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·论著·

MicroRNA–197调控上皮–间充质转化对乳腺癌侵袭和迁移的影响

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[摘　要]　**目的：**探讨微小RNA-197(miR-197)抑制乳腺癌细胞迁移和侵袭的能力，以及阻断上皮-间充质转化(epithelial-mesenchymal transition，EMT)过程的机制。**方法：**构建miR-197过表达载体(miR-197 mimics)，分别转染MDA-MB-231和MCF-7细胞，并设对照组；Real-time PCR分别检测以上各组细胞miR-197的表达水平变化；利用Transwell实验和划痕实验对乳腺癌细胞侵袭和迁移能力进行检测；Western印迹法检测过表达miR-197后对EMT相关标志物E-cadherin，snail和vimentin表达的影响。**结果：**MiR-197可以抑制乳腺癌细胞MDA-MB-231和MCF-7的迁移和侵袭能力；转染miR-197 mimics后，E-cadherin表达降低，snail和vimentin表达增加。**结论：**MiR-197有可能作为乳腺癌临床治疗的新靶点。

[关键词]　乳腺癌；微小RNA-197；上皮-间充质转化；侵袭；迁移

Effects of microRNA-197 regulating epithelial mesenchymal transition on invasion and migration in breast cancer

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Abstract　**Objective:** To investigate the ability of microRNA-197 (miR-197) to inhibit the migration and invasion of breast cancer cells and the mechanism of blocking the process of epithelial-mesenchymal transition (EMT). **Methods:** The miR-197 mimics vector was constructed, and transfected into MDA-MB-231 and MCF-7 cell, the control group were transfected with empty vectors. The expression of miR-197 was detected by real-time PCR. The transwell system and the wound healing were used to assess the invasion and migration ability of breast cancer cell line MDA-MB-231 and MCF-7. Western blot was used to assay the expression of E-cadherin, Snail and Vimentin, which were the markers of EMT. **Results:** MiR-197 inhibited the migration and invasion of breast cancer cells MDA-MB-231 and MCF-7. Transfection of miR-197 mimics reduced the expression of E-cadherin and increased the protein expression of snail and vimentin. **Conclusion:** MiR-197 may be a new target for clinical treatment of breast cancer.

Keywords　breast cancer; microRNA-197; epithelial-mesenchymal transition; invasion; migration

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乳腺癌是世界上最常见和最致命的恶性肿瘤之一，转移是导致乳腺癌患者死亡的主要原因。近50%接受化疗或激素治疗的乳腺癌患者仍然会发生远处转移[1-2]。因此，探索乳腺癌转移的分子机制对乳腺癌的发展和治疗起至关重要的作用。微小RNAs(microRNAs，miRNAs)的表达失调会导致肿瘤发生、发展及侵袭转移[3-5]。上皮-间充质转化(epithelial-mesenchymal transition，EMT)是细胞形态学发生变化，细胞与细胞间及细胞与基质之间的黏附性丢失及重塑，并获得迁徙和侵袭能力，是肿瘤发生转移的关键步骤之一。MiRNAs在调控肿瘤发生、侵袭转移及EMT等过程中发挥重要作用[6]。MiR-197与乳腺癌的发生密切相关[7]。然而，miRNA-197的异常表达对乳腺癌迁移和侵袭能力的影响及可能的调控机制尚不清楚。

本研究旨在探讨miR-197对乳腺癌细胞系MDA-MB-231和MCF-7迁移和侵袭能力的影响，以及与乳腺癌EMT之间的关系，为乳腺癌临床干预寻求新的治疗靶点。

1 材料与方法

1.1 细胞培养

人类乳腺癌细胞系MDA-MB-231和MCF-7为本实验室所保存。细胞培养于5%CO_2，37 ℃培养箱中，用含10%小牛血清的DMEM培养基进行培养。

1.2 实时荧光定量PCR

RNA提取试剂盒iScript®cDNA Synthesis Kit购自美国Bio-Rad公司。检测仪器Applied Biosystems® 7300 Real Time PCR System购自美国Thermo Fisher公司，内参为RNU6B，采用SYBR法检测。PCR引物E-cadherin为 5'-TGCACC-AACCCTCATGAGTG-3'，5'-GTCAGTATCAG-CCGCTTTCAG-3'；Snail：5'-TTCTCACTGCCAT-GGAATTCC-3'，5'-GCAGAGGACACAGAACC-AGAAA-3'；Vimentin：5'-CCTGAACCTGAGGG-AAACTAA-3'，5'-GCAGAAAGGCACTTG-AAAGC-3'；RNU6B：5'-CTCGCTTCGGCA-GCACA-3'，5'-AACGCTTCACGAATTTGCGT-3'；GAPHD：5'-AGGTCGGTGTGAACGGATTTG-3'，5'-GGGGTCGTTGATGGCAACA-3'。Real-time分析采用$2^{-\Delta\Delta Ct}$方法。故ΔΔCt=[(miR-197)CT-U6]$_{样品}$-[(miR-197)CT-U6]$_{空白对照组}$。

1.3 构建载体和si-miR-197细胞转染实验

用脂质体(Life Technologies)转染法对MDA-MB-231和MCF-7细胞进行miR-197 mimic (12.5 nmol/L)和对照组转染。24 h后收集细胞进行下一步实验。

1.4 Western印迹法

用PBS漂洗消化好的乳腺癌细胞，收集后用RIPA缓冲液裂解(美国Cell Signaling Technology公司)。用考马斯亮蓝法检测蛋白浓度(Invitrogen)。将变性蛋白按照50 μg进行10%或12%的SDS-PAGE胶进行电泳，结束后转膜，转膜结束后用含有5%脱脂牛奶的TBST封闭2 h，然后于4 ℃孵育一抗过夜。用辣根过氧化物酶偶联的二抗和ECL法显影。具体抗体为：E-cadherin(1∶1 000，美国Abcam公司)，Snail(1∶1 000，美国Abcam公司)，Vimentin (1∶1 000，美国Abcam公司)和β-actin (1∶5 000，武汉博士德生物工程有限公司)。

1.5 细胞侵袭和迁移实验

细胞侵袭实验：使用24孔的Transwell实验进行研究。实验前用预稀释的Matrigel(5 μg/μL；美国BD Biosciences公司)进行处理。细胞以每孔200 μL，5×10^4个细胞的密度接种于孔板中。其中上层室无血清培养基，下层培养基含有800 μL的10%FBS培养基。孵育24 h后，用棉签除去留在上层的细胞。对通过Matrigel侵入底部的细胞进行固定并使用Diff-Quik®染色(Sysmex，Hyogo，日本)。在高倍镜下观察迁移的细胞数量并进行计数。

细胞迁移实验：将细胞按照4×10^5个/孔的密度接种到6孔平板中。细胞融合达到90%聚集程度时，用无菌枪头直接划出划痕，除去漂浮细胞后继续培养。12 h后立即拍摄划痕处的图片并测量细胞的覆盖率。

1.6 统计学处理

采用SPSS 19.0软件进行数据分析，数据均以均数±标准差($\bar{x}\pm s$)表示，采用t检验，P<0.05为差异有统计学意义。

2 结果

2.1 MiR-197在乳腺癌细胞系中表达情况

采用qRT-PCR检测乳腺癌细胞系MDA-MB-231和MCF-7中miR-197的表达情况，以正常的乳腺上

皮细胞MCF-10A作为对照。结果显示miR-197在乳腺癌细胞系中的表达明显降低(图1)。

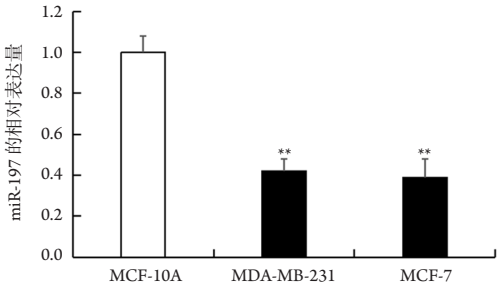

图1 MiR-197在乳腺癌细胞和正常乳腺细胞中的表达

Figure 1 Expression of miR-197 in the breast cancer cells and normal breast cells

$^{**}P<0.01$.

2.2 MiR-197 mimics对乳腺癌细胞迁移和侵袭能力的影响

在MDA-MB-231和MCF-7细胞中转染miR-197 mimics。然后用Transwell实验和划痕实验检测MDA-MB-231和MCF-7细胞迁移和侵袭情况。结果显示miR-197 mimics明显抑制了迁移和侵袭能力(图2)。

2.3 MiR-197异常表达对乳腺癌EMT相关因子表达的影响

过表达miR-197之后，分别检测两种乳腺癌细胞中EMT相关标志分子的mRNA和蛋白质水平。E-cadherin显著降低，而Snail和Vimentin表达明显增加(图3)。

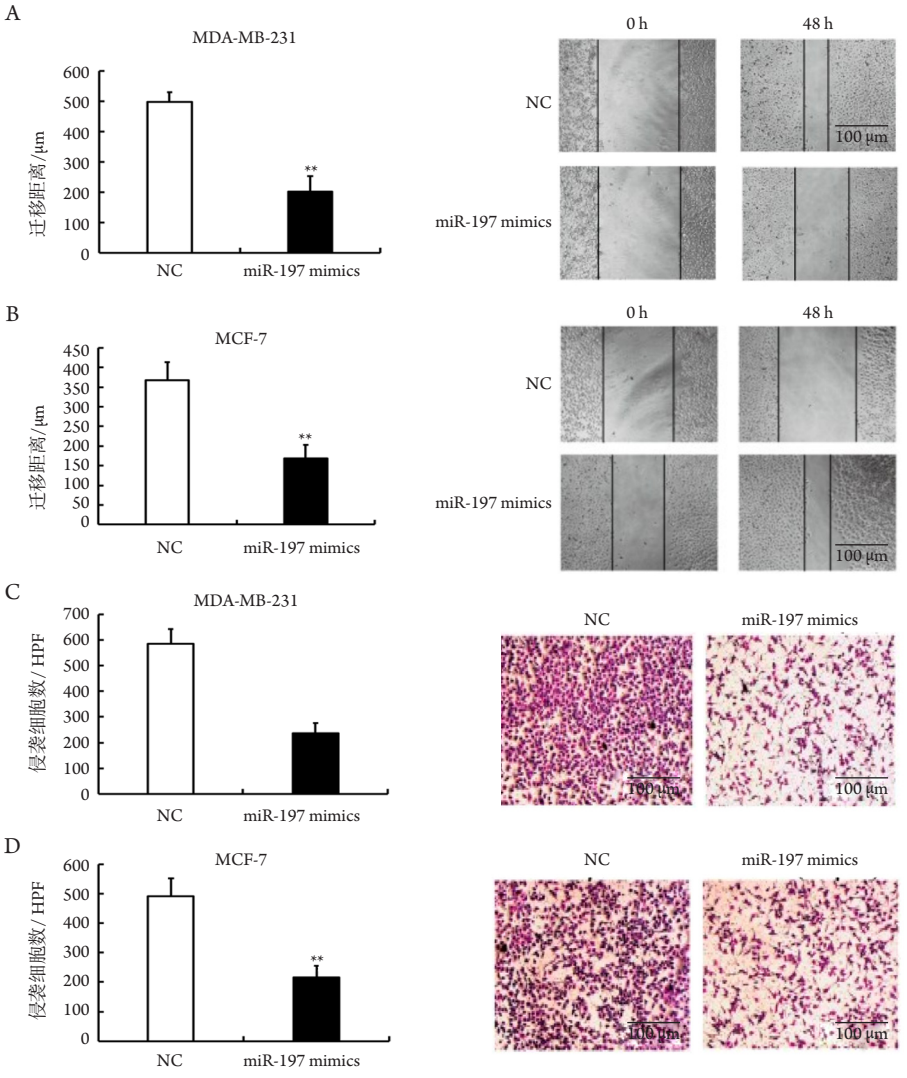

图2 MiR-197 mimics对乳腺癌细胞迁移(A，B)和侵袭(C，D)的影响

Figure 2 Effects of miR-197 mimics on the migration (A, B) and invasion (C, D) of breast cancer cells

$^{**}P<0.01$.

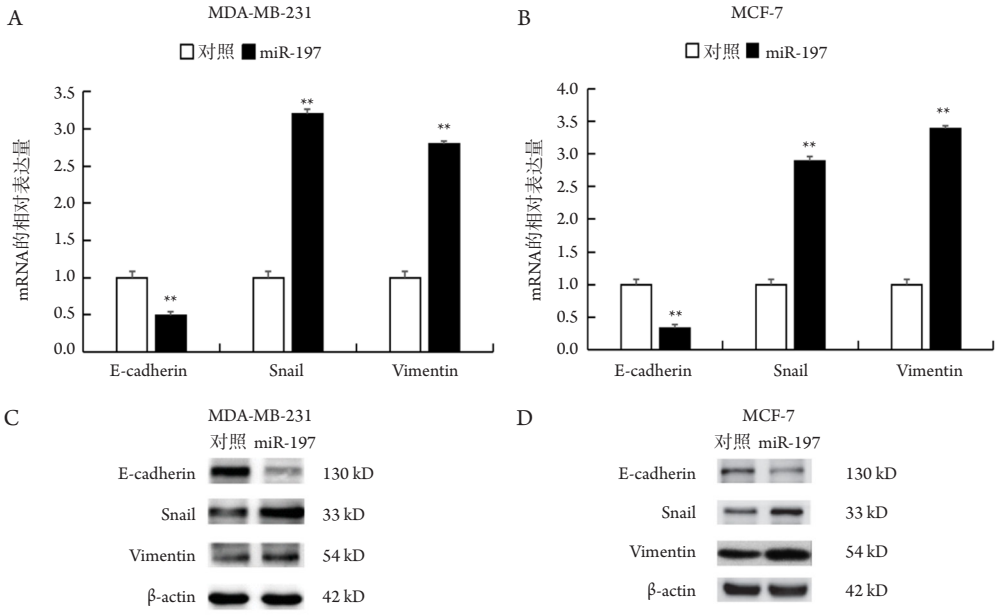

图3 miR-197 mimics对乳腺癌EMT相关因子mRNA表达(A，B)及蛋白表达(C，D)的影响

Figure 3 Effects of miR-197 mimics on the mRNA expression (A, B) and the protein expressions (C, D) of EMT markers in breast cancer cells

$^{**}P<0.01$.

3 讨论

MiRNAs是一种内源性小分子非编码RNA，通过与靶基因mRNA 3'-UTR结合，在翻译或转录后水平调控癌基因或抑癌基因的表达，从而参与肿瘤细胞周期、凋亡、增殖及迁移等生物学过程[8-9]。MiRNA作为关键因子参与调节肿瘤的发生发展已经成为一个热门的研究领域。在不同类型的癌症组织标本中，miRNA的异常表达与肿瘤细胞增殖、凋亡、细胞周期、迁移、侵袭和转移等病理生理过程有显著的关联性[10]。因此，miRNA可能作为在癌症发生发展以及预后、诊断和治疗的标志物，阐明miRNA的表达、功能和分子机制作用模式对癌症的临床治疗有重要的意义。

MiR-197在胶质母细胞瘤[11]、非小细胞肺癌[12]等肿瘤组织或细胞中表达呈现下调；过表达miR-197可诱导细胞凋亡，抑制细胞增殖和迁移[13-16]，从而抑制肿瘤发生。此外，miR-197可以抑制促癌基因FOXM1的表达[17-18]。本研究发现：与正常的乳腺上皮细胞相比较，miR-197在两个不同的乳腺癌细胞系中表达均降低，提示miR-197有可能作为乳腺癌特异性标志物。MiR-197在正常细胞和乳腺癌细胞中表达差异有统计学意义，具体调节肿瘤细胞的生物学功能呢？Wu等[19]证实miR-197在体外可以抑制子宫平滑肌瘤细胞的迁移和侵袭；miR-197的低表达在体外和体内均抑制肝癌的迁移和侵袭[13]。因此，本研究采用miR-197 mimics，检测过表达miR-197后，乳腺癌细胞侵袭和转移的功能变化。本研究中Transwell实验和划痕实验结果表明：过表达miR-197后，乳腺癌细胞的侵袭和转移能力显著减弱，由此推测miR-197可能通过抑制肿瘤细胞的转移和侵袭能力从而调节肿瘤的转移过程。

EMT过程是许多肿瘤细胞向远端器官转移扩散的关键步骤之一[20-21]。研究[22-24]表明miRNAs异常表达可以调控肿瘤EMT过程。MiR-200家族成员通过作用于E-cadherin的抑制诱导上皮细胞分化，或者直接靶向作用于EMT的激活子如ZEB1/2来抑制EMT过程[25-27]。Hamada等[28]研究表明：miR-197在胰腺癌细胞中靶向作用于p120 catenin诱导EMT。本研究结果发现：过表达miR-197后，E-cadherin表达升高、Vimentin和Snail表达降低，肿瘤细胞的间充质标志物丢失同时上皮标志物增加，表明miR-197参与阻断乳腺癌EMT的过程。

综上所述，miR-197具有抑制乳腺癌侵袭转移的能力，其机制可能涉及阻断乳腺癌细胞上皮细胞间充质转化过程。MiR-197可能作为癌症发生发展以及预后、诊断和治疗的标志物及新型靶点，为乳腺癌的临床治疗提供了新的策略和理论依据。

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