

## miR-125a-5p 通过靶向调控 GAB2 抑制乳腺癌细胞的迁移能力

徐新伟<sup>1)</sup>, 李佩瑞<sup>2)</sup>, 张增山<sup>2)</sup>, 王学文<sup>2)</sup>, 李洪利<sup>3)</sup>, 尹崇高<sup>4)</sup>\*

( 潍坊医学院<sup>1)</sup> 病理学教研室; <sup>2)</sup> 附属医院; <sup>3)</sup> 医学研究实验中心; <sup>4)</sup> 护理学院, 山东 潍坊 261053)

**摘要** miR-125a-5p 可负性调节 GAB2 表达,抑制胶质瘤细胞的侵袭和转移。本研究旨在证明 miR-125a-5p 抑癌作用的普遍性,即 miR-125a-5p 是否可通过靶向抑制 GAB2 抑制乳腺癌细胞的迁移。荧光素酶实验结果显示,miR-125a-5p 可特异识别 GAB2 的 3'-UTR,抑制报告酶的表达。荧光定量 PCR 结果揭示,与正常乳腺上皮细胞 MCF-10A 比较,miR-125a-5p 在乳腺癌细胞 MDA231 和 MCF-7 中的表达明显降低;与迁移能力相对较低的 MCF-7 细胞比较,miR-125a-5p 在迁移能力较高的 MDA231 细胞中的表达量更低。Western 印迹结果证明,与空载体(对照)和 anti-miR125a-5p 转染细胞比较,转染 miR-125a-5p 明显抑制 GAB2 蛋白在乳腺癌细胞中的表达。Transwell 结果显示,与空载体转染的对照细胞比较,转染 miR-125a-5p 的乳腺癌细胞穿过基质胶的细胞数明显减少;相反,转染 anti-miR125a-5p 的细胞穿过基质胶的细胞数却明显增多。上述结果提示,miR-125a-5p 在正常的乳腺细胞中高表达,而在乳腺癌细胞中低表达,其表达水平与癌细胞的迁移能力和 GAB2 表达呈反向关系。本研究结果还提示,miR-125a-5p 通过靶向负调控 GAB2 抑制乳腺癌细胞的迁移能力。总之,本研究证明,miR-125a-5p 在肿瘤中发挥抑癌作用。

**关键词** 乳腺癌; miR-125a-5p; GRB 相关结合蛋白 2; 迁移

**中图分类号** R737.9; Q2-33

## miR-125a-5p Inhibits the Migration of Breast Cancer Cells by Targeting GAB2

XU Xin-Wei<sup>1)</sup>, LI Pei-Rui<sup>2)</sup>, ZHANG Zeng-Shan<sup>2)</sup>, WANG Xue-Wen<sup>2)</sup>,  
LI Hong-Li<sup>3)</sup>, YIN Chong-Gao<sup>4)</sup>\*

(<sup>1)</sup> Department of Pathology; <sup>2)</sup> Affiliated Hospital; <sup>3)</sup> Medical Research Center;

<sup>4)</sup> College of Nursing, Weifang Medical University, Weifang 261053, Shandong, China)

**Abstract** It has been known that miR-125a-5p inhibits the invasion and metastasis of glioma cells through negatively regulating GRB associated-binding protein 2 (GAB2). To investigate its mechanism, luciferase reporter assays were carried out and results showed that miR-125a-5p directly targets GAB2. The qRT-PCR assays showed that the expression of miR-125a-5p in MDA231 and MCF-7 cells was lower than that of MCF-10A. The expression of miR-125a-5p in highly invasive cells MDA231 was lower than that of minimally invasive cells MCF-7. Western blotting showed that the levels of GAB2 in breast cancer

收稿日期: 2017-07-19; 修回日期: 2017-07-31; 接受日期: 2017-08-15

国家自然科学基金青年基金项目( No. 81402389, No. 81641111); 山东省自然科学基金( No. ZR2014HL077, No. ZR2015HL065) 和山东省高等学校科技计划( No. J12LK03, No. J13LK03) 资助

\* 通讯作者 Tel: 13506477591, E-mail: ycglihongli@163.com

Received: July 19, 2017; Revised: July 31, 2017; Accepted: August 15, 2017

Supported by National Natural Science Foundation of China ( No. 81402389, No. 81641111); Natural Science Foundation of Shandong Province ( No. ZR2014HL077, No. ZR2015HL065) and Foundation of Shandong Educational Committee ( No. J12LK03, No. J13LK03)

\* Corresponding author Tel: 13506477591; Email: ycglihongli@163.com

cells transfected with miR-125a-5p were significantly lower than that in breast cancer cells transfected with empty vectors or anti-miR-125a-5p. Transwell assays showed that the number of cells through Matrigel in MDA231 cells transfected with miR-125a-5p was significantly reduced than control MDA231 cells. The number of cells through Matrigel was significantly increased in MCF-7 cells transfected with anti-miR-125a-5p than control MCF-7 cells. The results above suggested that the expression level of miR-125a-5p in normal human mammary epithelial cells was higher than that in breast cancer cells. The expression level of miR-125a-5p negatively correlated with the migration ability of cancer cells and the levels of GAB2. Our results also suggested that miR-125a-5p could inhibit breast cancer cell migration by negatively targeting GAB2. In conclusion, our studies propose that miR-125a-5p was a repressive gene in tumor.

**Key words** breast cancer; miR-125a-5p; GRB associated-binding protein 2 (GAB2); migration

乳腺癌是威胁女性健康的主要疾病,尤其在中国,中年女性的发病率持续增高,年轻化趋势日益显著,每年发病率约占全世界的 12.2%<sup>[1]</sup>。目前,乳腺癌分子机制研究已受到高度重视,尤其是对 miRNAs 在乳腺癌中作用的研究<sup>[2]</sup>。已有研究表明,miRNAs 在多种恶性肿瘤的侵袭与转移过程中发挥重要作用<sup>[3]</sup>,研究显示,miR-125a-5p 与胃癌<sup>[4]</sup>、肺癌<sup>[5]</sup>等相关。本研究室先前实验发现,miR-125a-5p 能够通过调控 GAB2 抑制胶质瘤细胞的侵袭与转移<sup>[6]</sup>。

GRB 相关结合蛋白 2 (GRB associated-binding protein 2, GAB2) 作为高度保守的支架蛋白和重要的衔接蛋白,与细胞的生长、血细胞的分化<sup>[7]</sup>和成神经细胞瘤<sup>[8]</sup>等密切相关。本研究室先前研究发现, GAB2 能促进乳腺癌的侵袭与转移<sup>[9]</sup>。然而, miR-125a-5p 是否能够通过靶向调控 GAB2 进而影响乳腺癌细胞的迁移能力尚未见报道。本文通过改变 miR-125a-5p 在乳腺癌细胞中的表达,探讨 miR-125a-5p 在乳腺癌细胞迁移能力中的分子机制,为临床预防乳腺癌的迁移提供新的靶点。

## 1 材料与方法

### 1.1 材料

抗体 GAB2 与  $\beta$ -actin 均购自 Cell Signaling Technology 公司,双荧光素酶报告基因载体、miR-125a-5p 过表达质粒、miR-125a-5p 干扰质粒等均由吉凯基因构建合成,反转录试剂盒 Prime Script<sup>®</sup> RT Reagent Kit 和 SYBR<sup>®</sup> Prime Script<sup>®</sup> miRNA RT-PCR Kit 购自 TaKaRa 公司,Transwell 小室购自 Corning 公司,Lipofectamine2000 购于 Invitrogen 公司,正常乳腺上皮细胞 MCF-10A 和乳腺癌细胞 MDA231、MCF-7 等均由潍坊医学院医学研究实验中心提供。

### 1.2 细胞培养与转染

MDA231 培养于 10% FBS 的 RPMI1640 培养基,MCF-7 培养于 10% FBS 的 MEM 培养基。细胞转染按照 Lipofectamine 2000 说明书操作。1) MDA231 组:常规培养,不做任何处理;2) MDA231/NC 组:转入空载质粒;3) MDA231/miR-125a-5p 组:转入 miR-125a-5p 过表达质粒 (GV251-miR-125a-5p);4) MCF-7 组:常规培养,不做任何处理;5) Anti-NC/MCF-7 组:转入对照干扰质粒;6) Anti-miR-125a-5p/MCF-7 组:转入 miR-125a-5p 干扰质粒 (GV249-Anti-miR-125a-5p)。

### 1.3 荧光素酶检测

将野生型 GAB2 的 3'-UTR (Wt-GAB2-3'-UTR)、突变型 GAB2 的 3'-UTR (Mut-GAB2-3'-UTR) 分别与空载质粒、miR-125a-5p 过表达质粒共转染到乳腺癌细胞 MDA231 中,继续培养 48 h,双荧光素酶报告基因检测试剂盒进行检测。用 PBS 洗 3 遍,加 PLB 裂解细胞,于摇床摇 30 min,将细胞裂解液与 LAR II 混合吹匀,测萤火虫荧光素酶反应强度,再加入 Stop&Glo<sup>®</sup> Reagent 测海肾荧光素酶反应强度。

### 1.4 荧光定量 PCR

加入 Trizol 裂解并提取各组乳腺癌细胞的总 RNA,测 RNA 的纯度和浓度,加入 miR-125a-5p 的茎环引物等进行逆转录,以 U6 作为内参,进行 qRT-PCR,反应条件为:95 °C 30 s,1 个循环;95 °C 5 s,63 °C 30 s,72 °C 30 s,共 35 个循环。miR-125a-5p 上游引物:AGCGCGTCCCTGAGACCCTTTAAC,下游引物:ATCCAGTGCAGGGTCCGAGG,茎环结构:GTCGTATCCAGTGCAGGGTCCGAGGTATTGCGACTG GATACGACTCACAG。

### 1.5 Western 印迹实验

将各实验组细胞裂解后提取总蛋白质,配制 12% SDS-PAGE 凝胶电泳、转膜。封闭后加入一抗

GAB2(1:500),  $\beta$ -actin(1:1000) 4℃过夜, 次日加化学发光剂 ECL X 线胶片曝光, 显影液显影, 定影液定影, 水洗后晾干, 使用 image J 进行灰度扫描, 实验独立重复 3 次。

### 1.6 体外细胞迁移实验

将各组细胞悬液加入铺有 Matrigel 的小室上室, 下室加入胎牛血清, 置于培养箱中培养 24 h, 4% 多聚甲醛固定后, 用 Giemsa 染色, 随机取 5 个视野进行拍照、计数, 实验独立重复 3 次, 计算平均值。

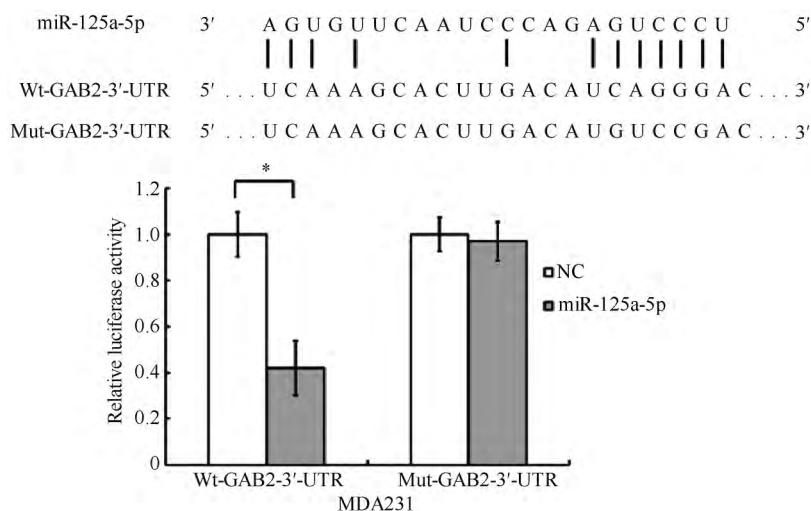
### 1.7 统计学方法

采用 SPSS 17.0 软件进行相关统计学分析, 计量资料采用独立样本 *t* 检验, 多组比较使用单因素方差分析。  $P < 0.05$  为差异有统计学意义。

## 2 结果

### 2.1 miR-125a-5p 与 GAB2 靶向结合

通过基因预测软件 TargetScan 测出 miR-125a-5p 与 GAB2 有结合位点, 提示 miR-125a-5p 靶向结合 GAB2。通过双荧光素酶实验检测 MDA231 细胞



**Fig. 1 miR-125a-5p directly targets GAB2** TargetScan predicts the binding sites of miR-125a-5p and GAB2. Wt-GAB2-3'-UTR and miR-125a-5p or empty vectors were co-transfected into MDA231 using Lipofectamine 2000 for 48 hours. Mut-GAB2-3'-UTR and miR-125a-5p or empty vectors were co-transfected into MDA231 using Lipofectamine 2000 for 48 hours. The relationship of miR-125a-5p and GAB2 was measured by Luciferase experiment assays. Bars, standard deviation. \*  $P < 0.05$ . The experiment is repeated at least three times

### 2.4 miR-125a-5p 负向调控 GAB2 蛋白的表达

通过 Western 印迹实验, 检测各组乳腺癌细胞中 GAB2 的表达量。Fig. 4 结果显示, 在 MDA231/miR-125a-5p 细胞组中, GAB2 的表达量明显低于 MDA231/NC 组和 MDA231 组 ( $P < 0.05$ ), 而在 Anti-miR-125a-5p/MCF-7 细胞组中, GAB2 的表达量

中 miR-125a-5p 与 GAB2 之间的关系。Fig. 1 结果显示, miR-125a-5p 能特异结合 GAB2 的 3'-UTR 端。

### 2.2 miR-125a-5p 在正常乳腺上皮细胞中高表达

通过 qRT-PCR 检测正常乳腺上皮细胞 MCF-10A 与乳腺癌细胞 MDA231、MCF-7 中 miR-125a-5p 的表达量。Fig. 2 结果显示, miR-125a-5p 在 MDA231 和 MCF-7 中表达量与 MCF-10A 相比均明显降低 ( $P < 0.05$ ); 与迁移能力相对较低的 MCF-7 细胞比较, miR-125a-5p 在迁移能力较高的 MDA231 细胞中的表达量更低 ( $P < 0.05$ )。结果表明, miR-125a-5p 可能在乳腺癌细胞中起抑癌基因的作用。

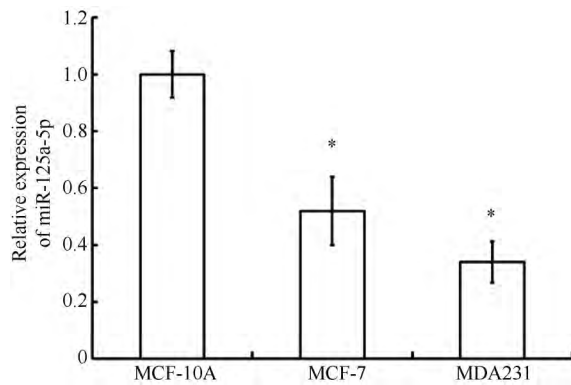
### 2.3 在乳腺癌细胞中过表达或者敲低 miR-125a-5p

通过 qRT-PCR 检测各组乳腺癌细胞中 miR-125a-5p 的表达量。Fig. 3 结果提示, 在 MDA231/miR-125a-5p 细胞组中, miR-125a-5p 的表达量明显高于 MDA231/NC 和 MDA231 细胞组中的表达量 ( $P < 0.05$ ), 而在 Anti-miR-125a-5p/MCF-7 中, miR-125a-5p 的表达量明显低于 Anti-NC/MCF-7 和 MCF-7 细胞组中的表达量 ( $P < 0.05$ )。结果表明转染成功。

明显高于 Anti-NC/MCF-7 组和 MCF-7 细胞组 ( $P < 0.05$ )。结果表明, miR-125a-5p 负向调控 GAB2 蛋白的表达。

### 2.5 miR-125a-5p 抑制乳腺癌细胞迁移能力

通过 Transwell 实验, 检测各组细胞的迁移能力。Fig. 5 结果显示, 在 MDA231/miR-125a-5p 细



**Fig. 2 miR-125a-5p is down-regulated in human breast cancer cells** Total RNA was isolated from MCF-10A, MDA231 and MCF-7 cells. The expression level of miR-125a-5p in various cells was examined by qRT-PCR. U6 was used as a control. Bars, standard deviation. \*  $P < 0.05$ . The experiment is repeated at least three times

胞组中,穿过基质胶的细胞数明显少于 MDA231/NC 中的细胞数 ( $P < 0.05$ ),而在 Anti-miR-125a-5p/MCF-7 中,穿过基质胶的细胞数明显多于 Anti-NC/MCF-7 中的细胞数 ( $P < 0.05$ )。结果表明,miR-125a-5p 能够显著抑制乳腺癌细胞的迁移能力。

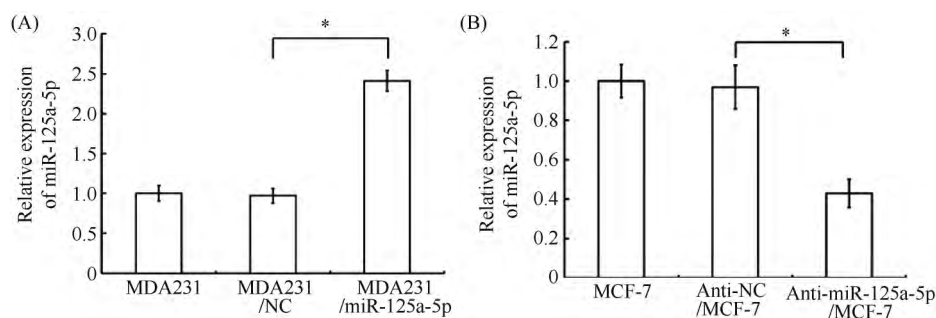
### 3 讨论

乳腺癌严重危害人类的健康,尽管目前的治疗水平不断提高,但其发病率仍居高不下。研究表明,侵袭和转移是引起乳腺癌患者死亡的关键因素<sup>[10]</sup>。因此,近年来对乳腺癌机制的研究备受关注,特别是如何控制乳腺癌的侵袭转移,如何预防和治疗乳腺癌以及如何提高患者生存率和生活质量等方面<sup>[11]</sup>。因此,研究乳腺癌的分子机制至

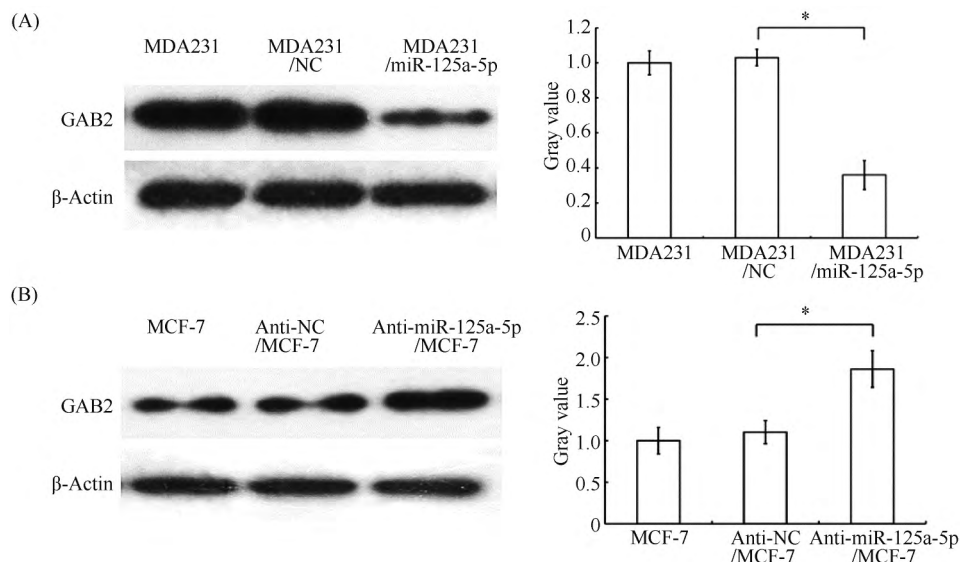
关重要。

GAB2 是一种非常重要的信号中介分子,在信号转导的放大和整合过程中,受多种生长因子及抗原等刺激而发挥作用<sup>[12]</sup>。GAB2 的高表达与各种恶性肿瘤,如卵巢癌<sup>[13]</sup>、结肠癌<sup>[14]</sup>密切相关。已有研究发现,GAB2 通过 GSK-3 $\beta$ /Snail 信号通路促进乳腺癌的上皮-间质转化<sup>[15]</sup>。本实验通过荧光素酶实验,检测 miR-125a-5p 与 GAB2 的关系。结果显示,miR-125a-5p 可特异性识别 GAB2 的 3'-UTR。荧光定量 PCR 结果发现,miR-125a-5p 在正常乳腺上皮细胞中的表达量较乳腺癌细胞中要高;与迁移能力相对较低的 MCF-7 细胞比较,miR-125a-5p 在迁移能力较高的 MDA231 细胞中的表达量更低。

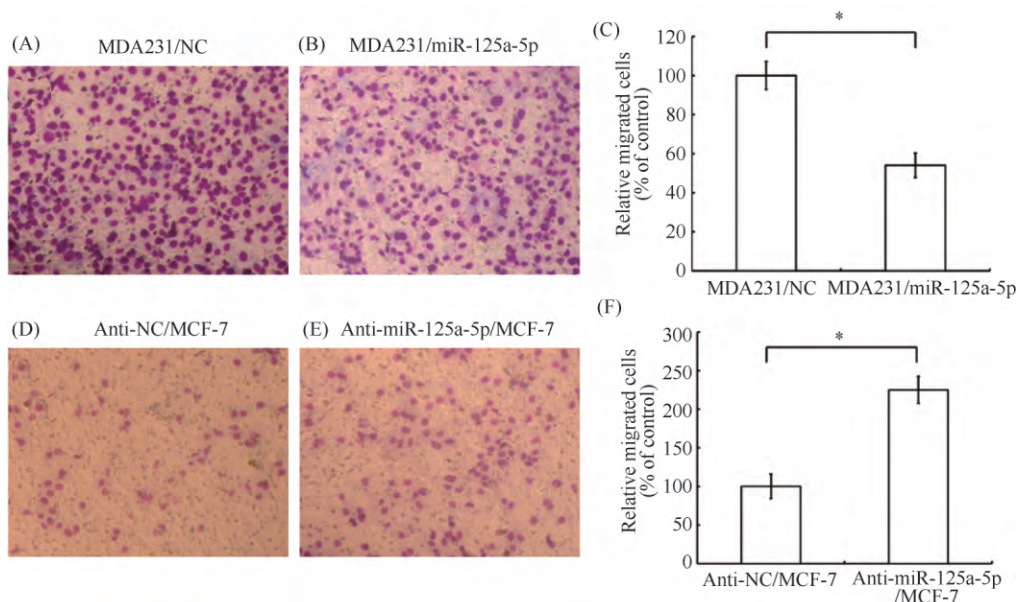
miRNAs 是一种非编码小 RNA,长度大约为 19~24 个核苷酸,通过清除 mRNA 或者抑制翻译过程而发挥作用<sup>[16]</sup>。多项研究表明,miR-125a-5p 与多种疾病关系密切,如白血病<sup>[17]</sup>、特发性关节炎<sup>[18]</sup>等。Zheng 等<sup>[19]</sup>发现,miR-125a-5p 可作为肝癌发展进程的标志物,在肝纤维化、肝癌中起监测作用。Cai 等<sup>[20]</sup>通过对甲状腺疾病的研究发现,miR-125a-5p 与免疫性甲状腺疾病相关。为了进一步验证 miR-125a-5p 与 GAB2 的关系,本实验发现,MDA231/miR-125a-5p 细胞组中 GAB2 的表达量明显减少,而在 Anti-miR-125a-5p/MCF-7 中 GAB2 的表达量明显增多,这进一步证明 miR-125a-5p 对 GAB2 负向调控作用。通过 Transwell 实验发现,MDA231/miR-125a-5p 细胞组中穿过基质胶的细胞数明显减少,而 Anti-miR-125a-5p/MCF-7 细胞组中穿过基质胶的细胞数的表达量明显增多,表明 miR-125a-5p 抑制乳腺癌细胞的迁移



**Fig. 3 Expression of miR-125a-5p in various breast cancer cells** Total RNA was isolated from various breast cancer cells. Expression levels of miR-125a-5p in various breast cancer cells were examined by qRT-PCR. U6 was used as a control. (A) Quantification analysis of the expression levels of miR-125a-5p in MDA231, MDA231/NC and MDA231/miR-125a-5p. (B) Quantification analysis of the expression levels of miR-125a-5p in MCF-7, anti-NC/MCF-7 and anti-miR-125a-5p/MCF-7. Bars, standard deviation \*  $P < 0.05$ . The experiment is repeated at least three times



**Fig. 4 miR-125a-5p negatively regulates GAB2** Total proteins were isolated from various breast cancer cells. The level of GAB2 was detected by Western blotting. (A) Quantification analysis of the level of GAB2 in MDA231, MDA231/NC and MDA231/miR-125a-5p. (B) Quantification analysis of the level of GAB2 in MCF-7, anti-NC/MCF-7 and anti-miR-125a-5p/MCF-7.  $\beta$ -Actin was used as a loading control. Bars, standard deviation. \*  $P < 0.05$ . The experiment is repeated at least three times



**Fig. 5 The migration capacity of various breast cancer cells** The migration capacity of various breast cancer cells were detected by Transwell after transfecting different plasmids for 24 hours. (A) Representative images of penetrated MDA231/NC. (B) Representative images of penetrated MDA231/miR-125a-5p. (C) Quantification analysis of Transwell assays on MDA231/miR-125a-5p and MDA231/NC. (D) Representative images of penetrated anti-NC/MCF-7 cells. (E) The representative pictures of penetrated anti-miR-125a-5p/MCF-7. (F) Quantification analysis of Transwell on anti-miR-125a-5p/MCF-7 and anti-NC/MCF-7 cells. Bars, standard deviation. \*  $P < 0.05$ . The experiment is repeated at least three times

能力。

综上所述,miR-125a-5p 通过与 GAB2 靶向结合,抑制乳腺癌细胞的迁移能力,为进一步研究乳腺癌侵袭与转移机制提供新的理论依据。

#### 参考文献(References)

- [1] Zhang JB, Song W, Wang YY, et al. Study on correlation between PKIB and pAkt expression in breast cancer tissues[J]. Eur Rev Med Pharmacol Sci, 2017, 21(6): 1264-1269

- [2] Fang H, Xie J, Zhang M, *et al.* miRNA-21 promotes proliferation and invasion of triple-negative breast cancer cells through targeting PTEN [J]. *Am J Transl Res*, 2017, **9**(3): 953-961
- [3] Xie Y, Zong P, Wang W, *et al.* Hypermethylation of potential tumor suppressor miR-34b/c is correlated with late clinical stage in patients with soft tissue sarcomas [J]. *Exp Mol Pathol*, 2015, **98**(3): 446-454
- [4] Dai J, Wang J, Yang L, *et al.* miR-125a regulates angiogenesis of gastric cancer by targeting vascular endothelial growth factor A [J]. *Int J Oncol*, 2015, **47**(5): 1801-1810
- [5] Jiang J, Gao Q, Wang T, *et al.* MicroRNA expression profiles of granulocytic myeloid-derived suppressor cells from mice bearing Lewis lung carcinoma [J]. *Mol Med Rep*, 2016, **14**(5): 4567-4574
- [6] Sun L, Zhang B, Liu Y, *et al.* MiR125a-5p acting as a novel Gab2 suppressor inhibits invasion of glioma [J]. *Mol Carcinog*, 2016, **55**(1): 40-51
- [7] Wang H, Nestor CE, Benson M, *et al.* GAB2 regulates type 2 T helper cell differentiation in humans [J]. *Cytokine*, 2017, **96**: 234-237
- [8] Zhang X, Dong Z, Zhang C, *et al.* Critical role for GAB2 in neuroblastoma pathogenesis through the promotion of SHP2/MYCIN cooperation [J]. *Cell Rep*, 2017, **18**(12): 2932-2942
- [9] 田红艳, 陈萍萍, 李笑, 等. Gab2 通过 PI3K/Akt/ARF5/MMP 途径影响乳腺癌的侵袭和转移 [J]. 中国药理学通报 (Tian HY, Chen PP, Li X, *et al.* Gab2 effects the invasion and metastasis of breast carcinoma through PI3K/Akt/ARF5/MMP signal pathway [J]. *Chin Pharmacol Bull*), 2015, **31**(7): 1014-1018
- [10] 孙志亮, 张胜超, 李洪利, 等. EBP50 通过 Wnt3a/ $\beta$ -catenin 信号通路抑制乳腺癌细胞的侵袭和转移 [J]. 中国生物化学与分子生物学报 (Sun ZL, Zhang SC, Li HL, *et al.* EBP50 inhibits invasion and migration of breast cancer cells through Wnt3a/ $\beta$ -catenin signaling pathway [J]. *Chin J Biochem Mol Biol*), 2016, **32**(9): 1027-1032
- [11] Li J, Zhang J, Wang Y, *et al.* Synergistic inhibition of migration and invasion of breast cancer cells by dual docetaxel/querceetin-loaded nanoparticles via Akt/MMP-9 pathway [J]. *Int J Pharm*, 2017, **523**(1): 300-309
- [12] Chen Y, Liu Q, Wu M, *et al.* GAB2 promotes cell proliferation by activating the ERK signaling pathway in hepatocellular carcinoma [J]. *Tumour Biol*, 2016, **37**(9): 11763-11773
- [13] Wang Y, Sheng Q, Spillman MA, *et al.* Gab2 regulates the migratory behaviors and E-cadherin expression via activation of the PI3K pathway in ovarian cancer cells [J]. *Oncogene*, 2012, **31**(20): 2512-2520
- [14] Ding C, Luo J, Yu W, *et al.* Gab2 is a novel prognostic factor for colorectal cancer patients [J]. *Int J Clin Exp Pathol*, 2015, **8**(3): 2779-2786
- [15] 田红艳, 李笑, 孙志亮, 等. Gab2 通过 GSK-3 $\beta$ /Snail 信号通路促进乳腺癌的上皮-间质转化 [J]. 中国癌症杂志 (Tian HY, Li X, Sun ZL, *et al.* Gab2 promotes epithelial-mesenchymal transition in breast cancer through GSK-3 $\beta$ /Snail signaling [J]. *China Oncol*), 2016, **26**(2): 134-139
- [16] Tu K, Zheng X, Dou C, *et al.* MicroRNA-130b promotes cell aggressiveness by inhibiting peroxisome proliferator-activated receptor gamma in human hepatocellular carcinoma [J]. *Int J Mol Sci*, 2014, **15**(11): 20486-20499
- [17] Rigolin GM, Saccenti E, Rizzotto L, *et al.* Genetic subclonal complexity and miR125a-5p down-regulation identify a subset of patients with inferior outcome in low-risk CLL patients [J]. *Oncotarget*, 2014, **5**(1): 140-149
- [18] Schuler GS, Fall N, Harley JB, *et al.* Monocyte microRNA expression in active systemic juvenile idiopathic arthritis implicates microRNA-125a-5p in polarized monocyte phenotypes [J]. *Arthritis Rheumatol*, 2016, **68**(9): 2300-2313
- [19] Zheng J, Zhou Z, Xu Z, *et al.* Serum microRNA-125a-5p, a useful biomarker in liver diseases, correlates with disease progression [J]. *Mol Med Rep*, 2015, **12**(1): 1584-1590
- [20] Cai T, Li J, An X, *et al.* Polymorphisms in MIR499A and MIR125A gene are associated with autoimmune thyroid diseases [J]. *Mol Cell Endocrinol*, 2017, **440**: 106-115