



· 论 著 ·

miR-193a-3p通过靶向TRIM14对乳腺癌干细胞迁移和侵袭的影响

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[摘要] **背景和目的:** 乳腺癌干细胞在癌症的发生、发展中发挥重要作用, 乳腺癌患者死亡率较高与癌症的复发及转移密切相关, 然而乳腺癌干细胞的自我更新和分化能力能够引发化疗耐药性, 进而影响肿瘤的复发和转移。本研究旨在探究miR-193a-3p通过靶向三结构域蛋白14 (tripartite motif-containing protein 14, TRIM14) 对乳腺癌干细胞迁移和侵袭的影响。**方法:** 选择人乳腺癌细胞T47D作为研究对象, 随机分组分为Control组、NC mimics组 (转染NC mimics)、miR-193a-3p mimics组 (转染miR-193a-3p mimics)、miR-193a-3p mimics+pcDNA-NC组 (转染miR-193a-3p mimics+pcDNA-NC) 和miR-193a-3p mimics+pcDNA-TRIM14组 (转染miR-193a-3p mimics+pcDNA-TRIM14)。采用流式细胞术分离干细胞并检测细胞成球能力; 通过细胞计数试剂盒-8 (cell counting kit-8, CCK-8) 实验检测细胞增殖; 使用transwell实验检测细胞迁移和侵袭; 通过流式细胞术检测细胞凋亡率; 采用蛋白质印迹法 (Western blot) 检测细胞中细胞周期蛋白D1 (cyclin D1)、基质金属蛋白酶-2 (matrix metalloproteinase-2, MMP-2)、Bcl-2相关X蛋白 (Bcl-2-associated X protein, Bax) 及TRIM14蛋白的表达; 采用双萤光素酶报告基因实验检测miR-193a-3p与TRIM14的相互作用。**结果:** T47D干细胞具有成球能力, 并随着时间推移增强, T47D干细胞球体积逐渐增大。与Control组、NC mimics组相比, miR-193a-3p mimics组细胞miR-193a-3p表达、凋亡率、Bax蛋白表达升高 ($P<0.05$), TRIM14 mRNA和蛋白表达、存活率、克隆数、迁移数、侵袭数、cyclin D1、MMP-2降低 ($P<0.05$)。与miR-193a-3p mimics组、miR-193a-3p mimics+pcDNA-NC组相比, miR-193a-3p mimics+pcDNA-TRIM14组细胞凋亡率、Bax蛋白表达降低 ($P<0.05$), TRIM14 mRNA和蛋白表达、存活率、克隆数、迁移数、侵袭数、cyclin D1、MMP-2升高 ($P<0.05$)。miR-193a-3p与TRIM14之间存在多个结合位点。与miR-NC+TRIM14-WT组相比, miR-193a-3p mimics+TRIM14-WT组双萤光素酶活性明显降低 ($P<0.05$)。**结论:** miR-193a-3p可能通过抑制TRIM14表达, 发挥抑制乳腺癌干细胞迁移和侵袭的作用。

[关键词] 乳腺癌; 干细胞; miR-193a-3p; 三结构域蛋白14; 迁移; 侵袭

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Impact of miR-193a-3p on migration and invasion of breast cancer stem cells through targeting TRIM14 WANG Xinrong, WANG Peixian, REN Haiqing, WANG Huan (Department of Laboratory Medicine, Xingtai People's Hospital, Xingtai 054000, Hebei Province, China)

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[Abstract] **Background and purpose:** Breast cancer stem cells play an important role in the occurrence and development of cancer. The high mortality of breast cancer patients is closely related to the recurrence and metastasis of cancer. However, the self-renewal and differentiation ability of breast cancer stem cells can lead to chemotherapy resistance, thus affecting the recurrence and metastasis of cancer. This study aimed to explore the impact of miR-193a-3p on the migration and invasion of breast cancer stem

cells by targeting the tripartite motif-containing protein 14 (TRIM14). **Methods:** Human breast cancer cell T47D was randomly assigned into control group, NC mimics group (transfected with NC mimics), miR-193a-3p mimics group (transfected with miR-193a-3p mimics), miR-193a-3p mimics+pcDNA-NC group (transfected with miR-193a-3p mimics+pcDNA-NC) and miR-193a-3p mimics+pcDNA-TRIM14 group (transfected with miR-193a-3p mimics+pcDNA-TRIM14). Separation of stem cells using flow cytometry and detection of cell spheroidization ability were carried out. Cell counting kit-8 (CCK-8) experiment was used to detect cell proliferation. Transwell experiment was used to measure cell migration and invasion. Flow cytometry was used to detect cell apoptotic rate. Western blot was used to detect the expressions of cyclin D1, matrix metalloproteinase-2 (MMP-2), Bcl-2-associated X protein (Bax), and TRIM14 protein in cells. Dual luciferase assay was used to detect the interaction between miR-193a-3p and TRIM14. **Results:** T47D stem cells had the ability to form spheroids, and with increasing time, the spheroid volume of T47D stem cells gradually increased. Compared with the Control group and NC mimics group, the miR-193a-3p mimics group showed increased miR-193a-3p expression, apoptotic rate, and Bax protein expression ($P<0.05$), and decreased TRIM14 mRNA and protein expression, survival rate, clone number, migration number, invasion number, cyclin D1 and MMP-2 ($P<0.05$). Compared with the miR-193a-3p mimics group and the miR-193a-3p mimics+pcDNA NC group, the miR-193a-3p mimics+pcDNA-TRIM14 group showed decreased cell apoptosis rate and Bax protein ($P<0.05$), and increased TRIM14 mRNA and protein expression, survival rate, clone number, migration number, invasion number, cyclin D1 and MMP-2 ($P<0.05$). There were multiple binding sites between miR-193a-3p and TRIM14. Compared with the miR-NC+TRIM14-WT group, the miR-193a-3p mimics+TRIM14-WT group showed a prominent decrease in dual luciferase activity ($P<0.05$). **Conclusion:** MiR-193a-3p may inhibit the migration and invasion of breast cancer stem cells through inhibiting TRIM14.

[**Key words**] Breast cancer; Stem cells; miR-193a-3p; Tripartite motif-containing protein 14; Migration; Invasion

乳腺癌是女性常见的恶性肿瘤, 在全球范围内发病率和死亡率较高, 严重危害女性健康及生命安全^[1]。随着分子生物学的不断深入, 肿瘤干细胞相关研究逐渐被关注, 有研究^[2]表明, 肿瘤干细胞具有无限增殖、自我更新的特点, 并在乳腺癌的发生、发展中占据关键地位。目前, 临床采用化疗、放疗等方式治疗乳腺癌, 但这些相关治疗可能引起严重的不良反应, 导致生存质量恶化^[3]。因此, 探寻新型并有针对性的靶向药物用于乳腺癌的治疗, 对改善疗效具有重要作用。近期研究^[4]发现, 微小RNA (microRNA, miRNA) 在癌症的发生、发展中发挥重要作用, 同时, miRNA在肿瘤干细胞中的研究逐渐成为热点。有研究^[5]发现, miR-193a-3p可参与多种癌症的发展, 并且在乳腺癌中呈低表达, 可促进癌细胞的恶性生物学行为。三结构域蛋白14 (tripartite motif-containing protein 14, TRIM14) 属于三结构域家族蛋白, 对机体免疫反应具有一定调控作用, 与癌症转移、凋亡等密切相关^[6]。有研究^[7]发现, TRIM14受到miR-363-5p的调控, 在乳腺癌中表达上调, 可增强癌细胞迁移、侵袭的能力, 具有一定的促癌作用。但是miR-193a-3p是否通过调控TRIM14对乳腺癌干细胞迁移、侵袭产生影响尚未可知, 因此, 本研究通过分析miR-193a-3p在乳腺癌干细胞中的作用, 进一步分析其对肿瘤干细胞的作用机制, 为临床治疗提供参考依据。

1 材料和方法

1.1 材料

人乳腺癌细胞T47D (货号: WN-42519) 购自武汉华尔纳生物科技有限公司, DMEM培养基 (批号: XB1) 购自广东环凯生物科技有限公司, 抗体细胞周期蛋白D1 (cyclin D1)、基质金属蛋白酶-2 (matrix metalloproteinase-2, MMP-2)、Bcl-2相关X蛋白 (Bcl-2-associated X protein, Bax)、TRIM14、羊抗兔二抗、甘油醛-3-磷酸脱氢酶 (glyceraldehyde-3-phosphate dehydrogenase, GAPDH) (批号: ab134175、ab181286、ab32503、ab181595、ab205718、ab181602) 均购自英国Abcam公司。

1.2 方法

1.2.1 细胞培养和鉴定

T47D细胞置于无血清DMEM培养基 [含1%青链霉素, 20 ng/mL胰岛素样生长因子-1 (insulin-like growth factor-1, IGF-1), 20 ng/mL表皮生长因子 (epidermal growth factor, EGF), 10 ng/mL碱性成纤维细胞生长因子 (basic fibroblast growth factor, bFGF)] 中, 在37 °C、CO₂体积分数为5%、100%相对湿度的条件下培养。2~3 d添加1次生长因子, 并半量换液, 连续7 d^[8]。分别在第3、5、7天时, 通过显微镜观察细胞球的形成, 并以CD44⁺/CD24⁻/CD49f⁺作为标志物, 采用流式细胞仪分离T47D

干细胞。

1.2.2 细胞分组

将细胞随机分为Control组、NC mimics组（转染NC mimics）、miR-193a-3p mimics组（转染miR-193a-3p mimics）、miR-193a-3p mimics+pcDNA-NC组（转染miR-193a-3p mimics+pcDNA-NC）和miR-193a-3p mimics+pcDNA-TRIM14组（转染miR-193a-3p mimics+pcDNA-TRIM14）。将对数生长期的T47D干细胞按照分组进行处理后，轻摇混匀静置48 h后，更换培养基进行阳性细胞筛选，随后加入孔板中进行后续实验。

1.2.3 实时荧光定量聚合酶链反应（real-time fluorescence quantitative polymerase chain reaction, RTFQ-PCR）检测T47D干细胞中miR-193a-3p、TRIM14 mRNA表达

采用TRIzol试剂盒从细胞中提取总RNA，配制逆转录反应体系进行逆转录反应，获取cDNA，随后通过RTFQ-PCR反应体系进行扩增，并检测目标基因转录本，分别以U6、GAPDH为内参，反应条件为：95 ℃ 10 min，95 ℃ 20 s，60 ℃ 60 s，共36个循环。相对表达采用 $2^{-\Delta\Delta Ct}$ 法计算，引物序列见表1。

1.2.4 CCK-8和细胞克隆形成实验检测T47D干细胞增殖

取各组细胞接种在96孔板上，加入10 μ L CCK-8试剂，培养2 h；随后通过酶标仪读取450 nm处的吸光度（D）值并计算存活率。此外，在6孔板上接种T47D干细胞，并在37 ℃、CO₂体积分数为5%的条件下培养，每3 d更换1次培养基，14 d后，经磷酸缓冲盐溶液（phosphate-buffered

saline, PBS）洗涤，固定液固定，结晶紫染色，观察并拍照，通过显微镜统计克隆细胞数。

1.2.5 Transwell实验检测T47D干细胞迁移和侵袭

各组T47D干细胞分别加入不含有Matrigel基质胶（迁移实验）或含有Matrigel基质胶（侵袭实验）的transwell小室的上室中，将含10%胎牛血清（fetal bovine serum, FBS）的DMEM培养基加入下室并培育24 h，随后采用4%多聚甲醛溶液固定30 min，结晶紫染色，使用显微镜观察，分别统计迁移数和侵袭数。

1.2.6 流式细胞术检测T47D干细胞凋亡

将T47D干细胞接种于6孔板上，用冷PBS洗涤，并加入结合缓冲液重悬。将膜联蛋白V-FITC和PI加入到远离光照的环境中温育15 min。再次加入结合缓冲液重悬，采用流式细胞仪检测60 min内的细胞凋亡率。

1.2.7 蛋白质印迹法（Western blot）检测蛋白质表达

采用RIPA裂解液提取T47D干细胞总蛋白，采用BCA试剂盒测定蛋白质浓度，加入缓冲液并煮沸至变性，配制10% SDS-PAGE凝胶进行电泳分离，转移到聚偏二氟乙烯（polyvinylidene fluoride, PVDF）膜上，用5%脱脂奶粉封闭1 h，立即转移到稀释后的一抗cyclin D1（1:1 000）、MMP-2（1:1 000）、Bax（1:1 000）、TRIM14（1:20 000）中，4 ℃下温育12 h。将膜洗涤并与二抗（1:2 000）温育1 h（常温），采用ECL试剂盒检测蛋白信号，以GAPDH（1:10 000）为内参，采用Image J软件对ECL信号进行密度分析。

表1 引物序列

Tab. 1 Primer sequence

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
miR-193a-3p	TTTGAGGGATATTAGAGTTT	AACCTAAAAACAACCTAACC
TRIM14	GGATTTGTGTCTCCGTCTG	TCTGTCTGCCTGGTATTCTG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
GAPDH	CACCCACTCCTCCACCTTTG	CCACCACCCTGTTGCTGTAG

1.2.8 双萤光素酶报告基因实验

将各组转染后的T47D干细胞接种在48孔板中并在37 ℃、CO₂体积分数为5%的条件下培养。当细胞融合为1层时进行双萤光素酶报告基因检测，将TRIM14-WT、TRIM14-MUT分别与miR-NC、miR-193a-3p mimics共转染细胞，48 h

后，使用双萤光素酶报告基因检测系统测定萤光素酶活性。

1.3 统计学处理

采用SPSS 25.0软件对数据进行统计学分析。计量资料采用Shapiro-Wilk检验进行正态性检验，采用Levene检验进行方差齐性检验。若符合正态

分布且方差齐, 则以 $\bar{x} \pm s$ 表示, 多组比较采用单因素方差分析, 若差异有统计学意义, 则进一步使用SNK法进行两两比较; 若不符合正态分布或方差不齐, 则采用非参数检验。所有显著性检验均为双侧检验, $P < 0.05$ 为差异有统计学意义。

2 结 果

2.1 乳腺癌T47D干细胞富集和分离

经观察, T47D干细胞具有成球能力, 并随着时间推移增强, T47D干细胞球体积逐渐增大(图1)。

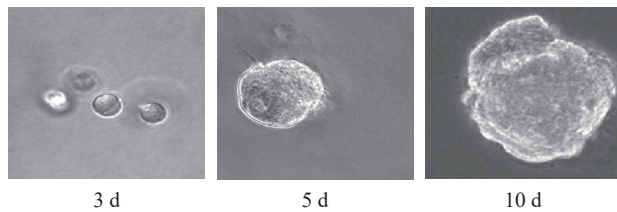


图1 T47D干细胞富集图($\times 400$)

Fig. 1 T47D stem cell enrichment diagram ($\times 400$)

2.2 过表达miR-193a-3p对T47D干细胞中miR-193a-3p、TRIM14 mRNA表达的影响

与Control组、NC mimics组相比, miR-193a-3p mimics组细胞miR-193a-3p表达升高($P < 0.05$), TRIM14 mRNA表达降低($P < 0.05$)。与miR-193a-3p mimics组、miR-

193a-3p mimics+pcDNA-NC组相比, miR-193a-3p mimics+pcDNA-TRIM14组细胞TRIM14 mRNA表达升高($P < 0.05$, 表2)。

2.3 过表达miR-193a-3p对T47D干细胞增殖的影响

与Control组、NC mimics组相比, miR-193a-3p mimics组细胞存活率、克隆数降低($P < 0.05$)。与miR-193a-3p mimics组、miR-193a-3p mimics+pcDNA-NC组相比, miR-193a-3p mimics+pcDNA-TRIM14组细胞存活率、克隆数升高($P < 0.05$, 图2, 表3)。

2.4 过表达miR-193a-3p对T47D干细胞迁移的影响

与Control组、NC mimics组相比, miR-193a-3p mimics组细胞迁移数降低($P < 0.05$)。与miR-193a-3p mimics组、miR-193a-3p mimics+pcDNA-NC组相比, miR-193a-3p mimics+pcDNA-TRIM14组细胞迁移数升高($P < 0.05$, 图3, 表4)。

2.5 过表达miR-193a-3p对T47D干细胞侵袭的影响

与Control组、NC mimics组相比, miR-193a-3p mimics组细胞侵袭数降低($P < 0.05$)。与miR-193a-3p mimics组、miR-193a-3p mimics+pcDNA-NC组相比, miR-193a-3p mimics+pcDNA-TRIM14组细胞侵袭数升高($P < 0.05$, 图4, 表5)。

表2 过表达miR-193a-3p对T47D干细胞中miR-193a-3p、TRIM14 mRNA表达的影响

Tab. 2 The effect of overexpression of miR-193a-3p on miR-193a-3p and TRIM14 mRNA expression in T47D stem cells

Group	miR-193a-3p	TRIM14
Control group	1.00 \pm 0.12	1.00 \pm 0.11
NC mimics group	0.98 \pm 0.10	0.99 \pm 0.10
miR-193a-3p mimics group	1.75 \pm 0.22 ^{ab}	0.37 \pm 0.05 ^{ab}
miR-193a-3p mimics+pcDNA-NC group	1.72 \pm 0.19	0.43 \pm 0.06
miR-193a-3p mimics+pcDNA-TRIM14 group	1.73 \pm 0.21	0.86 \pm 0.09 ^{cd}
F value	43.437	77.893
P value	0.000	0.000

^a: Compared with the Control group; ^b: Compared with the NC mimics group; ^c: Compared with the miR-193a-3p mimics group; ^d: Compared with the miR-193a-3p mimics+pcDNA NC group. The P value of SNK test is used.

2.6 过表达miR-193a-3p对T47D干细胞凋亡的影响

与Control组、NC mimics组相比, miR-193a-3p mimics组细胞凋亡率升高($P < 0.05$)。与miR-193a-3p mimics组、miR-193a-3p mimics+pcDNA-NC组相比, miR-193a-

3p mimics+pcDNA-TRIM14组细胞凋亡率降低($P < 0.05$, 图5, 表6)。

2.7 过表达miR-193a-3p对T47D干细胞中cyclin D1、MMP-2、Bax、TRIM14蛋白表达的影响

与Control组、NC mimics组相比, miR-193a-3p mimics组cyclin D1、MMP-2、TRIM14

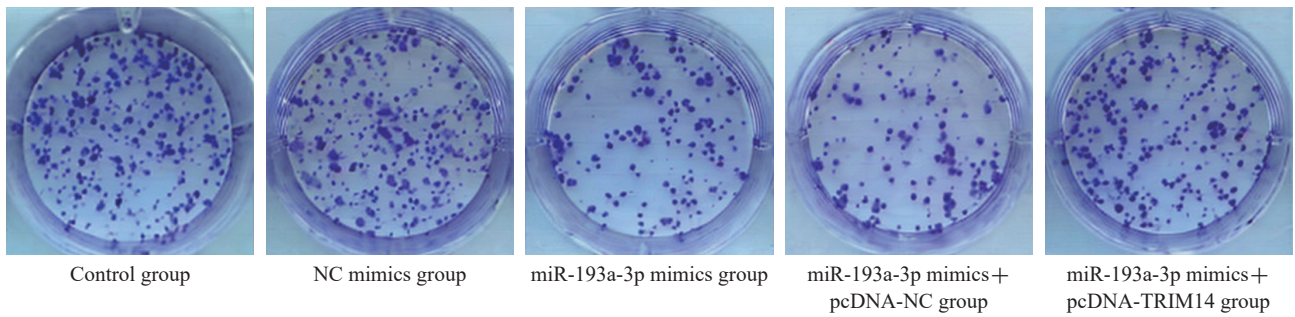


图2 克隆形成实验检测T47D干细胞的增殖

Fig. 2 Cloning formation experiment for detecting the proliferation of T47D stem cells

表3 过表达miR-193a-3p对T47D干细胞增殖的影响

Tab. 3 The effect of overexpression of miR-193a-3p on the proliferation of T47D stem cells

Group	survival rate(%)	Number of clones (pieces)
Control group	92.38 ± 0.57	128.67 ± 13.43
NC mimics group	87.52 ± 9.25	127.49 ± 13.06
miR-193a-3p mimics group	52.84 ± 5.69 ^{ab}	67.42 ± 7.38 ^{ab}
miR-193a-3p mimics + pcDNA-NC group	51.04 ± 5.22	68.94 ± 7.44
miR-193a-3p mimics + pcDNA-TRIM14 group	85.22 ± 9.03 ^{cd}	115.82 ± 12.34 ^{cd}
<i>F</i> value	53.552	46.983
<i>P</i> value	0.000	0.000

^a: Compared with the Control group; ^b: Compared with the NC mimics group; ^c: Compared with the miR-193a-3p mimics group; ^d: Compared with the miR-193a-3p mimics + pcDNA NC group. The *P* value of SNK test is used.

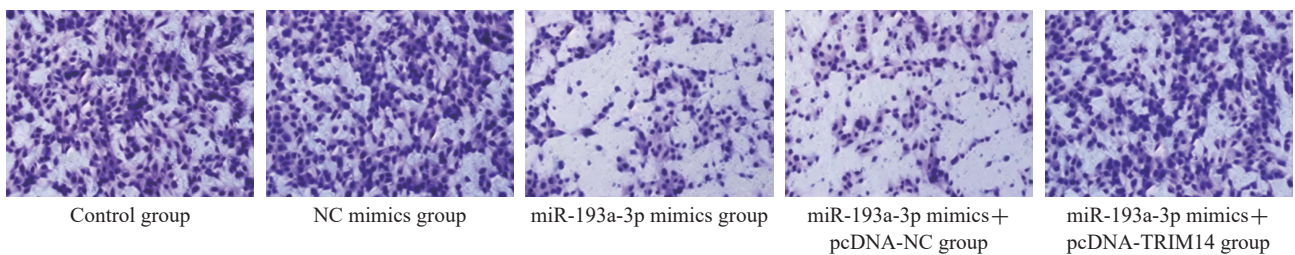


图3 Transwell实验检测T47D细胞的迁移 (×200)

Fig. 3 Transwell experiment detects the migration of T47D cells (×200)

表4 过表达miR-193a-3p对T47D干细胞迁移的影响

Tab. 4 The effect of overexpression of miR-193a-3p on the migration of T47D stem cells

Group	Number of migrations (pieces)
Control group	135.62 ± 14.92
NC mimics group	136.28 ± 14.76
miR-193a-3p mimics group	62.54 ± 7.38 ^{ab}
miR-193a-3p mimics + pcDNA-NC group	64.28 ± 7.14
miR-193a-3p mimics + pcDNA-TRIM14 group	129.63 ± 13.57 ^{cd}
<i>F</i> value	61.448
<i>P</i> value	0.000

^a: Compared with the Control group; ^b: Compared with the NC mimics group; ^c: Compared with the miR-193a-3p mimics group; ^d: Compared with the miR-193a-3p mimics + pcDNA NC group. The *P* value of SNK test is used.

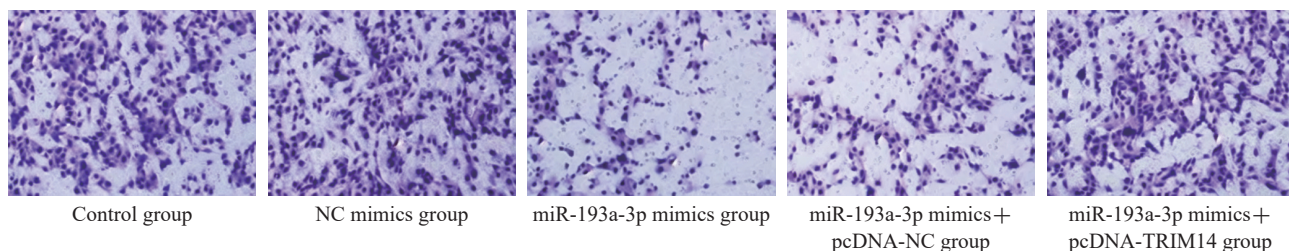
图4 Transwell实验检测T47D干细胞的侵袭 ($\times 200$)Fig. 4 Transwell experiment detects the invasion of T47D stem cells ($\times 200$)

表5 过表达miR-193a-3p对T47D干细胞侵袭的影响

Tab. 5 The effect of overexpression of miR-193a-3p on the invasion of T47D stem cells

Group	Number of invasions (pieces)
Control group	109.63 \pm 18.52
NC mimics group	105.62 \pm 11.27
miR-193a-3p mimics group	57.86 \pm 7.34 ^{ab}
miR-193a-3p mimics+pcDNA-NC group	60.24 \pm 7.59
miR-193a-3p mimics+pcDNA-TRIM14 group	95.38 \pm 10.05 ^{cd}
<i>F</i> value	27.324
<i>P</i> value	0.000

($\bar{x} \pm s, n=6$)

^a: Compared with the Control group; ^b: Compared with the NC mimics group; ^c: Compared with the miR-193a-3p mimics group; ^d: Compared with the miR-193a-3p mimics+pcDNA NC group. The *P* value of SNK test is used.

蛋白表达降低 ($P < 0.05$), Bax蛋白表达升高 ($P < 0.05$)。与miR-193a-3p mimics组、miR-193a-3p mimics+pcDNA-NC组相比, miR-193a-3p mimics+pcDNA-TRIM14组cyclin D1、MMP-2、TRIM14蛋白表达升高 ($P < 0.05$), Bax蛋白表达降低 ($P < 0.05$, 图6, 表7)。

2.8 双荧光素酶报告实验检测miR-193a-3p与TRIM14的靶向关系

通过StarBase数据库可知, miR-193a-3p与TRIM14之间存在多个结合位点。与miR-NC+TRIM14-WT组相比, miR-193a-3p mimics+TRIM14-WT组双荧光素酶活性明显降低 ($P < 0.05$, 表8)。

3 讨论

据报道, 乳腺癌是引发女性死亡的一大病因, 乳腺癌侵袭性高, 易发生转移, 导致患者预后不良, 严重威胁患者的生命安全^[9]。乳腺癌的发病因素较为复杂, 临床多采用手术切除或化疗等治疗方式, 近年来, 内分泌治疗及靶向治疗的进展使其在乳腺癌个体化综合治疗中的地位日益突出^[9-10]。但晚期患者对药物的耐药性及不良反应导致疗效无法达到预期, 临床仍缺乏新型有效的靶向药物^[11]。因此, 本研究旨在探究潜在治疗靶点, 为开发新型靶向药物及临床靶向治疗

提供参考依据。

miRNA作为单链非编码RNA, 可通过参与mRNA的转录、翻译, 进而调控靶基因的表达, 在多种生物学过程中发挥重要作用^[12]。有研究^[13]表明, miR-193a-3p在膀胱膜癌组织和细胞中呈低表达, 通过上调miR-193a-3p可发挥抑制癌细胞增殖、迁移的作用。Yang等^[14]研究发现, 在非小细胞肺癌中, miR-193a-3p受到circ_0020123的调控, 通过激活miR-193a-3p表达可介导癌细胞自噬、糖酵解等过程, 进而发挥抑癌作用。Xie等^[15]研究发现, 在乳腺癌中miR-193a-3p表达显著降低, 可能受到小核仁RNA宿主基因14 (Small nucleolar RNA host gene 14, SNHG14) 负调控, 与癌细胞增殖、侵袭等密切相关。相较于上述分析, 本研究创新性地通过检测肿瘤干细胞的恶性生物学行为发现, 过表达miR-193a-3p可抑制T47D干细胞存活率, 使克隆数、迁移数、侵袭数降低, 凋亡率升高, 结合上述分析可推测miR-193a-3p可能受到circ_0020123的调控作用, 通过参与糖酵解过程及癌细胞自噬, 进而降低乳腺癌干细胞增殖、迁移及侵袭能力, 促进细胞凋亡, 进而起到抑制癌症发展的作用。

TRIM14作为泛素化连接酶, 被鉴定出存在多种类型的多聚泛素化链, 能够促进肿瘤转

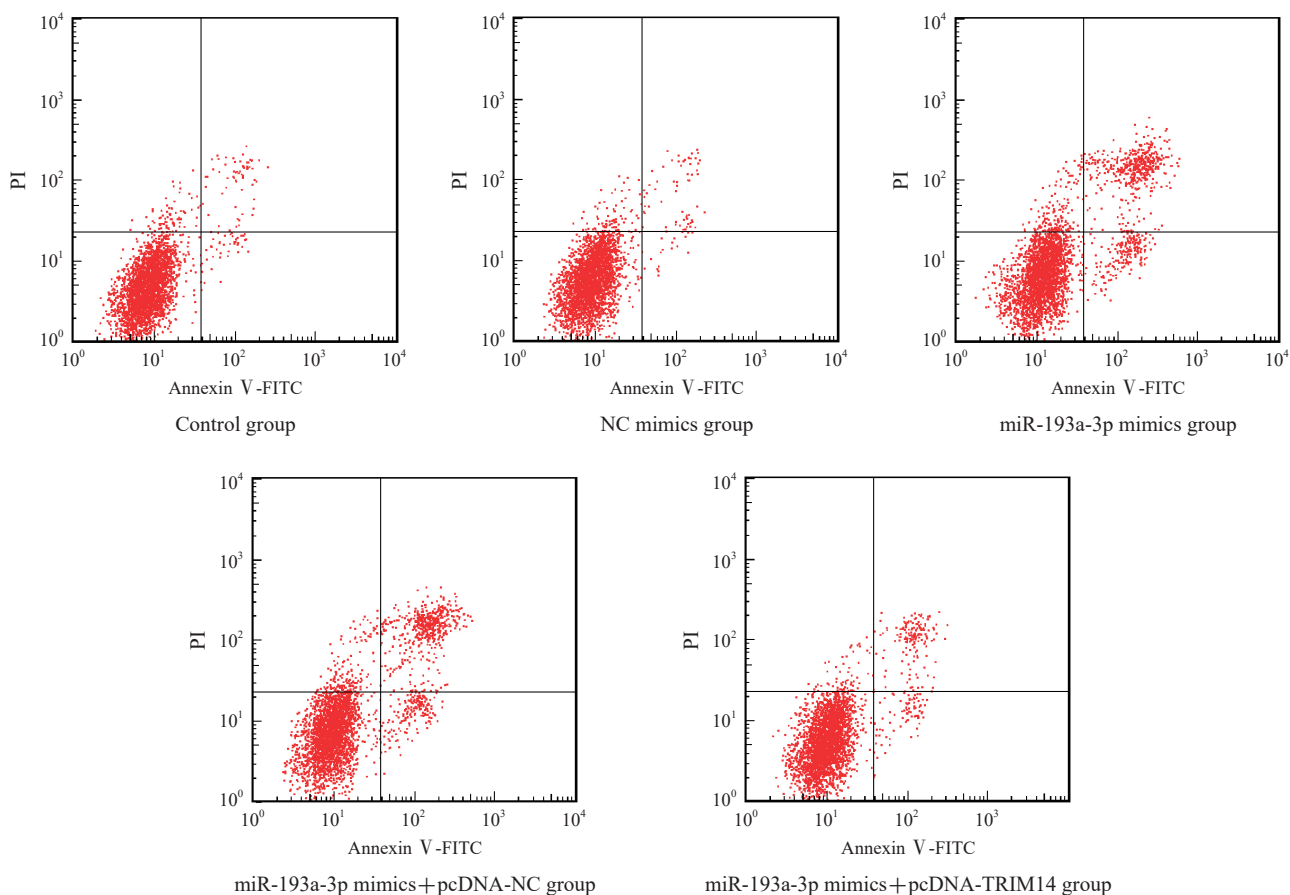


图5 流式细胞术检测T47D干细胞凋亡

Fig. 5 Flow cytometry detection of apoptosis in T47D stem cells

表6 过表达miR-193a-3p对T47D干细胞凋亡率的影响

Tab. 6 The effect of overexpression of miR-193a-3p on apoptosis rate of T47D stem cells

Group	Apoptosis rate (%)
Control group	1.69 ± 0.25
NC mimics group	1.65 ± 0.23
miR-193a-3p mimics group	28.06 ± 3.07ab
miR-193a-3p mimics + pcDNA-NC group	29.67 ± 3.13
miR-193a-3p mimics + pcDNA-TRIM14 group	10.38 ± 1.56cd
F value	261.810
P value	0.000

($\bar{x} \pm s, n=6$)

^a: Compared with the Control group; ^b: Compared with the NC mimics group; ^c: Compared with the miR-193a-3p mimics group; ^d: Compared with the miR-193a-3p mimics+pcDNA NC group. The P value of SNK test is used.

移、血管生成等^[16]。Chen等^[17]研究发现，在结肠癌细胞中，TRIM14表达明显升高，可通过促进细胞增殖、侵袭及上皮-间充质转化（epithelial-mesenchymal transition, EMT）等过程，促进癌症的进展。Xiao等^[18]研究发现，TRIM14在胃癌组织和细胞中均显著上调，过表达TRIM14可调控细胞自噬，并抑制细胞凋亡，进一步证实抑制TRIM14表达可缓解对细胞

自噬、凋亡的抑制作用。Xie等^[19]研究指出，TRIM14受到miR-1296-5p的靶向调控，通过抑制乳腺癌细胞中TRIM14表达，可抑制细胞迁移、侵袭能力，并促进细胞凋亡，可作为潜在靶点用于临床治疗相关研究中。基于上述分析发现，既往研究并未对miR-193a-3p与TRIM14的调控关系进行深入探究，因此，本研究通过分析发现，miR-193a-3p与TRIM14之间存在多个结合位点，

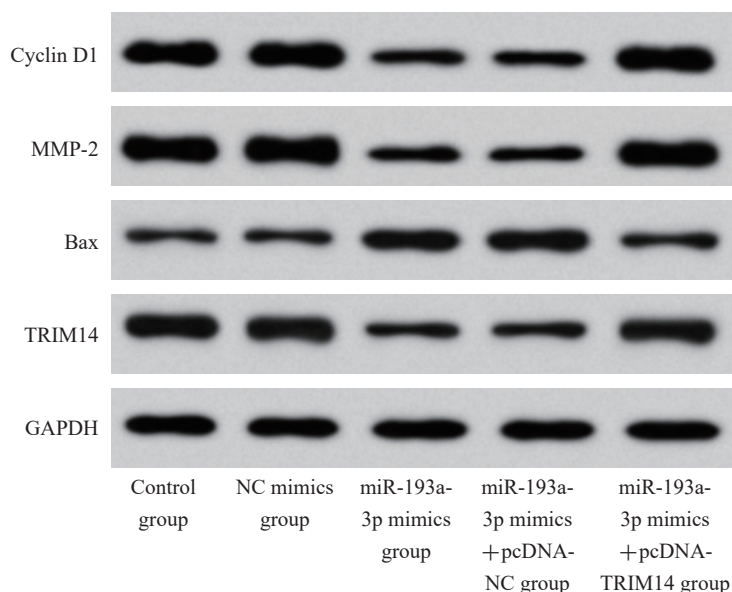


图6 WB检测T47D干细胞中cyclin D1、MMP-2、Bax、TRIM14蛋白表达

Fig. 6 WB detection of cyclin D1, MMP-2, Bax, TRIM14 protein expression in T47D stem cells

表7 过表达miR-193a-3p对T47D干细胞中cyclin D1、MMP-2、Bax、TRIM14蛋白表达的影响

Tab. 7 The effect of overexpression of miR-193a-3p on the expression of cyclin D1, MMP-2, Bax, and TRIM14 proteins in T47D stem cells

Group	($\bar{x} \pm s, n=6$)			
	Cyclin D1	MMP-2	Bax	TRIM14
Control group	1.59 ± 0.23	1.72 ± 0.28	0.42 ± 0.06	1.48 ± 0.21
NC mimics group	1.62 ± 0.24	1.68 ± 0.26	0.43 ± 0.07	1.52 ± 0.24
miR-193a-3p mimics group	0.49 ± 0.06 ^{ab}	0.55 ± 0.08 ^{ab}	1.37 ± 0.17 ^{ab}	0.56 ± 0.07 ^{ab}
miR-193a-3p mimics + pcDNA-NC group	0.53 ± 0.07	0.57 ± 0.09	1.41 ± 0.19	0.52 ± 0.05
miR-193a-3p mimics + pcDNA-TRIM14 group	1.54 ± 0.21 ^{cd}	1.63 ± 0.24 ^{cd}	0.46 ± 0.08 ^{cd}	1.43 ± 0.18 ^{cd}
<i>F</i> value	63.758	51.602	102.529	56.061
<i>P</i> value	0.000	0.000	0.000	0.000

^a: Compared with the Control group; ^b: Compared with the NC mimics group; ^c: Compared with the miR-193a-3p mimics group; ^d: Compared with the miR-193a-3p mimics+pcDNA NC group. The *P* value of SNK test is used.

表8 双萤光素酶活性比较

Tab. 8 Comparison of dual luciferase activity

Group	($\bar{x} \pm s, n=6$)	
	Dual luciferase activity	<i>P</i> value
miR-NC + TRIM14-WT group	1.00 ± 0.22	—
miR-193a-3p mimics + TRIM14-WT group	0.41 ± 0.16 ^a	0.000
miR-NC + TRIM14-MUT group	1.03 ± 0.23	0.822
miR-193a-3p mimics + TRIM14-MUT group	1.06 ± 0.25	0.668

All were compared with the miR-NC+TRIM14-WT group.

并且过表达miR-193a-3p后, TRIM14表达明显下调, 说明miR-193a-3p可靶向负调控TRIM14表达。为进一步验证miR-193a-3p对TRIM14的靶向调控作用, 本研究在过表达miR-193a-3p的基础上过表达TRIM14, 结果显示, 过表达TRIM14可部分逆转过表达miR-193a-3p对T47D干细胞增

殖、迁移、侵袭能力的抑制作用以及对凋亡的促进作用, 说明miR-193a-3p对乳腺癌干细胞迁移和侵袭的影响, 可能是通过抑制TRIM14表达而实现的。

综上所述, miR-193a-3p可能通过抑制TRIM14表达, 进而发挥抑制乳腺癌干细胞迁移

和侵袭的作用。同时本研究也存在一些局限性。首先, 缺乏动物模型及临床试验的相关探究, 导致本研究结果的可靠性不足。其次, 本研究仅通过观察肿瘤干细胞的恶性生物学行为分析miR-193a-3p的调控作用, 但是并未从其病理学机制方面进行深入探究。最后, 本研究仅分析miR-193a-3p调控TRIM14对该疾病的调控作用, 但由于癌症发展机制的复杂性, 还存在其他通路调控乳腺癌干细胞的恶性生物学行为, 并且缺乏TRIM14下游基因调控机制的相关分析。因此, 后续将补充动物实验及临床试验, 进一步深入探究相关机制。

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