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基于生物信息学分析研究 miR-182-5p 通过靶向调控 EP300 促进乳腺癌细胞的侵袭和转移

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摘要 近期研究表明，miR-182-5p 对多种癌症的侵袭和转移具有重要作用，但其在乳腺癌侵袭转移中的研究相对较少。本研究通过网上在线 microRNA 分析工具下载乳腺癌组织及正常乳腺组织表达比较的数据集，分析发现在 GSE4589、GSE38167、GSE61438 等 3 个数据库中，在乳腺癌组织中存在 26 个相同的 microRNA，其中 8 个上调，而我们实验验证发现 hsa-miR-182 在 8 例病理组织中的表达上调差异最显著($P=0.001$)，选定目的基因 hsa-miR-182；qRT-PCR 检测细胞中 miR-182-5p 的表达，结果显示，与 MCF-10A 相比，miR-182-5p 在 MDA-MB-231、T47D、MDA-MB-453、MCF-7 中表达上调($P<0.05$)；转染 miR-182-5p 干扰质粒，qRT-PCR 检测细胞中 miR-182-5p 的表达情况。结果显示，miR-182-5p 表达显著降低($P=0.003$)，提示转染成功；Transwell 侵袭结果显示，MDA-MB-231 细胞敲低 miR-182-5p，与对照组相比，体外侵袭能力明显降低($P=0.002$)；Western 印迹检测转染 miR-182-5p 干扰质粒时，MDA-MB-231 中上皮-间质转化(epithelial-mesenchymal transition，EMT)相关标志物的表达情况，结果显示，与对照组相比，敲低 miR-182-5p 使细胞中上皮-钙黏着蛋白(E-cadherin)表达上调，神经-钙黏着蛋白(N-cadherin)、波形蛋白(vimentin)表达下调。为研究探讨 miR-182-5p 的靶蛋白，采用在线预测软件预测可能与 miR-182-5p 结合的靶蛋白，cytoscape 构建蛋白质互作网络图并筛选出 *hub* 基因；双荧光素酶结果证实，miR-182-5p 可与 EP300 靶向结合($P=0.001$)；采用 qRT-PCR、Western 印迹检测转染 miR-182-5p 干扰质粒后 EP300 在 mRNA 及蛋白质水平的表达，结果显示，与对照组相比，在敲低 miR-182-5p 组中 EP300 在 mRNA 及蛋白质的表达上调($P=0.001$)。综上所述，miR-182-5p 可靶向调节 EP300，促进乳腺癌细胞的侵袭与转移。

关键词 乳腺癌；miR-182-5p；EP300；侵袭；转移
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miR-182-5p Promoted the Invasion and Metastasis of Breast Cancer Cells by Targeting EP300

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Abstract Recent studies have shown that miR-182-5p plays an important role in the invasion and metastasis of a variety of cancers, but reports regarding to its roles in the invasion and metastasis of breast cancer is relatively rare. This study analyzed 3 GEO data sets through online miRNAs analysis tools, and the results found in 26 miRNAs were commonly expressed in breast cancer tissues in these 3 data sets, eight of them was up-regulated, and hsa-miR-182 was the most significantly up-regulated one in breast cancer tissues ($P=0.001$) and was selected in this study. As compared with MCF-10 A cells, miR-182-5p was up-regulated in MDA-MB-231, T47D, MDA-MB-453, and MCF-7 breast cancer cells by qRT-PCR analysis ($P < 0.05$). When the cells were transfected with miR-182-5p interfering plasmid, the expression of miR-182-5p was significantly decreased ($P = 0.003$), suggesting that transfection was successful. Results from transwell invasion test showed that after miR-182-5p was knocked down, the invasion ability of MDA-MB-231 cells was significantly reduced as compared with the control group ($P=0.002$). Western blot assay detected the expression of epithelial-mesenchymal transition (EMT) related markers in MDA-MB-231 after transfection of miR-182-5p interfering plasmid. The results showed that when compared with the control group, the suppression of miR-182-5p up-regulated the expression of E-cadherin and down-regulated the expression of N-cadherin and Vimentin. To analyze the targets of miR-182-5p, online prediction software was used to predict the possible binding target proteins with miR-182-5p. Cytoscape was applied to construct the protein interaction network and screen the hub genes. The results of the double luciferase experiment confirmed that miR-182-5p could bind to EP300 ($P=0.001$). qRT-PCR and Western blot analysis were used to detect EP300 expression at mRNA and protein levels after transfection of miR-182-5p interfering plasmids. The results showed that compared with the control group, EP300 expression at mRNA and protein levels were up-regulated in the miR-182-5p knockdown group ($P = 0.001$). In conclusion, miR-182-5p can target EP300 and promote the invasion and metastasis of breast cancer cells.

Key words breast cancer; miR-182-5p; EP300; invasion; metastasis

乳腺癌在全球范围内是女性最常见的恶性肿瘤之一，在世界范围威胁着妇女的生命安全，且近年来的发病率及死亡率居高不下[1-2]。乳腺癌的高侵袭性及转移性是导致其死亡率高的主要原因[3]。因此，研究探讨乳腺癌侵袭和转移的分子机制，寻找靶向治疗乳腺癌的指标，对乳腺癌治疗具有重要意义。

MicroRNAs(miRNAs)是一种长度为 18～24 nt 的非编码核酸分子，对调控细胞的生长发育、增殖、凋亡过程中具有重要意义[4]。我们的前期研究表明，miR-182-5p 可通过下调 RAB27 A 来增强人胃癌细胞的活力、有丝分裂、迁移和侵袭[5]；在肝细胞癌中，miR-182-5p 激活 Wnt/β-catenin 信号，通过抑制 FOXO3a 进而抑制 β-联蛋白(β-catenin)退化并提高与 β-联蛋白之间的交互作用[6]；先前研究表明，miR-182-5p 可以通过靶向结合 FOXF2 促进三阴乳腺癌的增殖和转移[7,8]；而且 miR-182-5p 通过靶向结合 SMAD7 在 TGF-β 诱导的乳腺癌 EMT 及转移中发挥重要作用[9]。但 miR-182-5p 是否还可以通过其他方式促进乳腺癌的侵袭和转移还有待于进一步研究。

1 材料与方法

1.1 患者病历资料

收集潍坊医学院附属医院 2018～2019 年的乳腺癌组织及其癌旁相对正常组织(>5 cm)各 8 例作为研究对象，按照 WHO 标准进行分级，患者均为女性，术前未经过任何化疗和放疗，临床资料完整齐全。所有乳腺癌患者及其家属均已签署知情同意书。

1.2　通过网上在线 microRNA 分析工具确定目的基因

从网上在线 microRNA 分析工具(http://www.picb.ac.cn/dbDEMC/browse.php) 下载关于乳腺癌组织和正常乳腺组织的数据。利用 Log_2 变换统一取数据库中 microRNA 表达的交集,采用数据处理进行差异表达分析,利用 $Log_2FC > 1$ 鉴定乳腺癌患者的上调基因。

1.3　miR-182-5p 靶基因的预测及确定

采用 miRmap、miRTarBase、miRWalk、RNA22v2、TargetScan 网上预测软件,下载 miR-182-5p 的靶基因的数据集,并取其交集,筛选确定其共有的靶基因,构建蛋白质相互作用网络; 在线预测软件预测 miR-182-5p 可能与靶基因存在的结合位点。

1.4　材料

逆转录试剂盒及 SYBR Green real-time PCR Master Mix 购自大连 TaKaRa 公司; EP300、β-肌动蛋白(β-actin) 、E-钙黏着蛋白(E-cadherin) 、N-钙黏着蛋白(N-cadherin) 、波形蛋白(vimentin) 抗体均购自艾博抗(上海) 贸易有限公司; 双荧光素酶报告基因载体、miR-182-5p 干扰及其对照质粒由吉凯基因构建; 实验细胞系 MCF-10 A、293T、MDA-MB-231、T47D、MDA-MB-453 和 MCF-7 由潍坊医学院医学研究实验中心提供。

1.5　细胞培养

MCF-10 A 及 293T 使用 10% FBS 的 DMEM 培养基,MDA-MB-231、T47D、MDA-MB-453 使用 10% FBS 的 RPMI 1640 培养基,MCF-7 使用 10% FBS 的 MEM 培养基。细胞转染依照 Lipofectamine 2000 说明书进行操作。细胞经转染不同质粒后分组: (1) MDA-MB-231 组: 正常培养,不做任何处理; (2) Anti-NC/MDA-MB-231 组: 转入 miR-182-5p 的对照干扰质粒; (3) Anti-miR-182-5p/MDA-MB-231 组: 转入 miR-182-5p 的干扰质粒。

1.6　qRT-PCR

使用 TRIzol 试剂从组织、细胞中提取总 RNA。使用分光光度计评估各组 RNA 的质量,再使用逆转录试剂盒合成互补 DNA(cDNA) 。将合成的 cDNA 稀释,依照扩增指令与 SYBR Green real-time PCR Master Mix 进行实时聚合酶链反应(quantitative real-time polymerase chain reaction,qRT-PCR) 。U6 的上游引物为 GCTTCGGCAGCACATATACTAAAAT,下游引物为 CGCTTCACGAATTTGCGTGTCAT; EP300 上游引物: TCCGAGACATCTTGAGACGACAG,下游引物: GGGTTGCTGGAACTGGTTATGG; miR-182-5p 上游引物: TTGGCAATGGTAGAACTCACAC,下游引物: AGTCCTCGCCCCATAGTTG,茎环结构: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGTGTG; miR-182-5p 表达使用 U6 作为内参,使用 $2^{-\Delta\Delta Ct}$ 分析的方法分析。

1.7　Western 印迹检测

将各组细胞提取总蛋白质,检测蛋白质浓度并进行凝胶电泳、转膜、封闭、一抗 4 ℃过夜、TBST 洗膜、二抗孵育、显影、曝光。实验重复 3 次。抗体配制如下: β-肌动蛋白(1∶1 000) 、EP300(1∶500) 、E-钙黏着蛋白(1∶1 000) 、N-钙黏着蛋白(1∶1 000) 、波形蛋白(1∶1 000) 。

1.8　Transwell 侵袭实验

制备含有基质胶的 Transwell 小室,细胞接种后培养 48 h,用棉签取出内侧未侵入的细胞。用 4% 多聚甲醛固定,吉姆萨试剂染色,在光学显微镜下随机选取 3 个区域进行计数。所有实验均重复 3 次,取均值作为最终结果。

1.9　双荧光素酶检测

将 miR-182-5p 过表达质粒及其对照分别与 EP300 的 3′UTR 野生型(pGL3-EP300 3′UTR-WT) 报告载体和突变型(pGL3-EP300 3′UTR-MUT) 报告载体共转染入 293T 细胞中,转染报告载体 48 h,吸取培养基上清,再用 PBS 洗涤细胞; 向培养孔中加入 PLB 裂解液,裂解 15 min; 收集裂解液并进行荧光素酶活性测定,以萤火虫荧光素酶活性值与海肾荧光素酶活性值的比值作为相对荧光素酶活性,并与空载对照比较。

1.10　统计学方法

采用 SPSS22.0 软件进行统计学分析计量结果使用均数 ± 标准差($\bar{x} \pm s$) 表示,两组间均数比较采用独立样本 t 检验,多组比较用单因素方差分析,$P<0.05$ 被认为差异具有统计学意义。

2　结果

2.1　分析比较 microRNA 数据库,选定 hsa-miR-182 作为研究对象

通过分析网上在线 microRNA 数据库中乳腺癌组织及正常乳腺组织表达情况,发现在 3 个数据库 GSE4589、GSE38167、GSE61438 中有 26 个相同的 microRNA,其中有 8 个表达上调的 microRNA。利用 qRT-PCR 检测 8 例乳腺癌组织中 miRNAs 的表达。结果显示,hsa-miR-182 在 8 个表达上调的 microRNA

中表达差异最大，且具有统计学意义（Fig. 1），选定 hsa-miR-182 作为后续实验的研究对象。miRNAs 及 NC 在组织中的相对表达及 P 值：NC：1.00 ± 0.06；hsa-miR-130b：1.43 ± 0.08，P = 0.003；hsa-miR-183：1.29 ± 0.06，P = 0.005；hsa-miR-185：1.27 ± 0.07，P = 0.006；hsa-miR-182：1.76 ± 0.09，P = 0.001。

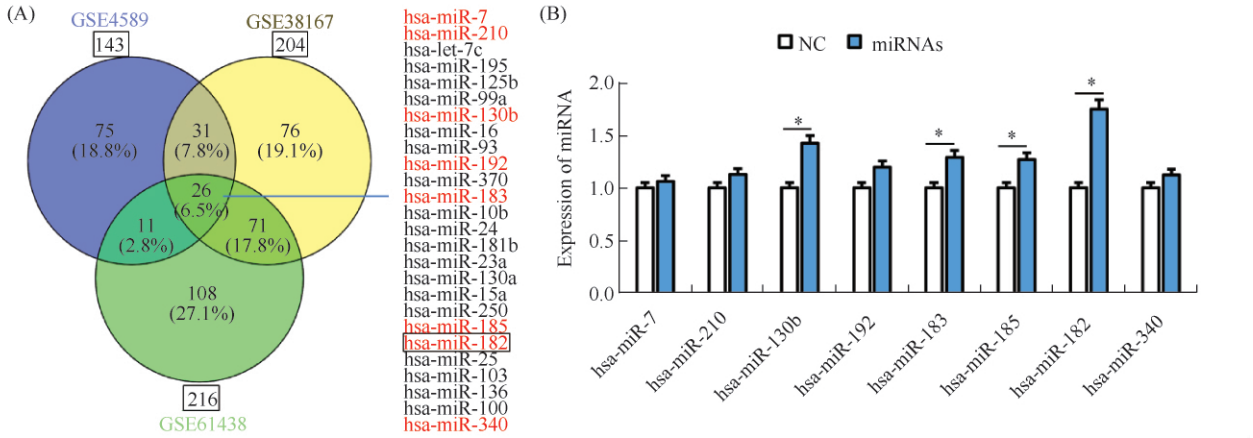

Fig.1 hsa-miR-182 was the most significantly upregulated miRNAs detected in 3 GEO data sets (A) By analyzing the expression of miRNAs in breast cancer tissues and normal breast tissues, the intersection of three GEO databases was taken to display the commonly expressed miRNAs, in which the red font represents the up-regulated miRNAs. (B) qRT-PCR was used to detect the expression of up-regulated miRNAs in 8 cases, * $P < 0.05$. The experiment was repeated three times

2.2 miR-182-5p 在乳腺癌细胞中的表达增高

使用 qRT-PCR 检测 miR-182-5p 在正常乳腺上皮细胞 MCF-10 A、乳腺癌细胞 MDA-MB-231、T47D、MDA-MB-453 和 MCF-7 中的表达情况。结果显示，在 MDA-MB-231、T47D、MDA-MB-453、MCF-7 细胞中 miR-182-5p 表达较高，在 MCF-10 A 中表达较低（Fig.2）。qRT-PCR 结果表明，miR-182-5p 在乳腺癌细胞中表达较高。miR-182-5p 在各组细胞中的相对表达及 P 值：MCF-10 A：1.00 ± 0.06；MDA-MB-231：2.53 ± 0.10，P = 0.000；T47D：1.79 ± 0.12，P = 0.002；MDA-MB-453：2.25 ± 0.10，P = 0.000；MCF-7：2.34 ± 0.13，P = 0.001。

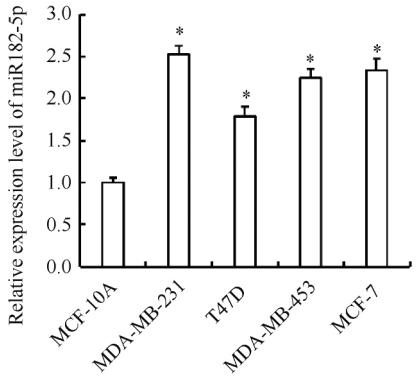

Fig.2 The expression of miR-182-5p in MCF-10 A, MDA-MB-231, T47D, MDA-MB-453 and MCF-7 cells was detected by qRT-PCR qRT-PCR was used to detect the expression of miR-182-5p in MCF-10 A normal breast epithelial cells, and breast cancer cells including MDA-MB-231, T47D, MDA-MB-453, and MCF-7 cells. miR-182-5p was highly expressed in MDA-MB-231 cells, so MDA-MB-231 cells were selected as cellular model of subsequent experiments. The value shows the mean ± standard deviation, * $P < 0.05$. The experiment was repeated three times

2.3 miR-182-5p 在乳腺癌细胞 MDA-MB-231 中的转染效率

转染 miR-182-5p 干扰质粒后，用 qRT-PCR 检测乳腺癌细胞 MDA-MB-231 中 miR-182-5p 的表达水平。结果显示，Anti-miR-182-5p/MDA-MB-231 组的 miR-182-5p 较 Anti-NC/MDA-MB-231 组表达明显降低（Fig.3）。结果表明转染成功。miR-182-5p

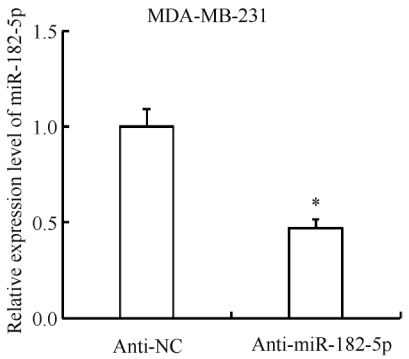

Fig.3 Transfection efficiency of miR-182-5p in MDA-MB-231 The control or interfering plasmids of miR-182-5p were transferred into MDA-MB-231 breast cancer cells. The expression of miR-182-5p was detected by qRT-PCR. U6 was used as the internal parameter. The value shows the mean ± standard deviation, * $P < 0.05$. The experiment was repeated three times

在乳腺癌细胞 MDA-MB-231 中的相对表达及 P 值: Anti-NC/MDA-MB-231 : 1.00 ± 0.09; Anti-miR-182-5p/MDA-MB-231 :0.47 ± 0.05, P = 0.003。

2.4　敲低 miR-182-5p 抑制乳腺癌细胞的侵袭能力

转染 miR-182-5p 干扰质粒后,利用 Transwell 侵袭实验检测 miR-182-5p 对乳腺癌细胞的侵袭能力的影响。结果显示, Anti-miR-182-5p/MDA-MB-231 组有更多的细胞穿过小室,且差异具有统计学意义(Fig.4) 。结果表明,敲低 miR-182-5p 可以抑制乳腺癌细胞的侵袭和迁移能力。转染 miR-182-5p 干扰质粒,乳腺癌细胞 MDA-MB-231 的相对侵袭情况及 P 值: Anti-NC/MDA-MB-231 : 100% ± 9%, Anti-miR-182-5p/MDA-MB-231 :48% ± 6%, P = 0.002。

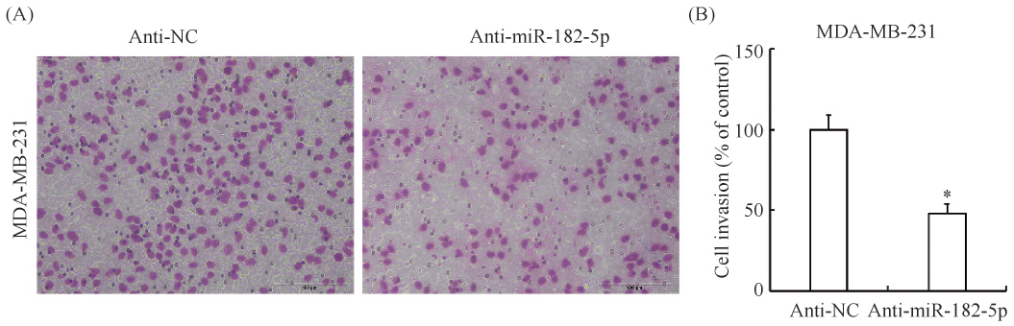

Fig.4　Knockdown of miR-182-5p inhibited invasion of breast cancer cells　After transfection of miR-182-5p interfering or control plasmids with Lipofectamine 2000, the cells were seeded for Transwell invasion assay. The number of cells passing through the basement membrane indicated their invasion ability, and 10 different sites were randomly observed. (A) Representative images of invaded cells transfected with miR-182-5p interfering or control plasmids. The scale bar is 100 micron meters. (B) Histogram was used to reflect the number of cells crossing the basement membrane. The value shows the mean ± standard deviation, * $P < 0.05$. The independent experiment was repeated three times

2.5　敲低 miR-182-5p 抑制乳腺癌细胞 EMT 的发生

为探究 miR-182-5p 对乳腺癌细胞 EMT 的影响,用 Western 印迹检测 Anti-miR-182-5p/ MDA-MB-231 与 Anti-NC/MDA-MB-231 中 E-钙黏着蛋白、N-钙黏着蛋白、波形蛋白的蛋白质表达水平。结果显示,与 Anti-NC/MDA-MB-231 组相比, Anti-miR-182-5p/MDA-MB-231 组中 E-钙黏着蛋白表达上调,而 N-钙黏着蛋白和波形蛋白的表达下调(Fig. 5) 。结果表明, miR-182-5p 可促进乳腺癌细胞的 EMT 的发生。

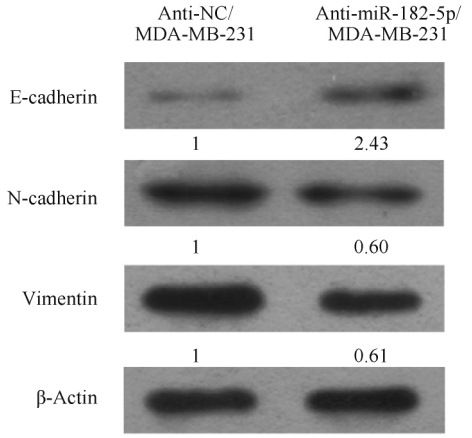

Fig.5　Knockdown of miR-182-5p inhibited EMT in breast cancer cells　After transfection, the cells in each group were cultured for 24 hours to extract total proteins, and the expression levels of E-cadherin, N-cadherin and Vimentin in Anti-miR-182-5p/MDA-MB-231 and Anti-NC/MDA-MB-231 were detected by Western blot assay. The experiment was repeated three times

2.6　miR-182-5p 与 EP300 mRNA 靶向结合

为探讨 miR-182-5p 通过哪种靶蛋白质影响乳腺癌细胞的侵袭能力,利用 miRmap、miRTarBase、miRWalk、RNA22v2、TargetScan 预测软件预测其靶蛋白质并取其交集。使用 cytoscape3. 7. 1 做 miR-182-5p 蛋白质互作网络,使用 cytoHubba 插件筛选 *hub* 基因,挑选 EP300 为研究对象,在线预测 miR-182-5p 与 EP300 存在的结合位点(Fig.6) 。

2.7　miR-182-5p 可靶向结合 EP300

采用双荧光素酶检测 miR-182-5p 能否与 EP300 靶向结合。结果显示,通过将 miR-182-5p 过表达质粒与 pGL3-EP300 3′-UTR-WT 报告载体共转染,荧光素酶活性显著降低(Fig. 7) 。结果证实, miR-182-5p 与 EP300 mRNA 的 3′UTR 可靶向结合。共转染后,各组细胞中荧光素酶相对活性及 P 值: pGL3-EP300 3′-UTR-WT/293T + miR-NC: 1.00 ± 0.08; pGL3-EP300 3′-UTR-WT/293T + miR-182-5p: 0.48 ± 0.06, P = 0.001; pGL3-EP300 3′-UTR-MUT/293T + miR-NC: 1.00 ± 0.07; pGL3-EP300 3′-UTR-MUT/ 293T+miR-182-5p: 1.08 ± 0.09。

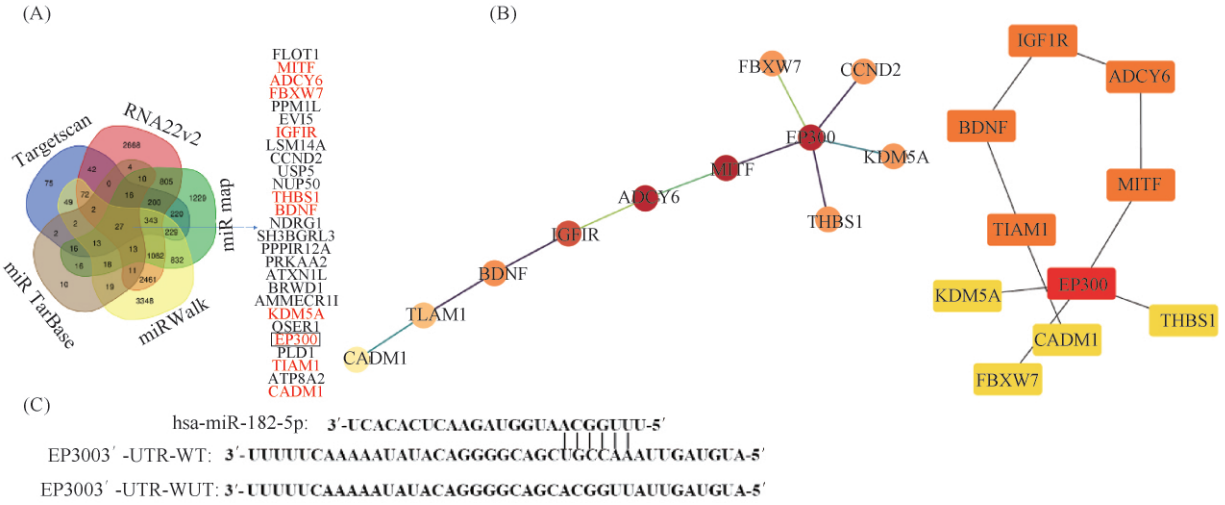

Fig.6 miR-182-5p binded to EP300 (A) miRmap, miRWalk, RNA22v2, miRTarBase, and TargetScan database were used to predict possible target proteins of miR-182-5p, and the target proteins of miR-182-5p were summarized; (B) A diagram of protein interaction network of miR-182-5p and a hub gene, EP300, was selected as the target protein; (C) Online prediction of the binding site of miR-182-5p and EP300

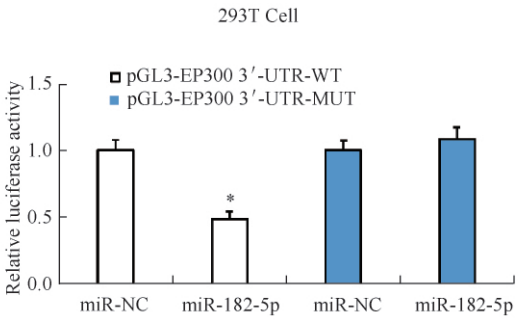

Fig.7 EP300 was a target of miR-182-5p Luciferase activity was investigated in 293T cells co-transfected with WT or MUT luciferase vectors, and combined with miR-182-5p or their negative control. The value shows the mean ± standard deviation, * $P<0.05$. The experiment was repeated three times

2.8 miR-182-5p 靶向结合 EP300

为进一步证实 miR-182-5p 可与 EP300 靶向结合，转染 miR-182-5p 干扰质粒，利用 qRT-PCR、Western 印迹检测 EP300 的表达水平。结果显示，EP300 在 Anti-miR-182-5p/MDA-MB-231 组中均表达上调(Fig.8)。结果表明，miR-182-5p 可靶向结合 EP300。转染 miR-182-5p 干扰质粒后，EP300 mRNA 在 MDA-MB-231 细胞中的相对表达及 P 值：Anti-NC/MDA-MB-231：1.00 ± 0.08；Anti-miR-182-5p/MDA-MB-231：1.76 ± 0.10，$P=0.001$。

3 讨论

乳腺癌是女性常见的激素依赖性肿瘤，约占女性恶性肿瘤的 23%，且以 3.1%的趋势逐年升高[1]。

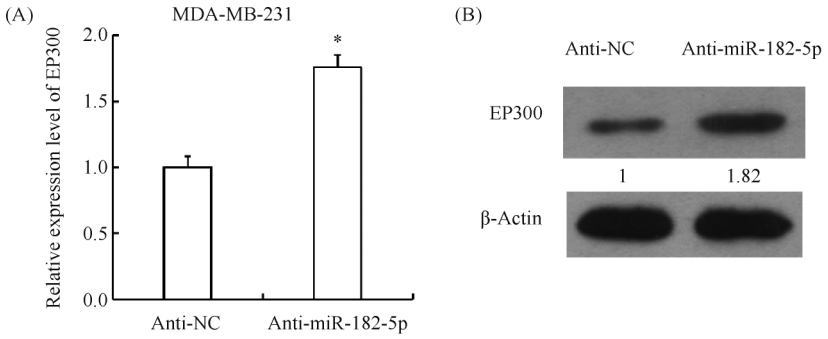

Fig.8 miR-182-5p targeted EP300 After transfection of miR-182-5p interference plasmid, qRT-PCR and Western blot results showed that knockdown of miR-182-5p promoted the expression of EP300. The value shows the mean ± standard deviation, * $P<0.05$. The experiment was repeated three times

与诸多肿瘤相似,乳腺癌死亡的患者 90%以上多因转移所致,乳腺癌的术后复发、高侵袭和转移性是乳腺癌高死亡率的重要原因[4]。随着对 microRNA 研究探讨的深入,为 microRNA 能否成为治疗乳腺癌的精准靶标,以及判断预后的标志物提供了重要依据。

MicroRNAs(miRNAs) 是一种小的非编码 RNA 分子,长约 22 个核苷酸,可通过沉默或降解靶 mRNA 在基因翻译表达过程中发挥重要的调控作用[11]。miRNA 几乎参与所有的生理过程,包括细胞的分化、增殖、代谢、止血、凋亡或炎症反应[12]。但 miRNA 的生物发生和功能受到严格的调控,其调控异常通常与癌症、心血管疾病或神经系统疾病等多种疾病的病理有关[13]。近年来,miRNA 被认为是预测疾病、治疗反应或进展预测的强有力的生物标志物[14]。近期研究表明,miR-182-5p 在肝细胞癌、结直肠癌、胃癌、卵巢癌等癌症的增殖、肿瘤形成、侵袭和转移中发挥重要作用[15-17]。因此,探究 miR-182-5p 在乳腺癌中发挥的重要作用便显得尤为重要。本文通过网上在线 microRNA 分析工具,分析及检测 hsa-miR-182 在乳腺癌组织中的表达并选定目的基因 hsa-miR-182。通过 qRT-PCR 实验证实,miR-182-5p 在乳腺癌细胞 MDA-MB-231 中表达较高($P < 0.05$) 。Transwell 检测证实,敲低 miR-182-5p 可有效抑制乳腺癌细胞 MDA-MB-231 的侵袭能力($P = 0.002$) 。结果表明,miR-182-5p 在乳腺癌的侵袭中发挥重要作用。

上皮-间质转化是参与乳腺癌侵袭与转移的重要环节,对乳腺癌的发生发展发挥重要作用,是指细胞失去上皮极性并向间充质细胞转变,从而使细胞的侵袭性与转移性提高的过程[4],在慢性炎症、肿瘤转移、伤口愈合及各种纤维化疾病中发挥重要作用[18]。前期研究结果表明,多种分子机制导致 EMT 发生过程中 E-钙黏着蛋白的表达降低[19],进而促进 EMT 的发生。在本研究中,Western 印迹结果表明,敲低 miR-182-5p 可有效抑制 EMT 的发生。

EP300 蛋白是一种组蛋白乙酰转移酶[20,21],通过染色质重构调控转录[22],在细胞增殖和分化过程中发挥重要的调节作用[23,24]。前期研究表明,EP300 和 E-钙黏着蛋白在非恶性肿瘤和癌症病例中均呈正相关,两者都可作为预测因子预测淋巴结转移,且优于单独使用 E-钙黏着蛋白[25]。EP300 能够上调 E-钙黏着蛋白的表达,对于维持细胞上皮表型,避免 EMT 的发生,抑制肿瘤转移发挥重要作用[26]。当 EP300 激活 E-钙黏着蛋白表达时,它可以作为肿瘤抑制因子。但是,如果 E-钙黏着蛋白表达被阻断,EP300 通过上调醛酮还原酶作为致癌基因[27]。本研究中,通过网上预测软件预测出 miR-182-5p 的蛋白质相互作用网络,并筛选出 *hub* 基因,双荧光素酶检测 miR-182-5p 与靶蛋白 EP300 之间存在结合位点($P = 0.001$) ,qRT-PCR 及 Western 印迹检测敲低 miR-182-5p 后 EP300 的表达情况,结果显示,EP300 在敲低 miR-182-5p 组均表达上调($P = 0.001$) 。结果表明,miR-182-5p 可靶向结合 EP300。

综上所述,本文研究证实,miR-182-5p 在乳腺癌细胞 MDA-MB-231 中高表达,且可靶向调控 EP300 促进乳腺癌细胞的侵袭转移。对于 miR-182-5p 是否可以通过其他分子靶向途径对乳腺癌细胞侵袭转移的产生影响,将会在本课题组后续研究中展开。我们目前的研究结果,为乳腺癌发生和发展的分子机制提供了新的见解。但是,miR-182-5p 是否可以作为乳腺癌的新型治疗靶标有待于更多的分子机制研究。有望通过研究控制 miR-182-5p 在乳腺癌中的表达,进而为治疗乳腺癌提供新的研究思路。

参考文献(References)

[1] DeSantis CE, Fedewa SA, Goding Sauer A, *et al*. Breast cancer statistics, 2015: Convergence of incidence rates between black and white women [J]. CA Cancer J Clin, 2016, **66**(1): 31-42

[2] Madhavan D, Peng C, Wallwiener M, *et al*. Circulating miRNAs with prognostic value in metastatic breast cancer and for early detection of metastasis [J]. Carcinogenesis, 2016, **37**(5): 461-470

[3] Li W, Zhang B, Li H, *et al*. TGF β1 mediates epithelial mesenchymal transition via β6 integrin signaling pathway in breast cancer [J]. Cancer Invest, 2014, **32**(8): 409-415

[4] Mansoori B, Mohammadi A, Ghasabi M, *et al*. miR-142-3p as tumor suppressor miRNA in the regulation of tumorigenicity, invasion and migration of human breast cancer by targeting Bach-1 expression [J]. J Cell Physiol, 2019, **234**(6): 9816-9825

[5] Li Y, Chen S, Shan Z, *et al*. miR-182-5p improves the viability, mitosis, migration, and invasion ability of human gastric cancer cells by down-regulating RAB27A [J]. Biosci Rep, 2017, **37**(3).pii: BSR20170136

[6] Cao MQ, You AB, Zhu XD, *et al*. miR-182-5p promotes hepatocellular carcinoma progression by repressing FOXO3a [J]. J Hematol Oncol, 2018, **11**(1): 12

[7] Zhang X, Ma G, Liu J, *et al*. MicroRNA-182 promotes proliferation and metastasis by targeting FOXF2 in triple-negative breast cancer [J]. Oncol Lett, 2017, **14**(4): 4805-4811

[8] Yu J, Shen W, Gao B, *et al*. MicroRNA-182 targets FOXF2 to promote the development of triple-negative breast cancer [J]. Neoplasma, 2017, **64**(2): 209-215

[9] Yu J, Lei R, Zhuang X, *et al*. MicroRNA-182 targets SMAD7 to potentiate TGFβ-induced epithelial- mesenchymal transition and metastasis of cancer cells [J]. Nat Commun, 2016, **7**: 13884

[10] Dai Y, Zhang X. MicroRNA Delivery with Bioreducible

Polyethylenimine as a Non-Viral Vector for Breast Cancer Gene Therapy [J]. Macromol Biosci, 2019, **19**(4): e1800445
[11] Almanza G, Rodvold JJ, Tsui B, *et al*. Extracellular vesicles produced in B cells deliver tumor suppressor miR-335 to breast cancer cells disrupting oncogenic programming in vitro and in vivo [J]. Sci Rep, 2018, **8**(1): 17581
[12] Fang R, Zhu Y, Hu L, *et al*. Plasma MicroRNA Pair Panels as Novel Biomarkers for Detection of Early Stage Breast Cancer [J]. Front Physiol, 2019, **9**: 1879
[13] Bellissimo T, Tito C, Ganci F, *et al*. Argonaute 2 drives miR-145-5p-dependent gene expression program in breast cancer cells [J]. Cell Death Dis, 2019, **10**(1): 17
[14] Borghei YS, Hosseini M. A New Eye Dual-readout Method for MiRNA Detection based on Dissolution of Gold nanoparticles via LSPR by CdTe QDs Photoinduction [J]. Sci Rep, 2019, **9**(1): 5453
[15] Wang X, Chen L, Jin H, *et al*. Screening miRNAs for early diagnosis of colorectal cancer by small RNA deep sequencing and evaluation in a Chinese patient population [J]. Onco Targets Ther, 2016, **9**: 1159-1166
[16] Sun H, Xi P, Sun Z, *et al*. Circ-SFMBT2 promotes the proliferation of gastric cancer cells through sponging miR-182-5p to enhance CREB1 expression [J]. Cancer Manag Res, 2018, **10**: 5725-5734
[17] Wang L, Zhu MJ, Ren AM, *et al*. A ten-microRNA signature identified from a genome-wide microRNA expression profiling in human epithelial ovarian cancer [J]. PLoS One, 2014, **9**(5): e96472
[18] Tang T, Yang Z, Zhu Q, *et al*. Up-regulation of miR-210 induced by a hypoxic microenvironment promotes breast cancer stem cells metastasis, proliferation, and self-renewal by targeting E-cadherin [J]. FASEB J, 2018, fj201801013R
[19] Guo XW, Xiang CX, Zhang Z, *et al*. Displacement of Bax by BMF Mediates STARD13 3′UTR-Induced Breast Cancer Cells Apoptosis in an miRNA-Depedent Manner [J]. Mol Pharm, 2018, **15**(1): 63-71
[20] Garcia-Carpizo V, Ruiz-Llorente S, Sarmentero J, *et al*. CREBBP/EP300 Bromodomain Inhibition Affects the Proliferation of AR-Positive Breast Cancer Cell Lines [J]. Mol Cancer Res, 2019, **17**(3): 720-730
[21] Peng Y, Wang Y, Tang N, *et al*. Andrographolide inhibits breast cancer through suppressing COX-2 expression and angiogenesis via inactivation of p300 signaling and VEGF pathway [J]. J Exp Clin Cancer Res, 2018, **37**(1): 248
[22] Grunstein M. Histone acetylation in chromatin structure and transcription [J]. Nature, 1997, **389**(6649): 349-352
[23] Bemanian V, Noone JC, Sauer T, *et al*. Somatic EP300-G211S mutations are associated with overall somatic mutational patterns and breast cancer specific survival in triple-negative breast cancer [J]. Breast Cancer Res Treat, 2018, **172**(2): 339-351
[24] Lucas J, Hsieh TC, Halicka HD, *et al*. Upregulation of PD-L1 expression by resveratrol and piceatannol in breast and colorectal cancer cells occurs via HDAC3/p300-mediated NF-κB signaling [J]. Int J Oncol, 2018, **53**(4): 1469-1480
[25] Mahmud Z, Asaduzzaman M, Kumar U, *et al*. Oncogenic EP300 can be targeted with inhibitors of aldo-keto reductases [J]. Biochem Pharmacol, 2019, **163**: 391-403
[26] Asaduzzaman M, Constantinou S, Min H, *et al*. Tumour suppressor EP300, a modulator of paclitaxel resistance and stemness, is downregulated in metaplastic breast cancer [J]. Breast Cancer Res Treat, 2017, **163**(3): 461-474
[27] Hu Y, Li K, Asaduzzaman M, *et al*. MiR-106b ~ 25 cluster regulates multidrug resistance in an ABC transporter-independent manner via downregulation of EP300 [J]. Oncol Rep, 2016, **35**(2): 1170-1178