·论　著·

miR-762在胰腺癌中的表达及对胰腺癌细胞生物学行为的影响

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［摘要］ **背景与目的：**miR-762在多种恶性肿瘤中存在表达异常，参与肿瘤的增殖、凋亡及侵袭转移。观察miR-762在胰腺癌组织和细胞系中的表达及对胰腺癌细胞增殖、侵袭转移的影响。**方法：**采用实时荧光定量聚合酶链反应（real-time fluorescence quantitative polymerase chain reaction，RTFQ-PCR）技术检测于河北医科大学第四医院行胰腺癌根治术的胰腺癌组织和细胞株中miR-762的表达。通过Lipofectamine^{TM}2000将miR-762模拟物（mimics）、miR-762抑制物（inhibitors）及其阴性对照序列（scramble序列）分别转染胰腺癌PANC-1细胞。采用细胞计数试剂盒（cell counting kit-8，CCK-8）实验检测细胞增殖；采用流式细胞术检测细胞凋亡；采用划痕实验和Transwell侵袭实验检测细胞侵袭转移能力；采用蛋白质印迹法（Western blot）检测上皮-间质转化（epithelial-mesenchymal transformation，EMT）相关分子标志物表达。**结果：**胰腺癌组织中miR-762 mRNA表达量显著高于癌旁组织（P<0.01）。胰腺癌细胞株BxPC-3、PANC-1、AsPC-1、SW-1990中miR-762 mRNA的表达量也显著高于正常胰腺上皮细胞HPDE（P<0.01）。转染miR-762 mimics后PANC-1细胞miR-762 mRNA表达量显著增加，而转染miR-762 inhibitors后PANC-1细胞miR-762 mRNA表达量显著降低（P<0.01）。同时miR-762 mimics组450 nm处的吸光度（D_{450}）值、细胞迁移距离和穿膜细胞数及间质表型细胞标志物N-钙黏蛋白（N-cadherin）、波形蛋白（vimentin）表达量显著增加，细胞凋亡率及上皮细胞标志物E-钙黏蛋白（E-cadherin）表达量显著降低；而miR-762 inhibitors组D_{450}、细胞迁移距离和穿膜细胞数及间质表型细胞标志物N-cadherin、vimentin表达量显著降低，细胞凋亡率及上皮细胞标志物E-cadherin表达量显著增加（P<0.05）。**结论：**miR-762在胰腺癌组织和细胞株中高表达，上调miR-762表达可能通过调控N-cadherin、vimentin、E-cadherin表达促进EMT进程，从而增强PANC-1细胞的增殖和侵袭转移能力。

［关键词］胰腺癌；miR-762；增殖；侵袭转移
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Expression of miR-762 in pancreatic cancer and its effect on biological behavior of pancreatic cancer cells

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［**Abstract**］ **Background and purpose:** The abnormally expressed miR-762 in various malignant tumors is involved in the proliferation, apoptosis, invasion and metastasis of tumors. Aim of the present study was to study the expression of miR-762 in pancreatic cancer tissues and cell lines and its effect on the proliferation, metastasis and invasion of pancreatic cancer cells. **Methods:** Real-time fluorescence quantitative polymerase chain reaction (RTFQ-PCR) was used to detect the expression of miR-762 mRNA in pancreatic cancer tissues who received radical resection of pancreatic cancer in the Fourth Hospital of Hebei Medical University and cell lines. The miR-762 mimics, miR-762 inhibitors and scramble sequences were transfected into PANC-1 cells with Lipofectamine^{TM}2000. The proliferation, apoptosis and migratory and invasive abilities of PANC-1 cells were detected with cell counting kit-8 (CCK-8) assay, flow cytometry, wound healing assay and Transwell assay. The expression of epithelial-mesenchymal transition (EMT) related markers were determined by Western blot. **Results:** The miR-762 expression was significantly up-regulated in pancreatic cancer tissues compared with paracancerous tissues (P<0.01). Similarly, miR-762 expressions of the four pancreatic

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cancer cell lines (BxPC-3, PANC-1, AsPC-1 and SW-1990) were significantly higher than those in the normal pancreatic ductal epithelial cells HPDE (P<0.01). miR-762 mRNA expression in PANC-1 cell line was obviously increased after transfection with miR-762 mimics, but markedly decreased after transfection with miR-762 inhibitors (P<0.01). Meanwhile, D_{450}, the healing rate, invasive cells and expressions of N-cadherin and vimentin proteins in miR-762 mimics group were significantly higher than those in negative control group after transfection, and apoptotic rate and E-cadherin protein expression were significantly lower than those in negative control group after transfection. D_{450}, the healing rate, invasive cells and expressions of N-cadherin and vimentin in miR-762 inhibitors group were significantly lower than those in negative control group after transfection, while apoptotic rate and E-cadherin expression were significantly higher than those in negative control group after transfection (P<0.05). **Conclusion:** The over-expression of miR-762 can effectively enhance the proliferation, metastasis and invasion ability in PANC-1 cells, in which EMT related markers including N-cadherin, vimentin and E-cadherin may play a role.

［**Key words**］ Pancreatic cancer；miR-762；Proliferation；Invasion and metastasis

胰腺癌是中国常见的恶性程度较高的消化道恶性肿瘤之一，危害极大[1-2]。因此，探索胰腺癌发生、发展的分子机制对于疾病防治具有重要的临床意义。miRNA是由18~22个核苷酸组成的内源性非编码RNA，能够与靶基因3’非编码区特异性结合，从而在转录后水平调控靶基因表达。研究发现，多种miRNA在肿瘤组织中异常表达[3-4]。其中miR-762已被证实在乳腺癌、卵巢癌等多种肿瘤组织中高表达，通过调控靶基因表达参与细胞增殖、凋亡、侵袭转移等过程[5-6]。但miR-762在胰腺癌发生、发展中的作用报道较少。本研究检测了miR-762在胰腺癌组织和细胞株中的表达情况，并分析了miR-762在胰腺癌细胞增殖、侵袭转移中的作用及机制。

1 材料和方法

1.1 组织标本及细胞株

收集2016年1月—2019年1月于河北医科大学第四医院行胰腺癌根治术的胰腺癌组织标本35例（包括癌组织及距离其3~5 cm的癌旁组织），术后均经病理学检查确诊，且术前未接受放化疗。人胰腺癌细胞株BxPC-3、PANC-1、AsPC-1、SW-1990和人正常胰腺上皮细胞HPDE购自中国科学院典型培养物保藏委员会细胞库。

1.2 主要试剂及仪器

RPMI-1640培养基、胎牛血清（fetal bovine serum，FBS）、TRIzol购自美国Gibco公司；miR-762模拟物（mimics）、miR-762抑制物（inhibitors）及其阴性对照序列（scramble序列）购自上海吉凯制药技术有限公司；Lipofectamine^{TM}2000购自美国Invitrogen公司；实时荧光定量聚合酶链反应（real-time fluorescence quantitative polymerase chain reaction，RTFQ-PCR）试剂盒、反转录试剂盒购自日本Takara公司；鼠抗人E-钙黏蛋白（E-cadherin）、N-钙黏蛋白（N-cadherin）、波形蛋白（vimentin）抗体购自美国Abcam公司。Chemi DocTM XRS+化学发光凝胶成像系统购自美国Bio-Rad公司；7300型RTFQ-PCR扩增仪购自美国ABI公司。

1.3 细胞培养

细胞用含10%FBS的RPMI-1640培养基，在37 ℃、CO_2体积分数为5%的培养箱中常规培养。取对数生长期的细胞接种于6孔板，待细胞融合达80%时采用Lipofectamine^{TM}2000将miR-762 mimics、miR-762 inhibitors及其scramble序列分别转染至PANC-1细胞中，不进行转染的细胞为空白对照组。实验重复3次。

1.4 CCK-8实验检测细胞活性

细胞消化后制成单细胞悬液，以2×10^{3}个/孔的密度接种于96孔板，每孔200 μL。分别于培养0、1、2、3、4 d时进行CCK-8实验，每孔加入10 μL CCK-8溶液，继续培养2 h，测定450 nm处的吸光度（D_{450}）值。实验重复3次。

1.5 流式细胞术检测细胞凋亡率

转染48 h后收集细胞，4 ℃ 1 000 r/min离心5 min，弃上清，PBS洗涤2次，重悬细胞，加入Annexin V和碘化丙啶（propidium iodide，PI）各5 μL，37 ℃避光染色20 min，采用流式细胞术检测细胞凋亡情况。实验重复3次。

1.6 划痕实验检测细胞迁移能力

转染48 h后，各组细胞接种于6孔板，待细胞融合达100%时用10 μL枪头在培养板底部做一均匀划痕，PBS洗涤3次，加入含2%FBS的新鲜培养基，在倒置显微镜下观察划痕后即刻、48 h时的细胞迁移情况，计算划痕愈合率。划痕愈合率（%）=（划痕后即刻划痕宽度 – 48 h划痕宽度）/划痕后即刻划痕宽度×100%。实验重复3次。

1.7 Transwell侵袭实验检测细胞侵袭能力

转染细胞48 h后，各组细胞制成1×10^5/mL的细胞悬液，取200 μL接种到预铺Matrigel基质胶的Transwell上室，下室加入200 μL含10%FBS的培养基，在37℃、CO_2体积分数为5%的培养箱中培养24 h后，用棉签刮去基质胶和未穿膜细胞，多聚甲醛固定，0.1%结晶紫染色，显微镜下随机选取5个视野计数穿膜细胞数，求平均值。实验重复3次。

1.8 RTFQ-PCR检测基因mRNA

TRIzol提取细胞总RNA，按照反转录试剂盒说明书合成cDNA。取2 μL cDNA为模板，在ABI 7300型RTFQ-PCR仪上进行PCR扩增。*U6*为内参照基因，采用$2^{-\triangle\triangle CT}$法定量目的基因mRNA相对表达量。*miR*-762及*U6*引物序列如下：*miR*-762上游引物5'-ACACGGGGCUGGGGCCGGGGCCGAGCGCCTC-3'，下游引物5'-CTCAGGGGCUGGGGCCGGGGCCGAGCCAGA-3'；*U6*上游引物5'-CTCGCTTCGGCAGCACA-3'，下游引物5'-AACGCTTCACGAATTTGCGT-3'。*E-cadherin*、*N-cadherin*、*vimentin*及甘油醛-3-磷酸脱氢酶（glyceraldehyde-3-phosphate dehydrogenase，*GAPDH*）基因引物序列如下：*E-cadherin*上游引物5'-TGCCCAGAAAATGAAAAAGG-3'，下游引物5'-GTGTATGTGGCAATGCGTTC-3'；*N-cadherin*上游引物5'-GAGAACTTTGCCGTTGAAGC-3'，下游引物5'-GTGTATGTGGCAATGCGTTC-3'；*Vimentin*上游引物5'-GAGAACTTTGCCGTTGAAGC-3'，下游引物5'-GCTTCCTGTAGGTGGCAATC-3'；*GAPDH*上游引物5'-CTCTGCTCCTCCTGTTCGAC-3'，下游引物5'-GCGCCCAATACGACCAAATC-3'。实验重复3次。

1.9 蛋白质印迹法（Western blot）检测蛋白表达

转染48 h后，提取细胞总蛋白，经10%十二烷基硫酸钠聚丙烯酰胺凝胶电泳（sodium dodecyl sulphate polyacrylamide gel electrophoresis，SDS-PAGE）分离后电转移至聚偏氟乙烯（polyvinylidene fluoride，PVDF）膜上，10%脱脂奶粉封闭2.0 h，加入特异性一抗温育过夜，TBST洗膜3次，加入二抗室温温育1.5 h，TBST洗膜3次。ECL化学发光法显色、定影，设GAPDH为内参照蛋白。实验重复3次。

1.10 统计学处理

采用SPSS 21.0软件包处理数据，计量资料采用$\bar{x}\pm s$表示。组间比较采用单因素方差分析，P<0.05为差异有统计学意义。

2 结　果

2.1 miR-762在胰腺癌组织和细胞株中的表达

RTFQ-PCR结果显示，胰腺癌组织中miR-762 mRNA表达量为0.91±0.28，显著高于癌旁组织（0.32±0.08）（t=12.30，P=0.000）。胰腺癌细胞株BxPC-3、PANC-1、AsPC-1、SW-1990中miR-762 mRNA的表达量分别为0.57±0.08、1.22±0.32、0.79±0.21、0.96±0.11，显著高于正常胰腺上皮细胞HPDE（0.31±0.08）（t=-4.61、-6.04、-4.67、12.03，P=0.010、0.004、0.010、0.000，图1）。

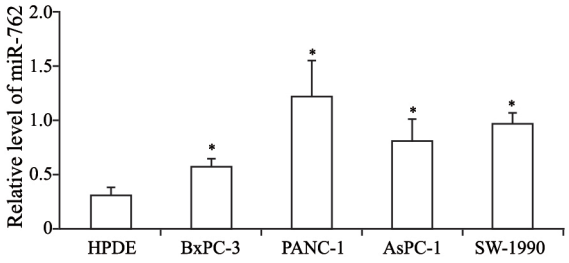

图 1 miR-762在胰腺癌和正常胰腺上皮细胞中的表达

Fig. 1 Expression of miR-762 in pancreatic cancer and normal pancreatic epithelial cell lines

Expression of miR-762 was tested by RTFQ-PCR. Each bar represents $\bar{x}\pm s$ of three independent experiments. *: P<0.01

2.2 转染miR-762 mimics、miR-762 inhibitors对PANC-1细胞miR-762表达的影响

RTFQ-PCR结果显示，转染24 h后，空白

对照组、阴性对照组、miR-762 mimics组、miR-762 inhibitors组miR-762 mRNA表达量分别为1.16±0.21、1.12±0.24、9.45±1.23、0.39±0.10。与阴性对照组比较，miR-762 mimics组miR-762 mRNA表达量显著增加，miR-762 inhibitors组miR-762 mRNA表达量显著降低（t=-13.70、5.10，P=0.000、0.007，图2）。

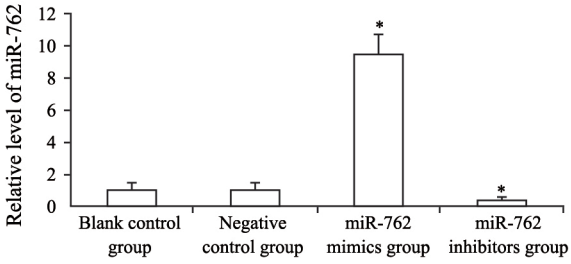

图 2 转染miR-762 mimics、miR-762 inhibitors对PANC-1细胞miR–762表达的影响

Fig. 2 Effects of miR-762 mimics and miR-762 inhibitors on expression of miR-762 in PANC-1 cells

Expression of miR-762 was tested by RTFQ-PCR. Each bar represents $\bar{x}\pm s$ of three independent experiments. *: P<0.01

2.3 miR–762对PANC–1细胞增殖的影响

CCK-8实验结果显示，转染后第2~4天，与阴性对照组比较，miR-762 mimics组D_{450}显著增加，miR-762 inhibitors组D_{450}显著降低（P < 0.01，图3）。

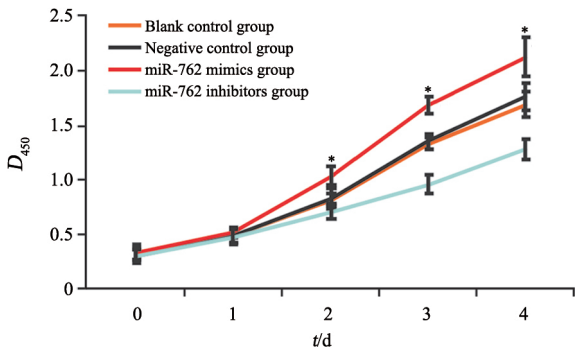

图 3 miR-762对PANC-1细胞增殖的影响（CCK-8实验）

Fig. 3 Effect of miR-762 on proliferation of PANC-1 cells (CCK-8 assay)

Cell activity was tested by CCK8 assay. Each line represents $\bar{x}\pm s$ of three independent experiments. *: P<0.01

2.4 miR–762对PANC-1细胞凋亡的影响

流式细胞术结果显示，转染48 h后，空白对照组、阴性对照组、miR-762 mimics组、miR-762 inhibitors组细胞凋亡率分别为（10.15±2.43）%、（10.00±1.95）%、（3.40±0.77）%、（22.64±3.71）%。与阴性对照组比较，miR-762 mimics组细胞凋亡率显著降低，miR-762 inhibitors组细胞凋亡率显著增加（t=7.95、-7.41，P=0.001、0.002，图4）。

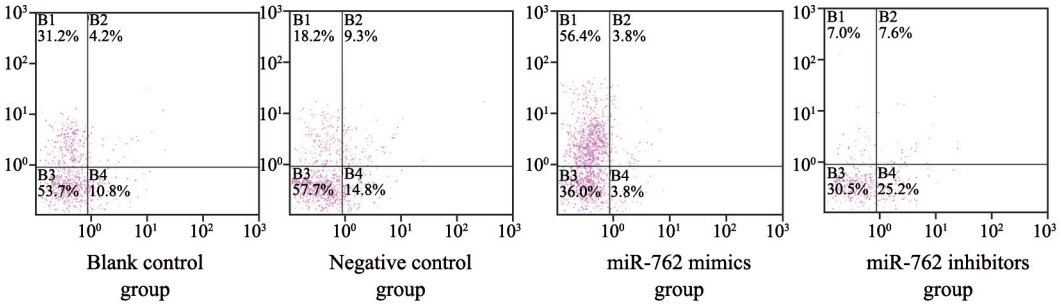

图 4 miR–762对PANC–1细胞凋亡的影响（流式细胞术）

Fig. 4 Effect of miR-762 on apoptosis of PANC-1 cells (flow cytometry)

2.5 miR-762对PANC-1细胞侵袭转移的影响

划痕实验结果显示，转染48 h后，空白对照组、阴性对照组、miR-762 mimics组、miR-762 inhibitors组细胞迁移距离分别为（1.04±0.27）μm、（1.00±0.24）μm、（1.79±0.16）μm、（0.34±0.10）μm。与阴性对照组比较，miR-762 mimics组细胞迁移距离显著延长，miR-762 inhibitors组细胞迁移距离显著缩短（t=-11.29、7.68，P=0.000、0.002）。Transwell侵袭实验结果显示，转染48 h后，空白对照组、阴性对照组、miR-762 mimics组、miR-762 inhibitors组穿膜细胞数分别为

（196.40±25.13）个、（188.20 ± 26.04）个、（279.40 ± 20.31）个、（92.80 ± 11.08）个。与阴性对照组比较，miR-762 mimics组穿膜细胞数显著增加，miR-762 inhibitors组穿膜细胞数显著减少（t=-4.91、6.67，P=0.008、0.003，图5）。

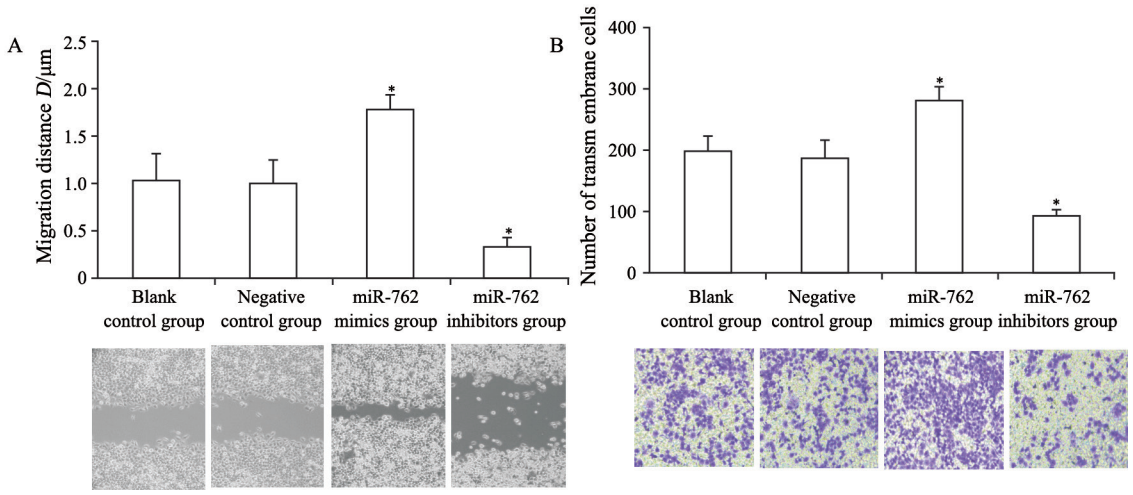

图 5　miR-762对PANC-1细胞侵袭转移的影响

Fig. 5　Effect of miR-762 on invasion and migration of PANC-1 cells

Migration of cells was tested by wound healing assay, and invasion of cells was tested by Transwell chamber assay. Each bar represents $\bar{x}\pm s$ of three independent experiments. *: P<0.01

2.6　miR-762对PANC-1细胞上皮-间质转化（epithelial-mesenchymal transformation，EMT）相关标志物的影响

Western blot结果显示，转染48 h后，与阴性对照组比较，miR-762 mimics组上皮细胞标志物E-cadherin表达量明显降低，间质表型细胞标志物N-cadherin、vimentin表达量明显增加（t=7.87、-6.11、-4.53，P=0.001、0.004、0.011）；而miR-762 inhibitors组上皮细胞标志物E-cadherin表达量明显增加，间质表型细胞标志物N-cadherin、vimentin表达量明显降低（t=4.13、5.31、6.78，P=0.014、0.006、0.002，图6）。

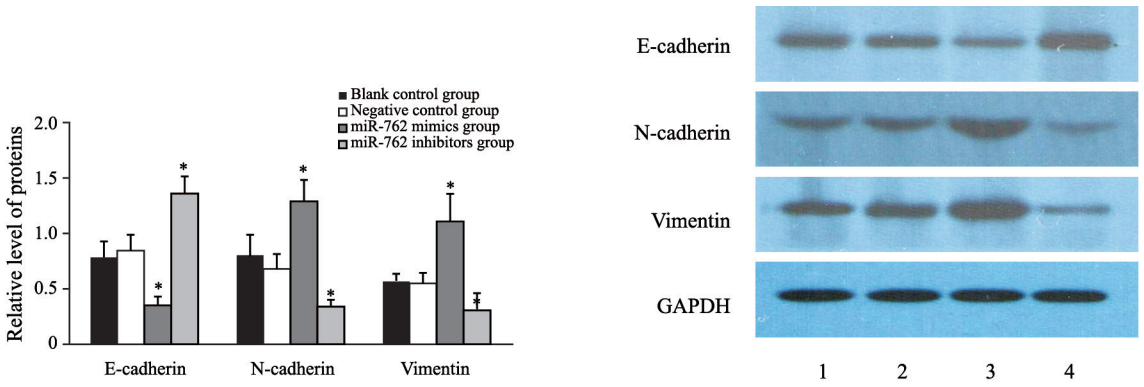

图 6　Western blot检测EMT相关蛋白表达

Fig. 6　Expression of EMT-associated proteins detected by Western blot

1: Blank control group; 2: Negative control group; 3: miR-762 mimics group; 4: miR762 inhibitors group. Equal amounts of lysates were analyzed by Western blot for protein levels. Each bar represents $\bar{x}\pm s$ of three independent experiments. *: P<0.01

3 讨 论

胰腺癌预后极差，与其早期易发生侵袭转移密切相关[7]。研究发现，多种miRNA表达失调在胰腺癌细胞增殖、凋亡及侵袭转移方面发挥重要作用[8-10]。因此，寻找与胰腺癌发生、发展密切相关的miRNA具有积极意义。miR-762与肿瘤关系密切，Hou等[5]发现卵巢癌组织高表达miR-762，与患者预后呈负相关，还发现抑癌基因*menin*是其下游靶基因。Li等[6]也证实乳腺癌组织和细胞株高表达miR-762，可靶向下调*IRF*7基因表达。但miR-762在调控胰腺癌发生、发展中的作用尚不清楚。本研究结果表明，胰腺癌组织及4种胰腺癌细胞株中miR-762呈高表达，提示miR-762可能参与了胰腺癌的形成及进展。在此基础上，本研究采用基因干扰技术在胰腺癌PANC-1细胞中过表达和抑制表达miR-762，并采用CCK-8、流式细胞术、划痕实验和Transwell侵袭实验检测调节miR-762表达对PANC-1细胞增殖、凋亡及侵袭转移的影响，结果发现，上调miR-762表达能够显著增强PANC-1细胞的增殖及侵袭转移能力，并抑制其凋亡；相反，下调miR-762表达能够显著抑制PANC-1细胞的增殖及侵袭转移能力，并促进其凋亡。

EMT是肿瘤耐药、干细胞样特性形成及获得更高恶性程度的关键环节[11-14]。在此过程中，上皮标志物E-cadherin表达量降低，而间质标志物N-cadherin、vimentin表达量升高。本研究结果表明，过表达miR-762能够显著上调胰腺癌细胞N-cadherin、vimentin表达，下调E-cadherin表达；而抑制miR-762表达能够显著下调胰腺癌细胞N-cadherin、vimentin表达，上调E-cadherin表达，证实miR-762可能参与调控胰腺癌细胞EMT进程。

综上所述，miR-762在胰腺癌组织和细胞株中异常高表达，通过影响EMT进程促进胰腺癌细胞的增殖和侵袭转移，提示miR-762有可能成为胰腺癌诊断和治疗的潜在靶点。然而，miR-762调控胰腺癌细胞生物学行为的具体靶点仍需深入研究。

[参 考 文 献]

[1] KLEEFF J, KORC M, APTE M, et al. Neoptolemos, pancreatic cancer [J]. Nat Rev Dis Primers, 2016, 2: 16022–16026.

[2] CHEN W, ZHENG R, BAADE P D, et al. Cancer statistics in China, 2015 [J]. CA Cancer J Clin, 2016, 66(2): 115–132.

[3] LI Y, SARKAR F H. MicroRNA targeted therapeutic approach for pancreatic cancer [J]. Int J Biol Sci, 2016, 12(3): 326–337.

[4] BROUWER J, KLUIVER J, DE ALMEIDA R C, et al. Small RNA sequencing reveals a comprehensive miRNA signature of BRCA1–associated high grade serous ovarian cancer [J]. J Clin Pathol, 2016, pii: jclinpath–2016–203679.

[5] HOU R, YANG Z, WANG S, et al. miR–762 can negatively regulate menin in ovarian cancer [J]. Onco Targets Ther, 2017, 10: 2127–2137.

[6] LI Y, HANG R, WANG L, et al. MicroRNA–762 promotes breast cancer cell proliferation and invasion by targeting IRF7 expression [J]. Cell Prolif, 2015, 48(6): 643–649.

[7] DARTA. Metastasis: CXCR2–targeted therapy for pancreatic cancer [J]. Nat Rev Cancer, 2016, 16(7): 411.

[8] HUANG S, GUO H, CAO Y, et al. MiR–708–5p inhibits the progression of pancreatic ductal adenocarcinoma by targeting Sirt3 [J]. Pathol Res Pract, 2019, 215(4): 794–800.

[9] SUN Y, ZHU Q, ZHOU M, et al. Restoration of miRNA–148a in pancreatic cancer reduces invasion and metastasis by inhibiting the Wnt/β–catenin signaling pathway via downregulating maternally expressed gene–3 [J]. Exp Ther Med, 2019, 17(1): 639–648.

[10] MOU T, XIE F, ZHONG P, et al. MiR–345–5p functions as a tumor suppressor in pancreatic cancer by directly targeting CCL8 [J]. Biomed Pharmacother, 2019, 111: 891–900.

[11] 李 鹏, 马晓莹, 李小嘉, 等. 一种结肠癌干细胞上皮–间质转化分层模型的建立和验证 [J]. 中国癌症杂志, 2019, 29(2): 100–110.

[12] LE LARGE T Y S, BIJLSMA M F, KAZEMIER G, et al. Key biological processes driving metastatic spread of pancreatic cancer as identified by multi–omics studies [J]. Semin Cancer Biol, 2017, 44: 153–169.

[13] NIETO M A, HUANG R Y, JACKSON R A, et al. EMT: 2016 [J]. Cell, 2016, 166(1): 21–45.

[14] KHOO B L, LEE S C, KUMAR P, et al. Short–term expansion of breast circulating cancer cells predicts response to anti–cancer therapy [J]. Oncotarget, 2015, 6(17): 15578–15593.

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