·ARTICLES· ·论 著·

DOI:10.11817/j.issn.1672-7347.2020.180459
http://xbyxb.csu.edu.cn/xbwk/fileup/PDF/202003280.pdf

# 微小RNA-92a-3p靶向PTEN调控胰腺癌细胞增殖和转移

刘宇，胡庆，敖晶晶，李红玲，李美梁

（湖南省人民医院病理科，长沙 410002）

[摘 要] **目的：**探讨微小RNA-92a-3p(microRNA-92a-3p，miR-92a-3p)靶向第10号染色体缺失的磷酸酶及张力蛋白同源基因(phosphatase and tension homolog deleted on chromosome ten，PTEN)调控胰腺癌细胞增殖和转移的作用机制。**方法：**采用Real-time PCR检测人正常胰腺导管上皮细胞株(HPDE6-C7)与胰腺癌细胞株(Panc-1，BxPC-3，AsPC-1，MIA Paca-2，Capan-2)中的miR-92a-3p表达；同时，采用蛋白质印迹法检测上述细胞中PTEN的表达。选取胰腺癌细胞株BxPC-3和Panc-1的细胞进行实验，分为转染对照模拟物组(NC mimics组)、miR-92a-3p模拟物组(miR-92a-3p mimics组)、对照拮抗剂组(NC inhibitor组)、miR-92a-3p拮抗剂(miR-92a-3p inhibitor组)，采用细胞计数试剂盒-8(cell counting kit-8，CCK-8)检测细胞增殖，Transwell小室检测细胞转移能力，蛋白质印迹法检测PTEN蛋白表达水平。将野生型PTEN的3'-非编码区(untranslated regions，UTR)(wt-PTEN 3'UTR)或突变体PTEN的3'-UTR(mut-PTEN 3'UTR)分别与NC mimics，miR-92a-3p mimics，NC inhibitor，miR-92a-3p inhibitor共转染293T细胞，采用双萤光素酶报告实验检测miR-92a-3p与PTEN的靶向结合关系。在BxPC-3细胞的回复实验中，实验又分NC inhibitor+si-NC组、miR-92a-3p inhibitor+si-NC组、NC inhibitor+si-PTEN组和miR-92a-3p inhibitor+si-PTEN组；在Panc-1细胞的回复实验中，实验又分为NC mimics+NC组、miR-92a-3p mimics+NC组、NC mimics+PTEN组和miR-92a-3p mimics+PTEN组，再分别采用CCK-8检测细胞增殖，Transwell小室检测细胞转移能力，蛋白质印迹法检测PTEN蛋白的表达。**结果：**与HPDE6-C7细胞相比，miR-92a-3p在胰腺癌细胞株中均呈高表达(均$P<0.01$)，PTEN在胰腺癌细胞株中均呈低表达(均$P<0.05$)。与NC mimics组相比，miR-92a-3p mimics组BxPC-3和Panc-1细胞活力均升高(均$P<0.01$)；与NC inhibitor组相比，miR-92a-3p inhibitor组BxPC-3和Panc-1细胞活力均降低(均$P<0.01$)。与NC mimics组相比，miR-92a-3p mimics组穿过微孔的BxPC-3和Panc-1细胞数均增多(均$P<0.01$)；与NC inhibitor组相比，miR-92a-3p inhibitor组穿过微孔的BxPC-3和Panc-1细胞数均减少(均$P<0.01$)。与NC mimics组相比，miR-92a-3p mimics组的wt-PTEN 3'UTR荧光素酶活性被抑制($P<0.01$)；与NC inhibitor组相比，miR-92a-3p inhibitor组的wt-PTEN 3'UTR荧光素酶活性被增强($P<0.01$)。与miR-92a-3p inhibitor+si-NC组相比，miR-92a-3p inhibitor+si-PTEN组恢复了miR-92a-3p inhibitor对BxPC-3细胞增殖和转移的影响；与miR-92a-3p mimics+NC组相比，miR-92a-3p mimics+PTEN组恢复了miR-92a-3p mimics对Panc-1细胞增殖和转移的影响。**结论：**MiR-92a-3p可靶向抑制PTEN的表达，促进胰腺癌细胞的增殖和转移。

[关键词] 胰腺癌；转移；增殖；微小RNA-92a-3p；10号染色体缺失的磷酸酶及张力蛋白同源物

**收稿日期(Date of reception)：**2018-08-03
**第一作者(First author)：**刘宇，Email: 27817498@qq.com, ORCID: 0000-0001-6734-3241
**通信作者(Corresponding author)：**刘宇，Email: 27817498@qq.com, ORCID: 0000-0001-6734-3241
**基金项目(Foundation item)：**湖南省人民医院仁术基金(RS201809)。This work was supported by the Renshu Foundation of Hunan Provincial People's Hospital, China (RS201809).

©Journal of Central South University (Medical Science). All rights reserved.

# Role of miR-92a-3p/PTEN axis in regulation of pancreatic cancer cell proliferation and metastasis

LIU Yu, HU Qing, AO Jingjing, LI Hongling, LI Meiliang

*(Department of Pathology, Hunan Provincial People's Hospital, Changsha 410002, China)*

**ABSTRACT**

**Objective:** To explore the effect of miR-92a-3p on the proliferation and metastasis of pancreatic cancer cells via targeting phosphatase and tension homolog deleted on chromosome ten (PTEN).

**Methods:** MiR-92a-3p expression and PTEN protein levels were quantified in a normal pancreatic cells (HPDE6-C7) and 5 pancreatic cancer cell lines (Panc-1, BxPC-3, AsPC-1, MIA Paca-2, and Capan-2) by real-time PCR and Western blotting, respectively. BxPC-3 and Panc-1 cells were selected for further experiment. After transfection of normal control (NC) mimics (NC mimics group), miR-92a-3p mimics (miR-92a-3p mimics group), NC inhibitor or miR-92a-3p inhibitor (NC inhibitor group or miR-92a-3p inhibitor group), the proliferation of BxPC-3 and Panc-1 cells was measured by cell counting kit-8 (CCK-8), and the migration of them was measured by Transwell assay, and the levels of PTEN protein were measured by Western blotting. In addition, wild-type PTEN 3'-UTR (wt-PTEN 3'UTR) and mutant-type PTEN 3'-UTR (mut-PTEN 3'UTR) luciferase reporter vectors were constructed and co-transfected with NC mimics, miR-92a-3p mimic, NC inhibitor or miR-92a-3p inhibitor into 293T tool cells, and then the dual luciferase reporter assay was performed to examine the regulative correlation between miR-92a-3p and PTEN. The BxPC-3 cells were divided into 4 groups: a NC inhibitor+si-NC group, a miR-92a-3p inhibitor+si-NC group, a NC inhibitor+si-PTEN group, and a miR-92a-3p inhibitor+si-PTEN group. The Panc-1 cells were also assigned into 4 groups: a NC mimics+NC group, a miR-92a-3p mimics+si-NC group, a NC mimics+ PTEN group, and a miR-92a-3p mimics+PTEN group. The proliferation of Panc-1 cells was measured by CCK-8; the cell migration was measured by Transwell assay, and the levels of PTEN protein were measured by Western blotting.

**Results:** The miR-92a-3p was highly expressed in pancreatic cancer cell lines (all $P<0.01$), while the PTEN protein levels were lower in pancreatic cancer cell lines (all $P<0.05$) compared with that in the HPDE6-C7 cells. Compared with the NC mimics group, the cell viability of BxPC-3 and Panc-1 cells were both increased in the miR-92a-3p mimics group (both $P<0.01$); compared with the inhibitor group, the cell viability of BxPC-3 and Panc-1 cells were both decreased in the miR-92a-3p inhibitor group (both $P<0.01$). Compared with the NC mimics group, the cell number of BxPC-3 and Panc-1 cells through micropores were increased in the miR-92a-3p mimics group (both $P<0.01$); compared with the inhibitor group, the cell number of BxPC-3 and Panc-1 cells through micropores were decreased in the miR-92a-3p inhibitor group (both $P<0.01$). Compared with NC mimics group, the activity of dual luciferaseof wt-PTEN3'-UTR was inhibited in the miR-92a-3p mimics group ($P<0.01$); compared with the NC inhibitor group, the activity of dual

©Journal of Central South University (Medical Science). All rights reserved.

luciferase of wt-PTEN3'-UTR was promoted in the miR-92a-3p inhibitor group ($P$<0.01). Compared with the miR-92a-3p inhibitor+si-NC group, the suppressive effects of miR-92a-3p on the proliferation and metastasis of BxPC-3 cells was restored in the miR-92a-3p inhibitor+si-PTEN group; while compared with the miR-92a-3p mimics+NC group, the positive effects of miR-92a-3p overexpression on the proliferation and metastasis of Panc-1 cells was restored in the miR-92a-3p mimics+PTEN group.

**Conclusion:** The highly expressed miR-92a-3p in pancreatic cancer cells can decrease the protein levels of PTEN, thereby enhancing the proliferation and metastasis activity of pancreatic cancer cells.

**KEY WORDS** pancreatic cancer; metastasis; proliferation; miR-92a-3p; phosphatase and tension homolog deleted on chromosome ten

胰腺癌(pancreatic cancer，PC)是一种早期确诊困难、恶性程度高、预后差的消化系统常见肿瘤，病死率高居恶性肿瘤的第4位。固然，PC所具有的高度恶性生物学特点是导致患者病死率高、生存期短的关键性因素，但该病发生和演进机制不明也是重要的原因[1-2]。

第10号染色体缺失的磷酸酶及张力蛋白同源基因(phosphatase and tensin homologue deleted on chromosome 10，PTEN)是一种重要的抑癌基因，可编码同时具脂质磷酸酶和酪氨酸磷酸酶活性的蛋白质[3]。现已证明在PC和前列腺癌、卵巢癌等不同实体肿瘤中均存在PTEN低表达、突变或缺失，进而导致其抑癌功能遭到削弱[4-6]。有研究[7]证实，微小RNA(microRNA，miR)在PTEN的表达调控和肿瘤发病机制中均扮演着重要角色。MiR是一种长约22 nt的单链非编码RNA，可通过抑制靶基因mRNA的翻译或诱使其降解，从而参与PC增殖、凋亡、迁移和侵袭等病理过程[8]。本研究通过癌症基因组图谱门户网站LinkedOmics(www.linkedomics.org)筛选，发现miR-92a-3p与PTEN在PC组织中存在负相关性，通过miR靶位点预测网站targetscan(www.targetscan.org/vert_72/)分析发现PTEN mRNA可能存在miR-92a-3p的结合位点。已有研究[9-11]报道miR-92a-3p可通过靶向抑制具有kazal基序的富含逆向诱导的半胱氨酸蛋白(reversion inducing cysteine rich protein with kazal mo-tifs，RECK)、含F-框WD重复域蛋白7(F-Box and WD repeat domain containing protein 7，FBXW7)、整合素α-5(integrin alpha-5，ITGA5)等基因的表达而促进肺癌、宫颈癌、卵巢癌等发生和发展，但miR-92a是否介导PTEN参与PC发病鲜见报道。因此，本研究拟进一步探究miR-92a-3p与PTEN在PC细胞增殖和转移中的功能作用。

## 1 材料与方法

### 1.1 材料

Panc-1，BxPC-3，AsPC-1，MIA Paca-2和Capan-2等PC细胞株均来源于美国模式培养物保藏中心(American Type Culture Collection，ATCC)；正常胰腺导管上皮细胞株HPDE6-C7来源于国家典型培养物保藏中心(National Type Culture Collection，NTCC)。RPMI-1640培养基、胎牛血清、Lipofectamine™ 2000购于美国Thermo Fisher Scientific公司；miR模拟物(mimics)[包括miR-92a-3p mimics和对照mimics(NC mimics)]、miR拮抗剂(包括miR-92a-3p inhibitor和NC inhibitor)、real-time PCR引物等均购于上海吉玛制药技术有限公司；PTEN和β-肌动蛋白(β-actin)等抗体购于美国Abcam公司；小片段干扰RNA(si-RNA)、si-PTEN及无关干扰片段(si-NC)购于广州锐博生物技术有限公司；细胞计数试剂盒-8(cell counting kit-8，CCK-8)购于日本Dojindo公司；Transwell小室购于美国Costar公司；双荧光素酶报告系统购于北京普洛麦格生物技术公司；细胞周期检测试剂盒、细胞培养、点突变试剂盒和蛋白质印迹法相关试剂购于上海碧云天生物技术有限公司。

### 1.2 方法

#### 1.2.1 细胞培养及转染

HPDE6-C7细胞株、PC细胞株等均使用含10%胎牛血清的RPMI-1640培养基，置于37 ℃，5%$CO_2$的细胞培养箱中培养。3~4 d更换1次培养基，待细胞长至培养瓶的85%左右时，使用0.25%的胰蛋白酶进行消化，并以1∶3的比例传代培养。取对数生长期

©Journal of Central South University (Medical Science). All rights reserved.

细胞，采用real-time PCR检测miR-92a-3p的表达，蛋白质印迹法检测PTEN蛋白的表达。

为了检测miR-92a-3p对细胞增殖、转移和PTEN表达的影响，取对数生长期Panc-1和BxPC-3细胞，分别转染NC mimics(NC mimics组)，miR-92a-3p mimics (miR-92a-3p mimics组)，NC inhibitor(NC inhibitor组)，miR-92a-3p inhibitor(miR-92a-3p inhibitor组)，转染6 h后进行换液，继续培养48 h，再用于后续实验。

为了检测PTEN对miR-92-3p作用的影响，取对数生长期BxPC-3细胞，随机分为如下组别：NC inhibitor+si-NC组、miR-92a-3p inhibitor+si-NC组、NC inhibitor+si-PTEN组和miR-92a-3p inhibitor+si-PTEN组。取对数生长期Panc-1细胞，随机分为如下组别：NC mimics+si-NC组、miR-92a-3p mimics+si-NC组、NC mimics+ si-PTEN组和miR-92a-3p mimics+si-PTEN组。

1.2.2 Real-time PCR实验

收集对数生长期的HPDE6-C7，Panc-1，BxPC-3，AsPC-1，MIA Paca-2和Capan-2等细胞，采用TRIzol法分别提取细胞总RNA，检测其浓度和纯度。使用U6-R与miR-92a-3p反转(RT)引物分别进行反转录，获得cDNA。遵照SYBR green qPCR检测试剂盒步骤进行操作，反应体系如下：SYBR green qPCR mix(2×)10 μL；正向引物0.4 μL；反向引物0.4 μL；模板cDNA 2 μL；去离子水7.2 μL。反应条件如下：95 ℃，5 min；95 ℃预变性5 min，95 ℃变性20 s，60 ℃延伸20 s，循环40次，60 ℃升温至95 ℃，升温速度1 ℃/15 s。以U6为内参，使用$2^{-\Delta\Delta Ct}$的方法计算相对表达水平，ΔCt为miR-92a-3p的Ct值减去U6的Ct值，ΔΔCt为PC细胞ΔCt减去HPDE6-C7细胞的ΔCt。引物序列信息见下表1。

**表1　引物序列信息**

**Tabel 1　Sequence of primer**

| 引物名称 | 引物序列 | 片段大小/bp |
|---|---|---|
| MiR-92a-3p-RT | 5'-GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACACAGGC-3' | |
| MiR-92a-3p-F | 5'-CAGTGCGTGTCGTGGAGTCGG-3' | 60 |
| MiR-92a-3p-R | 5'-TTGCACTTGTCCCGGCCTGTG-3' | |
| U6-F | 5'-CTCGCTTCGGCAGCACA-3' | 93 |
| U6-R | 5'-AACGCTTCACGAATTTGCGT-3' | |

RT：反转引物；F：正向引物；R：反向引物

1.2.3 蛋白质印迹法检测PTEN的表达

收集对数生长期的HPDE6-C7，Panc-1，BxPC-3，AsPC-1，MIA Paca-2和Capan-2细胞，二喹啉甲酸(bicinchoninic acid，BCA)法测定蛋白质浓度，进行十二烷基硫酸钠-聚丙烯酰胺凝胶电泳(sodium dodecyl sulfate salt-polyacrylamide gel electrophoresis，SDS-PAGE)。将蛋白质转移至聚偏乙二氟乙烯膜(polyviny-lidene fluoride，PVDF)。PVDF膜经5%的脱脂牛奶室温封闭2~3 h后，采用Tris-盐酸洗膜缓冲液(Tris-buffered saline Tween 20，TBST)洗膜3次，每次10 min。而后加入PTEN一抗(1∶1 000)，4 ℃孵育过夜，TBST洗膜3次后加入二抗(1∶5 000)室温孵育1 h，TBST洗膜3次后，化学发光试剂显色。使用β-actin作为内源性对照，通过灰度分析计算不同细胞PTEN蛋白质的表达水平。按照上述方法检测后续实验中各组BxPC-3细胞和Panc-1细胞中的PTEN表达。

1.2.4 CCK-8检测细胞的增殖

将NC mimics组、miR-92a-3p mimics组、NC inhibitor组、miR-92a-3p inhibitor组BxPC-3细胞接种于96孔细胞培养板，置于细胞培养箱中静置培养24，48和72 h。弃培养基后添加200 μL新鲜培养基和10 μL CCK-8溶液后，置于培养箱中孵育2 h，再使用酶标仪测定各孔在450 nm处的吸光度值，以了解上述各组BxPC-3细胞的增殖。同样，按照以上方法检测NC mimics组、miR-92a-3p mimics组、NC inhibitor组、miR-92a-3p inhibitor组Panc-1细胞中的吸光度值，以了解上述各组Panc-1细胞的增殖情况。

1.2.5 Transwell小室检测细胞的转移

将NC mimics组、miR-92a-3p mimics组、NC inhibitor组、miR-92a-3p inhibitor组BxPC-3细胞去血清饥饿处理约2 h后，使用0.25%胰蛋白酶消化。取200 μL细胞悬液接种于Transwell上层小室，下室内加入600 μL含10%胎牛血清的RPMI-1640培养基。于培养箱中培养约24 h后，PBS洗涤，乙醇棉擦去上室细胞，4%多聚甲醛固定约30 min，1%结晶紫染色15 min，PBS清洗后在显微镜下观察，拍照，以了解上述各组BxPC-3细胞的转移。同样，按照以上方法观察NC mimics组、miR-92a-3p mimics组、NC

©Journal of Central South University (Medical Science). All rights reserved.

inhibitor组和miR-92a-3p inhibitor组Panc-1细胞的转移。同样，按照上述方法检测NC inhibitor+si-NC组、miR-92a-3p inhibitor+si-NC组、NC inhibitor+si-PTEN组、miR-92a-3p inhibitor+si-PTEN组BxPC-3细胞的转移。使用PTEN过表达载体(PTEN)和阴性对照(NC)处理Panc-1细胞，按照上述方法检测NC mimics+NC组、miR-92a-3p mimics+NC组、NC mimics+PTEN组、miR-92a-3p mimics+PTEN组Panc-1细胞的转移活性。

1.2.6 双萤光素酶报告实验

将野生型PTEN-3'UTR (wt-PTEN)转染293T细胞，分为NC mimics+wt-PTEN组、miR-92a-3p mimics+wt-PTEN组、NC inhibitor+wt-PTEN组、miR-92a-3p inhibitor+wt-PTEN组，将突变型PTEN-3'UTR (mut-PTEN)转染293T细胞作为对照，分为NC mimics+mut-PTEN组、miR-92a-3p mimics+mut-PTEN组、NC inhibitor+mut-PTEN组、miR-92a-3p inhibitor+mut-PTEN组。细胞转染6 h后换液，随后继续培养48 h；最后采用双萤光素酶试剂盒测定各组中萤光素酶的相对活性。

### 1.3 统计学处理

所有数据采用由统计软件SPSS 21.0分析处理，最终数据以3次独立重复实验的均数±标准差($\bar{x}\pm s$)表示，组间比较采用One-wayANOVA分析法，两两比较使用LSD-$t$检验法，$P<0.05$为差异有统计学意义。

## 2 结 果

### 2.1 MiR-92a-3p促进PC细胞增殖

Real-time PCR结果显示：相较于HPDE6-C7细胞，Panc-1，BxPC-3，AsPC-1，MIA Paca-2和Capan-2细胞中miR-92a-3p表达均显著升高(均$P<0.01$)，其中在BxPC-3细胞中表达最高，在Panc-1细胞中表达最低(图1A)。与NC mimics组相比，miR-92a-3p mimics组BxPC-3和Panc-1细胞中的miR-92a-3p表达均显著升高(均$P<0.01$)；与NC inhibitor组相比，miR-92a-3p inhibitor组BxPC-3和Panc-1细胞中的miR-92a-3p表达均显著降低(均$P<0.01$；图1B，1C)。CCK-8结果显示：与NC mimics组相比，miR-92a-3p mimics组BxPC-3和Panc-1细胞的活力均显著升高(均$P<0.01$)；与NC inhibitor组相比，miR-92a-3p inhibitor组BxPC-3和Panc-1细胞的活力均显著降低(均$P<0.01$；图1D，1E)。

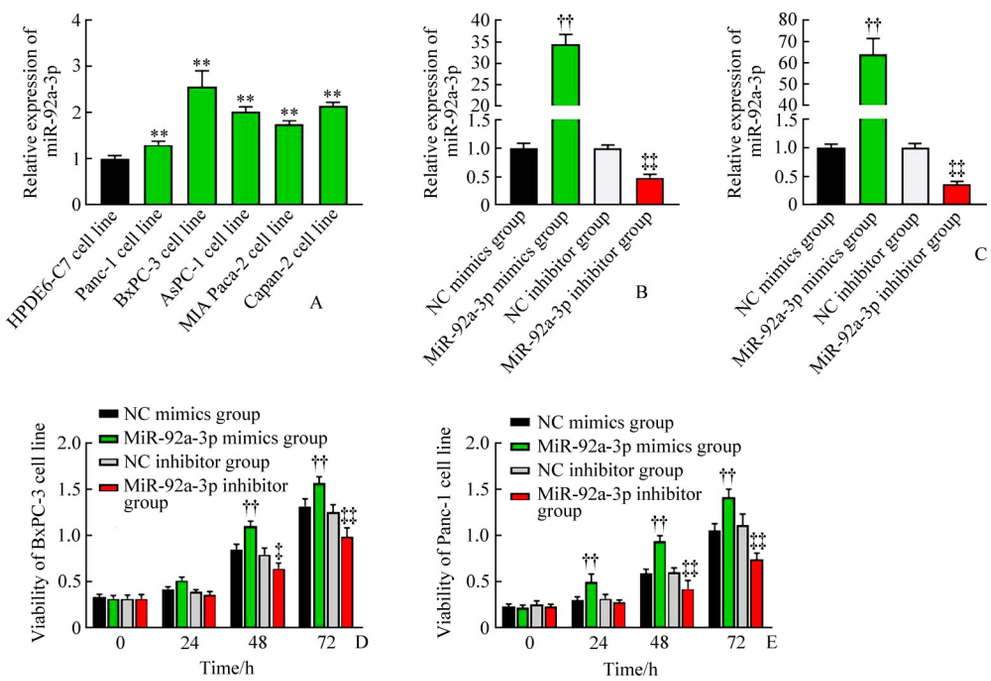


**图1 PC细胞中miR-92a-3p的表达及对细胞增殖的影响**

**Figure 1 Expression of miR-92a-3p in PC cells and its effect on cell proliferation**

A: Expression of miR-92a-3p in normal pancreatic cell line and PC cell lines by real-time PCR; B: Expression of miR-92a-3p in BxPC-3 cells by real-time PCR; C: Expression of miR-92a-3p in Panc-1 cells by real-time PCR; D: Viability of BxPC-3 cells by CCK-8; E: Viability of Panc-1 cells by CCK-8. **$P<0.01$ vs the HPDE6-C7 cell line; ††$P<0.01$ vs the NC mimics group; ‡$P<0.05$, ‡‡$P<0.01$ vs the NC inhibitor group

©Journal of Central South University (Medical Science). All rights reserved.

### 2.2 MiR-92a-3p促进PC细胞转移

Transwell小室检测结果显示：与NC mimics组相比，miR-92a-3p mimics组穿过微孔的BxPC-3和Panc-1细胞数均增多(均$P<0.01$)；与NC inhibitor组相比，miR-92a-3p inhibitor组穿过微孔的BxPC-3和Panc-1细胞数均减少(均$P<0.01$，图2)。

### 2.3 MiR-92a-3p抑制PC细胞PTEN蛋白的表达

对LinkedOmics的数据进行统计分析：发现PC组织中miR-92a-3p与PTEN表达呈负相关($r=-0.3418$，图3A)。蛋白质印迹法结果显示：与HPDE6-C7细胞株相比，Panc-1，BxPC-3，AsPC-1，MIA Paca-2和Capan-2细胞株中PTEN表达均显著降低(均$P<0.05$；图3B，3C)；与NC mimics组相比，miR-92a-3p mimics组BxPC-3和Panc-1细胞PTEN表达均显著降低(均$P<0.01$，图3D~3G)；与NC inhibitor组相比，miR-92a-3p inhibitor组BxPC-3和Panc-1细胞PTEN表达均显著升高(均$P<0.01$，图3D~3G)。

### 2.4 MiR-92a-3p直接靶向作用PTEN

通过Targetscan预测发现：miR-92a-3p与PTEN 3'UTR存在靶向关系，二者的相互结合位点如图所示(图4A)。双萤光素酶实验结果显示：在wt-PTEN 3'UTR实验中，与NC mimics组相比，miR-92a-3p mimics组萤光素酶活性显著降低($P<0.01$)，与NC inhibitor组相比，miR-92a-3p inhibitor组萤光素酶活性显著升高($P<0.01$)；突变型PTEN 3'UTR实验中，与NC mimics组相比，miR-92a-3p mimics组萤光素酶活性差异无统计学意义($P>0.05$)，与NC inhibitor组相比，miR-92a-3p inhibitor组萤光素酶活性差异无统计学意义($P>0.05$，图4B)。

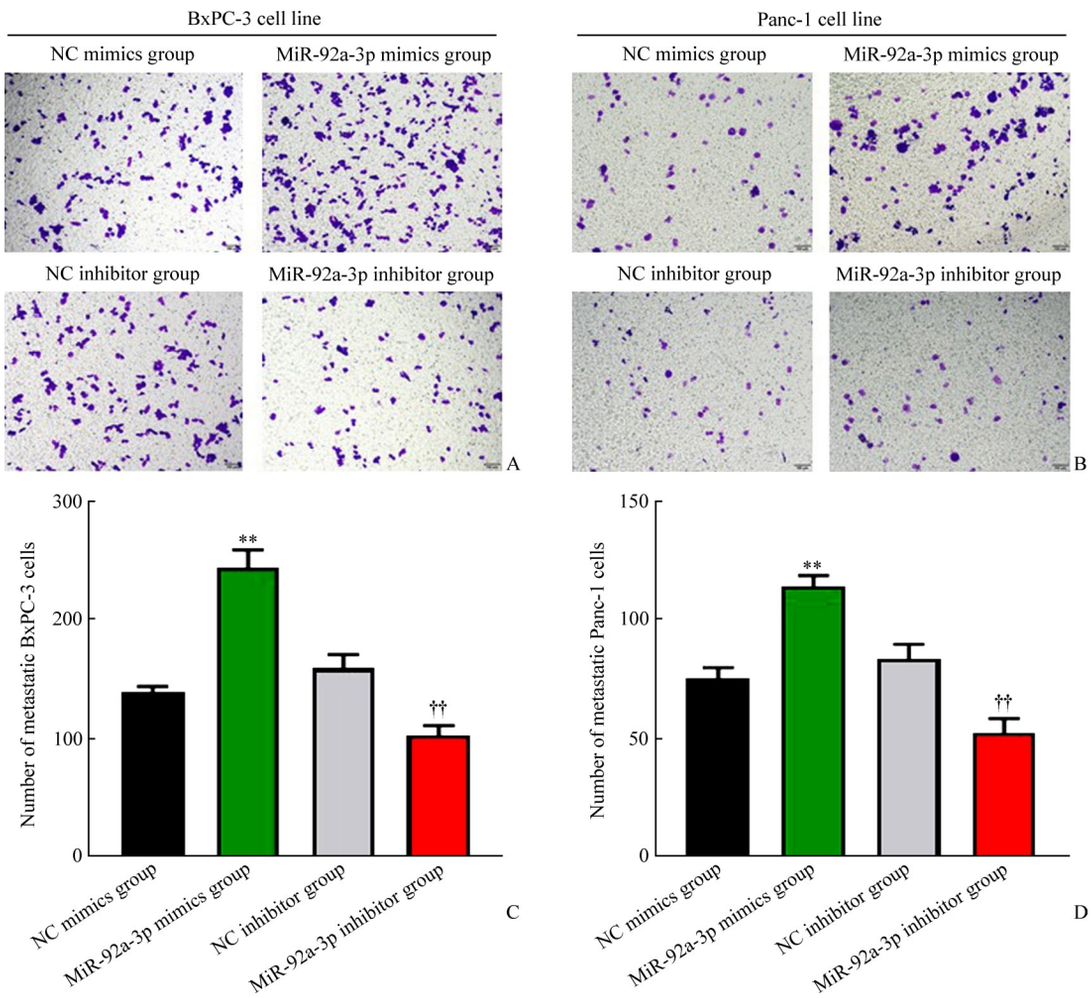


**图2　MiR-92a-3p对PC细胞转移的影响**

**Figure 2　Effect of miR-92a-3p on metastasis of PC cells**

A: Cell metastasis of BxPC-3 cell line by Transwell assay; B: Cell metastasis of Panc-1 cell line by Transwell assay; C: Histogram of cell metastasis of BxPC-3 cell line; D: Histogram of cell metastasis of Panc-1 cell line. **$P<0.01$ vs the NC mimics group; ††$P<0.01$ vs the NC inhibitor group

©Journal of Central South University (Medical Science). All rights reserved.

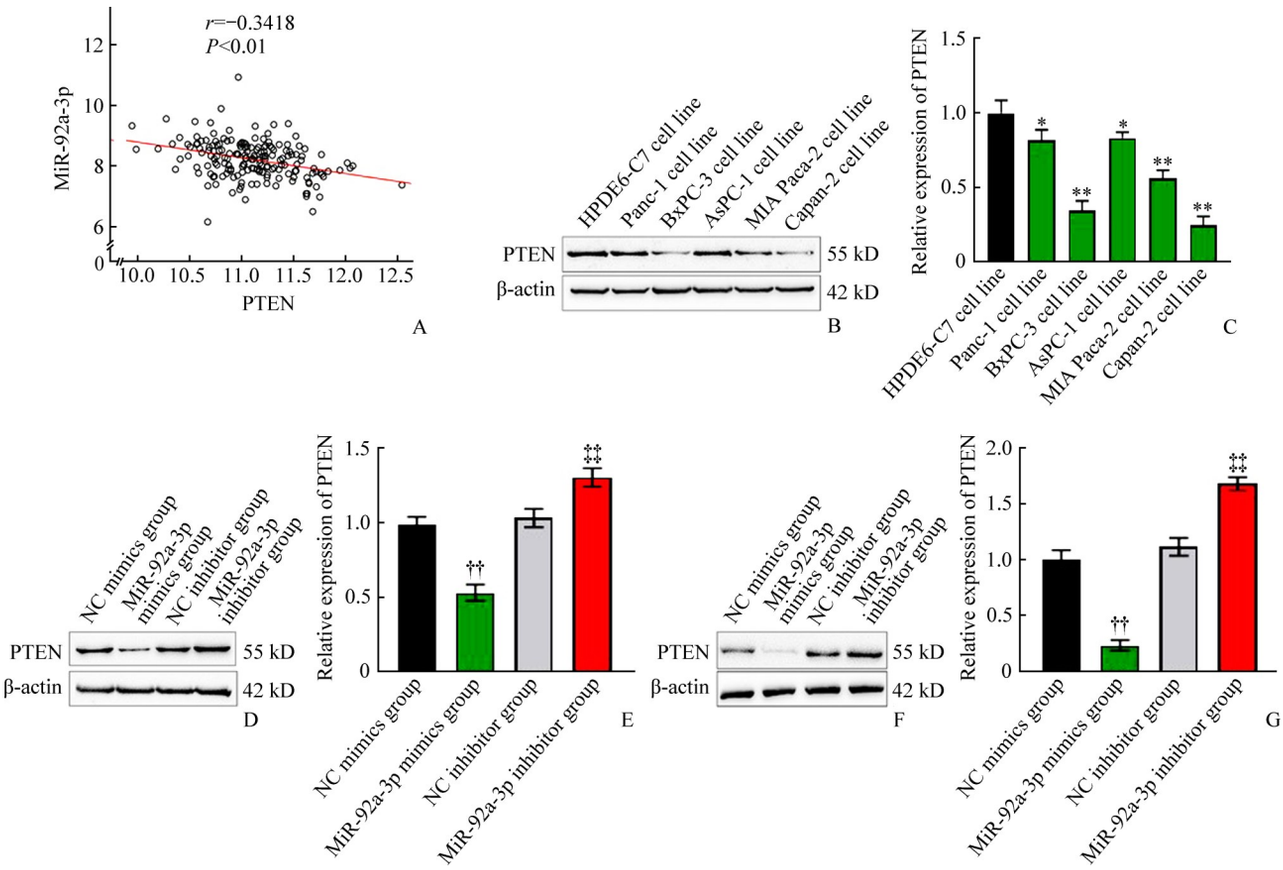


**图3 MiR-92a-3p调控PETN的表达**

**Figure 3 MiR-92a-3p regulates the expression of PETN**

A: Correlation between miR-92a-3p and PTEN; B: Expression of PTEN protein in normal pancreatic cell line and PC cell lines by Western blotting; C: Histogram of PTEN protein expression; D: Expression of PTEN protein in BxPC-3 cell line by Western blotting; E: Histogram of PTEN protein expression in BxPC-3 cell line; F: Expression of PTEN protein in Panc-1 cell line by Western blotting; G: Histogram of PTEN protein expression in Panc-1 cell line. *$P$<0.05, **$P$<0.01 vs the HPDE6-C7 cell line; ††$P$<0.01 vs the NC mimics group; ‡‡$P$<0.01 vs the NC inhibitor group

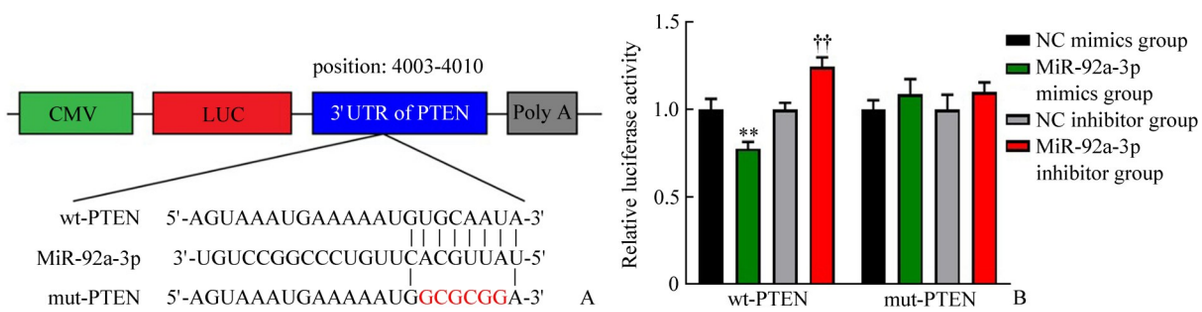


**图4 MiR-92a-3p与PTEN的靶向调控关系验证**

**Figure 4 Verification of the targeting relationship between miR-92a-3p and PTEN**

A: Schematic diagram of the binding site of miR-92a-3p and PTEN mRNA; B: Histogram of targeted regulative effect of miR-92a-3p on PTEN by dual luciferase reporter assay. **$P$<0.01 vs the NC mimics group; ††$P$<0.01 vs the NC inhibitor group

### 2.5 PTEN可回复miR-92a-3p对PC细胞的增殖

蛋白质印迹法结果显示：与NC inhibitor+si-NC组相比，NC inhibitor+si-PTEN组BxPC-3细胞PTEN表达降低，miR-92a-3p inhibitor+si-NC组BxPC-3细

©Journal of Central South University (Medical Science). All rights reserved.

胞PTEN表达升高($P$<0.05)；与miR-92a-3p inhibitor+si-NC组相比，miR-92a-3p inhibitor+si-PTEN组BxPC-3细胞中的PTEN表达降低($P$<0.01；图5A，5B)。与NC mimics+NC组相比，NC mimics+PTEN组Panc-1细胞中的PTEN表达升高，miR-92a-3p mimics+NC组Panc-1细胞中的PTEN表达降低(均$P$<0.01)；与miR-92a-3p mimics+NC组相比，miR-92a-3p mimics+PTEN组Panc-1细胞中的PTEN表达升高($P$<0.01；图5C，5D)。CCK-8结果显示：与NC inhibitor+si-NC组相比，NC inhibitor+si-PTEN组BxPC-3细胞的活力升高，miR-92a-3p inhibitor+si-NC组BxPC-3细胞的活力降低($P$<0.01)；与miR-92a-3p inhibitor+si-NC组相比，miR-92a-3p inhibitor+si-PTEN组BxPC-3细胞的活力升高($P$<0.01，图5E)。与NC mimics+NC组相比，NC mimics+PTEN组Panc-1细胞的活力降低，miR-92a-3p mimics+NC组Panc-1细胞的活力升高(均$P$<0.01)；与miR-92a-3p mimics+NC组相比，miR-92a-3p mimics+PTEN组Panc-1细胞的活力降低($P$<0.01，图5F)。

### 2.6 PTEN可回复miR-92a-3p对PC细胞的转移

Transwell小室检测结果显示：与NC inhibitor+si-NC组相比，NC inhibitor+si-PTEN组穿过微孔的BxPC-3细胞数增多，miR-92a-3p inhibitor+si-NC组穿过微孔的BxPC-3细胞数减少(均$P$<0.01)；与miR-92a-3p inhibitor+si-NC组相比，miR-92a-3p inhibitor+si-PTEN组穿过微孔的BxPC-3细胞数增多($P$<0.01)。与NC mimics+NC组相比，NC mimics+PTEN组穿过微孔的Panc-1细胞数减少，miR-92a-3p mimics+NC组穿过微孔的Panc-1细胞数增多(均$P$<0.01)；与miR-92a-3p mimics+NC组相比，miR-92a-3p mimics+PTEN组穿过微孔的Panc-1细胞数减少($P$<0.01，图6)。

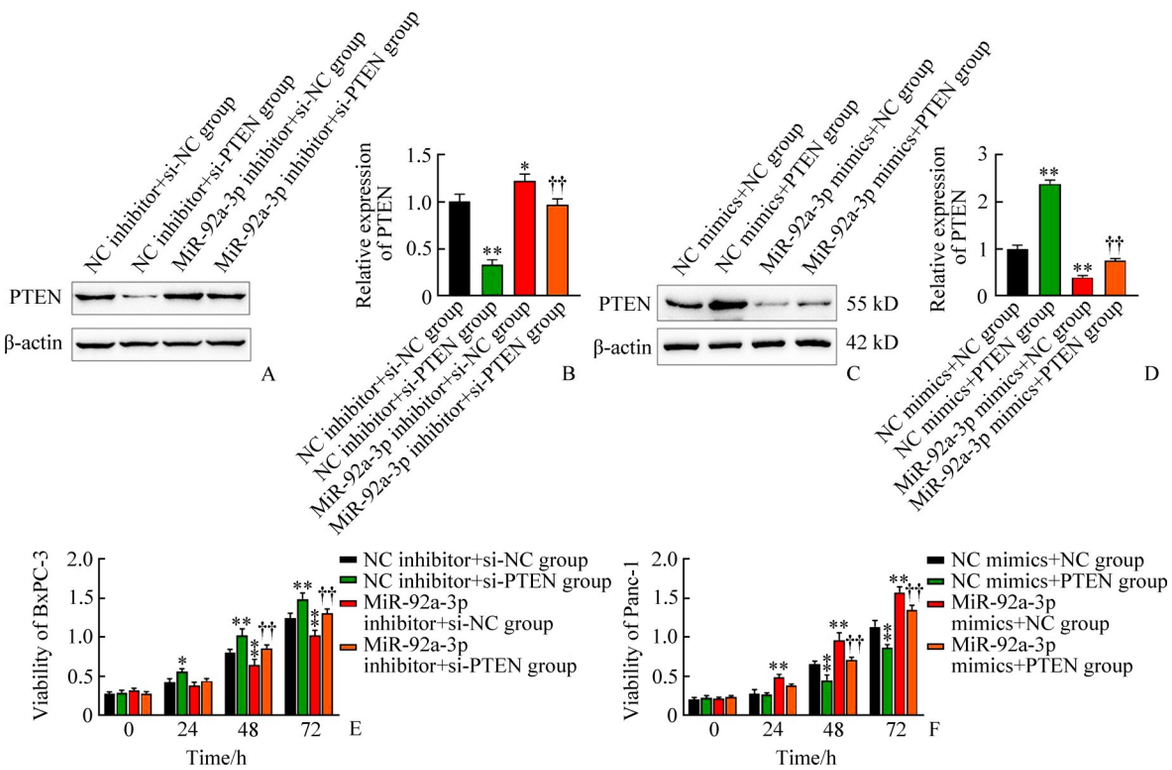


**图5　MiR-92a-3p调控PTEN影响PC细胞的增殖**

**Figure 5　MiR-92a-3p regulates PTEN and affects the proliferation of PC cells**

A: Expression of PTEN protein in BxPC-3 cell line by Western blotting; B: Histogram of PTEN protein expression in BxPC-3 cell line; C: Expression of PTEN protein in Panc-1 cell line by Western blotting; D: Histogram of PTEN protein expression in Panc-1 cell line; E: Viability of BxPC-3 cell line by CCK-8 assay; *$P$<0.05, **$P$<0.01 vs the NC inhibitor+si-NC group; ††$P$<0.01 vs the miR-92a-3p inhibitor+si-NC group. F: Viability of Panc-1 cell line by CCK-8 assay. **$P$<0.05 vs the NC mimics+NC group; ††$P$<0.01 vs the miR-92a-3p mimics+NC group

©Journal of Central South University (Medical Science). All rights reserved.

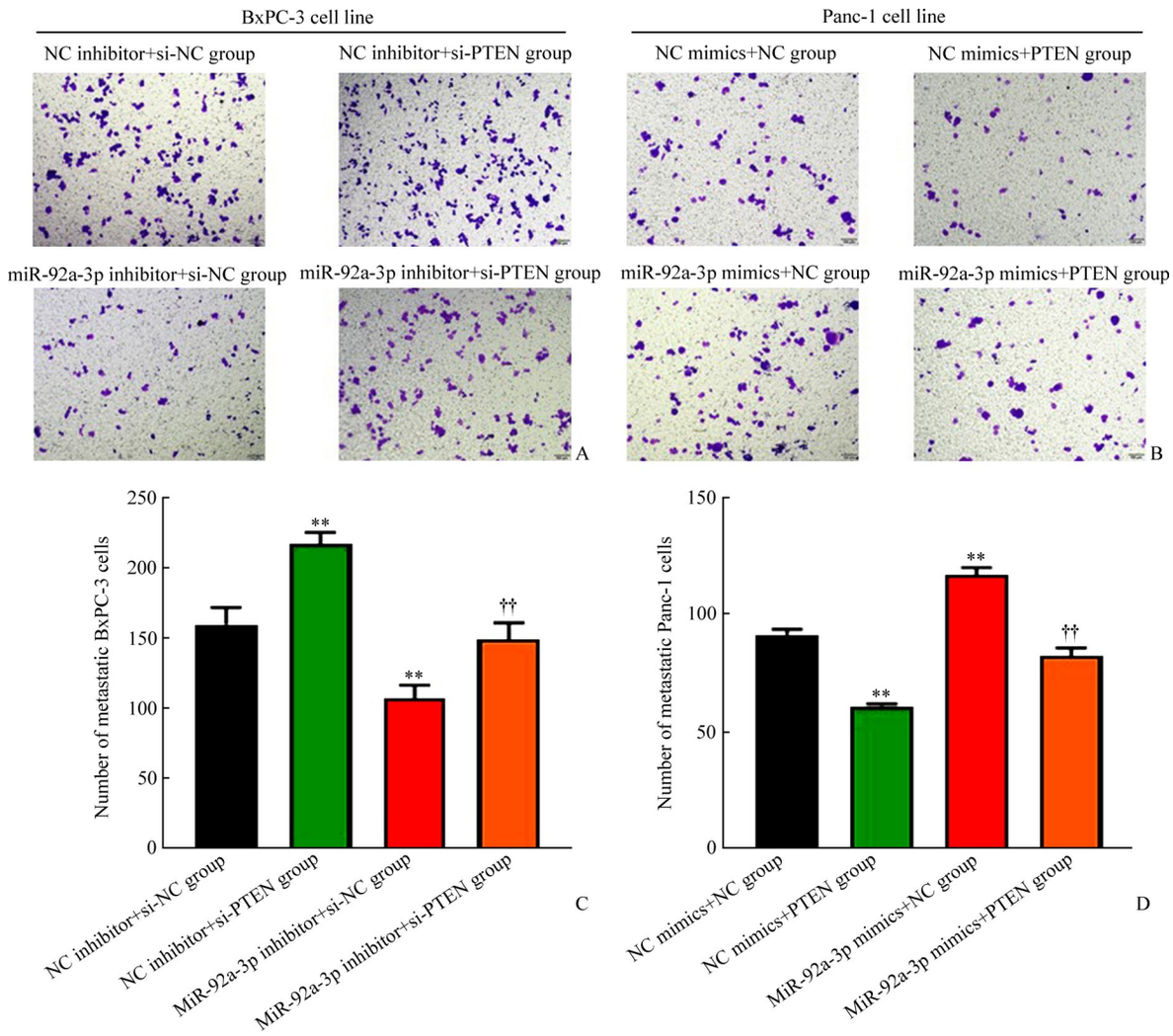


**图6 MiR-92a-3p调控PTEN影响PC细胞的转移**

**Figure 6 MiR-92a-3p regulates PTEN and affects the metastasis of PC cells**

A: Cell metastasis of BxPC-3 cell line by Transwell assay; B: Cell metastasis of Panc-1 cell line by Transwell assay; C: Histogram of metastasis of BxPC-3 cell line. **$P$<0.01 vs the NC inhibitor+si-NC group; ††$P$<0.01 vs the miR-92a-3p inhibitor+si-NC group. D: Histogram of metastasis of Panc-1 cell line. **$P$<0.01 vs the NC mimics+NC group; ††$P$<0.01 vs the miR-92a-3p minics+NC group

## 3 讨 论

癌症是现今世界共同面临的一个严峻的公共健康问题和挑战，作为其中较为常见且致死率较高的一种，PC不断升高的发病率，给社会造成的经济和健康负担日益沉重。研究[12-13]显示：我国的PC患者年确诊和死亡数已超过美国，占全球PC死亡人数的19.27%，因而进一步阐明PC的发病机制，有望为今后该病检测、诊断、治疗新方法和新药物的研发打下基础。

本研究首先通过功能实验证明：miR-92a-3p在5种不同PC细胞株中均表达上调，并可促进肿瘤细胞增殖和转移活性。借助基因功能研究手段，发现PC细胞中miR-92a-3p可抑制细胞中PTEN的表达，其后通过双萤光素酶报告实验证明miR-92a-3p与PTEN间存在靶向调控关系。通过在BxPC-3细胞中共转染miR-92a-3p inhibitor和si-PTEN，Panc-1细胞中共转染miR-92a-3p mimics和PTEN过表达载体，观察PC细胞的增殖和转移变化。本研究结果表明：miR-92a-3p促进PC细胞的增殖和转移，随后的回复实验进一步验证，干扰PTEN可回复miR-92a-3p inhibitor对癌细胞增殖和转移的抑制作用，过表达PTEN可回复miR-92a-3p mimics对癌细胞增殖和转移的促进作用。

近10年来对miRNA的研究[14]表明：该分子在血液、血清、新鲜乃至于石蜡包埋中的组织样本中均大量存在并可被检测到，在临床领域具有巨大的应用前景。MiR-92a-3p是miR-17-92家族的一员，由位于人染色体13q31.3上的基因编码而来。目前，miR-

©Journal of Central South University (Medical Science). All rights reserved.

92a-3p在肿瘤发生、发展中的作用已得到证实，如miR-92a-3p的表达在卵巢癌患者中显著上调，并可随着淋巴结受累和卵巢癌的恶化而增高[15]。而在接受根治性手术和辅助化学治疗的结肠癌患者中，miR-92a-3p可作为预测肿瘤是否复发的重要标志物[16]。而在PC中，miR-92a-3p已被证明与PC恶性程度密切相关，并可促进PC细胞增殖，本研究在此基础上深入挖掘miR-92a-3p在PC中的分子作用机制，明确miR-92a-3p与PTEN蛋白在PC发病机制中的重要作用。成鉴晓等[17]的研究表明：干扰miR-543可上调PTEN的表达，从而抑制胰腺癌细胞增殖、侵袭和上皮-间充质转化。PTEN作为一个重要的抑癌基因，早已被证明可通过将三磷酸磷脂酰肌醇(phosphatidylinositol trisphosphate，$PIP_3$)去磷酸化，阻断PI3K/AKT信号通路，从而抑制PC细胞增殖、侵袭、上皮-间质转化、耐药[18-19]。

将本研究的实验结果与相关报道结合分析可推测，PC细胞中高表达miR-92a-3p可能是通过抑制PTEN蛋白的表达，进而解除对PI3K/AKT信号通路的抑制，从而促进PC细胞的增殖和转移能力。研究miR-92a-3p调控PTEN在PC细胞增殖与转移中的作用机制，有利于进一步揭示miR-92a-3p促肿瘤的分子机制，有助于阐明PC发生和演进机制，也可为PC的早期诊断和预后提供新的理论基础。

**利益冲突声明：**作者声称无任何利益冲突。

## 参考文献

[1] Kamisawa T, Wood LD, Itoi T, et al. Pancreatic cancer[J]. Lancet, 2016, 388(10039): 73-85.

[2] Hosein AN, Beg MS. Pancreatic cancer metabolism: Molecular mechanisms and clinical applications[J]. Curr Oncol Rep, 2018, 20(7): 56.

[3] Lee YR, Chen M, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor: new modes and prospects[J]. Nat Rev Mol Cell Biol, 2018, 19(9): 547-562.

[4] Ferraldeschi R, Rodrigues DN, Riisnaes R, et al. PTEN protein loss and clinical outcome from castration-resistant prostate cancer treated with abiraterone acetate[J]. Eur Urol, 2015, 67(4): 795-802.

[5] Juric D, Castel P, Griffith M, et al. Convergent loss of PTEN leads to clinical resistance to a PI(3)Kα inhibitor[J]. Nature, 2015, 518(7538): 240-244.

[6] Leinonen KA, Saramäki OR, Furusato B, et al. Loss of PTEN is associated with aggressive behavior in ERG-positive prostate cancer[J]. Cancer Epidemiol Biomarkers Prev, 2013, 22(12): 2333-2344.

[7] Poliseno L, Pandolfi PP. PTEN ceRNA networks in human cancer[J]. Methods, 2015, 77-78: 41-50.

[8] Farazi TA, Hoell JI, Morozov P, et al. MicroRNAs in human cancer[J]. Adv Exp Med Biol, 2013, 774(2): 1-20.

[9] Zhou C, Shen L, Mao L, et al. MiR-92a is upregulated in cervical cancer and promotes cell proliferation and invasion by targeting FBXW7[J]. Biochem Biophys Res Commun, 2015, 458(1): 63-69.

[10] Lin HY, Chiang CH, Hung WC. STAT3 upregulates miR-92a to inhibit RECK expression and to promote invasiveness of lung cancer cells[J]. Br J Cancer, 2013, 109(3): 731-738.

[11] Chifumi OH, Kenjiro S, Shoji K, et al. MiR-92a inhibits peritoneal dissemination of ovarian cancer cells by inhibiting integrin α5 expression[J]. Am J Pathol, 2013, 182(5): 1876-1889.

[12] Lin QJ, Yang F, Jin C, et al. Current status and progress of pancreatic cancer in China[J]. World J Gastroenterol, 2015, 21(26): 7988-8003.

[13] Chu LC, Goggins MG, Fishman EK. Diagnosis and detection of pancreatic cancer[J]. Cancer J, 2017, 23(6): 333.

[14] Sethi S, Sethi S, Bluth MH. Clinical implication of MicroRNAs in molecular pathology: An update for 2018[J]. Clin Lab Med, 2018, 38(2): 237-251.

[15] Guo F, Tian J, Lin Y, et al. Serum microRNA-92 expression in patients with ovarian epithelial carcinoma[J]. J Int Med Res, 2013, 41(5): 1456-1461.

[16] Conev NV, Donev IS, Konsoulova-Kirova AA, et al. Serum expression levels of miR-17, miR-21, and miR-92 as potential biomarkers for recurrence after adjuvant chemotherapy in colon cancer patients[J]. Biosci Trends, 2015, 9(6): 393-401.

[17] 成鉴晓, 江月萍, 任琳琳, 等. lncRNA MEG3通过调控miR-543/PTEN分子轴抑制胰腺癌细胞增殖及转移的机制[J]. 肿瘤防治研究, 2019, 46(7): 588-593.
CHENG Jianxiao, JIANG Yueping, REN Linlin, et al. LncRNA MEG3 suppresses proliferation and metastasis of pancreatic cancer cells via regulating miR-543/PTEN axis[J]. Cancer Research on Prevention and Treatment, 2019, 46(7): 588-593.

[18] Zhang Y, Zhang J, Xu K, et al. PTEN/PI3K/mTOR/B7-H1 signaling pathway regulates cell progression and immuno-resistance in pancreatic cancer[J]. Hepatogastroenterology, 2013, 60(127): 1766-1772.

[19] Pan S, Deng Y, Fu J, et al. Decreased expression of ARHGAP15 promotes the development of colorectal cancer through PTEN/AKT/FOXO1 axis[J]. Cell Death Dis, 2018, 9(6): 673.

（本文编辑 傅希文）

**本文引用：**刘宇, 胡庆, 敖晶晶, 李红玲, 李美梁. 微小RNA-92a-3p靶向PTEN调控胰腺癌细胞增殖和转移[J]. 中南大学学报(医学版), 2020, 45(3): 280-289. DOI: 10.11817/j. issn. 1672-7347.2020.180459

***Cite this article as:*** LIU Yu, HU Qing, AO Jingjing, LI Hongling, LI Meiliang. Role of miR-92a-3p/PTEN axis in regulation of pancreatic cancer cell proliferation and metastasis[J]. Journal of Central South University. Medical Science, 2020, 45(3): 280-289. DOI:10.11817/j.issn.1672-7347.2020.180459

©Journal of Central South University (Medical Science). All rights reserved.