doi: 10.3978/j.issn.2095-6959.2022.09.002
View this article at: https://dx.doi.org/10.3978/j.issn.2095-6959.2022.09.002

沉默 lncRNA THAP9-AS1 对乳腺癌细胞增殖、凋亡、迁移及侵袭的影响

王枝红[1]，刘红[1]，何楠[1]，杨海松[2]

(1. 贵州省肿瘤医院乳腺外科，贵阳 550003；2. 贵州医科大学附属医院乳腺外科，贵阳 550004)

[摘　要]　**目的：**探讨沉默长链非编码RNA THAP9反义RNA1(lncRNA THAP9-AS1)对乳腺癌细胞增殖、凋亡、迁移及侵袭的影响。**方法：**选取贵州省肿瘤医院2017年6月至2020年6月25例乳腺癌患者的癌组织及癌旁组织，采用实时荧光定量PCR(real-time quantitative polymerase chain reaction，RT-qPCR)检测lncRNA THAP9-AS1和miR-505-3p表达水平。将MDA-MB-231细胞随机分为si-THAP9-AS1组、si-NC组、miR-505-3p组、miR-NC组、si-THAP9-AS1+anti-miR-NC组、si-THAP9-AS1+miR-505-3p inhibitor组。采用四甲基偶氮唑盐比色(methyl thiazolyl tetrazolium，MTT)法检测细胞活性，平板克隆实验检测细胞集落形成数，流式细胞术实验检测细胞凋亡，划痕实验和Transwell检测细胞迁移及侵袭，双荧光素酶报告实验检测lncRNA THAP9-AS1和miR-505-3p的靶向关系。**结果：**与癌旁组织相比，乳腺癌组织中lncRNA THAP9-AS1的表达水平升高，miR-505-3p的表达水平降低($P<0.05$)。沉默lncRNA THAP9-AS1或过表达miR-505-3p可降低细胞活性及迁移距离，减少集落形成数和侵袭细胞数，增加细胞凋亡率，增加E-cadherin蛋白表达水平升高，并降低N-cadherin蛋白表达水平($P<0.05$)。LncRNA THAP9-AS1靶向调控miR-505-3p；抑制miR-505-3p逆转了沉默lncRNA THAP9-AS1对MDA-MB-231增殖、迁移及侵袭的影响。**结论：**沉默lncRNA THAP9-AS1可通过增加miR-505-3p抑制乳腺癌细胞增殖、迁移及侵袭，并抑制凋亡。

[关键词]　THAP9-AS1；miR-505-3p；乳腺癌；增殖；迁移；侵袭

Effect of silencing lncRNA THAP9-AS1 on the proliferation, apoptosis, migration and invasion of breast cancer cells

WANG Zhihong[1], LIU Hong[1], HE Nan[1], YANG Haisong[2]

(1. Department of Breast Surgery, Guizhou Cancer Hospital, Guiyang 550003; 2. Department of Breast Surgery, Affiliated Hospital of Guizhou Medical University, Guiyang 550004, China)

Abstract　**Objective:** To explore the effect of silencing lncRNA THAP9-AS1 on the proliferation, apoptosis, migration and invasion of breast cancer cells. **Methods:** The cancer tissues and adjacent tissues were collected from 25 breast cancer patients at Guizhou Cancer Hospital from June 2017 to June 2020. The expression levels of lncRNA THAP9-AS1 and miR-505-3p were detected by real-time quantitative polymerase chain reaction (RT-qPCR). MDA-MB-231

收稿日期 (**Date of reception**)：2022–02–24
通信作者 (**Corresponding author**)：杨海松，Email: Yanghaisong609@sina.com

cells were randomly divided into the si-THAP9-AS1 group, the si-NC group, the miR-505-3p group, the miR-NC group, the si-THAP9-AS1 + anti-miR-NC group, and the si-THAP9-AS1 + miR-505-3p inhibitor group. Cell activity was detected by methyl thiazolyl tetrazolium (MTT) method; cell colony formation was detected by plate cloning assay; cell apoptosis was detected by flow cytometry assay; cell migration and invasion were detected by scratching assay and Transwell assay; dual luciferase reporting assay was used to detect the targeting relationship between lncRNA THAP9-AS1 and miR-505-3p. **Results:** The expression level of lncRNA THAP9-AS1 was increased in breast cancer tissues compared with adjacent tissues, while the expression level of miR-505-3p was decreased (P<0.05). Silencing lncRNA THAP9-AS1 or miR-505-3p overexpression could inhibit cell viability and migration, decrease the number of colonies and invaded cells, increase the rate of apoptosis and the protein level of E-cadherin, and reduce the protein level of N-cadherin (P<0.05). Moreover, lncRNA THAP9-AS1 targeted miR-505-3p, and inhibition of miR-505-3p reversed the effects of lncRNA THAP9-AS1 silence on the proliferation, migration and invasion of MDA-MB-231 cells. **Conclusion:** Silencing lncRNA THAP9-AS1 can inhibit the proliferation, migration and invasion and promote apoptosis of breast cancer cells by up-regulating miR-505-3p.

Keywords THAP9-AS1; miR-505-3p; breast cancer; proliferation; migration; invasion

乳腺癌是全世界成年女性中最常见的癌症，随着对乳腺癌发病机制认识的加深，分子靶向治疗成为乳腺癌治疗的新方式[1-2]。研究[3]表明：lncRNA在乳腺癌的进展和转移中具有重要作用，有望成为乳腺癌诊断的预后生物标志物。LncRNA BCRT1通过靶向miR-1303/PTBP3轴促进乳腺癌的生长和转移[4]。LncRNA DLEU1在乳腺癌中高表达，通过靶向miR-300/RAB22A促进乳腺癌细胞及迁移侵袭[5]。研究[6]发现：在胃癌组织中，lncRNA THAP9-AS1高表达，上调lncRNA THAP9-AS1可促进胃癌细胞增殖和迁移。而lncRNA THAP9-AS1对乳腺癌细胞的研究尚不清楚。LncRNA DLX6-AS1通过miR-505-3p/RUNX2轴促进乳腺癌的增殖和侵袭[7]。骨转移性前列腺癌组织中miR-505-3p明显下调，miR-505-3p的上调抑制了前列腺癌细胞的侵袭和迁移能力[8]。然而lncRNA THAP9-AS1与miR-505-3p调控作用对乳腺癌的进展尚不清楚。因此，本研究拟探究lncRNA THAP9-AS1通过调控miR-505-3p对乳腺癌细胞增殖、凋亡、迁移及侵袭的影响。

1 材料与方法

1.1 材料

1.1.1 标本来源

选取2017年6月至2020年6月贵州省肿瘤医院收治的25例乳腺癌患者的癌组织及癌旁组织(距离组织>3 cm)，患者年龄39~62(45.54±6.88)岁；淋巴结转移13例，无淋巴结转移12例，且术前均未进行过放疗、化疗。本研究经贵州省肿瘤医院医学伦理委员会批准同意(审批号：201705-16)，患者均签署知情同意书。

1.1.2 主要试剂

MDA-MB-231细胞株购自上海奥陆生物科技有限公司；TRIzol试剂购自美国Genmed公司；实时荧光定量PCR试剂盒购自天根生化科技(北京)有限公司；RPMI-1640培养基购自上海雅吉生物科技有限公司；总蛋白提取试剂盒购自大连美仑生物技术有限公司；四甲基偶氮唑盐比色(methyl thiazolyl tetrazolium，MTT)试剂盒购自上海晶抗生物工程有限公司；双荧光素酶报告基因检测试剂盒购自美国BioAssay Systems公司；Transwell小室、Matrigel购自美国Corning公司。

1.2 方法

1.2.1 细胞处理与分组

使用RPMI-1640培养基培养MDA-MB-231细胞，将si-NC、si-THAP9-AS1、miR-NC、miR-505-3p mimic转染至MDA-MB-231细胞中，记为si-NC组、si-THAP9-AS1组、miR-505-3p组、miR-NC组；将si-THAP9-AS1分别与anti-miR-NC、miR-505-3p inhibitor转染至细胞，记为si-THAP9-AS1+anti-miR-NC组、si-THAP9-AS1+miR-505-3p inhibitor组。细胞转染6 h后更换细胞培养基，然后按照Lipofectamine 3000试剂盒操作进行。

1.2.2 实时荧光定量 PCR 法检测 lncRNA THAP9-AS1 和 miR-505-3p 的表达水平

提取乳腺癌组织、癌旁组织及细胞总RNA，合

成cDNA后进行PCR，相对表达量用$2^{-\Delta\Delta Ct}$法计算。以GAPDH和U6为内参，lncRNA THAP9-AS1正向引物序列为5'-ACTGGCTGCTATGGAAAAAGT-3'，反向引物序列为5'-TCCCTTCCCCTCCTGTCTGC-3'；GAPDH正向引物序列为5'-GCACCGTCAAGGCTGAGAAC-3'，反向引物序列为5'-ATGGTGGTGAAGACGCCAGT-3'。miR-505-3p正向引物序列为5'-CTACGTGGGTCACCCCCTC-3'，反向引物序列为5'-CCAAAGGAGACCTCGTAGT-3'；U6正向引物序列为5'-CTCGCTTCGGCAGCACA-3'，反向引物序列为5'-AACGCTTCACGAATTTGCGT-3'。

1.2.3 MTT 法检测细胞增殖活性

各组细胞培养24 h、48 h、72 h时，加MTT溶液，继续孵育4 h后，加入DMSO溶液，用酶标仪检测490 nm处吸光度(OD)值。

1.2.4 平板克隆实验检测细胞集落形成数

将各组细胞接种于6孔板中，培养2周后看到克隆形成后，用吉姆萨染色30 min，在低倍光学显微镜下计数>50个细胞。

1.2.5 划痕实验检测细胞迁移距离

取各组对数生长期细胞，在6孔培养板上接种培养(每孔中接种2×10^5个/mL)，用10 μL枪头在细胞表面划痕，换液后继续培养，在0 h和24 h时分别拍照观察，计算划痕距离。

1.2.6 Transwell 检测细胞侵袭

在Transwell小室上室加入Matrigel胶，十字摇匀，凝固后加100 μL无血清悬浮的细胞，培养24 h，用结晶紫染色30 min，PBS漂洗并干燥后，用光学显微镜)随机选择5个视野拍照并计数。

1.2.7 流式细胞术检测细胞凋亡

取各组细胞使用预冷PBS冲洗细胞，然后将细胞沉淀物加结合缓冲液中，根据凋亡试剂盒说明书步骤，加Annexin V-FITC与PI试剂，避光反应15 min，上流式细胞仪检测细胞凋亡情况。

1.2.8 蛋白质印迹法检测蛋白表达

提取细胞总蛋白质，进行SDS-PAGE，转膜，用5%的脱脂奶粉封闭；加入E-cadherin、N-cadherin(稀释1:800)、GAPDH(稀释1:2 500)一抗4 ℃过夜，加入二抗(稀释1:5 000)室温培养2 h，加ECL化学试剂用于显影，Image-Pro Plus分析蛋白质条带的灰度水平，计算蛋白相对表达水平。

1.2.9 双荧光素酶报告实验

构建lncRNA THAP9-AS1的野生型和突变型荧光素酶载体(wt-THAP9-AS1、mut-THAP9-AS1)。收集MDA-MB-231细胞并接种24孔板上，细胞融合度达到80%，分别与miR-NC和miR-505-3p共转染至细胞中，转染48 h后收集细胞，按照试剂盒说明检测荧光素酶活性。

1.3 统计学处理

采用SPSS 20.0统计学软件进行数据分析，计量资料以均数±标准差($\bar{x}\pm s$)表示，两组比较行t检验。以$P<0.05$为差异有统计学意义。

2 结果

2.1 LncRNA THAP9-AS1 和 miR-505-3p 在乳腺癌组织中的表达

与癌旁组织相比，lncRNA THAP9-AS1的表达在乳腺癌组织中升高，miR-505-3p的表达水平下降($P<0.05$，图1)。

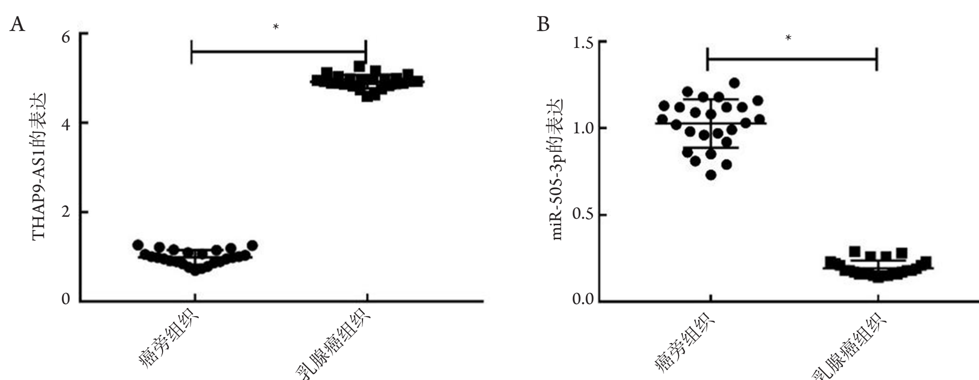

图1 LncRNA THAP9-AS1和miR-505-3p的表达

Figure 1 Expression of lncRNA THAP9-AS1 and miR-505-3p

(A)LncRNA THAP9-AS1的表达；(B)MiR-505-3p的表达。与癌旁组织比较，*$P<0.05$。

(A) Expression of lncRNA THAP9-AS1; (B) Expression of miR-505-3p. Compared with paracancer tissue, *$P<0.05$.

2.2 沉默lncRNA THAP9-AS1对MDA-MB-231增殖、凋亡、迁移及侵袭的影响

与si-NC组相比，si-THAP9-AS1组THAP9-AS1表达水平、细胞活性、迁移距离降低，miR-505-3p的表达水平升高，集落形成数及侵袭细胞数减少，凋亡率、E-cadherin表达水平升高，N-cadherin表达水平降低(P<0.05；图2、3，表1)。

2.3 LncRNA THAP9-AS1和miR-505-3p靶向关系验证

Starbase预测lncRNA THAP9-AS1和miR-505-3p存在相互结合序列(图4)。与miR-NC相比，miR-505-3p组中wt-THAP9-AS1细胞荧光素酶活性降低(P<0.05)；而mut-THAP9-AS1与miR-505-3p共转染后的细胞荧光素酶活性相较于与miR-NC共转染的细胞无显著差异(P>0.05，表2)。

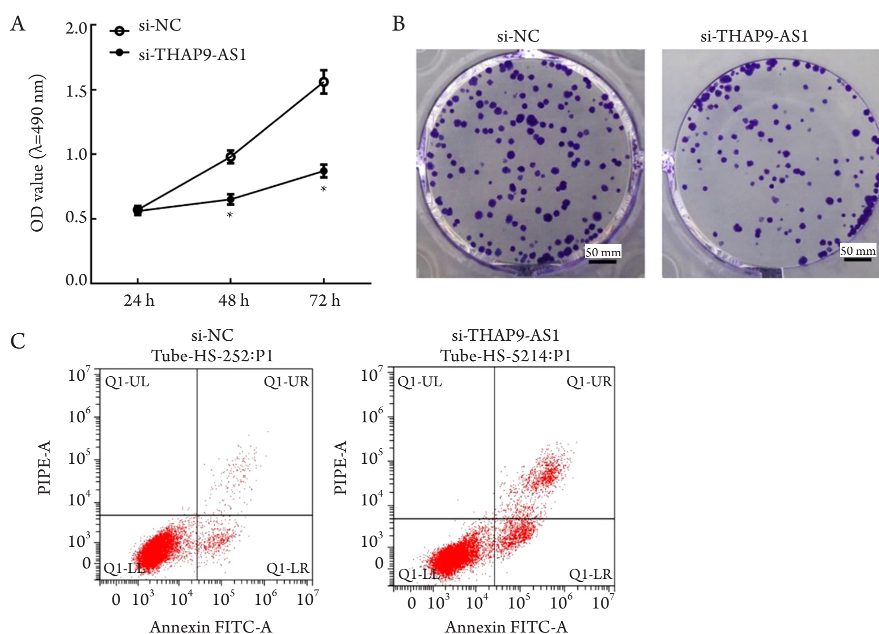

图2 沉默lncRNA THAP9-AS1对MDA-MB-231细胞活性和凋亡的影响

Figure 2 Effect of silencing lncRNA THAP9-AS1 on the activity and apoptosis of MDA-MB-231 cells

(A)沉默lncRNA THAP9-AS1对MDA-MB-231细胞活性的影响；(B)沉默lncRNA THAP9-AS1对MDA-MB-231细胞克隆形成的影响(吉姆萨染色，×50)；(C)沉默lncRNA THAP9-AS1对MDA-MB-231细胞凋亡的影响。与si-NC组相比，*P<0.05。

(A) Effect of silencing lncRNA THAP9-AS1 on the activity of MDA-MB-231 cells; (B) Effect of silencing lncRNA THAP9-AS1 on the clone formation of MDA-MB-231 cells (Giemsa staining, ×50); (C) Effect of silencing lncRNA THAP9-AS1 on the apoptosis of MDA-MB-231 cells. Compared with si-NC group, *P<0.05.

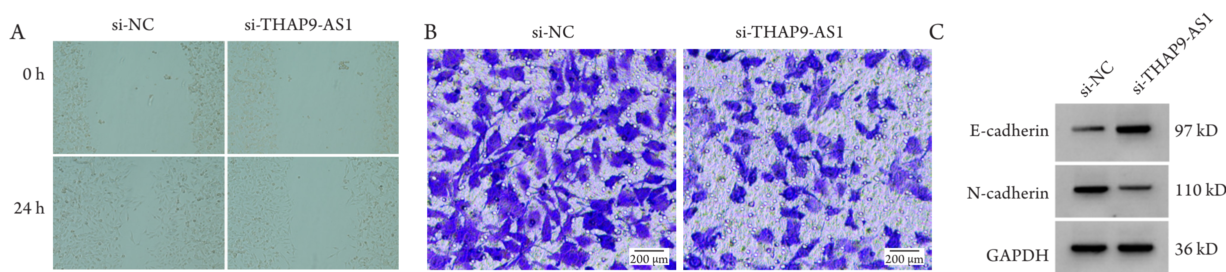

图3 沉默lncRNA THAP9-AS1对MDA-MB-231细胞迁移侵袭及E-cadherin、N-cadherin蛋白表达的影响

Figure 3 Effect of silencing lncRNA THAP9-AS1 on MDA-MB-231 cell migration and invasion, and E-cadherin and N-cadherin protein expression

(A)沉默lncRNA THAP9-AS1对MDA-MB-231细胞迁移的影响(×100)；(B)沉默lncRNA THAP9-AS1对MDA-MB-231细胞侵袭的影响(结晶紫染色，×200)；(C)沉默lncRNA THAP9-AS1对MDA-MB-231细胞E-cadherin、N-cadherin蛋白表达的影响。

(A) Effect of silencing lncRNA THAP9-AS1 on MDA-MB-231 cell migration; (B) Effect of silencing lncRNA THAP9-AS1 on MDA-MB-231 cell invasion (crystal violet staining, ×200); (C) Effect of silencing lncRNA THAP9-AS1 on E-cadherin and N-cadherin protein expression in MDA-MB-231 cell.

表1 沉默lncRNA THAP9-AS1对MDA-MB-231集落形成、凋亡、迁移及侵袭的影响(*n*=3)

Table 1 Effects of silencing lncRNA THAP9-AS1 on colony formation, apoptosis, migration and invasion of MDA-MB-231 (*n*=3)

组别	THAP9-AS1	miR-505-3p	集落形成数/个	迁移距离/μm	侵袭细胞数/个	E-cadherin	N-cadherin	凋亡率/%
si-NC组	1.00 ± 0.00	1.00 ± 0.00	137.33 ± 3.86	181.00 ± 10.68	156.67 ± 6.34	0.20 ± 0.02	0.68 ± 0.05	6.99 ± 0.72
si-THAP9-AS1组	0.26 ± 0.02*	4.07 ± 0.08*	75.67 ± 1.70*	93.82 ± 3.81*	88.33 ± 2.05*	0.82 ± 0.07*	0.21 ± 0.02*	19.47 ± 2.54*
t	64.086	66.467	25.321	13.317	17.765	14.751	15.117	8.188
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

与si-NC组相比，*$P<0.05$。

Compared with si-NC group, *$P<0.05$.

wt-THAP9-AS1 5' CUGUGACUACUCA--UGUUGACU 3'
miR-505-3p 3' UCCUUUGGUCGUUCACAACUGC 5'
mut-THAP9-AS1 5' CUGUGACUACUCA--CCAGCGGU 3'

图4 LncRNA THAP9-AS1和miR-505-3p互补序列

Figure 4 Complementary sequence of lncRNA THAP9-AS1 and miR-505-3p

表2 双荧光素酶报告实验(*n*=3)

Table 2 Double luciferase report experiment (*n*=3)

组别	wt-THAP9-AS1	mut-THAP9-AS1
miR-NC组	1.01 ± 0.10	1.03 ± 0.09
miR-505-3p组	0.29 ± 0.02*	1.05 ± 0.07
t	12.229	0.304
P	<0.001	0.776

与miR-NC组相比，*$P<0.05$。

Compared with miR-NC group, *$P<0.05$.

2.4 MiR-505-3p对MDA-MB-231细胞增殖、凋亡、迁移及侵袭的影响

与miR-NC组相比，miR-505-3p组miR-505-3p的表达水平、凋亡率、E-cadherin表达水平升高，N-cadherin表达水平降低，细胞活性、迁移距离降低，集落形成数和侵袭细胞数减少($P<0.05$；表3，图5、6)。

2.5 抑制miR-505-3p可以逆转沉默lncRNA THAP9-AS1处理的MDA-MB-231增殖、凋亡、迁移及侵袭的影响

与si-THAP9-AS1+anti-miR-NC组相比，si-THAP9-AS1+miR-505-3p inhibitor组miR-505-3p的表达水平降低，细胞活性、迁移距离、N-cadherin表达水平增加，集落形成数和侵袭细胞数增加，凋亡率、E-cadherin表达水平降低($P<0.05$；图7、8，表4)。

表3 MiR-505-3p对MDA-MB-231集落形成、凋亡、迁移及侵袭的影响(*n*=3)

Table 3 Effects of miR-505-3p on colony formation, apoptosis, migration and invasion of MDA-MB-231 (*n*=3)

组别	miR-505-3p	集落形成数/个	迁移距离/μm	侵袭细胞数/个	E-cadherin	N-cadherin	凋亡率/%
miR-NC组	1.00 ± 0.00	138.67 ± 4.03	185.43 ± 8.54	156.33 ± 6.60	0.20 ± 0.02	0.68 ± 0.05	7.22 ± 0.35
miR-505-3p组	5.32 ± 0.11*	65.33 ± 2.05*	77.63 ± 3.33*	69.00 ± 1.41*	0.92 ± 0.06*	0.13 ± 0.01*	20.33 ± 2.11*
t	7.438	28.095	20.370	22.412	19.718	18.683	10.617
P	0.002	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

与miR-NC组相比，*$P<0.05$。

Compared with miR-NC group, *$P<0.05$.

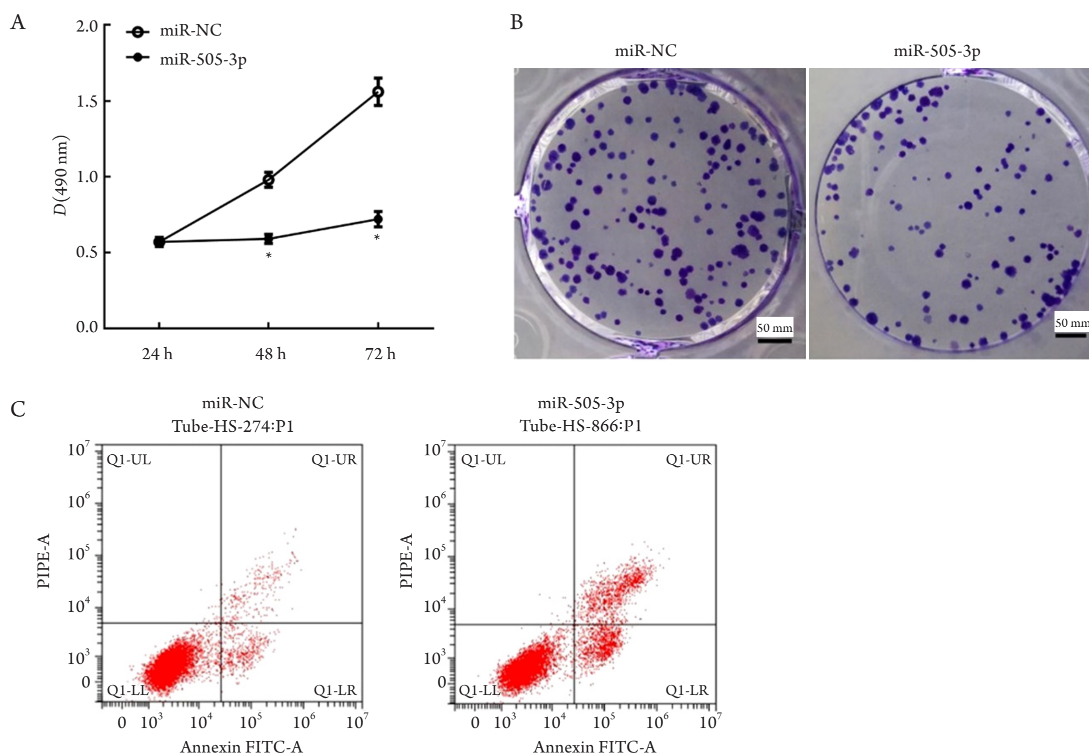

图5 MiR-505-3p对MDA-MB-231细胞活性和凋亡的影响

Figure 5 Effect of miR-505-3p on the activity and apoptosis of MDA-MB-231 cells

(A)MiR-505-3p对MDA-MB-231细胞活性的影响；(B)MiR-505-3p对MDA-MB-231细胞克隆形成的影响(吉姆萨染色，×50)；(C)MiR-505-3p对MDA-MB-231细胞凋亡的影响。与miR-NC组相比，$^*P$<0.05。

(A) Effect of miR-505-3p on the activity of MDA-MB-231 cells; (B) Effect of miR-505-3p on the clone formation of MDA-MB-231 cells (Giemsa staining, ×50); (C) Effect of miR-505-3p on the apoptosis of MDA-MB-231 cells. Compared with miR-NC group, $^*P$<0.05.

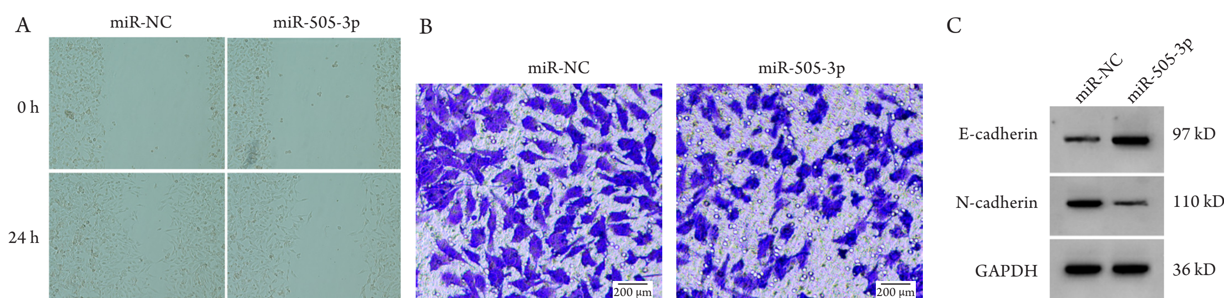

图6 MiR-505-3p对MDA-MB-231细胞迁移侵袭及E-cadherin、N-cadherin蛋白表达的影响

Figure 6 Effect of miR-505-3p on the migration and invasion of MDA-MB-231 cells and the expression of E-cadherin and N-cadherin proteins

(A)MiR-505-3p对MDA-MB-231细胞迁移的影响(×100)；(B)MiR-505-3p对MDA-MB-231细胞侵袭的影响(结晶紫染色，×200)；(C)MiR-505-3p对MDA-MB-231细胞E-cadherin、N-cadherin蛋白表达的影响。

(A) Effect of miR-505-3p on the migration of MDA-MB-231 cells (×100); (B) Effect of miR-505-3p on the invasion of MDA-MB-231 cells (crystal violet staining, ×200); (C) Effect of miR-505-3p on the expression of E-cadherin and N-cadherin proteins in MDA-MB-231 cells.

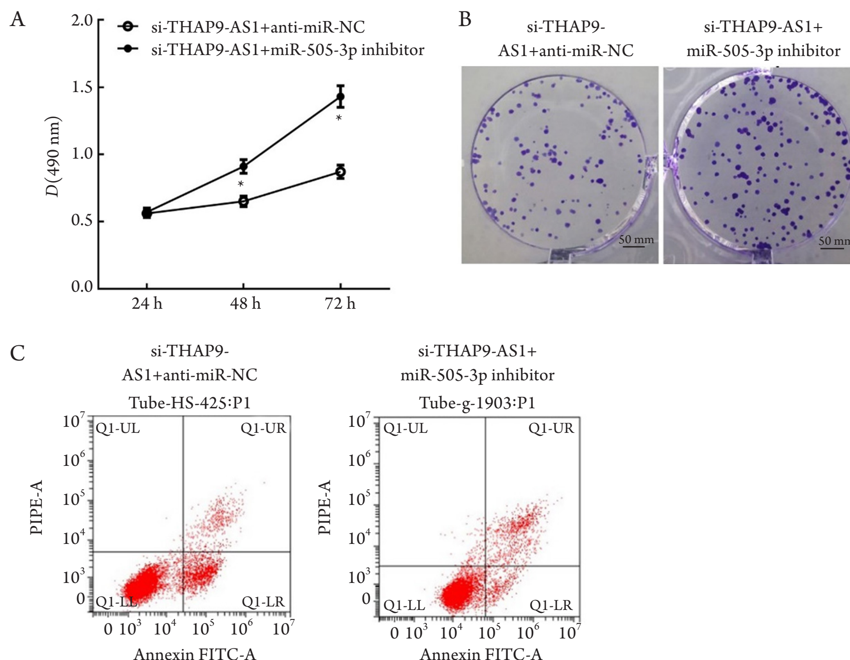

图7 抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231细胞活性和凋亡的影响

Figure 7 Effect of miR-505-3p inhibition on the viability and apoptosis of MDA-MB-231 cells treated with silenced lncRNA THAP9-AS1

(A)抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231细胞活性的影响；(B)抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231细胞克隆形成的影响(吉姆萨染色，×50)；(C)抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231细胞凋亡的影响。与si-THAP9-AS1+anti-miR-NC组相比，*P<0.05。

(A) Effect of miR-505-3p inhibition on the viability of MDA-MB-231 cells treated with silenced lncRNA THAP9-AS1; (B) Effect of miR-505-3p inhibition on the clone formation of MDA-MB-231 cells treated with silenced lncRNA THAP9-AS1 (Giemsa staining, ×50); (C) Effect of miR-505-3p inhibition on the apoptosis of MDA-MB-231 cells treated with silenced lncRNA THAP9-AS1. Compared with si-THAP9-AS1+anti-miR-NC group, *P<0.05.

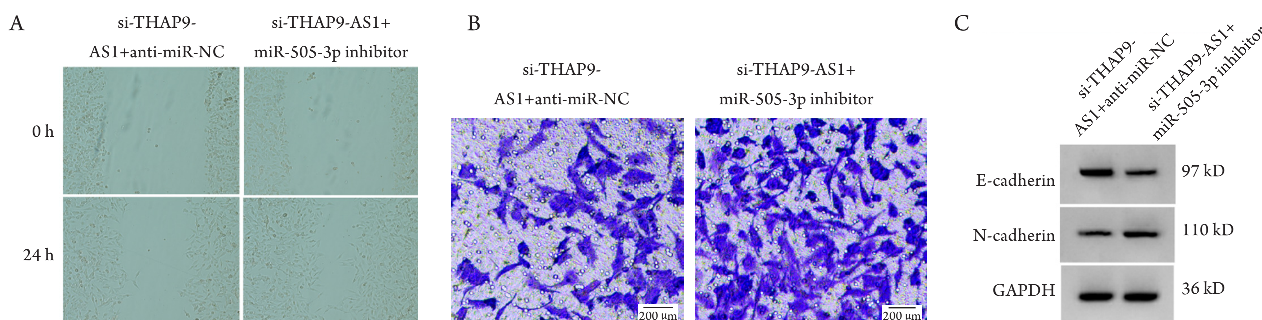

图8 抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231细胞迁移侵袭及E-cadherin、N-cadherin蛋白表达的影响

Figure 8 Effect of inhibiting miR-505-3p on the MDA-MB-231 cells active, migration, invasion and the expression of E-cadherin and N-cadherin proteins treated with silent lncRNA THAP9-AS1

(A)抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231细胞迁移的影响(×100)；(B)抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231细胞侵袭的影响(结晶紫染色，×200)；(C)抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231细胞E-cadherin、N-cadherin蛋白表达的影响。

(A) Effect of inhibiting miR-505-3p on the MDA-MB-231 cells migration treated with silent lncRNA THAP9-AS1 (×100); (B) Effect of inhibiting miR-505-3p on the MDA-MB-231 cells invasion treated with silent lncRNA THAP9-AS1 (crystal violet staining, ×200); (C) Effect of inhibiting miR-505-3p on the expression of E-cadherin and N-cadherin proteins treated with silent lncRNA THAP9-AS1 in MDA-MB-231 cells.

表4 抑制miR-505-3p对沉默lncRNA THAP9-AS1处理的MDA-MB-231集落形成、凋亡、迁移及侵袭的作用(*n*=3)

Table 4 Effects of miR-505-3p inhibition on colony formation, apoptosis, migration and invasion of MDA-MB-231 treated with silenced lncRNA THAP9-AS1 (*n*=3)

组别	miR-505-3p	集落形成数/个	迁移距离/μm	侵袭细胞数/个	E-cadherin	N-cadherin	凋亡率/%
si-THAP9-AS1+anti-miR-NC组	4.10 ± 0.12	75.67 ± 2.05	93.70 ± 3.83	86.33 ± 3.09	0.82 ± 0.07	0.21 ± 0.02	20.14 ± 2.28
si-THAP9-AS1+miR-505-3p inhibitor组	1.34 ± 0.05*	115.33 ± 4.92*	167.74 ± 6.39*	141.33 ± 4.64*	0.26 ± 0.02*	0.57 ± 0.04*	13.58 ± 1.39*
t	36.773	12.888	17.214	17.088	13.323	13.943	4.255
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.013

与si-THAP9-AS1+anti-miR-NC组相比，*P<0.05。

Compared with si-THAP9-AS1 + anti-miR-NC group, *P<0.05.

3 讨论

乳腺癌占女性癌症的30%，且其发病率和病死率可能会继续升高[9]。乳腺癌的治疗手段包括手术、放疗、化疗和靶向治疗等。靶向治疗在乳腺癌的治疗中显出独特的优势，可提高患者的生存质量和生存率[10-11]。研究[12]证实lncRNA参与调控乳腺癌的进展，如沉默lncRNA PART1可抑制乳腺癌细胞的增殖、迁移及侵袭。LncRNA FGF14-AS2通过使miR-205-5p变海绵状抑制乳腺癌的增殖、迁移、侵袭，并诱导凋亡[13]。LncRNA MORT在乳腺癌组织和细胞系中表达下调，低水平的lncRNA MORT预示着较高的远处转移率，过表达lncRNA MORT可抑制乳腺癌细胞的增殖率和迁移率，而敲除lncRNA MORT则效果相反[14]。在乳腺癌组织和细胞中，lncRNA MALAT1的高表达被认为是预后不良的预测因子，下调lncRNA MALAT1可抑制乳腺癌的进展[15]。沉默lncRNA DRAIC可抑制乳腺癌细胞增殖、迁移及侵袭，促进凋亡[16]。LncRNA PCAT6在乳腺癌组织和细胞系中过度表达，其高表达反映乳腺癌患者不良预后，lncRNA PCAT6敲除在体外可抑制乳腺癌细胞增殖，促进细胞凋亡及细胞周期发展，在体内也抑制移植瘤生长[17]。在胰腺导管腺癌患者中，lncRNA THAP9-AS1过表达与患者的不良预后显著相关；在体外和体内，lncRNA THAP9-AS1均可促进胰腺导管腺癌细胞的生长[18]。本研究结果显示：乳腺癌组织中lncRNA THAP9-AS1表达水平被上调；沉默lncRNA THAP9-AS1后乳腺癌MDA-MB-231细胞活性降低，集落形成数和侵袭细胞数减少，迁移距离缩小，凋亡率升高，E-cadherin表达水平升高，N-cadherin表达水平降低，表明沉默lncRNA THAP9-AS1可抑制乳腺癌细胞增殖、迁移及侵袭，并促进凋亡。

研究[18]表明：lncRNA THAP9-AS1可作为竞争性内源RNAs调控miR-484表达影响胰腺导管腺癌细胞恶性行为。本研究结果显示lncRNA THAP9-AS1与miR-505-3p间存在互补核苷酸序列；双荧光素酶报告基因实验显示，在wt-THAP9-AS1中，转染miR-505-3p的荧光素酶活性显著降低，而mut-THAP9-AS1中转染miR-NC、miR-505-3p的光素酶活性无显著差异。MiR-505-3p在肿瘤进展中发挥抑癌基因作用。在胶质瘤中，miR-505-3p的表达降低，与胶质瘤患者的临床预后差和预后有关；过表达miR-505-3p抑制神经胶质瘤细胞增殖、迁移及侵袭[19]。LncRNA KTN1-AS1通过负调控miR-505-3p促进神经胶质瘤细胞增殖和侵袭[20]。LncRNA ZEB1-AS1通过调节miR-505-3p/TRIB2轴促进胰腺癌的进展[21]。与上述研究结果相似，本研究结果显示：miR-505-3p在乳腺癌组织中表达水平降低，过表达miR-505-3p降低了乳腺癌细胞活性、集落形成数和侵袭细胞数，增加凋亡率，升高E-cadherin蛋白水平，降低迁移距离以及N-cadherin蛋白水平，说明miR-505-3p可抑制乳腺癌的增殖、迁移及侵袭，并促进凋亡，在乳腺癌中也起抑癌基因作用。进一步功能回复实验结果显示：抑制miR-505-3p表达部分逆转了沉默lncRNA THAP9-AS1对MDA-MB-231细胞增殖、凋亡、迁移及侵袭的影响，提示lncRNA THAP9-AS1可通过调控miR-505-3p影响MDA-MB-231细胞的增殖、凋亡和迁移侵袭能力。

综上所述，lncRNA THAP9-AS1在乳腺癌中

表达增加，沉默lncRNA THAP9-AS1可通过靶向上调miR-505-3p表达抑制MDA-MB-231细胞增殖、迁移及侵袭，并诱导细胞凋亡。沉默lncRNA THAP9-AS1对乳腺癌的影响仅限于体外实验，其在体内的作用及其分子调控机制还有待进一步研究与验证。

参考文献

1. 赵晶, 吴楠, 张世超, 等. 乳腺癌靶向治疗新进展[J]. 中华肿瘤杂志, 2020, 42(5): 353-361.
   ZHAO Jing, WU Nan, ZHANG Shichao, et al. New advances in targeted therapy for breast cancer[J]. Chinese Journal of Oncology, 2020, 42(5): 353-361.
2. Meisel JL, Venur VA, Gnant M, et al. Evolution of targeted therapy in breast cancer: where precision medicine began[J]. Am Soc Clin Oncol Educ Book, 2018, 38: 78-86.
3. Yousefi H, Maheronnaghsh M, Molaei F, et al. Long noncoding RNAs and exosomal lncRNAs: Classification, and mechanisms in breast cancer metastasis and drug resistance[J]. Oncogene, 2020, 39(5): 953-974.
4. Liang Y, Song X, Li Y, et al. LncRNA BCRT1 promotes breast cancer progression by targeting miR-1303/PTBP3 axis[J]. Mol Cancer, 2020, 19(1): 85.
5. Wang C, Xie XX, Li WJ, et al. LncRNA DLEU1/microRNA-300/RAB22A axis regulates migration and invasion of breast cancer cells[J]. Eur Rev Med Pharmacol Sci, 2019, 23(23): 10410-10421.
6. Jia W, Zhang J, Ma F, et al. Long noncoding RNA THAP9-AS1 is induced by Helicobacter pylori and promotes cell growth and migration of gastric cancer[J]. Onco Targets Ther, 2019, 12: 6653-6663.
7. Zhao P, Guan H, Dai Z, et al. Long noncoding RNA DLX6-AS1 promotes breast cancer progression via miR-505-3p/RUNX2 axis[J]. Eur J Pharmacol, 2019, 865: 172778.
8. Tang Y, Wu B, Huang S, et al. Downregulation of miR-505-3p predicts poor bone metastasis-free survival in prostate cancer[J]. Oncol Rep, 2019, 41(1): 57-66.
9. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020[J]. CA Cancer J Clin, 2020, 70(1): 7-30.
10. 侯虹丽, 侯基铭, 张凤栖, 等. 乳腺癌靶向治疗的现状及展望[J]. 世界最新医学信息文摘, 2019, 19(12): 56-57.
    HOU Hongli, HOU Jiming, ZHANG Fengqi, et al. Current situation and prospect of breast cancer targeted therapy[J]. World Latest Medicine Information, 2019, 19(12): 56-57.
11. 郑曼俐, 马金柱. 乳腺癌靶向药物的研究及临床应用进展[J]. 实用药物与临床, 2019, 22(3): 325-329.
    ZHENG Manli, MA Jinzhu. Advances in research and clinical application of targeted molecular drugs for breast cancer[J]. Practical Pharmacy and Clinical Remedies, 2019, 22(3): 325-329.
12. Zhang L, Zhang J, Ni C. Silencing of lncRNA PART1 inhibits proliferation, invasion and migration of breast cancer cells and promotes the efficacy of cisplatin in breast cancer cells[J]. Gen Physiol Biophys, 2020, 39(4): 343-354.
13. Yang Y, Xun N, Wu JG. Long non-coding RNA FGF14-AS2 represses proliferation, migration, invasion, and induces apoptosis in breast cancer by sponging miR-205-5p[J]. Eur Rev Med Pharmacol Sci, 2019, 23(16): 6971-6982.
14. Yang Q, Yang D, Zhu HB, et al. LncRNA MORT negatively regulates FGF1 to suppress malignant progression of breast cancer[J]. Eur Rev Med Pharmacol Sci, 2021, 25(20): 6179-6186.
15. Shao J, Zhang Q, Wang P, et al. LncRNA MALAT1 promotes breast cancer progression by sponging miR101-3p to mediate mTOR/PKM2 signal transmission[J]. Am J Transl Res, 2021, 13(9): 10262-10275.
16. Li S, Jia H, Zhang Z, et al. DRAIC promotes growth of breast cancer by sponging miR-432-5p to upregulate SLBP[J/OL]. Cancer Gene Ther, 2021, Epub ahead of print.
17. Zhu Q, Wang S, Shi Y. LncRNA PCAT6 activated by SP1 facilitates the progression of breast cancer by the miR-326/LRRC8E axis[J]. Anticancer Drugs, 2022, 33(2): 178-190.
18. Li N, Yang G, Luo L, et al. lncRNA THAP9-AS1 promotes pancreatic ductal adenocarcinoma growth and leads to a poor clinical outcome via sponging miR-484 and interacting with YAP[J]. Clin Cancer Res, 2020, 26(7): 1736-1748.
19. Cheng Z, Wang B, Zhang C. MicroRNA-505-3p inhibits development of glioma by targeting HMGB1 and regulating AKT expression[J]. Oncol Lett, 2020, 20(2): 1663-1670.
20. Mu Y, Tang Q, Feng H, et al. lncRNA KTN1-AS1 promotes glioma cell proliferation and invasion by negatively regulating miR-505-3p[J]. Oncol Rep, 2020, 44(6): 2645-2655.
21. Wei G, Lu T, Shen J, et al. LncRNA ZEB1-AS1 promotes pancreatic cancer progression by regulating miR-505-3p/TRIB2 axis[J]. Biochem Biophys Res Commun, 2020, 528(4): 644-649.

本文引用：王枝红, 刘红, 何楠, 杨海松. 沉默lncRNA THAP9-AS1对乳腺癌细胞增殖、凋亡、迁移及侵袭的影响[J]. 临床与病理杂志, 2022, 42(9): 2054-2062. doi: 10.3978/j.issn.2095-6959.2022.09.002
Cite this article as: WANG Zhihong, LIU Hong, HE Nan, YANG Haisong. Effect of silencing lncRNA THAP9-AS1 on the proliferation, apoptosis, migration and invasion of breast cancer cells[J]. Journal of Clinical and Pathological Research, 2022, 42(9): 2054-2062. doi: 10.3978/j.issn.2095-6959.2022.09.002