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# MiR-125a regulates chemo-sensitivity to gemcitabine in human pancreatic cancer cells through targeting A20

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## Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most deadly human malignant diseases and the sixth leading cause of cancer-related deaths in China. Gemcitabine is the only first-line chemotherapeutic agent used for the palliative treatment of patients with PDAC, but chemo-resistance limits their efficacy. Here, we showed that miR-125a was up-regulated in chemo-resistant SW1990GZ cells when compared with SW1990 cells. Over-expression of miR-125a increased the chemo-resistance to gemcitabine in SW1990 cells, while down-regulation of miR-125a in SW1990GZ cells increased chemo-sensitivity to gemcitabine. By using bioinformatics analysis tool (Targetscan), the 3′ untranslated region (3′UTR) of *A20* gene was found to be a target of miR-125a. Luciferase reporter assay further confirmed that A20 3′UTR is a direct target of miR-125a. Over-expression of A20 in SW1990 cells increased chemo-sensitivity to gemcitabine, while knockdown of A20 in SW1990 cells promoted the chemo-resistance to gemcitabine. Finally, the expression level of miR-125a in pancreatic cancer tissues from chemo-sensitive patients was significantly lower than that from chemo-resistant patients, and was inversely correlated with the *A20* mRNA levels. In conclusion, our results suggest that miR-125a promotes chemo-resistance to gemcitabine in pancreatic cells through targeting A20, which may provide novel therapeutic targets or molecular biomarkers for cancer therapy and improve tumor diagnosis or predictions of therapeutic responses.

**Key words:** PDAC, gemcitabine, miR-125a, A20, chemo-resistance

## Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the most deadly human malignant diseases and the sixth leading cause of cancer-related deaths in China [1]. With an overall cumulative 5-year survival rate of 1%–3%, pancreatic cancer has an extremely poor prognosis [1]. Gemcitabine is the only first-line chemotherapeutic agent used for the palliative treatment of patients with PDAC, and it has been used in patients with PDAC for more than a decade [2]. Unfortunately, in a substantial number of patients, PDAC are chemo-resistant to gemcitabine [3]. Several lines of studies have demonstrated the possible chemo-resistant mechanisms related to the metabolism and molecular targets of gemcitabine [4,5]. Thus, gemcitabine in combination with a novel targeted therapeutic strategy may provide better management of gemcitabine-resistant pancreatic cancer.

MicroRNAs (miRNAs) are a class of single-stranded, small, non-coding RNAs, and they negatively regulate gene expression by inducing mRNA degradation or translational repression by targeting the 3′ untranslated region (3′UTR) of mRNAs [6]. Numerous studies have shown that miRNAs in cancer can function as either an activator or a suppressor by regulating the expression of cancer-specific genes

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[7]. Dysregulation of miRNAs has been shown to be associated with proliferation, invasion, chemo-sensitivity, and prognosis in PDAC [8,9]. For example, let-7 expression is significantly lower in pancreatic cancer cells than in normal pancreatic tissue; the restoration of let-7 expression inhibits cell proliferation and down-regulates signal transducers and activators of transcription phosphorylation in pancreatic cancer cells by increasing suppressor of cytokine signaling 3 [10]. Moreover, miR-217 functions as a tumor suppressor in PDAC by directly targeting GTPase KRas [11]. On the other hand, miR-21 is up-regulated in pancreatic cancer cells, and it has several targets including phosphatase and tensin homolog, programmed cell death protein 4, and tropomyosin alpha-1 chain, which leads to the inhibition of apoptosis [12]. Studies also showed that miR-33a-mediated down-regulation of Pim-3 kinase expression is associated with the sensitivity of human pancreatic cancer cells to gemcitabine [13]. The tumor suppressive role of miR-125a has been found in many types of cancers, including liver cancer [14], colon cancer [15], and breast cancer [16]. A recent study also suggested that miR-125a may be a potential novel marker in pancreatic cancer patients [17]. The detailed mechanism of miR-125a in PDAC is still unclear. Therefore, a full understanding of the role of miR-125a in PDAC may provide novel therapeutic targets or molecular biomarkers for cancer therapy and improve tumor diagnosis or predictions of therapeutic responses.

A20, a zinc finger family protein, is a key endogenous modulator of NF-κB signaling [18]. In contrast to its anti-inflammatory function that has been well documented, A20 has been shown to have anti- or pro-apoptotic functions, which depends on the cell type or apoptotic stimulus [19,20]. The role of A20 in anticancer resistance has been slowly unraveled in the past decade. Studies have revealed that the decreased A20 level promotes the resistance to O6-alkylating agents in human glioblastomas [21]. However, the role of A20 in chemo-resistance to gemcitabine in PDAC is largely unknown.

Here, we showed that miR-125a was up-regulated in chemo-resistant SW1990GZ cells when compared with SW1990 cells. Over-expression of miR-125a increased the chemo-resistance to gemcitabine in SW1990 cells, while down-regulation of miR-125a in SW1990GZ cells increased chemo-sensitivity to gemcitabine. By using bioinformatics analysis tool (Targetscan), the 3′UTR of *A20* gene was found to be a target of miR-125a. Luciferase reporter assay further confirmed that *A20* 3′UTR is a direct target of miR-125a. Over-expression of A20 in SW1990 cells increased the chemo-sensitivity to gemcitabine, while knockdown of A20 in SW1990 cells increased the chemo-resistance to gemcitabine. Finally, the expression level of miR-125a in pancreatic cancer tissues from chemo-sensitive patients was found to be significantly lower than that from chemo-resistant patients, and the expression level of miR-125a was inversely correlated with the mRNA level of *A20*.

## Materials and Methods

### Cell culture

SW1990 and HEK293T cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, USA) supplemented with 10% fetal bovine serum. Cells were incubated in a humidified atmosphere with 5% $CO_2$ at 37°C. A gemcitabine-resistant pancreatic cancer cell line (SW1990GZ) was established by incubating SW1990 cells with serially escalating doses of gemcitabine as previously reported [22]. Initially, SW1990 cells were cultured with 10 nM gemcitabine for 72 h with a defined drug-free interval. When cells adapted to the initial dose, the concentration of gemcitabine was doubled. Finally, SW1990 cells were adapted to 100 nM gemcitabine.

### Tissue sample

A total of 28 patients with pancreatic cancer (14 chemo-sensitive cases and 14 chemo-resistant cases) who had routine surgery at the Northern Jiangsu People's Hospital (Yangzhou, China) were recruited in this study. The age range for the chemo-sensitive group was from 52 to 73 years with a median of 67.5. The age range for the chemo-resistant group was from 45 to 75 years with a median of 62.0. All patients received 6–8 cycles of chemotherapy regimen (gemcitabine) after surgery. The cancer is identified as chemo-sensitive if there is no cancer recurrence within 6 months of the initial chemotherapy, and as chemo-resistant if there is cancer recurrence within 6 months of the initial chemotherapy. Tissue samples taken from these 28 patients were snap-frozen in liquid nitrogen for further real-time polymerase chain reaction (PCR) analysis. The study was approved by the Ethics Review Committee of Northern Jiangsu People's Hospital, and informed consents were obtained from all patients.

### Cell transfection

Cells (SW1990 and SW1990GZ) were seeded in six-well plates and cultured for 24 h. Then, the cells were transfected with miR-125a mimic, miR-125a inhibitor, A20 siRNA, or negative control (NC) using Lipofectamine 2000 (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Co-transfections of (a) miR-125a NC and pGEM-T easy, (b) miR-125a mimic and plasmid pGEM-T easy, and (c) miR-125a NC and pGEM-T easy-A20 were performed according to the similar procedure. MiR-125a mimic, miR-125a inhibitor, siRNA against A20, and the NC were synthesized by GenePharma (Shanghai, China). The sequences were listed in Table 1. A20 over-expression plasmid pGEM-T easy-A20 and its NC plasmid pGEM-T easy were purchased from Generay Biotechnology (Shanghai, China).

### RNA preparation and quantitative real-time PCR (qRT-PCR) analysis

Total RNAs were isolated from cells or tissues using Trizol reagent (Invitrogen). The TaqMan Reverse Transcription Kit (Takara, Dalian, China) was used to obtain cDNA for mRNA detection, whereas TaqMan MicroRNA Reverse Transcription Kit (Takara) was used to reverse transcribe RNA for miRNA detection. For *miR-125a* and *A20* mRNA, qRT-PCR was performed using miRscript SYBR Green PCR Kit and SYBR Green PCR Kit (Takara) according to the manufacturer's instructions, respectively. *U6* and *GAPDH* were used as internal controls for *miR-125a* and *A20* mRNA analysis, respectively. The primers used were listed in Table 2. Data were expressed as fold

**Table 1. Sequence of miRNAs used in this study**

| miRNA | Sequence |
|---|---|
| miR-125a mimic | 5′-UCCCUGAGACCCUUUAACCUGUGA-3′ |
| miR-125a mimic NC | 5′-UUCUCCGAACGUGUCAGGUTT-3′ |
| miR-125a inhibitor | 5′-UCACAGGUUAAAGGGUCUCAGGGA-3′ |
| miR-125a inhibitor NC | 5′-UUGUACUACACAAAAGUACUG-3′ |
| A20 siRNA | 5′-AGACACACGCAACTTTAAA-3′ |
| A20 siRNA NC | 5′-CCAUCCUGAUGUCGCAAUGCCGAA A-3′ |

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**Table 2. Sequence of primers used in real-time qRT-PCR analysis**

| Gene | Sequence |
|---|---|
| *miR-125a* | Forward: 5′-GGGTTCCTTGGGGAGGAG-3′ |
| | Reverse: 5′-ATTCCCCAGGTGTGTGGTT-3′ |
| *A20 mRNA* | Forward: 5′-AACTCCAAGCCGGGCCCTGA-3′ |
| | Reverse: 5′-AGGCTTGGCACTTCCCGGGA-3′ |
| *U6* | Forward: 5′- CTCGCTTCGGCAGCACATATACT-3′ |
| | Reverse: 5′- ACGCTTCACGAATTTGCGTGTC-3′ |
| *GAPDH* | Forward: 5′-CTGGTAAAGTGGATATTGTTGCCAT-3′ |
| | Reverse: 5′- TGGAATCATATTGGAACATGTAAACC-3′ |

changes relative to U6 or *GAPDH* calculated based on the following formula: RQ = 2-ΔΔCt.

### Western blot analysis

Twenty-four hours after transfection, proteins were extracted from whole cell lysates and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, then transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking, membranes were incubated with the following primary antibodies: mouse anti-A20 (1:2000; Abcam, Cambridge, USA) and mouse anti-β-actin (1:6000; Abcam). Membranes were then incubated with the horseradish peroxidase-conjugated secondary antibody (1:4000; Abcam). Finally, membranes were detected by using an ECL kit (Bio-Rad, Hercules, USA) according to the manufacturer's protocol, and the blots were analyzed with the Image J program (Bio-Rad).

### Cell proliferation assay

For cell viability analysis, the cells (SW1990 and SW1990GZ) were seeded in 96-well plates ($5 \times 10^3$ cells per well) and incubated for 24 h, and then transfected with miR-125a mimic, miR-125a inhibitor, or their respective scrambled siRNA (NC). In the A20 siRNA transfection study, cells were transfected with A20 siRNA-125a or its NC. Twenty-four hours after transfection, the cells were treated with gemcitabine (0, 1, 1.5, 3, 6, 12, 24 μM) for 24 h. Twenty-four hours after co-transfection of (a) miR-125a NC and pGEM-T easy, (b) miR-125a mimic and plasmid pGEM-T easy, and (c) miR-125a NC and pGEM-T easy-A20, the cells were treated with gemcitabine (24 μM) for another 24 h. CCK-8 kit (Beyotime, Beijing, China) was used to detect cell proliferation index according to the manufacturer's instructions.

### Luciferase reporter assay

The pmirGLO vectors containing wild-type or mutant miR-125a-binding site of *A20* 3′ UTR were synthesized by Ribobio (Guangzhou, China). HEK293T cells were seeded into 24-well plates. After 24 h, wild-type or mutant luciferase vector (50 ng) and miR-125a mimic or NC (20 μM) were co-transfected into HEK293T cells. Forty-eight hours after transfection, the luciferase activities were measured by using the Dual-luciferase Reporter Assay System (Promega, Madison, USA).

### Statistical analysis

All data were expressed as mean ± standard error of the mean (SEM). All statistical analysis was performed using GraphPad Prism version 6 (GraphPad Prism version 6.0, Inc., La Jolla, USA). The differences among groups were analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests or *t*-test, when appropriate. Differences were considered significant when $P < 0.05$.

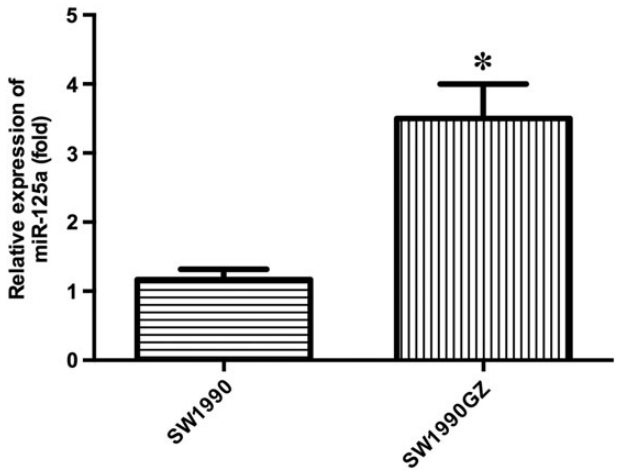


**Figure 1. Relative expression of miR-125a in SW1990 and SW1990GZ cell lines** The expression levels of miR-125a were verified by qRT-PCR. Data represents the mean ± SEM ($n = 3$). *$P < 0.05$ (*t*-test).

## Results

### The effect of miR-125a on the chemo-sensitivity to gemcitabine in pancreatic cancer cells

The relative expression levels of miR-125a in the pancreatic cancer cell line SW1990 and the chemo-resistant pancreatic cancer cell line SW1990GZ were analyzed by qRT-PCR. Results showed that the relative expression level of miR-125a in SW1990GZ was significantly higher than that in SW1990 cells (**Fig. 1**).

To further confirm the effect of miR-125a on the chemo-sensitivity to gemcitabine in SW1990 cells, miR-125a mimic and miR-125a mimic NC were transfected into SW1990 cells. qRT-PCR results showed that there was no difference in the miR-125a expression level between SW1990 cells and SW1990 cells transfected with miR-125a mimic NC. At 24, 48, and 72 h post-transfection, the expression level of miR-125a in SW1990 cells transfected with miR-125a mimic was significantly higher when compared with that in SW1990 cells (**Fig. 2A**). We further examined the effect of miR-125a on chemo-sensitivity to gemcitabine in the transfected cells. Results showed that gemcitabine treatment decreased the cell viability in SW1900 cells, SW 1990 cells transfected with miR-125a mimic or miR-125a NC (**Fig. 2B**). MiR-125a mimic-transfected cells showed a higher cell viability with the treatment of gemcitabine (6, 12, 24 μM) compared with the control cells and NC-transfected cells (**Fig. 2B**).

Furthermore, SW1990GZ cells were transfected with miR-125a inhibitor or miR-125a inhibitor NC. qRT-PCR results showed that there was no difference in the miR-125a expression levels between SW1990GZ cells and SW1990GZ cells transfected with miR-125a inhibitor NC. At 24, 48, and 72 h post-transfection, the expression level of miR-125a in SW1990GZ cells transfected with miR-125a inhibitor was significantly lower when compared with that in SW1990GZ cells (**Fig. 2C**). Gemcitabine was found to decrease the cell viability in a concentration-dependent manner in chemo-resistant SW1990GZ cells, or in SW1990GZ cells transfected with miR-125a inhibitor or miR-125a inhibitor NC. MiR-125a inhibitor transfection further decreased the cell viability in SW1990GZ cells treated with gemcitabine (3, 6, 12, 24 μM) (**Fig. 2D**).

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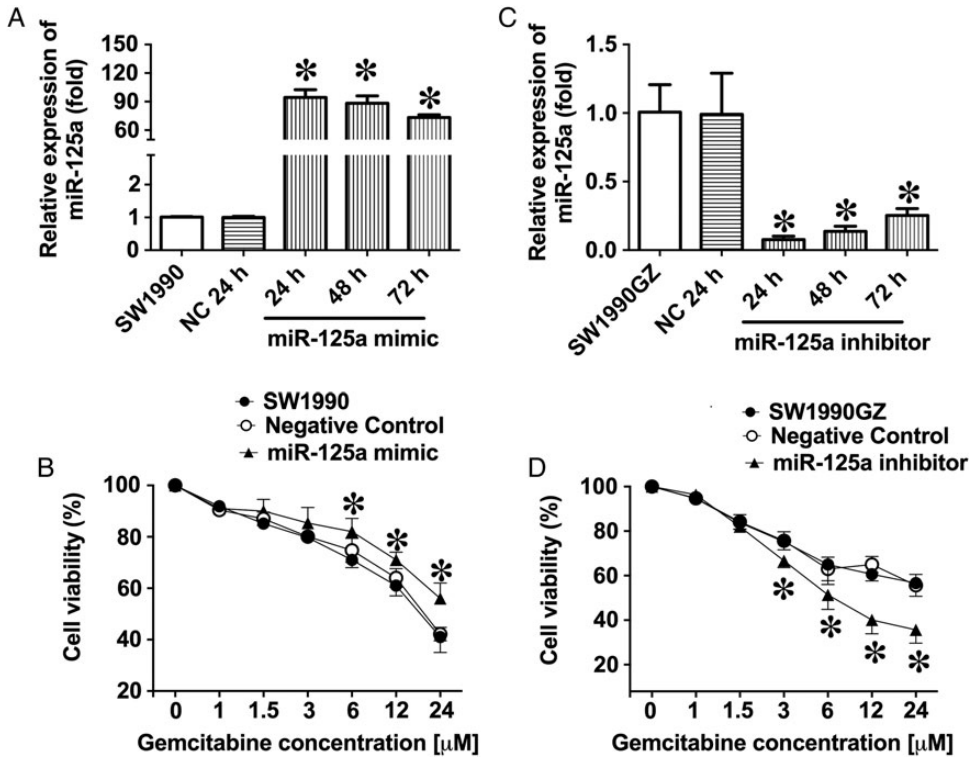


**Figure 2. Effects of miR-125a on chemo-sensitivity in SW1990 and SW1990GZ cells** (A) Relative expression of miR-125a was analyzed by qRT-PCR in SW1990 cells, SW1990 cells transfected with miR-125a mimic at 24, 48, and 72 h, or miR-125a NC at 24 h; (B) CCK-8 kit was used to analyze the effect of gemcitabine (0–24 μM) on the cell viability in SW1990 cell, SW1990 cells transfected with miR-125a mimic or miR-125a NC; (C) Relative expression of miR-125a was analyzed by qRT-PCR in SW1990GZ cells after transfected with miR-125a inhibitor at 24, 48, and 72 h or miR-125a NC at 24 h; (D) CCK-8 kit was used to analyzed the effect of gemcitabine (0–24 μM) on the cell viability in SW1990 GZ cells, SW1990GZ cells transfected with miR-125a inhibitor, or miR-125a inhibitor NC. Data represent the mean ± SEM ($n = 3$). Significant differences comparing with NC group are indicated as $^*P < 0.05$ (one-way ANOVA followed by Bonferroni's multiple comparison tests).

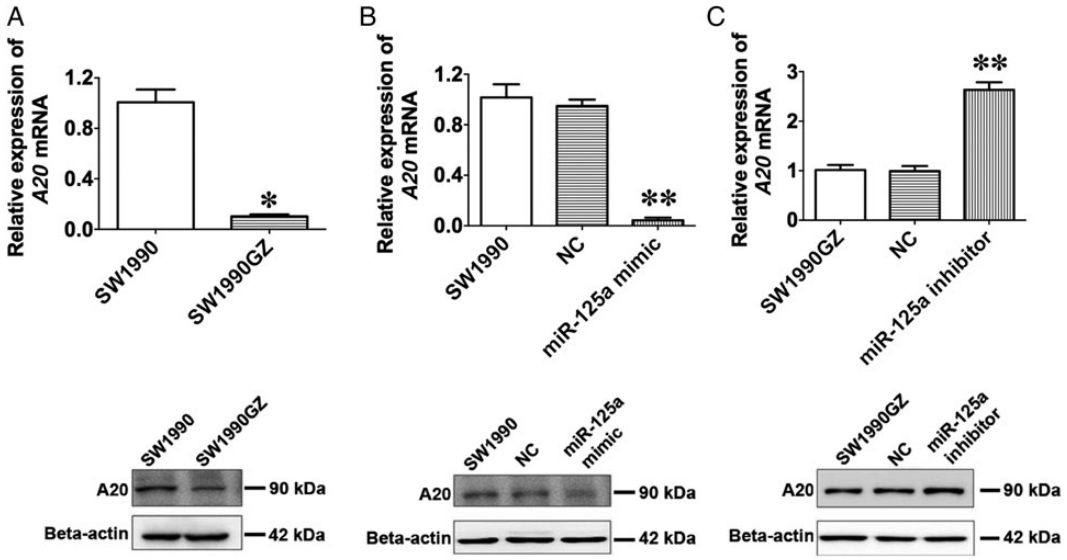


**Figure 3. Effects of miR-125a on the expression levels of A20 mRNA and protein in SW1990 and SW1990GZ cells** (A) Expression levels of A20 mRNA and protein were analyzed by qRT-PCR and western blot in SW1900 and SW1990GZ cells; (B) Expression levels of A20 mRNA and protein were analyzed by qRT-PCR and western blot in SW1990 cells, and SW1990 cells transfected with miR-125a mimic or miR-125a NC; (C) Expression levels of A20 mRNA and protein were analyzed by qRT-PCR and western blot in SW1990GZ cells, and SW1990GZ cells transfected with miR-125a inhibitor or miR-125a NC. Data represent the mean ± SEM ($n = 3$). Significant differences comparing with NC group are indicated as $^*P < 0.05$, $^{**}P < 0.01$ (one-way ANOVA followed by Bonferroni's multiple comparison tests).

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## A20 is a direct target of miR-125a and regulates chemo-sensitivity to gemcitabine in pancreatic cancer cells

To examine the relationship between miR-125a and A20, qRT-PCR and western blotting were performed to examine the effect of miR-125a on the mRNA and protein levels of A20 in SW1990 and SW1990GZ cells. The expression levels of A20 mRNA and protein in SW1990GZ were significantly lower than those in SW1990 cells (**Fig. 3A**). There was no difference in the expression levels of A20 mRNA and protein between SW1990 cells and SW1990 cells transfected with miR-125a mimic NC. MiR-125a mimic transfection significantly decreased the expression levels of A20 mRNA and protein in SW1990 cells compared with NC group (**Fig. 3B**). Further results showed that there was no difference in the expression levels of A20 mRNA and protein between SW1990GZ cells and SW1990GZ cell transfected with miR-125a inhibitor NC. Transection of miR-125a inhibitor in SW1990GZ cells led to an increase in the expression levels of A20 when compared with its NC (**Fig. 3C**).

MiRNAs normally target the 3′UTR of mRNAs to regulate the gene expression. As predicted by TargetScan, there were complementarities between miR-125a and the 3′ UTR of *A20* (**Fig. 4A**). To validate whether *A20* is a target of miR-125a, the 3′UTR fragment of *A20* containing wild-type or mutant miR-125a-binding sequence was sub-cloned into the downstream of the Renilla luciferase reporter gene. When miR-125a mimic and miR-125a mimic NC were co-transfected with the reporter plasmid, the relative luciferase activity of the reporter containing the 3′ UTR of wild-type *A20* was significantly suppressed, while the luciferase activity of the reporter containing the 3′UTR of mutant A20 was unaffected (**Fig. 4B**). Co-transfection of miR-125a mimic and pGEM-T easy plasmid significantly increased the cell viability in SW1990 cells received 24 μM gemcitabine when compared with the control group (MiR NC + pGEM-T easy plasmid) (**Fig. 4C**), while co-transfection of miR-125a NC and pGEM-T easy plasmid-A20 significantly decreased the cell viability when compared with the control group (MiR NC + pGEM-T easy plasmid) (**Fig. 4C**).

The effect of A20 knockdown was examined in SW1990 cells treated with gemcitabine (0–24 μM). A20 siRNA transfection significantly inhibited the expression level of A20 protein in SW1990 cells compared with A20 NC siRNA transfection (**Fig. 5A**). Transfection of A20 siRNA increased the cell viability in SW1990 cells treated with gemcitabine (3, 6, 12, 24 μM) when compared with cells transfected with A20 NC siRNA (**Fig. 5B**).

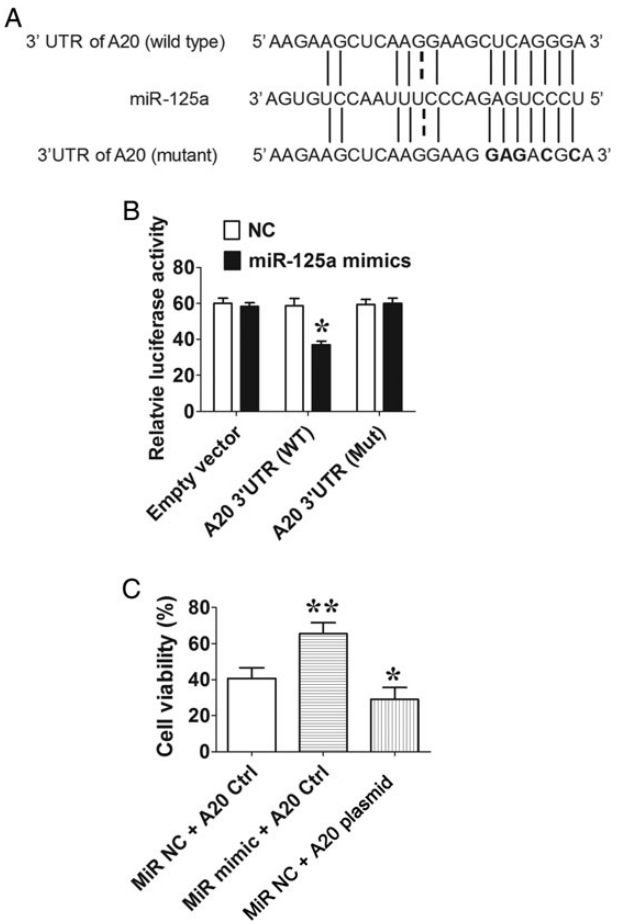


**Figure 4. Luciferase report assay and effect of A20 on chemo-sensitivity in SW1990 cells** (A) The seed sequence of miR-125a targets the 3′UTR of A20 (wild type and mutant); (B) HEK-293 T cells were co-transfected with miR-125a or its NC with wild-type (WT) or mutant (Mut) A20 3′ UTR, and the luciferase activity was detected; and (C) SW1900 cells were co-transfected with miR-125a mimic or miR-125a NC and pGEM-T easy-A20 or pGEM-T easy as indicated. The cell viabilities were determined by CCK-8 assay in the presence of gemcitabine (24 μM); data represent the mean ± SEM ($n = 3$). Significant differences comparing to NC are indicated as $^*P < 0.05$, $^{**}P < 0.01$ (unpaired *t*-test or one-way ANOVA followed by Bonferroni's multiple comparison tests). MiR NC, miR-125a NC; MiR mimic, miR-125a mimic; A20 Ctrl, plasmid pGEM-T easy; A20 plasmid, plasmid pGEM-T easy-A20.

## Inverse relationship between the expression *A20* mRNA and miR-125a in pancreatic cancer tissues

The expression level of miR-125a and *A20* mRNA in pancreatic cancer tissues from 14 chemo-sensitive patients and 14 chemo-resistant

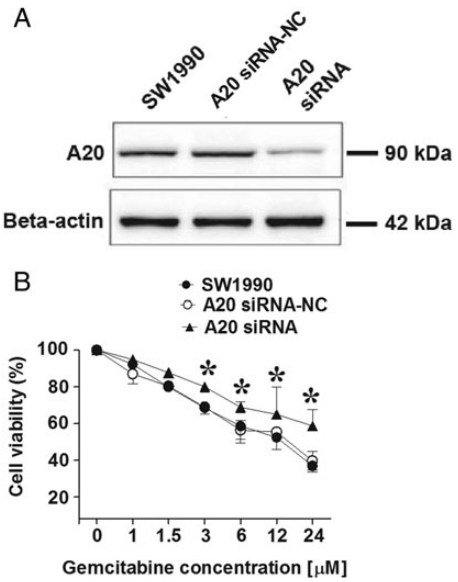


**Figure 5. Effects of A20 on chemo-sensitivity in SW1990 cells** (A) Expression levels of A20 protein were analyzed by western blot analysis in SW1990 cells, A20 siRNA transfected SW1990 cells, and A20 siRNA NC transfected SW1990 cells; (B) CCK-8 kit was used to analyze the effect of gemcitabine (0–24 μM) on the cell viability in SW1990 cells, A20 siRNA transfected SW1990 cells, and A20 siRNA NC transfected SW1990 cells. Significant differences comparing to NC group are indicated as $^*P < 0.05$ (one-way ANOVA followed by Bonferroni's multiple comparison tests).

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patients was examined by qRT-PCR. The expression level of miR-125a in pancreatic cancer tissues from chemo-sensitive patients was significantly lower than that from chemo-resistant patients (**Fig. 6A**), whereas the expression level of *A20* mRNA in pancreatic cancer tissues from chemo-sensitive patients was significantly higher than that from chemo-resistant patients (**Fig. 6B**). Spearman's correlation analysis revealed a significant negative correlation between the expression of miR-125a and A20 in pancreatic cancer tissues from both chemo-sensitive patients and chemo-resistant patients (**Fig. 6C,D**).

## Discussion

Several miRNAs have been reported to be associated with drug resistance against gemcitabine, including miR-21, miR-220b, miR-15a, miR-320c, and members of the let-7 family [23–26]. The role of miR-125a in association with drug resistance against gemcitabine is largely unknown, although lots of studies have shown the functional role of miR-125a in other types of cancers. Down-regulation of miR-125a increases hepatocellular carcinoma cells proliferation, invasion, and metastasis via up-regulation of matrix metallopeptidase 11 and vascular endothelial growth factor A [14]. Meanwhile, it has been shown that miR-125a can suppress cell growth by down-regulating human antigen R protein level in breast cancer cell lines [16]. MiR-125a has also been shown to regulate the chemo-resistance in colon cancer through up-regulating aldehyde dehydrogenase 1 family, member A2 and myeloid cell leukemia sequence 1 gene expression [15]. In this study, we demonstrated that miR-125a was up-regulated in chemo-resistant SW1990GZ cells compared with SW1990 cell. The effect of miR-125a on chemo-sensitivity to gemcitabine was confirmed by either over-expression of miR-125a in SW1990 cells or inhibition of miR-125a in SW1990GZ cells.

A20, a member of the zinc finger protein family, is a key endogenous modulator of NF-κB signaling [18]. In addition to A20's role in regulation of innate and adaptive immunities, A20 has been recognized as anti- or pro-apoptotic protein, depending on cell type or apoptotic stimulus [19,20]. However, little is known about miR-125a that targets this kinase. Based on bioinformatics analyses, miR-125a was identified and confirmed as a novel miRNA that directly binds to 3′ UTR of A20. Moreover, over-expression of miR-125a in SW1990 cells led to a significant down-regulation of A20 mRNA and protein expression, while inhibition of miR-125a in SW1990GZ cells led a significant up-regulation of A20 mRNA and protein expression. A recent study showed that enhanced expression of A20 could contribute to tumor resistance in glioblastoma tissues to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), which was characterized as a selective apoptosis-inducing agent in transformed cells [27]; and the ability of A20 to bind and post-translationally modify the expression or activation of apical caspase-8 or RIP1 has been identified as the key to this process [28,29]. In contrast to that over-expression of A20 supports tumor resistance to TRAIL, down-regulation of A20 has been shown to promote resistance to O6-alkylating agent (temozolomide) in glioblastoma cells via modulating NF-κB signaling pathway

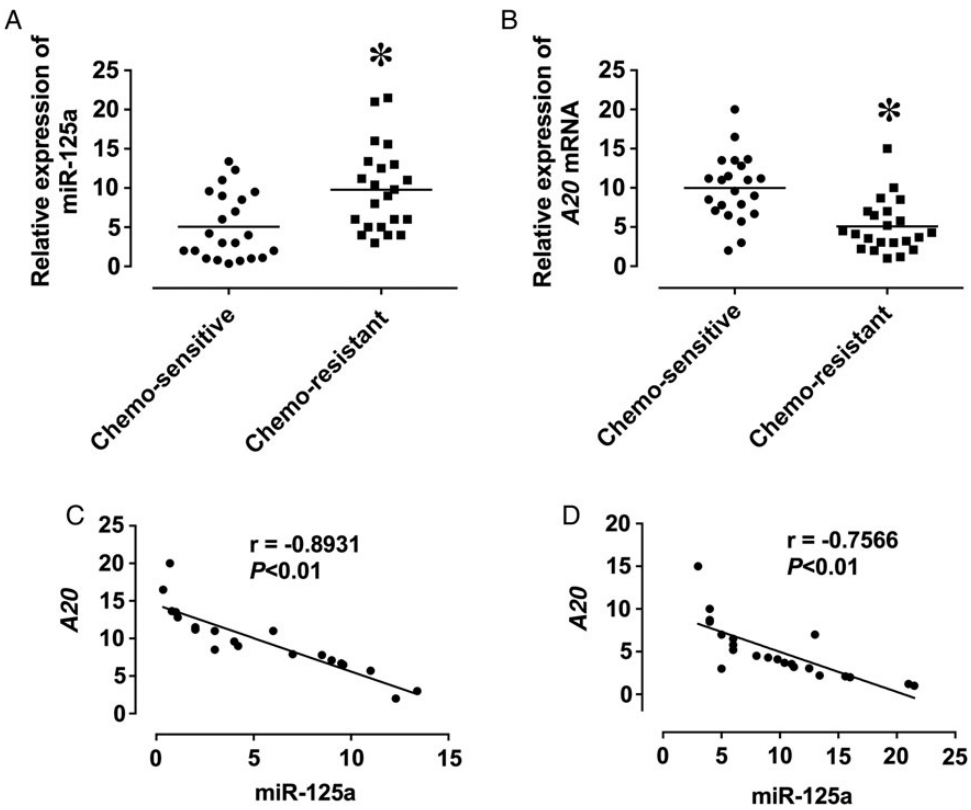


**Figure 6. Inverse correlation of miR-125a with *A20* levels in pancreatic cancer tissues** (A) Relative expression levels of miR-125a in pancreatic cancer tissues from chemo-sensitive and chemo-resistant patients ($n = 14$) were determined by qRT-PCR. (B) Relative expression levels of *A20* mRNA in pancreatic cancer tissues from chemo-sensitive and chemo-resistant patients ($n = 14$) were determined by qRT-PCR. Data represent the mean ± SEM of 14 determinations, (C) Correlation of miR-125a levels to *A20* mRNA levels in pancreatic cancer tissues from chemo-sensitive patients, and (D) correlation of miR-125a levels to *A20* mRNA levels in pancreatic cancer tissues from chemo-resistant patients. Correlation coefficient was calculated using Prism 6. Significant differences between groups are indicated as $^*P < 0.05$ (unpaired *t*-test).

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[21]. In this study, over-expression of A20 was found to increase the chemo-sensitivity in SW1900 cells, while knockdown of A20 promoted the chemo-resistance to gemcitabine in SW1990 cells. These results suggest that A20 may act as a modulator of tumor resistance to anticancer drugs, depending not only on the cell type but also on the chemotherapeutic agents.

By analyzing the miR-125a levels in chemo-sensitive and chemo-resistant pancreatic cancer tissues, we found that the expression level of miR-125a was inversely correlated with the expression level of *A20* mRNA, which further validated our findings in SW1990 and SW1990GZ cell lines. However, the mechanism of miR-125a regulation remained to be determined in future studies. Studies have shown that multiple mechanisms, such as abnormal DNA methylation, histone modifications, biogenesis machinery, and the recruitment of specific transcription factors, may lead to deregulation of miR-125a in pancreatic cancer [30]. In the future study, the impact of A20 on NF-κB signaling pathway will be investigate, which may in turn affect the chemo-sensitivity in pancreatic cancer.

In summary, our results suggest that miR-125a may promote chemo-resistance to gemcitabine in pancreatic cell lines through targeting A20, which may provide novel therapeutic targets or molecular biomarkers for cancer therapy and improve tumor diagnosis or predictions of therapeutic responses. To the best of our knowledge, this is the first study to (i) provide evidence that miR-125a decreases the sensitivity of pancreatic cancer cells to gemcitabine, (ii) identify A20 as a direct binding target of miR-125a, (iii) demonstrate that miR-125a down-regulates A20 to increase chemo-resistance to gemcitabine in pancreatic cancer, and (iv) demonstrate that miR-125a levels could be a potential biomarker for human pancreatic cancer.

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