静脉注射si-DNMT1)、联合对照组(尾静脉注射si-DNMT1和anti-NC)、联合沉默组(尾静脉注射si-DNMT1和anti-miR-377-3p),每组6只。

接种后35 d麻醉处死小鼠,取出肿瘤组织,称重后于4%多聚甲醛中固定,经石蜡包埋、切片、脱蜡、水化后进行苏木精-伊红染色,显微镜下观察肿瘤形态变化。

1.9 统计学分析

采用SPSS 25.0统计软件分析数据,数据经*Shapiro-Wilk*检验符合正态分布,以均数±标准差($\bar{x}\pm s$)表示,2组间比较采用配对t检验,多组间比较采用单因素方差分析,两两比较采用*SNK-q*检验。$P<0.05$表示差异有统计学意义。

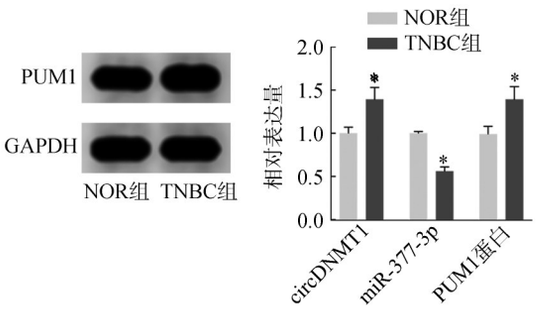
表1 引物序列

基因	正向引物	反向引物
miR-377-3p	5′-ATCACACAAAGGCACTTTGT-3′	5′-GGTGCAGGTCGAGGTAT-3′
U6	5′-GCTTCGGCAGCACATATACTAAAAT-3′	5′-CGCTTCACGAATTTGCGTGTCAT-3′
circDNMT1	5′-GAGGAGGGCTACCTGGCTAA-3′	5′-CTCCATCGGACTTGCTCCTC-3′
GAPDH	5′-GGAGAGTGTTTCCTCGTCCC-3′	5′-TTACTCCTTGGAGGCCATGTAG-3′

2　结果

2.1　circDNMT1、miR-377-3p、PUM1在组织和细胞中的表达

与NOR组比较，TNBC组组织中circDNMT1、PUM1表达显著升高（$P<0.05$），miR-377-3p表达显著降低（$P<0.05$）；与HC11细胞比较，JC、Eph41424及4T1细胞中circDNMT1、PUM1表达显著上调（$P<0.05$），miR-377-3p表达显著下调（$P<0.05$），且在4T1细胞中表达差异最显著，因此本研究选择4T1细胞进行后续实验，见图1、2。



*：与NOR组比较，$P<0.05$

图1　circDNMT1、miR-377-3p、PUM1在TNBC组织中的表达

2.2　circDNMT1、miR-377-3p、PUM1在4T1细胞中的表达

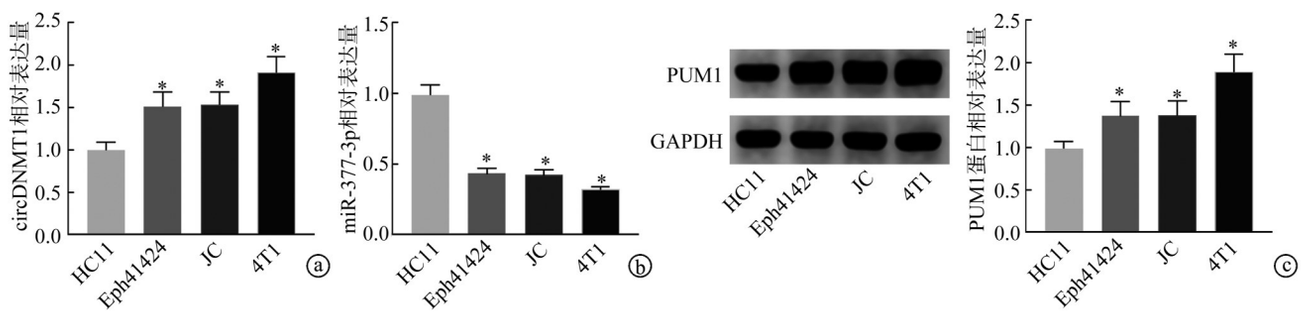
与4T1组、si-NC组比较，si-DNMT1组细胞中circDNMT1水平以及PUM1蛋白表达水平显著降低（$P<0.05$），miR-377-3p表达显著升高（$P<0.05$）；与si-DNMT1组、si-DNMT1+anti-NC组比较，si-DNMT1+anti-miR-377-3p组细胞PUM1蛋白表达水平显著升高（$P<0.05$），miR-377-3p表达显著降低（$P<0.05$），见图3。

2.3　沉默circDNMT1或miR-377-3p对4T1细胞增殖的影响

与4T1组、si-NC组比较，si-DNMT1组细胞24 h、48 h的OD值显著降低（$P<0.05$）；与si-DNMT1组、si-DNMT1+anti-NC组比较，si-DNMT1+anti-miR-377-3p组细胞24 h、48 h的OD值显著升高（$P<0.05$），见图4。

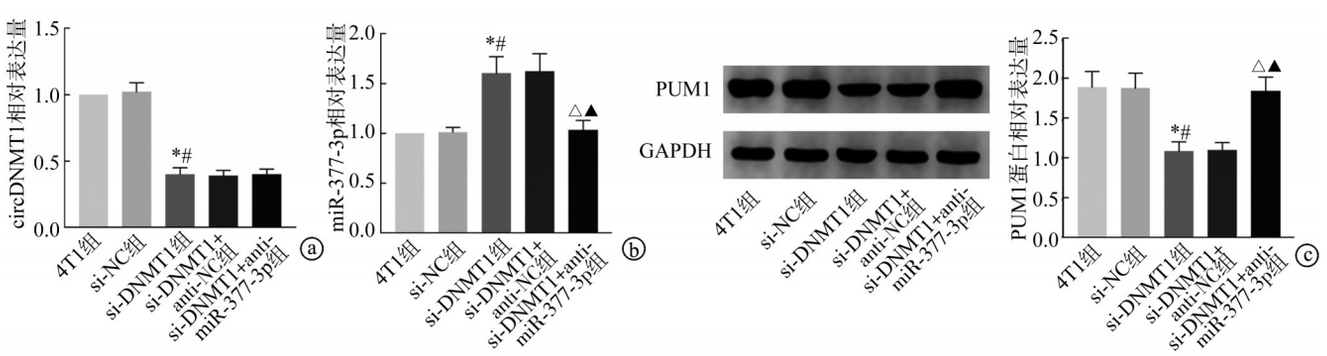
2.4　沉默circDNMT1或miR-377-3p对4T1细胞凋亡的影响

与4T1组、si-NC组比较，si-DNMT1组4T1细胞凋亡率及Bax、cleaved caspase-3蛋白表达水平显著升高（$P<0.05$），Bcl-2蛋白表达水平显著降低（$P<0.05$）；与si-DNMT1组、si-DNMT1+anti-NC组比较，si-DNMT1+anti-miR-377-3p组细胞凋亡率及Bax、cleaved caspase-3蛋白表达水平显著下降（$P<0.05$），Bcl-2蛋白表达水平显著升高（$P<0.05$），见图5。



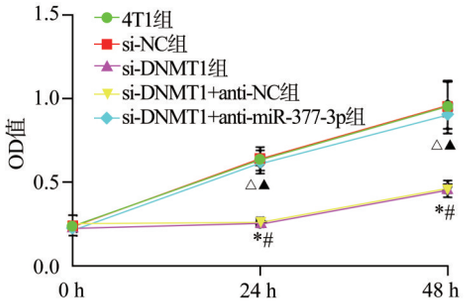
a：circDNMT1表达；b：miR-377-3p表达；c：PUM1蛋白表达　*：与HC11细胞比较，$P<0.05$

图2　circDNMT1、miR-377-3p、PUM1在各细胞中的表达



a：circDNMT1表达；b：miR-377-3p表达；c：PUM1蛋白表达　*：与4T1组比较，$P<0.05$；#：与si-NC组比较，$P<0.05$；△：与si-DNMT1组比较，$P<0.05$；▲：与si-DNMT1+anti-NC组比较，$P<0.05$

图3　circDNMT1、miR-377-3p、PUM1在各组4T1细胞中的表达



*:与4T1组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-DNMT1组比较,$P<0.05$;▲:与si-DNMT1+anti-NC组比较,$P<0.05$

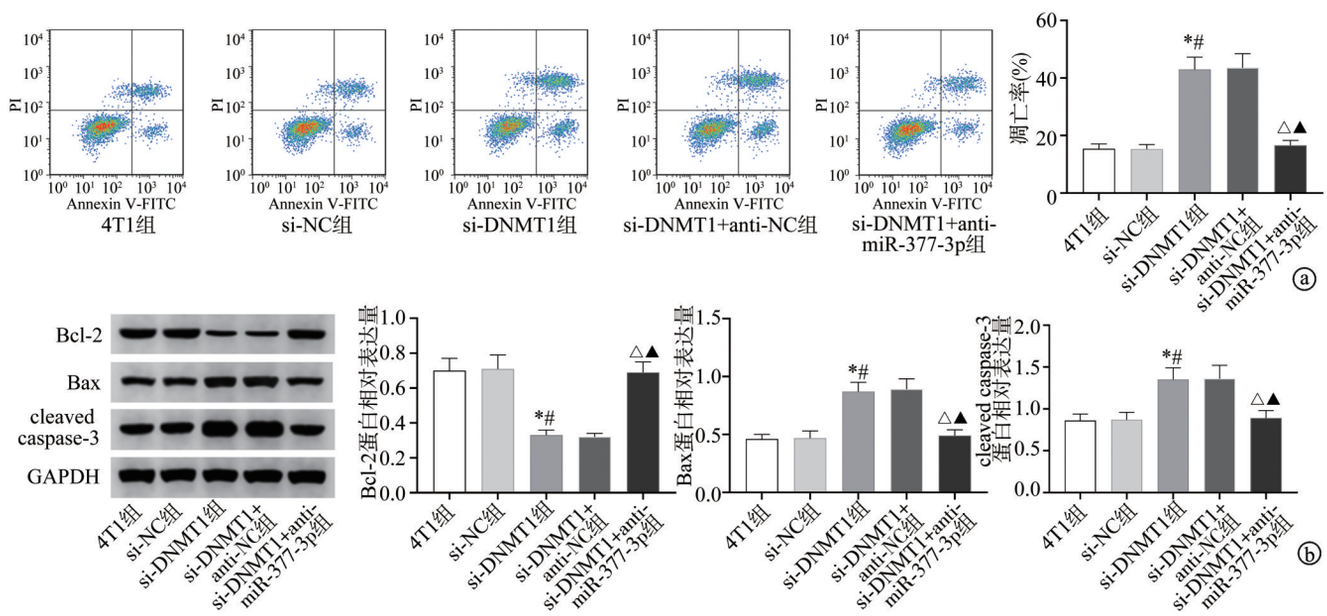
图4　各组4T1细胞增殖能力比较

2.5　沉默circDNMT1或miR-377-3p对4T1细胞EMT的影响

与4T1组、si-NC组比较,si-DNMT1组4T1细胞E-cadherin蛋白表达水平显著升高($P<0.05$),N-cadherin、Vimentin蛋白表达水平显著降低($P<0.05$);与si-DNMT1组、si-DNMT1+anti-NC组比较,si-DNMT1+anti-miR-377-3p组4T1细胞E-cadherin蛋白表达水平显著下降($P<0.05$),N-cadherin、Vimentin蛋白表达水平显著上升($P<0.05$),见图6。

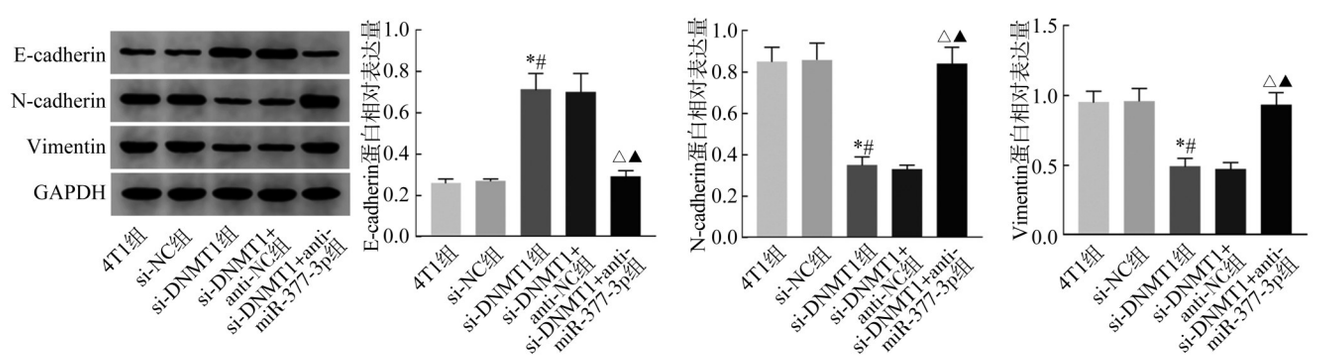
2.6　circDNMT1靶向调控miR-377-3p/PUM1轴

通过TargetScan网站预测发现,miR-377-3p与circDNMT1、PUM1均存在结合位点,见图7a。与miR-NC+WT-circDNMT1组比较,miR-377-3p mimic+WT-circDNMT1组细胞的荧光素酶活性显著降低($P<0.05$);与miR-NC+MUT-circDNMT1组比较,miR-377-3p mimic+MUT-circDNMT1组细胞的荧光素酶活性无显著变化($P>0.05$);与miR-NC+WT-PUM1组比较,miR-377-3p mimic+WT-PUM1组细胞的荧光素酶活性显著降低($P<0.05$);而与miR-NC+MUT-PUM1组比较,miR-377-3p mimic+MUT-PUM1组细胞



a:细胞凋亡情况检测;b:Bcl-2、Bax、cleaved caspase-3蛋白检测　*:与4T1组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-DNMT1组比较,$P<0.05$;▲:与si-DNMT1+anti-NC组比较,$P<0.05$

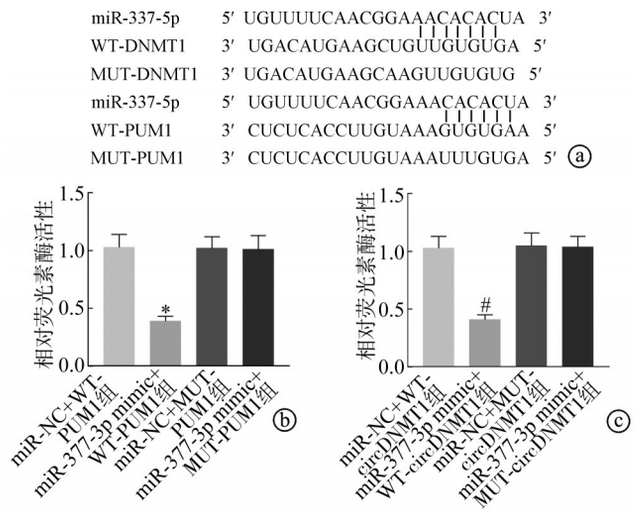
图5　各组4T1细胞凋亡率及凋亡相关蛋白表达



*:与4T1组比较,$P<0.05$;#:与si-NC组比较,$P<0.05$;△:与si-DNMT1组比较,$P<0.05$;▲:与si-DNMT1+anti-NC组比较,$P<0.05$

图6　各组4T1细胞中E-cadherin、N-cadherin、Vimentin蛋白表达

的荧光素酶活性无显著变化(P>0.05),见图7b、c。



a:TargetScan网站预测miR-377-3p与circDNMT1、PUM1的结合位点;b、c:双荧光素酶报告实验验证miR-377-3p与PUM1、circDNMT1的靶向关系 *:与miR-NC+WT-PUM1组比较,P<0.05;#:与miR-NC+WT-circDNMT1组比较,P<0.05

图7 circDNMT1靶向调控miR-377-3p/PUM1轴

2.7 沉默circDNMT1或miR-377-3p对4T1在小鼠体内增殖的影响

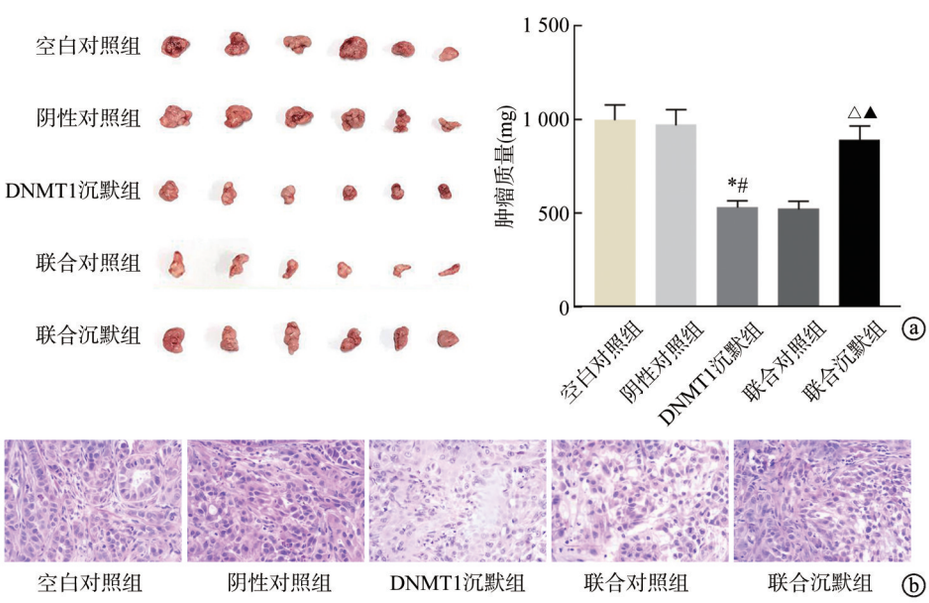
与空白对照组、阴性对照组比较,DNMT1沉默组小鼠肿瘤质量显著减少(P<0.05);与DNMT1沉默组、联合对照组比较,联合沉默组小鼠肿瘤质量显著增加(P<0.05),见图8a。

空白对照组、阴性对照组小鼠肿瘤细胞排列密集,边界清晰;DNMT1沉默组、联合对照组细胞排列疏松,胞核固缩,细胞碎片增多;联合沉默组组小鼠肿瘤细胞排列比较密集,边界趋于清晰,见图8b。

3 讨论

乳腺癌是女性常见的癌症,是全球癌症相关死亡的主要原因之一,而TNBC是最具侵袭性的亚型之一[1]。TNBC常见的治疗方法包括手术、化疗、放疗和免疫疗法[9]。然而,由于局部复发和转移的发生率较高,晚期TNBC患者的预后并不理想。因此,亟需寻找新的靶点开发新的有效治疗策略[10]。CircRNAs具有末端共价连接、稳定性高、组织特异性表达模式等特点,是人类疾病诊断、治疗和预后的理想生物标志物[11]。研究发现,circRNA可以作为竞争性内源RNA、蛋白质支架或翻译模板,从而在TNBC中发挥重要作用[12]。此外,上调circDNMT1可以通过激活自噬来促进乳腺癌的进展[13],而下调circDNMT1可以抑制乳腺癌的进展[5]。本研究也发现,circDNMT1在TNBC组织和细胞中表达上调,说明circDNMT1可能具有促进TNBC进展的作用。

上皮标志物E-cadherin表达降低及间质标志物N-cadherin、Vimentin表达升高是EMT的典型特征[14]。细胞增殖和凋亡对于TNBC细胞的生长极其重要,上调促凋亡蛋白Bax、caspase-3和下调抗凋亡蛋白Bcl-2可以促进TNBC细胞凋亡,从而起到治疗效果[15]。本研究结果显示,与4T1组、si-NC组比较,si-DNMT1组细胞miR-377-3p表达,4T1细胞凋亡率,Bax、cleaved caspase-3、E-cadherin蛋白表达水平显著升高,OD值及Bcl-2、N-cadherin、Vimentin蛋白表达水平均显著降低,



a:沉默circDNMT1或miR-377-3p对肿瘤生长的影响;b:沉默circDNMT1或miR-377-3p对荷瘤小鼠移植瘤组织形态的影响(×200)
*:与空白对照组比较,P<0.05;#:与阴性对照组比较,P<0.05;△:与DNMT1沉默组比较,P<0.05;▲:与联合对照组比较,P<0.05

图8 沉默circDNMT1或miR-377-3p对4T1在小鼠体内增殖的影响

提示沉默DNMT1可抑制4T1细胞增殖、EMT,促进其凋亡。

hsa_circ_0131242可以通过海绵化hsa-miR-2682促进TNBC的进展[16]。而circRNA/miRNA/mRNA轴在TNBC中的研究也有报道[17]。本研究通过TargetScan网站预测发现,circDNMT1与miR-377-3p存在结合位点。研究显示,下调miR-377-3p会促进TNBC的进展[7,18]。本研究结果亦显示,在TNBC组织和细胞中miR-377-3p下调,沉默circDNMT1后miR-377-3p表达显著升高;且双荧光素酶报告实验显示,circDNMT1与miR-377-3p存在靶向关系。因此,我们推测沉默circDNMT1可能通过上调miR-377-3p来抑制TNBC的进展。为了进一步验证该猜测,我们对4T1细胞同时转染si-DNMT1和anti-miR-377-3p,结果显示,下调miR-377-3p会逆转沉默circDNMT1对4T1细胞增殖、EMT的抑制作用和对凋亡的促进作用。PUM1是乳腺癌的生物标志物[19],下调PUM1会抑制TNBC的进展[8]。本研究通过双荧光素酶报告实验发现,miR-377-3p与PUM1也存在靶向关系,PUM1在TNBC组织和细胞中上调,且沉默circDNMT1后PUM1蛋白表达水平显著下降,下调miR-377-3p后PUM1蛋白表达水平显著上升,表明沉默circDNMT1可能通过上调miR-377-3p来抑制PUM1表达,进而抑制4T1细胞增殖、EMT,促进细胞凋亡。此外,本研究通过荷瘤小鼠模型验证circDNMT1对4T1移植瘤的影响,结果发现,si-DNMT1组小鼠肿瘤质量显著减少,肿瘤细胞排列疏松,胞核固缩,细胞碎片增多,进一步证明circDNMT1可能是TNBC患者的潜在治疗靶点。

综上,在TNBC中沉默circDNMT1可能通过上调miR-377-3p来抑制PUM1表达,进而抑制细胞增殖、EMT,促进细胞凋亡。

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