

miR-17-5p Inhibitor Enhances Chemosensitivity to Gemcitabine Via Upregulating Bim Expression in Pancreatic Cancer Cells

Hai-jiao Yan · Wen-song Liu · Wen-hui Sun ·
Jun Wu · Mei Ji · Qi Wang · Xiao Zheng ·
Jing-ting Jiang · Chang-ping Wu

Received: 25 June 2012 / Accepted: 28 August 2012 / Published online: 22 September 2012
© Springer Science+Business Media, LLC 2012

Abstract

Background miR-17-5p is reported to be overexpressed in pancreatic cancer, and it plays an important role in carcinogenesis and cancer progression. Gemcitabine is the standard first-line chemotherapeutic agent for pancreatic cancer, however the chemoresistance limits the curative effect.

Aims In the present study, we investigated whether inhibition of miR-17-5p could enhance chemosensitivity to gemcitabine in pancreatic cancer cells.

Methods miR-17-5p inhibitor was transfected to pancreatic cancer cell lines Panc-1 and BxPC3, and then cell proliferation, cell apoptosis, caspase-3 activation, and chemosensitivity to gemcitabine were measured in vitro.

Results Our data showed that Panc-1 and BxPC3 cells transfected with miR-17-5p inhibitor showed growth inhibition, spontaneous apoptosis, higher caspase-3 activation, and increased chemosensitivity to gemcitabine. In addition,

miR-17-5p inhibitor upregulated Bim protein expression in a dose-dependent manner without changing the Bim mRNA level, and it increased the activity of a luciferase reporter construct containing the Bim-3' untranslated region.

Conclusions These results prove that miR-17-5p negatively regulates Bim at the posttranscriptional level. We suggest that miR-17-5p inhibitor gene therapy would be a novel approach to chemosensitization for human pancreatic cancer.

Keywords miR-17-5p · Pancreatic cancer · Gemcitabine · Drug resistance

Introduction

Pancreatic cancer is one of the most common invasive malignancies, and it is the fourth major cause of cancer-related deaths in the world [1]. Gemcitabine has been considered the first-line treatment for patients with advanced pancreatic cancer over the past two decades, and it has offered some relief of clinical symptoms, however, even the combination therapy using gemcitabine with other agents has not been successful in increasing the 5-year survival rate [2, 3]. Drug resistance remains the major clinical obstacle to chemotherapy and results in poor prognosis. More understanding of the drug-resistance mechanisms is necessary in order to improve the current chemotherapy effect. Thus, it remains an urgent task to develop new effective treatment for pancreatic cancer.

MicroRNAs (miRNAs) are small noncoding RNAs of 14–24 nucleotides regulating gene expression posttranscriptionally, and it plays critical roles in diverse biological processes. Recently, increasing studies have indicated that aberrant miRNA expression is strongly involved in anti-cancer drug resistance phenotype. Xu et al. [4] reported

Hai-jiao Yan and Wen-song Liu contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s10620-012-2400-4) contains supplementary material, which is available to authorized users.

H. Yan · W. Sun · J. Wu · M. Ji · Q. Wang · X. Zheng ·
J. Jiang (✉) · C. Wu (✉)
Department of Tumor Biological Treatment, The Third
Affiliated Hospital of Soochow University, Changzhou 213003,
People's Republic of China
e-mail: jjtnew@163.com

C. Wu
e-mail: wcpzlk@163.com

W. Liu
Department of Hepatobiliary Surgery, The Third Affiliated
Hospital of Soochow University, Changzhou 213003
People's Republic of China

that overexpression of miR-122 by adenovirus vector could render cells sensitive to adriamycin and vincristine in hepatocellular carcinoma cells by inhibiting expression of MDR. miR-15b, -16, and -181b were reported to modulate multidrug resistance by targeting BCL2 in human gastric cancer cells [5, 6]. miRNA involvement in tumor cell response to chemotherapeutic agents was confirmed by many studies [7–9]. Previous miRNA microarray data revealed that miR-17-5p, a member of the miR-17-92 cluster, is overexpressed in pancreatic carcinoma [10]. Yu et al. [11] reported that miR-17-5p plays an important role in pancreatic carcinogenesis and cancer progression, and it is also associated with poor prognosis of pancreatic cancer. So we proposed that inhibition of miR-17-5p expression may inhibit cell proliferation, and enhance apoptosis and chemosensitivity in pancreatic cancer.

In the present study, the miR-17-5p inhibitor was transfected to pancreatic cancer cell lines Panc-1 and BxPC3. The changes of cell proliferation, cell apoptosis, caspase-3 activation, and chemosensitivity to gemcitabine *in vitro* were investigated.

Materials and Methods

Materials

Gemcitabine was purchased from Eli Lilly (Indianapolis, IN, USA). miR-17-5p inhibitor and scramble (control miR) were from GenePharma (Shanghai, China). Monoclonal antibody specific to Bim and caspase-3 was from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and Cell Signaling Technology (Beverly, MA, USA) respectively. QuikChange II Site-Directed Mutagenesis Kit was from Stratagene (La Jolla, CA, USA). PGL3-control vector and Dual-Luciferase Reporter Assay System were purchased from Promega (Madison, WI, USA).

Cell and Cell Culture

Panc-1 and BxPC3 cells were obtained from the American Type Culture Collection and cultured in DMEM culture medium supplemented with 10 % fetal bovine serum (Gibco), and maintained at 37 °C in a humidified atmosphere with 5 % CO₂. All cell lines were grown under identical conditions.

miRNA Transfection

Cells in exponential phase were plated in 60-mm plates at 1×10^6 cells/plate and cultured for 16 h, and then transfected with the miR-17-5p inhibitor or scramble (400 nM) using LipofectamineTM 2000 (Invitrogen Life Technologies,

Carlsbad, CA, USA), according to the manufacturer's protocol. The effects of miR-17-5p inhibitor on Bim and caspase-3 expression were examined 48 h after transfection.

Cell Viability Assay

Twenty-four hours after transfection with miR-17-5p inhibitor, cells were plated in triplicate in 96-well plates at 3×10^3 viable cells/well overnight. The cells were then incubated with medium containing 500 nmol/l gemcitabine for 24 h. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. The absorbance at 490 nm was read on a spectrophotometer.

Apoptosis Assay by Flow Cytometry

Panc-1 and BxPC3 cells were seeded in six-well plates (5×10^5 /well) and transfected with miR-17-5p inhibitor or scramble. Twenty-four hours later, gemcitabine was added with a final concentration of 500 nmol/l. After another 24 h, cells were harvested, washed in cold PBS, and double-stained with FITC-conjugated Annexin V and propidium iodide (PI), and then analyzed by flow cytometry (Coulter Biosciences).

Acridine Orange/Ethidium Bromide (AO/EB) Fluorescence Staining

Cellular morphological changes were investigated by AO/EB staining and observed by a fluorescence microscopy. Panc-1 and Bxpc3 cells were transfected with miR-17-5p inhibitor for 24 h, and then exposed to 500 nmol/l gemcitabine for another 24 h. Cells were harvested, washed, and resuspended in 200 μ l of medium. Then, 8 μ l mixture of fluorescent dye containing 100 μ g/ml AO and 100 μ g/ml EB (Sigma) was gently added to the cells. The cells were finally visualized under a fluorescent microscope (100 \times) (Leica Microsystems GmbH, Wetzlar, Germany) with excitation at 488 nm and emission at 520 nm.

Western-Blot Analysis

After 48 h of transfection with miR-17-5p inhibitor or scramble (400 nM), Panc-1 and BxPC3 cells were harvested, and then 80 μ g of total protein extract was separated by 10 % SDS-PAGE gels and transferred to PVDF membranes (BD). The membrane was probed with monoclonal anti-Bim and anti-Bcl-2 (1:500, Santa Cruz), anti-caspase-3 (1:500, Cell Signaling) used to recognize the cleaved (active) caspase-3 (17 kDa) and full-length (FL)-caspase-3 (35 kDa), and anti-GAPDH (1:1,000, Sigma). The membrane was further probed with peroxidase-conjugated secondary antibodies at optimized concentrations,

and the protein bands were visualized using enhanced chemiluminescence (Amersham Pharmacia Corp, Piscataway, NJ, USA).

Luciferase Reporter Assay

The 3'-UTR of Bim mRNA containing the miR-17-5p binding site was amplified by PCR, and the product was inserted to the XbaI site of pGL3 vector (Promega). The primers used for the amplification were 5' BimFL (TTTTGTCGACCAGGTTCTTTGCGGAGCC) and 3' BimFL (TTTTGCGGCCGCATTGCACAAGTAAAGTGGCAATTA). A mutant with a deletion of 5 bp from the fully complementary site was also generated by using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA). Wild-type and mutant inserts were confirmed by sequencing. Twenty-four hours before transfection, Panc-1 cells were plated at 1.5×10^5 cells/well in 24-well plates. Eight hundred ng of pGL3-Bim-3'-UTR or pGL3-mutBim-3'-UTR plus 16 ng of pGL4.73 (Promega) were transfected alone or in combination with 50nM of miR-17-5p inhibitor to the cells using Lipofectamine 2000. Forty-eight hours after transfection, luciferase activity was assayed by using the Dual-Luciferase Reporter Assay System (Promega).

Statistical Analysis

Each experiment was repeated at least three times. The results were calculated using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA), and analysis of variance (ANOVA) was used to determine the statistical differences among the groups. Differences were considered statistically significant when $p < 0.05$.

Results

miR-17-5p Inhibitor Enhances Chemosensitivity to Gemcitabine in Pancreatic Cancer Cells

We investigated the chemosensitivity to gemcitabine in human pancreatic carcinoma cells transfected with miR-17-5p inhibitor in vitro. Cells were either treated with miR-17-5p inhibitor (400 nmol/l) alone for 48 h or added with gemcitabine (500 nmol/l) for 24 h, and cell viability was determined by MTT assay. We noticed that treatment with miR-17-5p inhibitor for 48 h or with gemcitabine for 24 h alone caused 20.67 ± 3.26 and 34.15 ± 4.27 % loss of viability respectively in Panc-1 cells, but miR-17-5p inhibitor combined with gemcitabine caused 62.87 ± 5.93 % loss of viability (Fig. 1a). It caused 19.45 ± 3.41 and 32.36 ± 4.19 % loss of viability, respectively, in BxPC-3

cells after treatment with miR-17-5p inhibitor for 48 h or with gemcitabine alone for 24 h, but the combined therapy caused 60.36 ± 6.27 % loss of viability (Fig. 1b). These data showed that inhibition of miR-17-5p could markedly decrease the proliferation and enhanced the chemosensitivity of pancreatic cancer cells.

miR-17-5p Inhibitor Enhances Cell Apoptosis with Gemcitabine

No obvious cell apoptosis was detected in blank control or negative control cells, but it was detected in 11.08 ± 1.34 % of Panc-1 cells and 9.36 ± 1.09 % of BxPC3 cells, respectively, after transfection with miR-17-5p inhibitor. After 24 h with gemcitabine, the apoptosis rates of Panc-1 cells in the blank control group, negative control group, and positive experiment group were 31.65 ± 3.82 , 30.86 ± 4.03 , and 53.46 ± 5.89 %, respectively. The results were similar in BxPC3 cells, which were 34.15 ± 3.71 , 35.46 ± 4.26 , and 51.39 ± 5.53 %, respectively (Figs. 2, 3). These data showed that inhibition of miR-17-5p could induce the spontaneous apoptosis of both panc-1 and BxPC3 cells, and it could significantly enhance cell apoptosis with gemcitabine chemotherapy.

Cell Morphology Validates Cell Apoptosis Using AO-EB Staining

AO/EB staining distinguished apoptosis and necrosis of cancer cells. Early and late apoptotic cells showed cell shrinkage, bright green or orange nuclei, and condensed or fragmented chromatin, respectively. Necrotic cells lost their selective permeability and produced a red intact nuclei. After 48 h exposure to miR-17-5p inhibitor or with gemcitabine for 24 h, Panc-1 and BxPC3 cells showed typical apoptotic characteristics, while uniformly green live cells with normal morphology were observed in the control group. Our results showed that miR-17-5p inhibitor was able to induce marked apoptotic morphology in both Panc-1 and BxPC3 cells. miR-17-5p inhibitor could also significantly enhance gemcitabine-induced apoptosis and necrosis ($p < 0.05$) (Fig. 4).

miR-17-5p Inhibitor Regulates Bim Expression

To gain insight into the molecular mechanisms underlying the phenotypes observed in Panc-1 and BxPC3 cells, we searched for miR-17-5p targeting genes predicted by TargetScan, and focused on genes known to be involved in apoptosis. Our attention was drawn to Bcl2l11/Bim, a pro-apoptotic gene. Bim belong to a small group of genes that

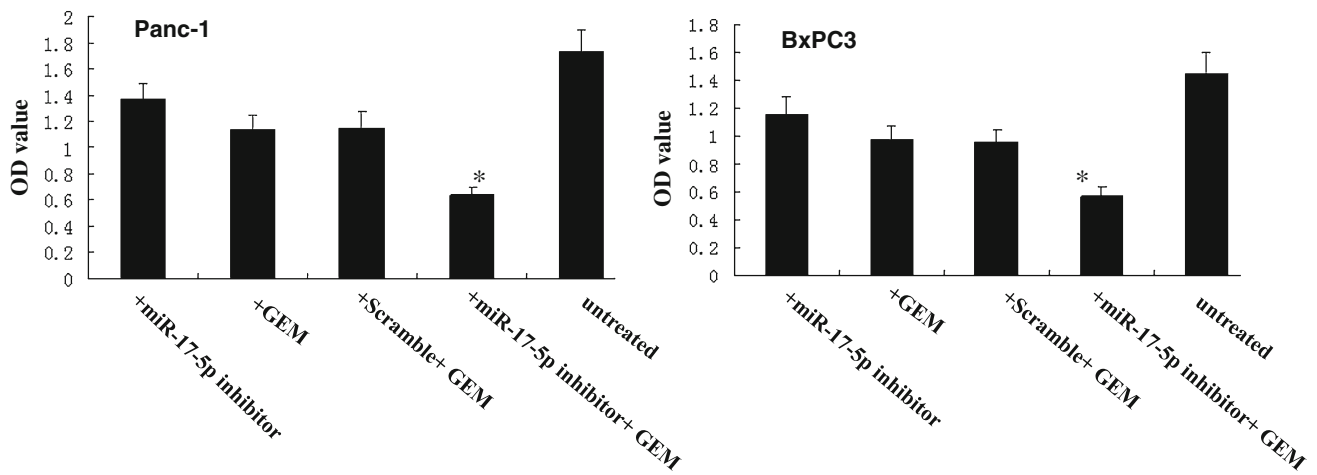


Fig. 1 Cell viability was assessed by MTT assays. The results indicate that miR-17-5p inhibitor can inhibit cell viability of both Panc-1 and BxPC3 ($p < 0.05$). After chemotherapy with gemcitabine for 24 h, miR-17-5p inhibitor can significantly enhance the inhibition rates of proliferation to 62.87 ± 5.93 and 60.36 ± 6.27 % in Panc-1 and BxPC3, respectively ($*p < 0.01$)

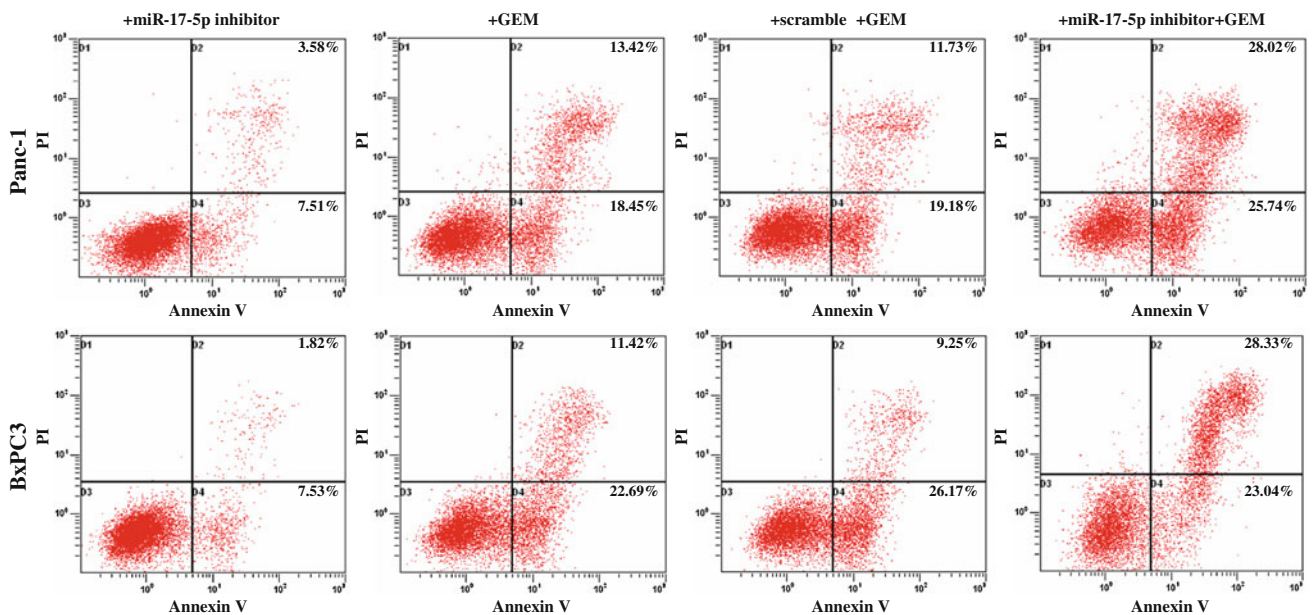


Fig. 2 Panc-1 and BxPC3 cell apoptosis detected by flow cytometry. **a** Cancer cells transfected with miR-17-5p inhibitor; **b** cancer cells with gemcitabine; **c** cancer cells with scramble + gemcitabine; **d** cancer cells with miR-17-5p inhibitor + gemcitabine

has a strong potential for coordinate regulation by miR-17-5p. Our data showed that Bim protein level was elevated when transfected with miR-17-5p inhibitor in Panc-1 or BxPC3 cells, however Bcl-2 protein level was downregulated ($p < 0.05$). Meanwhile, we found that miR-17-5p inhibitor led to significant caspase-3 activation by Western-blot assay, as evidenced by increased ratios of cleaved caspase-3 fragment (17 kDa) to FL-caspase-3 (35 kDa) ($p < 0.05$) (Fig. 5a). The Bim protein level was increased in a dose-dependent manner when pancreatic cancer cells were transfected with 0–400 nM of miR-17-5p inhibitor

($p < 0.05$) (Fig. 5b), without any change on Bim mRNA level.

miR-17-5p Regulates Bim by Targeting Its 3' Untranslated Region

To determine whether the 3' untranslated region (UTR) of Bim mRNA is a functional target of miR-17-5p, we cloned the putative miR-17-5p targets (Fig. 6) in the Bim 3'-UTR into a luciferase construct. The construct was cotransfected with miR-17-5p inhibitor to Panc-1 cells, and then

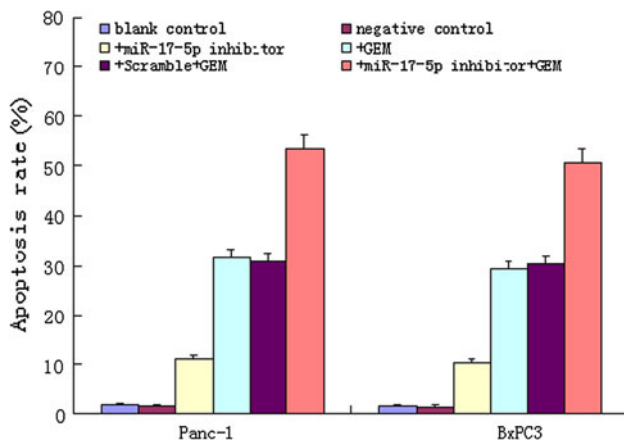


Fig. 3 Cell apoptosis detected by flow cytometry. Forty-eight hours after transfection, miR-17-5p inhibitor induce spontaneous apoptosis to 11.08 ± 2.34 and 9.36 ± 1.09 % in Panc-1 and BxPC3 cells, respectively, while there was no obvious cell apoptosis in the blank control or the negative control group ($p < 0.05$). Meanwhile, miR-17-5p inhibitor significantly promoted gemcitabine-induced apoptosis to 53.46 ± 5.89 and 51.39 ± 5.53 % in Panc-1 and Bxpc3 cells, respectively, compared with chemotherapy alone ($p < 0.05$)

luciferase activity was significantly increased ($p < 0.05$; Fig. 7). This effect was abolished with the mutation of the putative miR-17-5p target sites in Bim 3'-UTR. These results suggested that miR-17-5p binds to Bim 3'-UTR and

regulates Bim expression at the posttranscriptional level in pancreatic cancer cells.

Discussion

In this study, we demonstrated that transfection with miR-17-5p inhibitor led to growth inhibition, spontaneous apoptosis, higher caspase-3 activation and increased chemosensitivity to gemcitabine in both Panc-1 and BxPC3 cells. Meanwhile, we found that miR-17-5p inhibitor upregulated Bim protein expression in a dose-dependent manner without changing the Bim mRNA level, and it also could increased the activity of a luciferase reporter construct containing the Bim-3' UTR. These results prove that miR-17-5p negatively regulates Bim at the posttranscriptional level by binding to its 3' UTR.

miRNAs encoded by the miR-17-92 polycistron and its paralogs are known as oncogenes [12]. The miR-17-92 cluster promotes cancer cell proliferation, suppresses cell apoptosis, and induces tumor angiogenesis [13]. Of the more than 1,500 miR-17-92 targets predicted by TargetScan program, ten targets (E2F1, E2F3, Pten, AML1, Bim, AIB1, TGFBR2, Tsp1, CTGF, and p130) have been identified [14–17] to be key players in hematopoiesis, apoptosis, cell-cycle progression, angiogenesis, and tumorigenesis. As a member

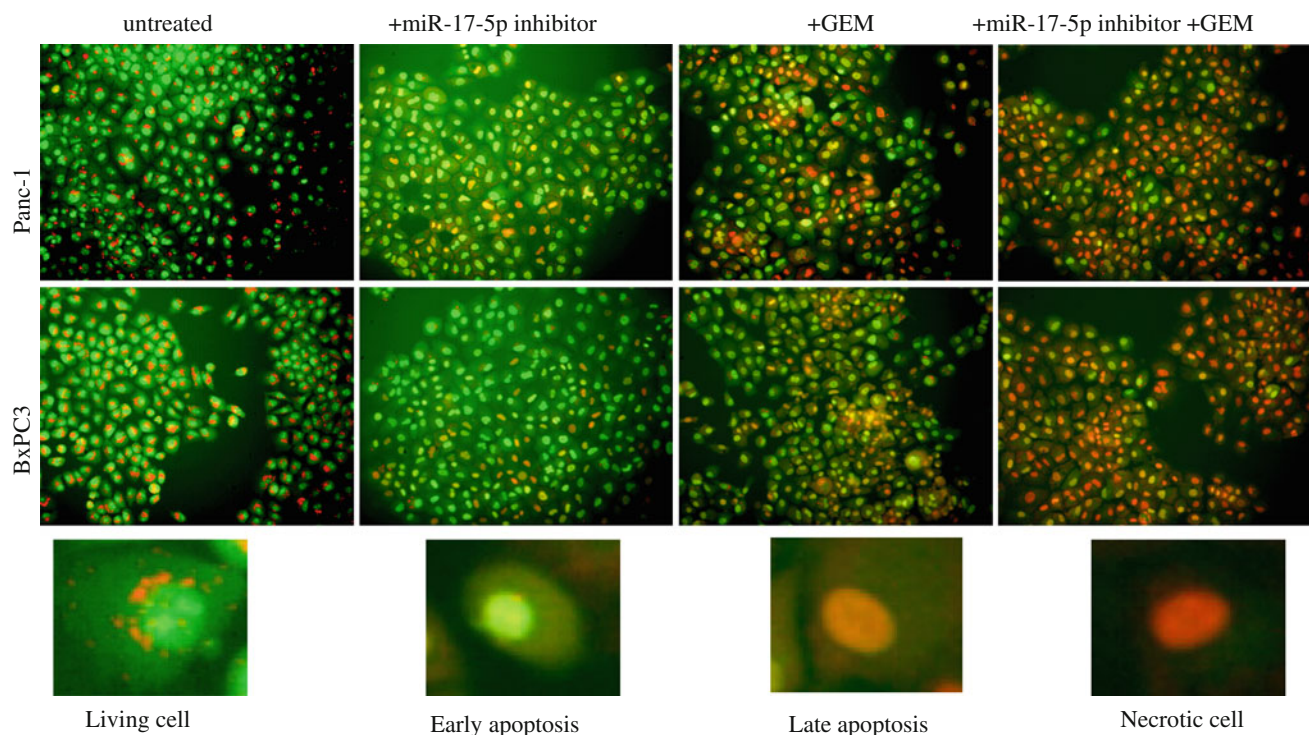


Fig. 4 Apoptotic morphological changes in Panc-1 and BxPC3 cells detected by AO/EB staining under fluorescence microscope. Uniformly green cells with normal morphology were observed in the control group. Apoptotic cells with cell shrinkage, chromatin

condensation, and bright green or orange nuclei were observed in the miR-17-5p-treated cells. miR-17-5p can significantly enhance gemcitabine-induced apoptosis and necrosis ($p < 0.05$)

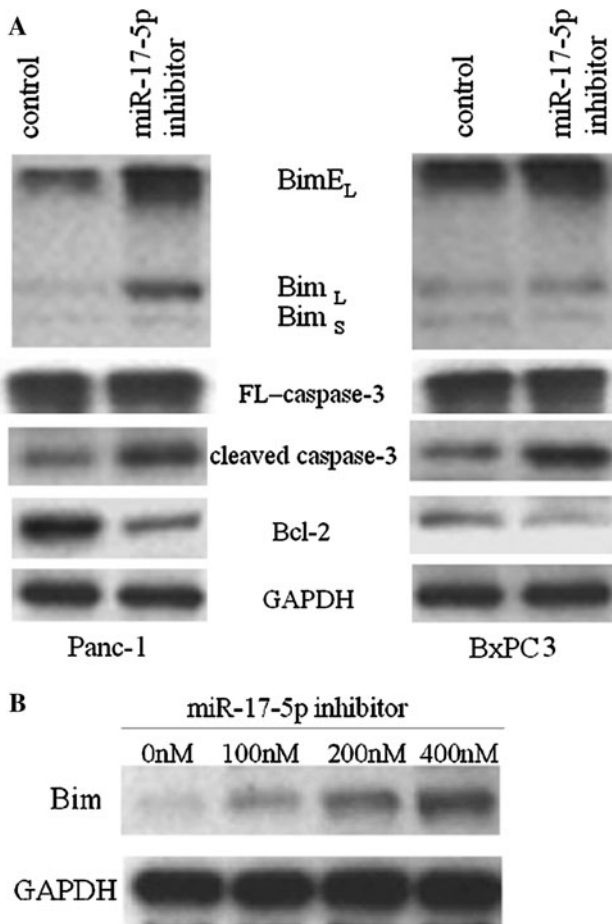


Fig. 5 Protein expression level detected by Western-blot analysis. **a** miR-17-5p inhibitor upregulated three major isoforms of Bim and downregulated bcl-2 protein levels 48 h after transfection. miR-17-5p inhibitor led to significant caspase-3 activation, as evidenced by increased ratios of cleaved caspase-3 fragment (17 kDa) to FL-caspase-3 (35 kDa). **b** Bim protein level was increased in a dose-dependent manner with miR-17-5p inhibitor transfection ($p < 0.05$)

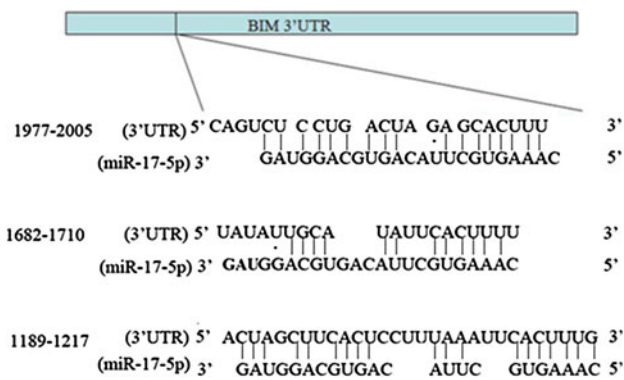


Fig. 6 Predicted binding site of miR-17-5p in Bim-3' UTR by using TargetScan. Sequence alignment of the Bim-3' UTR with three conserved target sites matched for miR-17-5p

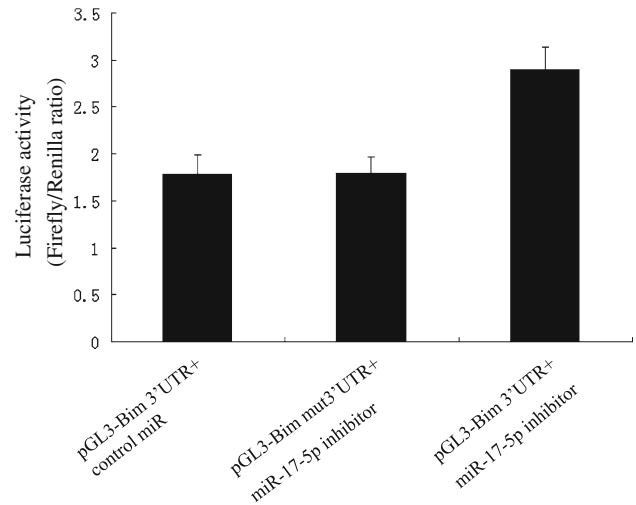


Fig. 7 Luciferase reporter assay. Panc-1 cells were co-transfected with Bim-3' UTR vector or Bim-mut3' UTR luciferase reporter and miR-17-5p inhibitor. Luciferase activity was significantly increased in the cells cotransfected with Bim-3' UTR luciferase reporter ($p < 0.05$). This effect was abolished in the cells cotransfected with Bim-mut3' UTR one. It suggested miR-17-5p directly targets the Bim-3' UTR at the posttranscriptional level

of the miR-17-92 cluster, miR-17-5p is reported to be overexpressed in pancreatic carcinoma, and it plays an important role in pancreatic carcinogenesis and cancer progression [11]. So we proposed that the downregulation of miR-17-5p may suppress pancreatic cancer progression. In the present study, our data confirmed that transfection of miR-17-5p inhibitor could inhibit cell proliferation, induce spontaneous apoptosis, and enhance chemosensitivity to gemcitabine in both Panc-1 and BxPC3 cells.

The Bcl-2 family consists of prosurvival proteins and two factions of proapoptotic proteins that include multidomain Bak and the BH3-only proteins (Bad, Bim, Bid, Puma, and Noxa). Resistance of the tumors to cytotoxic chemotherapy might be due to the frequent expression of prosurvival Bcl-2 proteins (Bcl-2, Bcl-xL, Mcl-1, and Bcl-w) that are potent inhibitors of the intrinsic mitochondrial apoptotic pathway [18]. BH3 proteins are induced by cellular stress and bind to the prosurvival Bcl-2 family proteins to neutralize them, allowing apoptosis to occur [19, 20]. Bim critically regulates apoptosis in a variety of tissues by activating proapoptotic molecules like Bax and Bad and antagonizing antiapoptotic molecules like Bcl-2 and BclXL [21]. A fine balance of Bim and its partner proteins in the intracellular concentration is crucial to properly regulate apoptosis. Notably, Bim is the key downstream apoptotic effector of the TGF β pathway, and its down-modulation abrogates TGF β -dependent apoptosis in Snu-16 cells [22]. Bim also mediates tumor cell death induced by radiotherapy or chemotherapy including paclitaxel, which is among the most effective chemotherapeutic agents for patients with lung, prostate, and breast cancer [23–25]. These

studies indicate that Bim can act as a tumor suppressor and that cancer cells may be driven to undergo apoptosis by increasing the intracellular concentration of Bim.

Thus, we searched for effectors of apoptosis by the TargetScan database, and we identified BCL2L11 (Bim) as the only strong candidate out of 18 human genes harboring putative binding sites for miR-17-5p. Bim is induced by Myc in acute B cell leukemia and it mediates apoptosis, and inactivation of even a single allele accelerates Myc-induced development of tumors in mice [26]. Thus, we wanted to verify whether Bim was a direct target of miR-17-5p. Our results showed that Panc-1 and BxPC3 cells expressed all the three major isoforms of Bim, named Bim EL, Bim L, and Bim S. Intriguingly, transfection of miR-17-5p inhibitor induced an upregulation of all the three isoforms of Bim in both cell lines, without any change on Bim mRNA. Furthermore, miR-17-5p inhibitor could have increased the activity of a luciferase reporter construct containing the Bim-3' UTR instead of the mutant one, which indicates that miR-17-5p negatively regulated Bim expression by binding directly to the 3' UTR of Bim mRNA. Caspase-3 activation is one of the final key steps of cell apoptosis [27], and we found that miR-17-5p inhibitor led to significant caspase-3 activation, as evidenced by increased ratios of cleaved caspase-3 fragment to FL-caspase-3. We also found that Bcl-2 protein level was down-regulated at the same time, which was probably in part because of Bim neutralization. Our studies are consistent with another report that treatment with antagomir-17-5p abolishes the growth of therapy-resistant neuroblastoma through upregulation of BIM [28].

In summary, our study indicates that transfection of miR-17-5p inhibitor could inhibit cell proliferation and enhance chemosensitivity to gemcitabine in pancreatic carcinoma cells in vitro, and the effects are probably caused by upregulation of Bim protein expression at the posttranscriptional level, followed by caspase-3 activation. Together, we suggest that inhibition of miR-17-5p would be a potential approach to chemosensitization for pancreatic carcinoma in the future.

Acknowledgments This work was supported by the National Natural Science Foundation of China (Grant 81201676, 30972703, 81171653), and the science and technology project for young talent by Changzhou Municipal Health Bureau (Grant QN201103).

Conflict of interest None.

References

- Assifi MM, Hines OJ. Anti-angiogenic agents in pancreatic cancer: a review. *Anticancer Agents Med Chem.* 2011;11:464–469.
- Liu WS, Yan HJ, Qin RY, et al. siRNA directed against survivin enhances pancreatic cancer cell gemcitabine chemosensitivity. *Dig Dis Sci.* 2009;54:89–96.
- Kato H, Seto K, Kobayashi N, et al. CCK-2/gastrin receptor signaling pathway is significant for gemcitabine-induced gene expression of VEGF in pancreatic carcinoma cells. *Life Sci.* 2011;89:603–608.
- Xu Y, Xia F, Ma L, et al. MicroRNA-122 sensitizes HCC cancer cells to adriamycin and vincristine through modulating expression of MDR and inducing cell cycle arrest. *Cancer Lett.* 2011;310:160–169.
- Xia L, Zhang D, Du R, et al. miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. *Int J Cancer.* 2008;123:372–379.
- Zhu W, Shan X, Wang T, et al. miR-181b modulates multidrug resistance by targeting BCL2 in human cancer cell lines. *Int J Cancer.* 2010;127:2520–2529.
- Li Y, Zhu X, Gu J, et al. Anti-miR-21 oligonucleotide sensitizes leukemic K562 cells to arsenic trioxide by inducing apoptosis. *Cancer Sci.* 2010;101:948–954.
- Galluzzi L, Morselli E, Vitale I, et al. miR-181a and miR-630 regulate cisplatin-induced cancer cell death. *Cancer Res.* 2010;70:1793–1803.
- Hummel R, Hussey DJ, Haier J. MicroRNAs: predictors and modifiers of chemo- and radio-therapy in different tumour types. *Eur J Cancer.* 2010;46:298–311.
- O'Donnell KA, Wentzel EA, Zeller KI, et al. c-Myc-regulated microRNAs modulate E2F1 expression. *Nature.* 2005;435:839–843.
- Yu J, Ohuchida K, Mizumoto K, et al. MicroRNA miR-17-5p is overexpressed in pancreatic cancer, associated with a poor prognosis, and involved in cancer cell proliferation and invasion. *Cancer Biol Ther.* 2010;10:748–757.
- Osada H, Takahashi T. let-7 and miR-17-92: small-sized major players in lung cancer development. *Cancer Sci.* 2011;102:9–17.
- Bonauer A, Dimmeler S. The microRNA-17-92 cluster: still a miRacle? *Cell Cycle.* 2009;8:3866–3873.
- Tagawa H, Karube K, Tsuzuki S, et al. Synergistic action of the microRNA-17 polycistron and Myc in aggressive cancer development. *Cancer Sci.* 2007;98:1482–1490.
- Hossain A, Kuo MT, Saunders GF. mir-17-5p regulates breast cancer cell proliferation by inhibiting translation of AIB1 mRNA. *Mol Cell Biol.* 2006;26:8191–8201.
- Doebele C, Bonauer A, Fischer A, et al. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. *Blood.* 2010;115:4944–4950.
- Wang Q, Li YC, Wang J, et al. miR-17-92 cluster accelerates adipocyte differentiation by negatively regulating tumor-suppressor Rb2/p130. *Proc Natl Acad Sci USA.* 2008;105:2889–2894.
- Zivny J, Klener P Jr, Pytlík R, et al. The role of apoptosis in cancer development and treatment: focusing on the development and treatment of hematologic malignancies. *Curr Pharm Des.* 2010;16:11–33.
- Ola MS, Nawaz M, Ahsan H. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. *Mol Cell Biochem.* 2011;351:41–58.
- Happo L, Strasser A, Cory S. BH3-only proteins in apoptosis at a glance. *J Cell Sci.* 2012;125:1081–1087.
- Ren D, Tu HC, Kim H, et al. BID, BIM, and PUMA are essential for activation of the BAX- and BAK-dependent cell death program. *Science.* 2010;330:1390–1393.
- Ohgushi M, Kuroki S, Fukamachi H, et al. Transforming growth factor beta-dependent sequential activation of Smad, Bim, and caspase-9 mediates physiological apoptosis in gastric epithelial cells. *Mol Cell Biol.* 2005;25:10017–10028.

23. Li R, Moudgil T, Ross HJ, et al. Apoptosis of non-small-cell lung cancer cell lines after paclitaxel treatment involves the BH3-only proapoptotic protein Bim. *Cell Death Differ.* 2005;12:292–303.
24. Kutuk O, Letai A. Displacement of Bim by Bmf and Puma rather than increase in Bim level mediates paclitaxel-induced apoptosis in breast cancer cells. *Cell Death Differ.* 2010;17:1624–1635.
25. Gillings AS, Balmanno K, Wiggins CM, et al. Apoptosis and autophagy: BIM as a mediator of tumour cell death in response to oncogene-targeted therapeutics. *FEBS J.* 2009;276:6050–6062.
26. Egle A, Harris AW, Bouillet P, et al. Bim is a suppressor of Myc-induced mouse B cell leukemia. *Proc Natl Acad Sci USA.* 2004;101:6164–6169.
27. Lakhani SA, Masud A, Kuida K, et al. Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. *Science.* 2006;311:847–851.
28. Fontana L, Fiori ME, Albini S, et al. Antagomir-17-5p abolishes the growth of therapy-resistant neuroblastoma through p21 and BIM. *PLoS One.* 2008;3:e2236.