IFN-γ induces apoptosis in gemcitabine-resistant pancreatic cancer cells

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Abstract. Pancreatic ductal adenocarcinoma (PDAC) is the most prevalent and aggressive form of pancreatic cancer. Gemcitabine (GEM), the first-line treatment for PDAC, which alleviates symptoms and enhances the quality of life of patients. However, it is prone to lead to the development of drug resistance during treatment. Interferon (IFN)-γ exhibits antitumor and immunomodulatory properties. The present study aimed to explore the impact of IFN-γ on the viability, migration and apoptosis of GEM-resistant pancreatic cancer cells. Firstly, a GEM-resistant pancreatic cancer cell line, named PANC-1/GEM, was constructed. Hematoxylin and eosin staining analyzed the cell morphology, whereas reverse transcription-quantitative PCR (RT-qPCR) assessed the expression levels of the drug-resistance genes multidrug resistance-associated protein (MRP) and breast cancer resistance protein (BCRP). The MTT assay and cell counting techniques were used to determine the appropriate concentration of IFN-γ and its effects on cell viability. The IFN-γ-induced apoptosis of PANC-1/GEM cells was assessed using an Apoptosis Detection Kit, whereas the impact of IFN-γ on the migration of these cells was evaluated using a wound-healing assay. The MTT assay revealed a resistance index of 22.4 in the PANC-1/GEM cell line. RT-qPCR indicated that, compared with in wild-type cells, the PANC-1/GEM resistant strain exhibited lower MRP and higher BCRP mRNA expression levels. The optimal concentration of IFN-γ for affecting PANC-1/GEM cells was determined to be 0.3 μg/ml. At this concentration, IFN-γ induced PANC-1/GEM cell apoptosis, along with a notable reduction in migration. Following treatment of PANC-1/GEM cells with IFN-γ, MRP expression increased whereas BCRP mRNA expression decreased, indicating a reversal in their drug-resistance gene expression. In conclusion, IFN-γ exhibited antitumor immune properties by upregulating MRP and downregulating BCRP expression, reversing drug-resistance gene expression, and reducing cell viability and migration, while promoting apoptosis in PANC-1/GEM cells. IFN-γ could potentially serve as a treatment option for patients with GEM-resistant pancreatic cancer.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer in the world (1); these tumors are highly aggressive, with PDAC projected to be the second leading cause of cancer-related death by 2030 (2). Worldwide, the incidence of PDAC is estimated to be fifteenth among all malignant tumors, with a 5-year survival rate of <5% (3).

Gemcitabine (GEM) is the first-line chemotherapeutic drug for patients with pancreatic cancer, as it can improve their quality of life. However, numerous PDACs are highly resistant to chemotherapeutic agents, resulting in the failure of pancreatic cancer chemotherapy. Thus, GEM does not significantly improve the overall prognosis and survival rate of patients with pancreatic cancer (4). Identification of the mechanism underlying the resistance of PDAC to treatment with GEM may therefore provide clues to more effective therapeutic methods.

Immunotherapy is a treatment method for the management and elimination of cancer, which works by restarting and maintaining the tumor immune cycle and re-establishing the normal antitumor immune response in the body (5). Present research on pancreatic cancer has primarily concentrated on early diagnosis, and the exploration of drug combinations or new treatment targets (6). GEM has been a first-line treatment for pancreatic cancer for a number of years and holds a significant position in clinical therapy; however, its associated drug resistance notably limits its long-term efficacy (7). There is a relative lack of research on the treatment of pancreatic cancer or the improvement of the drug-resistant immune microenvironment after drug resistance. Interferon (IFN)-γ, a member of the type II IFN family, is a pleiotropic molecule with anti-proliferative, pro-apoptotic and antitumor activities (8).

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Currently, to the best of our knowledge, no studies have assessed the impact of IFN-γ on GEM-resistant pancreatic cancer. To investigate the impact of IFN-γ on the cell viability, apoptosis and migration of GEM-resistant pancreatic cancer cells, the present study constructed GEM-resistant cells from the PANC-1 pancreatic cancer cell line, named PANC-1/GEM, and assessed the effects of IFN-γ on drug resistance.

Materials and methods

Reagents and materials. The human pancreatic cancer cell line PANC-1 (lot no. SNL-100) was purchased from the American Type Culture Collection. GEM was obtained from Qilu Pharmaceutical Co., Ltd. The MTT Cell Proliferation and Cytotoxicity Assay Kit (cat. no. C0009S) and the BCA protein quantification kit (cat. no. P0009) were obtained from Beyotime Institute of Biotechnology; DMSO (cat. no. D8370) was from Beijing Solarbio Science & Technology Co., Ltd.; the Apoptosis Detection Kit (cat. no. CA1120) was from Solarbio Co., Ltd.; IFN-γ (cat. no. 106-06) was from Shanghai Puxin Biotechnology Co., Ltd.; TRIzol® reagent, DMEM and fetal bovine serum (FBS) were purchased from Thermo Fisher Scientific, Inc.; HiScript III RT SuperMix for qPCR (+gDNA wiper) reverse transcription (RT) kit (cat. no. R323-01) and ChamQ SYBR Color qPCR Master Mix (cat. no. Q411-02) were from Vazyme Biotech Co., Ltd.; hematoxylin and eosin (H&E) staining reagent (cat. no. HEH-020) was from BaSO Biotech; RIPA lysis buffer (cat. no. G2002-100ML) was from Wuhan Servicebio Technology Co., Ltd.; ColorMixed Protein Marker (cat. no. RM19001) and anti-β-actin (cat. no. AC026) were from ABclonal Biotech Co., Ltd.; anti-multidrug resistance-associated protein (MRP; cat. no. DF8801) and anti-breast cancer resistance protein (BCRP; cat. no. AF5177) were from Affinity Biosciences. The BSA (cat. no. GC310001) was from Servicebio Technology Co., Ltd.; the Goat Anti-Rabbit IgG (H+L) HRP (cat. no. S0001) was from Affinity Biosciences Co., Ltd.

Study design. The number of repetitions and the duplicate samples for all cell experiments of the present study were determined according to the methodologies of other academic studies (9,10). The sample size calculation for the experiment was validated using GPower 3.1 software (Düsseldorf University); the cellular experimental samples were triplicated, achieving a test efficiency of 0.9. The software was also employed for power analysis, which was employed to ascertain the required number of experimental replicates.

Cell viability assay. PANC-1 cells were cultured in DMEM containing 10% FBS at 37˚C in an atmosphere containing 5% CO_2, with the medium replaced every 72 h. Cells were subcultured when their density reached 80-90%. The cells were trypsinized, their concentrations were adjusted by counting, and 6,000 cells in 100 µl medium were added to each well of a 96-well plate. After 48 h, the medium was removed and fresh DMEM containing 0, 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 µg/ml GEM was added to the wells, incubate at a constant temperature of 37˚C in a cell incubator. The culture medium was replaced after 48 h, maintaining the same GEM concentration as before (11-13). Each concentration of GEM was evaluated in triplicate. After culturing in a GEM-containing medium for 96 h, 10 µl of MTT reagent was added to each well, and DMSO was added to dissolve the formazan after 4 h. After an additional 4 h, the absorbance of each well was measured at 570 nm using a microplate reader, and the half-maximal inhibitory concentration (IC_{50}) of GEM was determined using the Kärber method (14-16): $IogIC_{50}$ = Xm-I [P-(3-Pm-Pn)/4]; where 'Xm' represents the Ig maximum dose, 'I' indicates the Ig or maximum dose/near dose, 'P' represents the sum of the positive reaction rate, 'Pm' indicates the maximum positive reaction rate, and 'Pn' refers to the minimum positive reaction rate.

The effects of GEM on PANC-1 cell viability were determined by measuring cell counts at 24, 48 and 72 using the MTT method. According to the results of the IC_{50} calculation, the cell viability of PANC-1 cells with and without GEM (0.8 µg/ml) was assessed. Briefly, 2,000 cells in 100 µl medium were added to each well of a 96-well plate. The GEM-resistant strain was designated as PANC-1/GEM cells. The MTT analysis was also performed to assess the viability of PANC-1/GEM cells and to evaluate the impact of IFN-γ on viability, using the aforementioned procedure. The concentrations of IFN-γ used were 0, 0.15625, 0.3125, 0.625, 1.25, 2.5 or 5.0 µg/ml, respectively. MTT detection was performed after 6 days of constant-temperature culture at 37˚C. % Inhibition = (1-OD value at each concentration/OD value at concentration 0) x100.

Induced drug-resistant strains. Drug resistance was induced in PANC-1 cells using a GEM concentration gradient, as described previously (17,18). Briefly, 2-6x10^6 PANC-1 cells were cultured in each of two culture plates containing 5 ml DMEM supplemented with 10% FBS and 0.8 µg/ml GEM. The concentrations added to both culture dishes were identical. If the cell density in a single dish was too low, the cells from both dishes were combined to increase the cell density and facilitate normal cell proliferation. Cell death was assessed daily under a light microscope, and the medium was removed after 48 h. To each plate, DMEM plus 10% FBS without GEM was added and the cells were cultured until the bottom of the plates was completely covered. The medium was then removed and the cells were cultured in medium containing 0.8 µg/ml GEM. After 48 h, the medium was replaced with medium containing 0.8 µg/ml GEM. After the cells repopulated the dish, the procedure was repeated. After the cells became adapted to 0.8 µg/ml GEM, the concentration was gradually increased to a maximum concentration of 15 µg/ml.

H&E staining. Each well of a 6-well plate was seeded with 1x10^6 PANC-1 or PANC-1/GEM cells. After 24 h, the medium was removed and the cells were washed with phosphate-buffered saline (PBS). Cells were then fixed in absolute ethanol for 20 min at 37˚C and were washed twice with PBS. Subsequently, the cells were stained with an H&E staining kit at room temperature; with hematoxylin for 5 min and eosin for 1 min. The cells were then detected by light microscopy and images were captured using the NIS-Elements software (v.5.21.00; Nikon Corporation).

RT-quantitative PCR (RT-qPCR). Total RNA was extracted from 1x10^6 PANC-1 and PANC-1/GEM cells in the logarithmic growth phase using TRIzol reagent. After discarding the culture

Table I. Sequences of primers used for reverse transcription-quantitative PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Product length, bp	NCBI reference no.	Corresponding cDNA sequence, bp
MRP	GCGAGTGTCTCCCTCAAACG	TCCTCACGGTGATGCTGTTC	118	NM_004996	2,000-2,117
BCRP	GATATGGATTTACGGCTTTGC	CGATGCCCTGCTTTACCAA	135	NM_004827	2,217-2,351
β-actin	AGTGTGACGTGGACATCCGC AAAG	ATCCACATCTGCTGGAAGG TGGAC	220	NM_001101	935-1,154

BCRP, breast cancer resistance protein; MRP, multidrug resistance-associated protein.

medium from PANC-1/GEM cells, the cells were rinsed once with pre-cooled sterile PBS, the supernatant was discarded and then 1 ml single-phase lysis solution was added for cell lysis. A pipette tip was utilized to ensure thorough mixing for complete cell lysis. Preparations with an OD260/280 nm ratio of 1.9-1.95 and with no obvious degradation were regarded as being suitably pure for further experiments. The RNA was reverse transcribed to obtain 20 μl aliquots of cDNA, following the manufacturer's instructions. The mRNA expression levels of MRP, BCRP and β-actin were determined using the ChamQ SYBR Color qPCR Master Mix using specific primer sequences (Table I), with each assay performed in triplicate. The qPCR thermal cycling conditions were as follows: Initial denaturation at 95˚C for 30 sec; cycling reaction at 95˚C for 10 sec, then 60˚C for 30 sec; melting curve at 95˚C for 15 sec, then 60˚C for 60 sec and 95˚C for 15 sec (40 cycles). qPCR was performed according to the manufacturer's protocol and each group was tested three times. Analytical data were acquired using CFX Manager software version 2.0 (Bio-Rad Laboratories, Inc.), with the mRNA expression levels of MRP and BCRP determined relative to β-actin mRNA using the $2^{-\Delta\Delta Cq}$ method (19).

PANC-1/GEM cells in the logarithmic growth phase were cultured in DMEM containing 0.3 μg/ml IFN-γ for 30 days, with the medium changed every 2 days; the control group consisted of PANC-1 cells. Total cell RNA was extracted from the cells with TRIzol reagent, cDNA was synthesized, and the levels of MRP, BCRP and β-actin mRNAs were analyzed by fluorescence quantitative PCR using specific primers for each gene (Table I), with each assay performed in triplicate. Levels of MRP and BCRP mRNA relative to those of β-actin mRNA were calculated using the $2^{-\Delta\Delta Cq}$ method, as aforementioned.

Cell count analysis. PANC-1/GEM cells in the logarithmic growth phase were digested with trypsin, plated at 5,000 cells/well in 96-well plates, and cultured in DMEM for 24 h. The medium was then removed and replaced with medium containing 0, 0.15625, 0.3125, 0.625, 1.25, 2.5 or 5.0 μg/ml IFN-γ, with triplicate wells used for each concentration. The cells were cultured in a 37˚C cell culture incubator for 6 days, with the medium containing IFN-γ being renewed every 48 h. Subsequently, the medium was removed, 100 μl DMEM without IFN-γ was added to each well, images were captured using the NIS-Elements program.

Hoechst staining. Aliquots containing $2.5x10^5$ PANC-1/GEM cells in the logarithmic growth phase were added to each well of a 24-well plate, and the cells were cultured in DMEM containing 10% FBS for 24 h. After the cells adhered to the plate, the medium was aspirated, and the cells were cultured in medium containing 0.3 μg/ml IFN-γ for 72 h at 37˚C. The supernatant was then removed, and 1 ml cell staining buffer, 5 μl Hoechst staining solution (cy5 dye) and 5 μl PI staining solution (DAPI dye) were added at 4˚C (these are all from the kit). The plates were incubated at 4˚C for 4 h and images were captured under a fluorescence microscope (Olympus Corporation). Weak red plus weak green staining indicated normal cells; weak red plus strong blue staining indicated apoptotic cells; and strong red plus strong blue staining indicated necrotic cells.

Wound-healing assay. Aliquots containing $1x10^6$ PANC-1/GEM cells were added to each well of a 6-well plate, and the cells were cultured for 24 h. Subsequently, upon reaching 90-100% confluence in the culture plate wells, a monolayer of cells in each well was mechanically injured using a pipette tip (1,000-μl). The cells in three wells were then cultured in medium containing 0.3 μg/ml IFN-γ, whereas the cells in the other three wells were cultured in medium alone for 72 h. All cells in the culture were maintained in serum-free media. The images were then captured using an optical microscope (Nikon Corporation) at 0, 24, 48 and 72 h, and subsequent analysis was performed using ImageJ software (version 1.8.0; National Institutes of Health). The ImageJ software was employed to detect the wound area and analyze the percentage of the healed wound area. Wound healing (%)=(1-unhealed area/initial wound area) x100. The 'unhealed area' refers to the exposed region within the wound area measured at 24, 48 and 72 h, whereas the 'initial wound area' represents the area at 0 h (19). In addition, the scratch width was quantified using Adobe Photoshop 2023 (v24.7.1.741; Adobe Systems, Inc.). Wound healing % relative to 0 h is shown is presented.

Western blotting. The PANC-1 cell and PANC-1/GEM cell were lysed in cold RIPA lysis buffer with 1% protease inhibitor cocktail and proteasome inhibitors. The supernatant was collected post-centrifugation (13,800 x g at 4˚C for 10 min), and protein concentration was determined using the BCA protein quantification kit. The protein samples were then loaded with a marker (20 μg protein/lane) and separated on 8% gels using SDS-PAGE, followed by transfer to a PVDF membrane. The membrane was blocked using 5% BSA in TBS with 0.1% Tween (TBST) for 1 h at room temperature, then incubated overnight with the following primary antibodies: MRP (1:1,000), BCRP (1:500) and β-actin (1:50,000). After washing three times

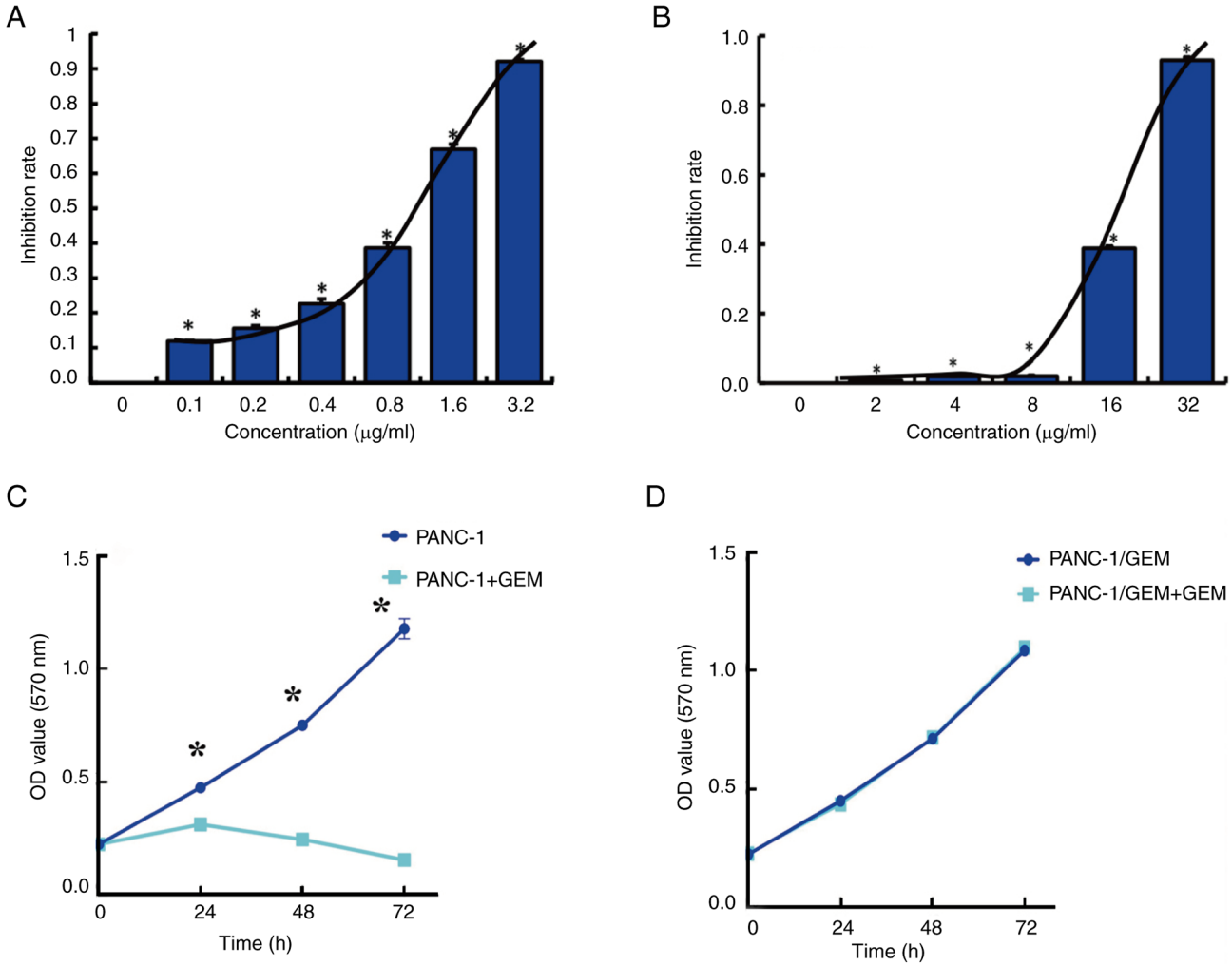

Figure 1. Construction of GEM pancreatic cancer-resistant cell line PANC-1/GEM. Effects of GEM on the proliferation of (A) PANC-1 and (B) PANC-1/GEM cells. GEM had a median IC_{50} value of 0.8 μg/ml in PANC-1 cells and 18 μg/ml in PANC-1/GEM cells (n=3). Viability of (C) PANC-1 cells with and without GEM (0.8 μg/ml) and (D) PANC-1/GEM cells with and without GEM (15 μg/ml) (n=3). Data are presented as mean ± SD. *P<0.05 vs. PANC-1 + GEM. GEM, gemcitabine.

with TBST (5-min intervals), the membrane was incubated with the secondary antibody for 1 h at room temperature. Chemiluminescence detection was performed using a western blot detection system (Shanghai Tianneng Life Sciences Co., Ltd.), and the chemiluminescence signal was semi-quantified with Image-Pro Plus 6.0 software (Media Cybernetics, Inc.).

Statistical analysis. Data are presented as the mean ± SD. Experiments were performed in triplicate and repeated three times with similar results. Statistical analyses were conducted using SPSS version 26.0 (IBM Corp.). Parametric data were analyzed using unpaired t-test or one-way analysis of variance, Subsequently, upon detecting significant differences between groups, Tukey's honestly significant difference test was employed for post hoc analysis. (two-tailed P≤0.05 was considered to indicate a statistically significant difference.

Results

Effect of GEM on the proliferation of PANC-1 and PANC-1/GEM cells as determined by MTT assay. The MTT assay showed that GEM had a median IC_{50} of 0.8 μg/ml in PANC-1 cells (Fig. 1A), as calculated using the Kärber formula. In addition, the MTT assay showed that GEM had a median IC_{50} of 18 μg/ml in PANC-1/GEM cells (Fig. 1B) and that the resistance index, defined as the IC_{50} of GEM in PANC-1/GEM cells divided by the IC_{50} in PANC-1 cells, was 22.4. The proliferation of PANC-1 cells was subsequently determined with or without treatment with the IC_{50} concentration of GEM (0.8 μg/ml). Proliferation

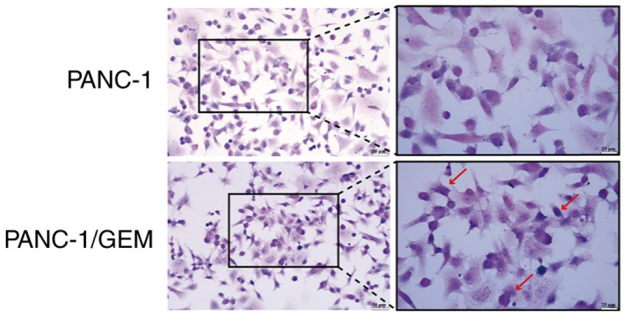

Figure 2. Hematoxylin and eosin staining of PANC-1 and PANC-1/GEM cells. GEM, gemcitabine. Scale bar, 25 μm.

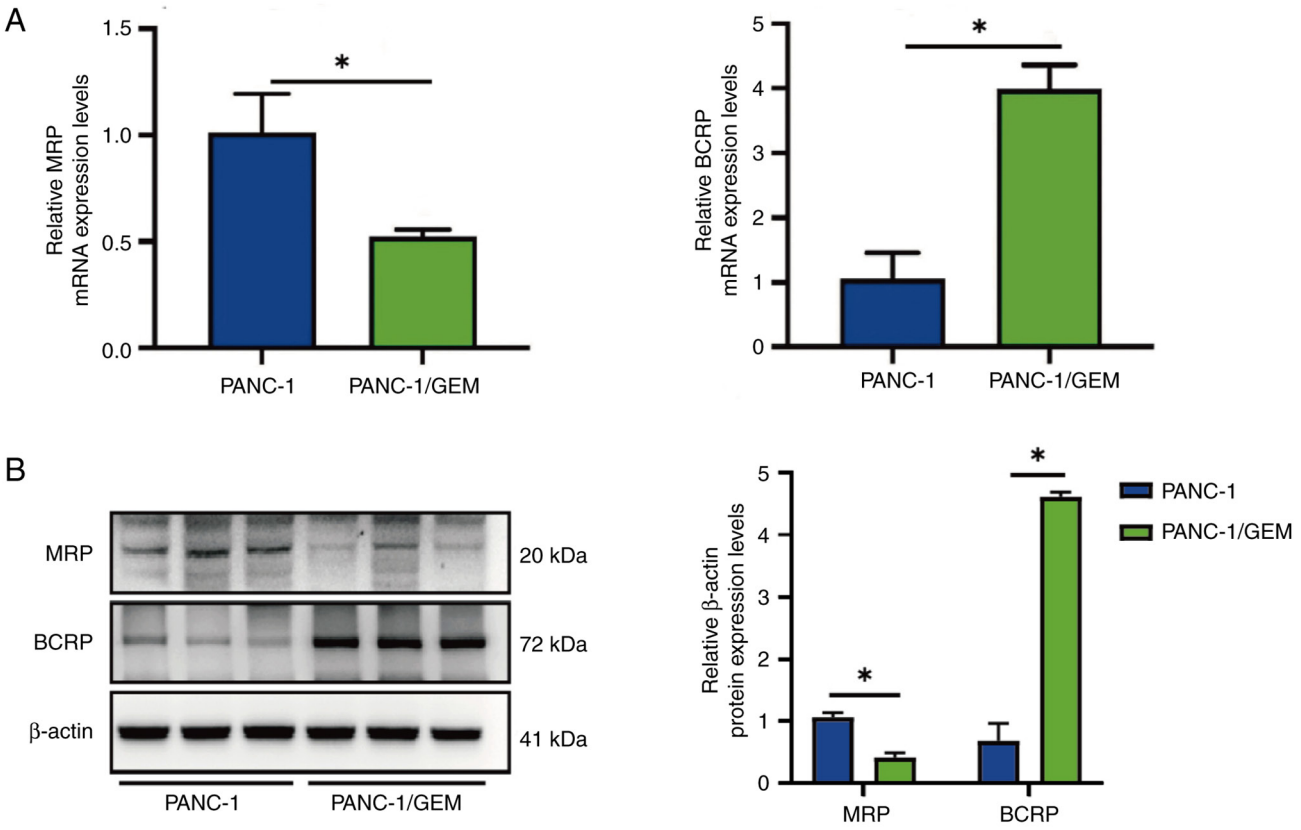

Figure 3. Assessment of MRP and BCRP expression in PANC-1 and PANC-1/GEM cells. (A) Reverse transcription-quantitative PCR analysis of MRP and BCRP mRNA expression levels in PANC-1 and PANC-1/GEM cells (n=3). (B) Western blot analysis of MRP and BCRP protein expression in PANC-1 and PANC-1/GEM cells (n=3). Data are presented as the mean ± SD. *P<0.05. BCRP, breast cancer resistance protein; GEM, gemcitabine; MRP, multidrug resistance-associated protein.

of untreated PANC-1 cells exhibited a time-dependent increase, whereas PANC-1 cells treated with GEM exhibited inhibited cell proliferation. This suggests that PANC-1 cells were in optimal condition, ensuring the reliability of the MTT assay results (Fig. 1C). Notably, the viability of PANC-1/GEM cells remained unchanged in response to GEM (18 μg/ml), thus indicating the resistance of these cells to GEM (Fig. 1D).

Cell morphology. Hematoxylin stained the nuclei of PANC-1 cells a vivid blue, whereas eosin stained the eosinophilic granules in the cytoplasm brilliant red with significant light reflection, and the cytoplasm was stained various colors ranging from pink to peach. H&E staining of PANC-1/GEM cells showed an altered morphology, with the appearance of irregular forms, such as circles; however, there were no marked differences in their nucleocytoplasmic ratios (Fig. 2).

Effects of GEM on the mRNA expression levels of MRP and BCRP. RT-qPCR showed that the mRNA expression levels of MRP were 2-fold lower, whereas the mRNA expression levels of BCRP were 4-fold higher in PANC-1/GEM cells compared with those in PANC-1 cells; both differences were statistically significant (Fig. 3A). In addition, western blotting revealed a decrease in MRP protein expression and an increase in BCRP protein expression after PANC-1 cells developed resistance to GEM. The findings imply that PANC-1 cells developed resistance to GEM due to changes in the expression of resistance-related genes (Fig. 3B). This result indicated the successful establishment of GEM-resistant pancreatic cancer cells, denoted as PANC-1/GEM cells.

Effect of IFN-γ on PANC-1/GEM cell viability. Treatment of PANC-1/GEM cells with 0.16-5.0 μg/ml IFN-γ for 6 days inhibited cell viability, as shown by MTT assays, with the lowest effective concentration being 0.31 μg/ml (Fig. 4A). To exclude the influence of the MTT method on cell viability, the numbers of cells were counted directly. The findings indicated that a concentration of 0.31 μg/ml effectively inhibited PANC-1/GEM cell viability (Fig. 4B). In order to exclude poor cellular activity or inactivation of the IFN-γ protein from interfering with the accuracy of the MTT results, the viability of PANC-1/GEM cells was detected with and without the addition of IFN-γ. The results revealed that 0.31 μg/ml IFN-γ significantly reduced PANC-1/GEM cell viability, in contrast to the viability of cells without IFN-γ treatment (Fig. 4C).

Effects of IFN-γ on MRP and BCRP mRNA expression levels in PANC-1/GEM cells. RT-qPCR assays showed that treatment of PANC-1/GEM cells with IFN-γ resulted in 1.61-fold higher expression levels of MRP mRNA and 2.5-fold lower expression levels of BRCP mRNA than in the control PANC-1/GEM cells (Fig. 5A). Furthermore, following IFN-γ treatment of PANC-1/GEM cells, western blotting demonstrated an increase in MRP protein expression and a decrease in BCRP protein expression (Fig. 5B). These findings indicated that IFN-γ treatment reversed the effects of GEM on GEM-resistant cells.

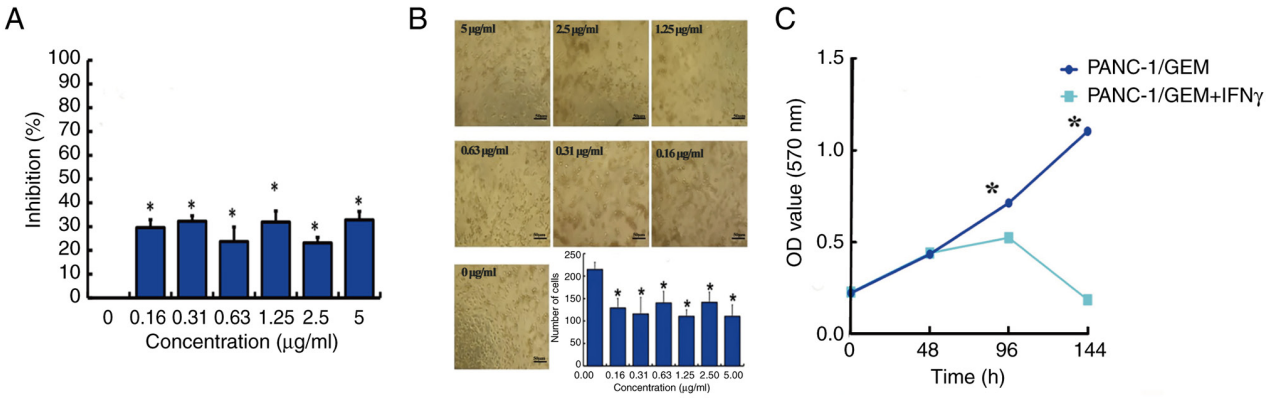

Figure 4. Effect of IFN-γ on PANC-1/GEM cells. (A) MTT assay showed that IFN-γ inhibited the viability of PANC-1/GEM cells. The optimal concentration of IFN-γ was 0.31 μg/ml (n=3). *P<0.05 vs. 0 μg/ml. (B) Direct counting of PANC-1/GEM cells treated with IFN-γ for 6 days (n=6). Scale bar, 50 μm. (C) Viability of PANC-1/GEM cells following treatment with or without IFN-γ (0.3 μg/ml) for 6 days (n=3). *P<0.05 vs. PANC-1/GEM + IFNγ. Data are presented as the mean ± SD. GEM, gemcitabine; IFN-γ, interferon-γ.

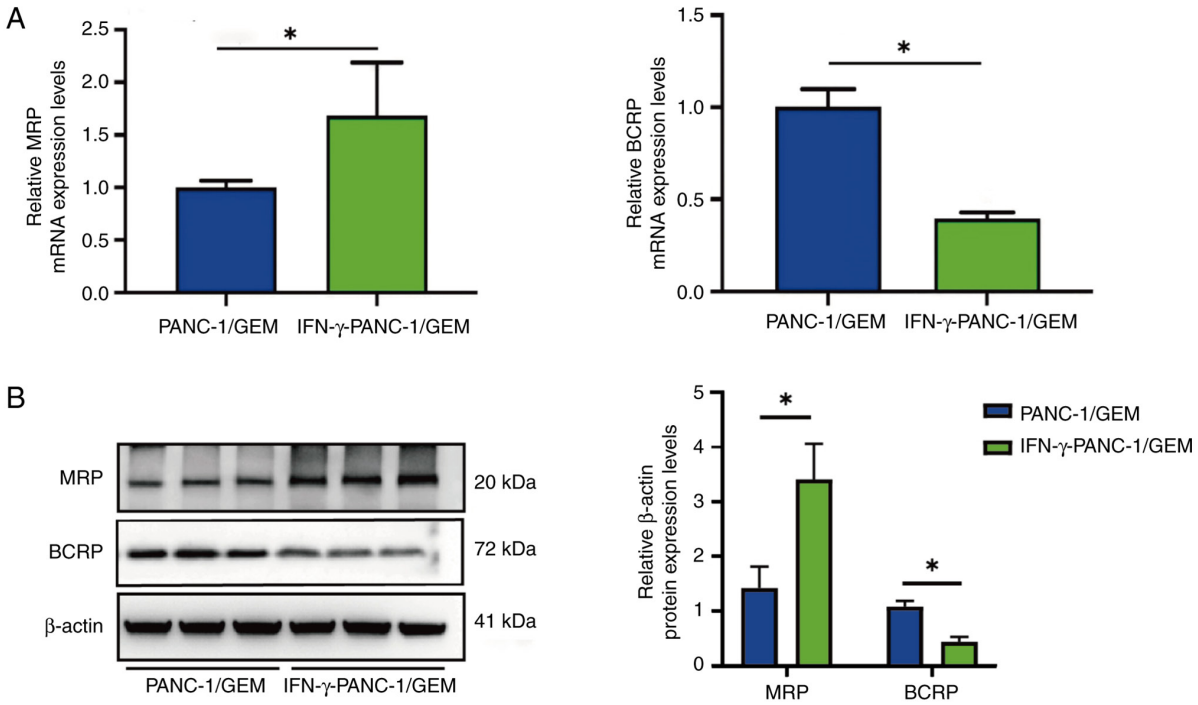

Figure 5. Assessment of MRP and BCRP expression in PANC-1/GEM cells before and after IFN-γ treatment. (A) Reverse transcription-quantitative PCR analysis of MRP and BCRP mRNA expression levels in PANC-1/GEM cells cultured in the presence or absence of IFN-γ (n=3). (B) Western blot analysis of MRP and BCRP protein expression in PANC-1/GEM cells cultured in the presence or absence of IFN-γ (n=3). Data are presented as the mean ± SD. *P<0.05. BCRP, breast cancer resistance protein; GEM, gemcitabine; IFN-γ, interferon-γ; MRP, multidrug resistance-associated protein.

Effects of IFN-γ on cell apoptosis. PANC-1/GEM cells were treated with 0.3 μg/ml IFN-γ for 6 h, and cell apoptosis was determined using a cell apoptosis detection kit (Fig. 6A). The apoptotic count of PANC-1/GEM cells increased by ~4.3 times following treatment with IFN-γ compared with that in the untreated group, and this difference was statistically significant (Fig. 6B). These findings indicated that drug-resistant PANC-1/GEM cells can undergo IFN-γ-induced apoptosis.

IFN-γ exhibits the ability to inhibit the migration of PANC-1/GEM cells. In the control group, the initial wound width of PANC-1/GEM cells was 1.26 mm, reducing to 0.60 mm at 72 h. Meanwhile, the IFN-γ-treated group exhibited a wound width of 1.25 mm at 0 h and 1.03 mm at 72 h (Fig. 7A). Additionally, statistical analysis revealed that, over time, the area of the wound in the IFN-γ-treated group was significantly higher than that in the control group at 48 and 72 h, suggesting that cell migration was inhibited in the IFN-γ-treated group (Fig. 7B). These findings suggested that IFN-γ has the potential to inhibit the migration of PANC-1/GEM cells.

Discussion

Most patients with PDAC exhibit local progression and/or metastasis at diagnosis, preventing surgical resection of the primary tumor. Early diagnosis is hampered by the highly

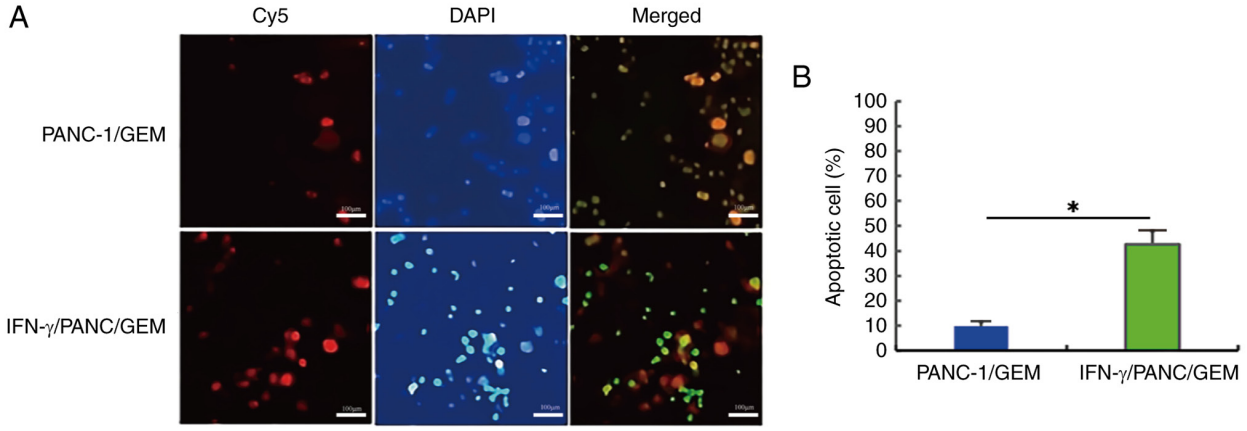

Figure 6. IFN-γ induces the apoptosis of PANC-1/GEM cells. (A) Apoptosis Detection Kit was used to detect the apoptosis of PANC-1/GEM cells. Scale bar, 100 μm. (B) Quantitative analysis of apoptotic PANC-1/GEM cells (n=3). Data are presented as mean ± SD. *P<0.05. GEM, gemcitabine; IFN-γ, interferon-γ.

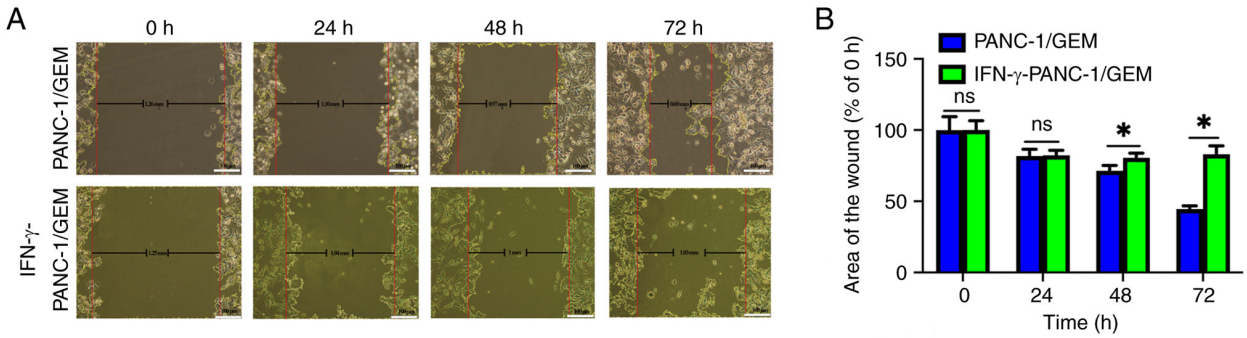

Figure 7. Effects of IFN-γ on the migration of PANC-1/GEM cells. (A) Wound-healing assay images. Scale bar, 100 μm. (B) Wound-healing rate at each time point (n=3). *P<0.05. GEM, gemcitabine; IFN-γ, interferon-γ; ns, not significant.

aggressive nature of these tumors and the lack of screening techniques for early identification (20,21). Although patients with both resectable and non-resectable PDAC can benefit from chemotherapy, these benefits are minor due to the high prevalence of drug resistance (22). Therefore, it is crucial to identify agents that can successfully treat PDAC following the development of chemotherapeutic drug resistance. Immunotherapy, which targets mechanisms of carcinogenesis, may be successful in treating these patients. Recent studies have demonstrated a significant correlation between immunotherapy-related biomarkers, including NLRP3, PARP1, NOX1, NOX2, eNOS and iNOS, and the treatment outcomes, prognoses and survival rates of patients with pancreatic cancer (23,24). Since the cytokine IFN-γ has been shown to promote cell apoptosis and limit cell proliferation, lung, breast and several other types of cancer are treated with this protein, which serves a key role in activating cellular immunity and stimulating antitumor immune responses (25). Consequently, IFN-γ may improve the treatment of patients with pancreatic cancer resistant to GEM.

The present study investigated the effects of IFN-γ on a PANC-1 pancreatic cancer cell line that had developed resistance to the chemotherapeutic agent GEM, and quantified the impact of IFN-γ on PANC-1/GEM cell resistance. These findings may establish a basis for the treatment of patients with cancer who develop resistance to chemotherapeutic agents. Relative to PANC-1 cells, PANC-1/GEM cells had a resistance index of 22.4.

H&E staining was performed to compare the morphological characteristics of PANC-1 and PANC-1/GEM cells. The findings showed that cells became round and irregular in shape after becoming resistant to GEM, which is consistent with the hallmarks of cellular malignancy (26). GEM may rapidly induce resistance by altering the expression levels or inducing mutations in resistance-related genes in cancer stem cells (27,28). RT-qPCR showed that tumor cell resistance to GEM was associated with a significant reduction in MRP mRNA expression levels (29) and a significant increase in BCRP mRNA expression levels (30). This further supports the credibility of the constructed PANC-1/GEM cells in the present study.

IFN-γ is a key immune response controller, which has a major effect on tumors by regulating and activating the cellular immune responses, thereby activating tumor-killing activity (8). IFN-γ may have a greater impact on GEM-resistant tumor cells as its therapeutic mechanism differs from that of GEM. Screening of IFN-γ concentrations to treat PANC-1/GEM cells showed that 0.3 μg/ml IFN-γ exhibited optimal activity against drug-resistant cells. Because genes associated with tumor cell resistance have been frequently associated with tumor resistance to chemotherapeutic agents, PANC-1/GEM cells were cultured for 1 month in the presence of 0.3 μg/ml IFN-γ, and it

was revealed that IFN-γ treatment reversed the effects of GEM on the mRNA expression levels of MRP and BCRP. IFN-γ has been reported to enhance the antigenicity of tumor cells by upregulating the expression of major histocompatibility complex class Ia (31), suggesting that the antitumor activity of IFN-γ against PANC-1/GEM cells may be due to its enhancement of the antigenicity of drug-resistant tumor cells and its immunomodulatory action. Thus, the drug resistance of these tumor cells was partially reversed, and their susceptibility to chemotherapeutic agents was enhanced. This could explain the observed reversal in the expression of resistance-related genes in PANC-1/GEM cells after IFN-γ treatment, as compared to the post-resistance period in the present study.

Both direct cell counting and MTT assays showed that IFN-γ greatly decreased the viability of PANC-1/GEM cells, suggesting that IFN-γ has a considerable impact on the viability of pancreatic cancer cells, even after those cells have developed resistance to GEM. Thus, IFN-γ may be an additional and/or alternative option for the treatment of patients with pancreatic cancer for whom chemotherapy has failed. Moreover, IFN-γ was able to promote the apoptosis of drug-resistant pancreatic cancer cells and to markedly decrease the migration of PANC-1/GEM cells, suggesting that IFN-γ may reduce the invasiveness of GEM-resistant pancreatic cancer cells. Based on previous studies, we propose that IFN-γ, acting directly on GEM-resistant pancreatic cancer cells, may influence their proliferation, migration and apoptosis by impacting the cell cycle and chemotaxis.

In conclusion, the present study showed that IFN-γ could reduce the migration and viability, and enhance the apoptosis of GEM-resistant pancreatic cancer cells. The reversal of resistance-related gene expression in PANC-1/GEM cells following IFN-γ treatment suggests that IFN-γ may have reversed the resistance of pancreatic cancer cells to chemotherapy. These findings suggested that IFN-γ may improve the condition of patients with GEM-resistant pancreatic cancer.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XK and DC were responsible for the study design, development and molecular biology experiments. XX, YZ and XL conducted molecular biology experiments and participated in data analysis. WP participated in the experimental design and supervision of the experiment. All authors read and approved the final manuscript. WP and XK confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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