# Aspirin increases the efficacy of gemcitabine in pancreatic cancer by modulating the PI3K/AKT/mTOR signaling pathway and reversing epithelial-mesenchymal transition

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**Abstract.** Gemcitabine is regarded as a standard medication for patients with pancreatic cancer. The aim of the present study was to investigate the impact of aspirin (ASA) on the efficacy of gemcitabine in pancreatic cancer and the potential mechanism. The SW1990 and BxPC-3 human pancreatic cell lines were treated with 2 mmol/l ASA and/or 1 mg/l gemcitabine. The effects of the treatments were tested on the viability, migration and invasion of the cells using MTT, wound healing and Transwell invasion assays. In addition, cell apoptosis was evaluated via flow cytometry with Annexin V-FITC/PI and the western blotting of Bax and Bcl-2. The expression of epithelial-mesenchymal transition (EMT)-associated proteins and activation of the PI3K/AKT/mTOR pathway were also assessed using western blotting. The results reveal that ASA increased the efficacy of gemcitabine in reducing the proliferation, migration and invasion of pancreatic cancer cells and increasing their apoptosis. These effects are associated with inhibition of the PI3K/AKT/mTOR pathway and the reversal of EMT. Thus, the combined use of ASA and gemcitabine is suggested to be a potential therapeutic strategy for patients with pancreatic cancer.

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## Introduction

Pancreatic cancer is associated with early metastases and an extremely poor prognosis, and ranks as the eighth most common cause of cancer-associated deaths globally. Less than 9% of people survive for 5 years on average (1). When diagnosed, the majority of patients have localized, regional or distant metastases (2). Gemcitabine is currently a standard first-line agent for the treatment of pancreatic cancer.

The PI3K/AKT/mTOR signaling pathway is established as being crucial for tumor formation and is frequently active in pancreatic cancer (3). AKT has been shown to contribute to cancer development when activated by various stimuli, including PI3K and growth factors, while downstream effectors such as mTOR lead to signal transduction (4). PI3K is a downstream effector of oncogenic KRAS, which is nearly ubiquitous in pancreatic cancer (5). A study that analyzed 32 cancer types in The Cancer Genome Atlas identified that KRAS exerts a pro-tumorigenic effect via activation of the PI3K/AKT/mTOR pathway in pancreatic cancer (6). There is evidence suggesting that an interaction exists between KRAS and the PI3K/AKT/mTOR pathway. In a study of bladder tumors, PI3K catalytic subunit α (PIK3CA) mutations were found to be associated with mutated KRAS (7). In another study, patient-derived xenograft models of PIK3CA-mutated metaplastic breast cancers treated with a combination of PI3K and MEK inhibitors were shown to undergo a durable remission (8). A study conducted by Candido *et al* (9) suggested that patients with melanoma who are treated with agents targeting the MAPK signaling pathway may experience tumor progression due to the development of resistance to the targeted therapy; as mutations affecting the PI3K-AKT

pathway appeared to favor drug resistance, crosstalk may occur between the MAPK and PI3K-AKT pathways.

The efficacy of gemcitabine in pancreatic cancer has been linked with mTOR activation (10). The high aggressiveness of pancreatic cancer may be partly attributed to the chemotherapy-resistant characteristics of pancreatic cancer cells, which are associated with the epithelial-mesenchymal transition (EMT) phenotype and cancer stem cells (11). EMT is a process that alters cell-cell and cell-extracellular matrix (ECM) interactions, inducing a fibroblast-like appearance in cancer tissue and enabling neoplastic cells to migrate and invade (12,13). EMT is essentially the transition between two types of completely differentiated and mature cells (14). E-cadherin and other epithelial cell phenotype marker proteins are expressed at reduced levels and mesenchymal cell phenotype marker proteins including vimentin and N-cadherin are expressed at increased levels in tumor cells. Additionally, homocellular tight junctions and cell polarity are lacking (15,16). Notably, some studies have suggested that EMT alters the sensitivity of neoplastic cells to chemotherapy agents (17,18). Therefore, therapy aimed at inhibiting or minimizing EMT may increase the efficacy of chemotherapy.

Several types of chemotherapeutic agents are widely used as first-line treatments for pancreatic cancer, including 5-fluouracil (5-FU), according to National Comprehensive Cancer Network (NCCN) guidelines (2021.V2) (19). In the NCCN Guidelines, gemcitabine is included in first-line therapy or the preferred neoadjuvant therapy regimens for resectable/borderline resectable disease, adjuvant therapy, locally advanced disease and metastatic disease, i.e., gemcitabine is a standard first-line chemotherapeutic drug for pancreatic cancer. Other types of local therapies, including radiofrequency ablation, irreversible electroporation, high-intensity focused ultrasound, microwave ablation and local anti-KRAS therapy using a small interfering RNA targeting KRA G12D in a biodegradable polymeric matrix are under investigation (20). Numerous researchers are focused on the development of cell therapies, antitumor vaccines and new biotechnological drugs, which have shown promising results in preclinical studies (21). According to NCCN guidelines (2021.V2) (19), FOLFIRINOX, which includes 5-FU, irinotecan, oxaliplatin and taxanes, is also an important first-line treatment (22,23), but it is not recommended for patients with a low performance status (24). Moreover, the majority of patients experience side effects when treated with FOLFIRINOX, which restricts the use of aspirin (ASA). Adding ASA to chemotherapy may increase the risk of bleeding, which is a serious issue that could lead to death and commonly causes anemia; therefore, (ASA) was not combined with FOLFIRINOX in the present study. However, ASA has been reported to inhibit the proliferation of pancreatic cancer cells via inhibition of the PI3K/AKT/mTOR signaling pathway and to show therapeutic potential via the targeted inhibition of tumor growth and angiogenesis (25). Considering these findings, gemcitabine may be more suitable than 5-FU, irinotecan, oxaliplatin, taxanes and other chemotherapeutic agents for investigation in combination with ASA.

ASA is a weak organic acid that shows promise as a cancer chemoprevention agent due to its anti-inflammatory properties (26). A pooled analysis of 25,570 patients in eight trials reported that the daily use of ASA reduced the deaths associated with several common cancers, including pancreatic cancer, with the greatest benefit observed after 5 years of the scheduled treatment (27), while in a clinic-based case-control study, the use of ASA was associated with a lowered risk of pancreatic cancer development (28). Furthermore, in China, a population-based study of 761 cases and 794 control subjects showed that regular use of ASA was associated with a reduced risk of pancreatic cancer: Odds ratio, 0.54; 95% CI, 0.40-0.73 (29). However, the evidence that ASA lowers the risk of pancreatic cancer is conflicting and the ability of ASA to improve the efficacy of gemcitabine in pancreatic cancer remains unclear, as does the molecular mechanism by which ASA may provide a benefit. Therefore, these were investigated in the present study.

## Materials and methods

*Cell lines and cell culture*. Human pancreatic cancer cell lines SW1990 and BxPC-3 were obtained from the Cell Bank of Type Culture Collection of The Chinese Academy of Sciences and Changhai Hospital Affiliated to the Second Military Medical University, respectively. Mycoplasma testing was performed for both cell lines. Cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum (FBS) (Biological Industries) and maintained in a humid atmosphere at 37˚C with 5% $CO_2$. The cell medium was changed every 2 or 3 days. Cell passage was performed with trypsin when the confluence of monolayer cells reached 70-80%.

*Main reagents*. ASA was purchased from Sigma-Aldrich (Merck KGaA) and was dissolved in dimethyl sulfoxide (DMSO; SRL Chemical) to make a 5-M stock solution. This solution was stored at -20˚C. Gemcitabine was obtained from Eli Lilly and Company and dissolved in a sterile saline solution to make a 1g/l stock solution. This solution was stored at -20˚C. Even though different solvents were used for gemcitabine and ASA due to their different solubilities, this was not anticipated to significantly influence the results obtained. In addition, the concentration of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Merck KGaA) was set to 5 mg/ml. The Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit was obtained from R&D Systems, Inc.

*Cell proliferation assay*. Cells were cultured in RPMI-1640 medium containing 10% FBS and maintained in a humid atmosphere at 37˚C with 5% $CO_2$ for 72 h. The cells were then harvested and inoculated into 96-well plates with $3x10^3$ cells/well 24 h before treatment. After that, the cells were divided into four groups as follows: i) RPMI-1640 containing 10% FBS and 2 mmol/l ASA; ii) RPMI-1640 containing 10% FBS and 1 mg/l gemcitabine; iii) RPMI-1640 medium containing 10% FBS, 2 mmol/l ASA and 1 mg/l gemcitabine; and iv) negative control comprising RPMI-1640 with 10% FBS. After 24 h of culture, the cells were incubated at 37˚C with 5% $CO_2$ for 0, 24, 48 and 72 h. Then, 10% MTT (5 mg/ml) was added to each well. After 4 h of treatment with MTT, 150 $\mu$l DMSO was added to each well and the samples were vibrated for 10 min. The optical density at 570 nm was then measured using a microplate reader. Each cell proliferation experiment

was repeated three times and the readings were averaged for statistical analysis.

*Wound healing assay*. BxPC-3 and SW1990 cells were seeded in 6-well plates. When the cell confluence was ~100%, scratches were made on the monolayer cell surface with 200-$\mu$l pipette tips. After washing twice with phosphate-buffered saline (PBS), the cells were divided into four groups as described in the cell proliferation assay. The four groups include the combination of ASA and gemcitabine, ASA or gemcitabine alone, and the control group, with RPMI-1640 containing 1% FBS.

Images of the scratches and the cells on each side were captured with an inverted microscope at 0 and 24 h. Finally, the migration was analyzed in three randomly selected fields of view in images captured at x100 magnification under an inverted microscope (Olympus Corporation). The width (W) of the scratch was measured and the percentage of the wound remaining was calculated as follows: $W_{24\ h}/W_{0\ h}$ x100. All experiments were performed in triplicate.

*Transwell assay*. Transwell assays were performed using 6.5-mm diameter Transwell chambers each with an 8-$\mu$m pore polycarbonate membrane insert (Corning, Inc.). BxPC-3 and SW1990 cells ($3x10^4$) were plated on the upper chambers. The upper chambers were coated at 4˚C with 60 $\mu$l Matrigel (Corning, Inc.) in serum-free medium and then left overnight at 37˚C. RPMI-1640 containing 10% FBS was added to the lower chambers. After incubation with the aforementioned treatments for 24 h at 37˚C, the cells were fixed with 4% paraformaldehyde at 25˚C for 30 min before being stained with crystal violet solution at 25˚C for 30 min. Then counted under a light microscope (x200 magnification). The assay was repeated three times in duplicate. The numbers of cells counted in five random fields were averaged.

*Cell apoptosis*. BxPC-3 and SW1990 cells were seeded in 6-well plates and cultured with RPMI-1640 medium containing 10% FBS. When the cells had attached to the plate for 24 h, they were divided into four groups as described in the cell proliferation assay and cultured for 24 h at 37˚C. The cells were then collected and washed with PBS solution twice or thrice. In order to count the number of apoptotic cells, the cells were stained with annexin V-FITC and propidium iodide (PI). The stained cells were analyzed using FlowJo_V10 (FlowJo, LLC). All experiments were carried out in triplicate.

*Western blot analysis*. Cells from the aforementioned four groups were harvested after culture for 24 h, lysed with RIPA lysis buffer (Thermo Fisher Scientific, Inc.) and quantified by bicinchoninic acid analysis (Beyotime Institute of Biotechnology). Next, the proteins released from the cells were separated on 10% gels using SDS-PAGE,and then transferred to PVDF membranes for western blotting. The membranes were blocked with 5% non-fat milk at 25˚C for 1 h. Next, the membranes were incubated with primary antibodies against β-actin, Bax, Bcl-2, E-cadherin, vimentin, PI3K, phospho (p)-PI3K, AKT, p-AKT, mTOR and p-mTOR at a dilution of 1:1,000 at 4˚C overnight. Monoclonal antibodies against human E-cadherin (cat. no. 14-3249-82; Thermo Fisher Scientific, Inc.), vimentin (cat. no. 14-9897-82; Thermo Fisher Scientific, Inc.), Bcl-2 (cat. no. MA5-11757; Thermo Fisher Scientific, Inc.), Bax (cat. no. MA5-14003; Thermo Fisher Scientific, Inc.), PI3K (cat. no. 4249; Cell Signaling Technology, Inc.), p-PI3K (cat. no. ab32089; Abcam), AKT (cat. no. 60203-2-Ig; ProteinTech Group, Inc.), p-AKT (cat. no. 66444-1-Ig; ProteinTech Group, Inc.), mTOR (cat. no. 66888-1-Ig; ProteinTech Group, Inc.), p-mTOR (cat. no. 67778-1-Ig; ProteinTech Group, Inc.) and β-actin (cat. no. AF0003; Beyotime Institute of Biotechnology). Horseradish peroxidase (HRP)-labeled goat-anti-rabbit and goat-anti-mouse IgG secondary antibodies were obtained from Abcam (cat. nos. ab205719 and ab205718). The membranes were incubated with the HRP-labeled secondary antibody at a dilution of 1:5,000 at 37˚C for 1 h. The western blots were visualized using Immobilon Western Chemiluminescent HRP Substrate (MilliporeSigma) followed by film exposure. β-actin was used as the internal reference. Using ImageJ v1.8.0 (National Institutes of Health), the gray levels were analyzed, and the results were calculated as the gray level of the target protein divided by the gray level of the internal reference.

*Statistical analysis*. All data are expressed as the mean ± standard deviation. SPSS version 20.0 (IBM Corp.) was used to perform the statistical analyses. One-way analysis of variance followed by Dunnett's post hoc test was used for the analysis of multiple groups. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Combination of ASA and gemcitabine inhibits cell proliferation more than ASA or gemcitabine alone*. As shown in Fig. 1, the viability of SW1990 cells was more strongly reduced after treatment with a combination of 2 mmol/l ASA and 1 mg/l gemcitabine than with gemcitabine or ASA alone ($P<0.05$). Similar results were observed with BxPC-3 cells, in which the combination of ASA and gemcitabine inhibited cell viability more strongly compared with ASA or gemcitabine alone. These results indicate that ASA increases the antiproliferative efficacy of gemcitabine in SW1990 and BxPC-3 cells.

*Combination of ASA and gemcitabine inhibits pancreatic cell migration and invasion*. Wound healing and Transwell assays were performed to examine the effect of ASA and gemcitabine on cell migration and invasion, respectively. The migration and invasion of the cells were significantly reduced following treatment with the combination of ASA and gemcitabine compared with either ASA or gemcitabine alone ($P<0.05$; Fig. 2). This indicates that treatment with a combination of ASA and gemcitabine strongly suppressed the cell migration and invasion abilities of SW1990 and BxPC-3 pancreatic cancer cells.

*Effect of the combination of ASA and gemcitabine on the apoptosis of pancreatic cancer cells*. The proportion of apoptotic cells in the two pancreatic cancer cell lines was significantly higher when they were treated with the combination of ASA and gemcitabine than when they were treated with ASA or gemcitabine alone ($P<0.05$; Fig. 3A). In the control SW1990 and BxPC3 cells, the percentages of apoptosis were

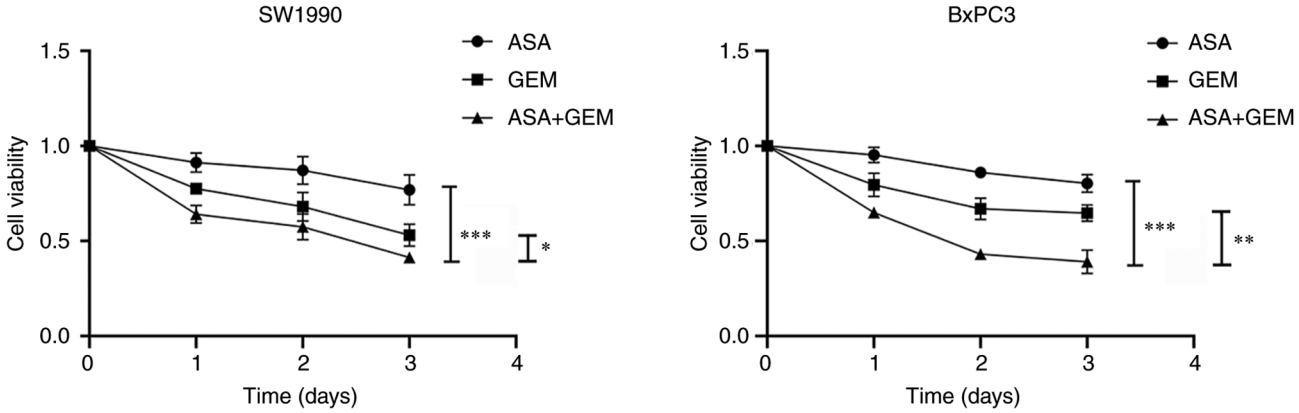


Figure 1. ASA combined with GEM inhibits cell proliferation more strongly compared with ASA or gemcitabine alone. SW1990 and BxPC-3 cells were treated with ASA (2 mmol/l) and/or GEM (1 mg/l) for different time intervals and the cell viability was calculated. The experiments were performed in triplicate and the values are expressed as the mean ± SD of three replicates. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. ASA, aspirin; GEM, gemcitabine.

4.13±0.88 and 4.79±0.65%, respectively. In cells treated with ASA alone, these values were 7.84±2.35 and 6.12±0.23%, respectively; in cells treated with gemcitabine alone, they were 24.68±1.53 and 7.31±0.83%, respectively; and in cells treated with the combination of ASA and gemcitabine, they were 32.13±1.95 and 11.32±0.98%, respectively. Furthermore, western blotting revealed that the protein expression level of Bcl-2 was markedly reduced and that of Bax was increased in the cells treated with the combination of ASA and gemcitabine, and the increase in the Bax/Bcl-2 ratio in cells treated with the combination was significantly greater than that obtained with either ASA or gemcitabine alone (P<0.05; Fig. 3B). These experimental results indicate that the combination of ASA and gemcitabine induced the apoptosis of pancreatic cancer cells to a greater extent than ASA or gemcitabine alone. These findings suggest that ASA promoted the antitumor effect of gemcitabine in SW1990 and BxPC-3 cells.

*Combination of ASA and gemcitabine significantly increases the expression of E-cadherin and decreases the expression of vimentin*. The expression of EMT biomarkers was assessed in the pancreatic cancer cells *in vitro*, and the results revealed that the expression of E-cadherin was significantly increased and that of vimentin was significantly decreased in the ASA and gemcitabine combination group compared with the ASA and gemcitabine alone groups (P<0.05). These results indicate that the cell apoptosis and growth inhibition induced by the combination treatment may be closely associated with EMT (Fig. 4).

*Combination of ASA and gemcitabine downregulates PI3K/AKT/mTOR signaling*. The pancreatic cancer cells presented a significant reduction in the levels of p-PI3K and p-AKT after treatment with the combination of ASA (2 mmol/l) and gemcitabine (1 mg/l) for 24 h compared with those observed in untreated control cells. The p-mTOR protein level of the combination treatment group was also significantly reduced by the combination treatment. These findings suggest that downregulation of PI3K/AKT/mTOR pathway signaling may be involved in the cell apoptosis and growth inhibition induced by the combination treatment (Fig. 5).

## Discussion

The present study revealed the potential of ASA combined with gemcitabine against pancreatic cancer *in vitro*. The findings support those from a previous study, in which the non-steroidal anti-inflammatory drug ASA was shown to prevent the growth of pancreatic cancer by blocking the mTOR signaling pathway (30). The regular use of ASA has been observed to be associated with a decreased risk of various types of cancer, including hepatocellular carcinoma and breast cancer (31,32). In addition, ASA has been shown to inhibit the activation of GSK-3β in the pancreatic cancer cell lines Capan-1 and PANC-1 when combined with gemcitabine (33). Another study demonstrated that a combination of ASA and metformin significantly inhibited pancreatic cancer cell growth *in vitro* and *in vivo* via the regulation of pro- and anti-apoptotic Bcl-2 gene family members (34). Studies have also indicated that adenomas in the large bowel can be prevented to a certain extent by low-dose ASA, as patients who have had colorectal cancer in the past are significantly less likely to develop colorectal adenomas when they take ASA every day (35,36). Other studies have shown that ASA decreases various tumor characteristics, such as tumor cell migration, and lowers the risk of cancer initiation and development (37,38). However, to the best of our knowledge, no detailed study has been published in which the effect of ASA on gemcitabine monotherapy-associated chemotherapy in pancreatic cancer cells has been assessed. The upregulation of ECM quantity in pancreatic cancer prevents drugs from penetrating the tumor (39), which may be associated with a requirement for an increased ASA concentration to treat the disease. However, scientists have been exploring several approaches to address this issue. Collagenase nanoparticles have been suggested to improve the access of medications to pancreatic cancer tissue. The thick collagen stroma of pancreatic cancer can be disassembled by pretreatment with a proteolytic-enzyme nanoparticle system, which promotes the penetration of medication into the tumor (40). Furthermore, to improve drug penetration, a dendrimer-camptothecin conjugate was developed that actively penetrated deep into pancreatic cancer via γ-glutamyl transpeptidase-triggered

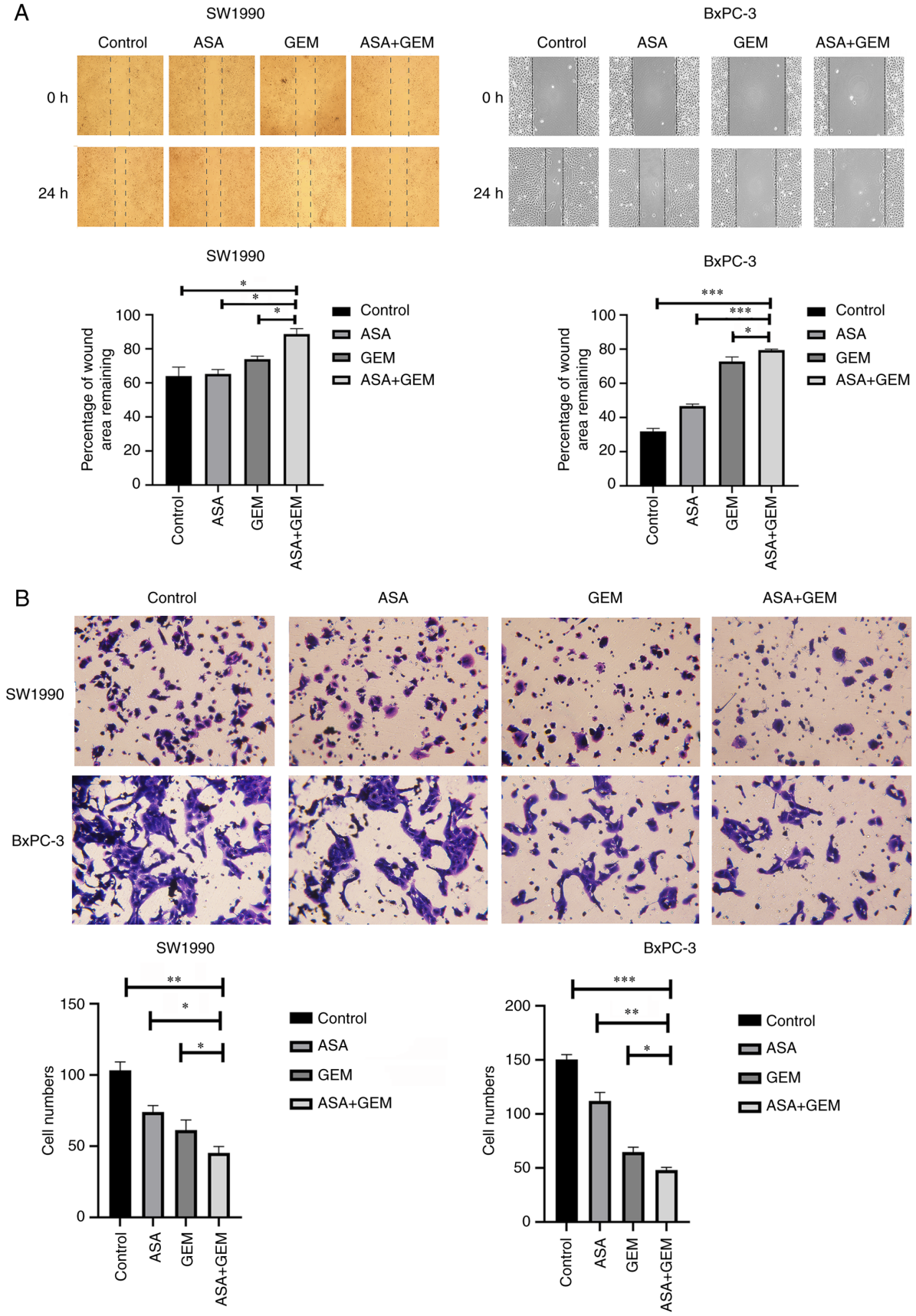


Figure 2. Migration and invasion of SW1990 and BxPC-3 cells treated with ASA and/or GEM. (A) The combination of ASA (2 mmol/l) and GEM (1 mg/l) inhibited pancreatic cell migration. The migratory ability of SW1990 and BxPC-3 cells following treatment with ASA and/or GEM for 24 h was evaluated using a wound healing assay (magnification, x100). The percentage of the wound area remaining was calculated using the following formula: $W_{24\,h}/W_{0\,h}$ x100. The experiments were performed in triplicate and the results are presented as the mean ± SD. (B) The invasion of SW1990 and BxPC-3 cells following treatment with ASA and/or GEM for 24 h was evaluated by Transwell assay (magnification, x200). The experiments was repeated three times in duplicate, and the numbers of cells counted in five random fields were averaged. The results are presented as the mean ± SD. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. ASA, aspirin; GEM, gemcitabine; W, width of the scratch.

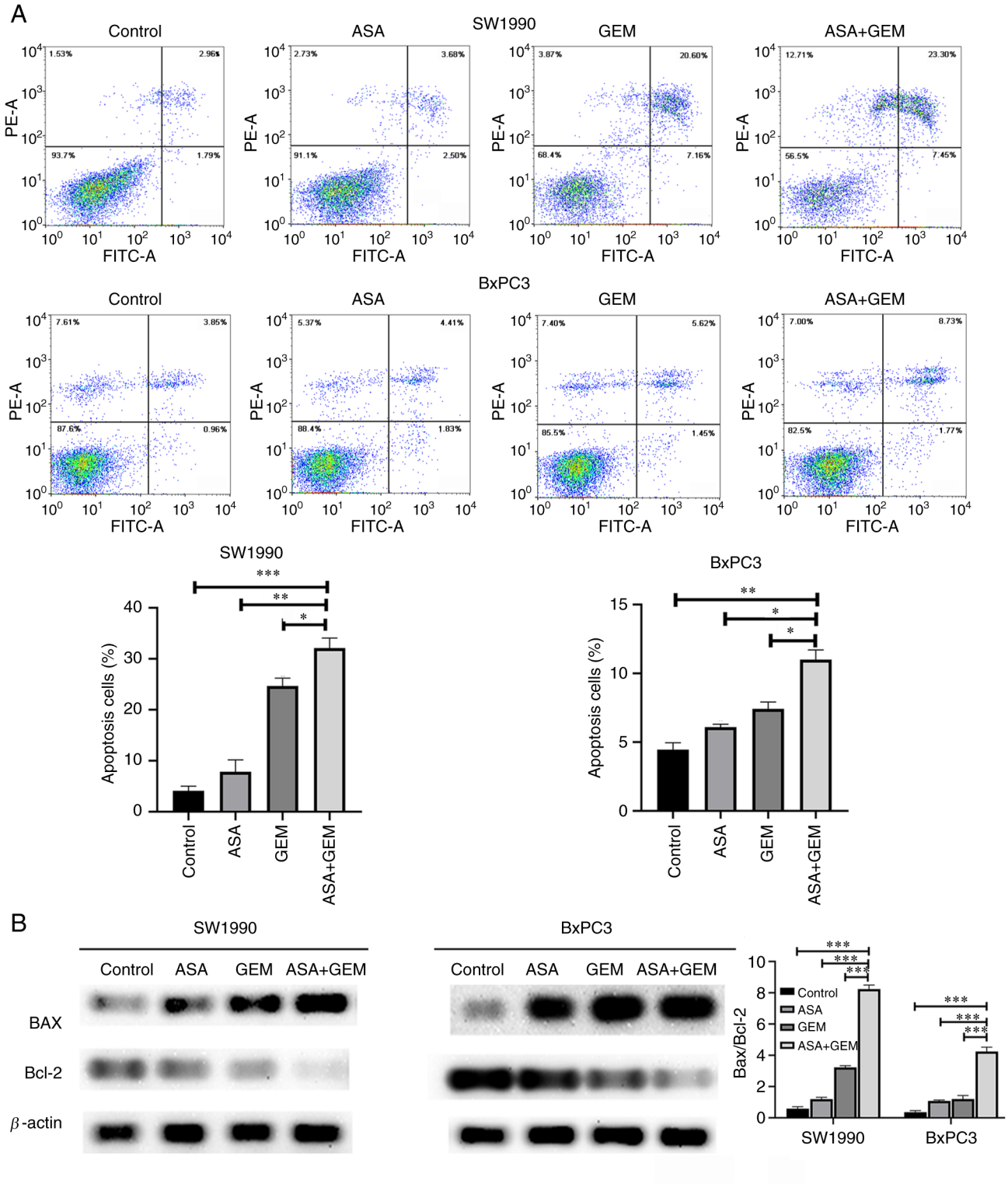


Figure 3. Effect of ASA and GEM on the apoptosis of pancreatic cancer cells. ASA (2 mmol/l) combined with gemcitabine (1 mg/l) increases pancreatic cancer cell apoptosis in pancreatic cancer cell lines treated with GEM and/or ASA for 48 h. (A) Cell apoptosis was detected using an Annexin V-FITC/PI staining assay. Representative flow cytometry plots and quantitative analysis of the apoptotic cell numbers are shown. (B) Western blot analysis was used to evaluate the expression of the apoptosis-associated proteins Bcl-2 and Bax. The data shown represent the mean ± SD from three independent experiments with similar results. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. ASA, aspirin; GEM, gemcitabine.

cell endocytosis and transcytosis. This conjugate exhibited high antitumor activity in multiple pancreatic cancer xenograft mouse models compared with gemcitabine (41). In summary, advancements in materials science, biology, pharmacology and other domains are necessary to increase the concentration of medications in the pancreatic cancer microenvironment.

The ability of ASA to inhibit the tumorigenesis and development of pancreatic cancer and the underlying mechanism remain unclear. The goal of the present study was to evaluate

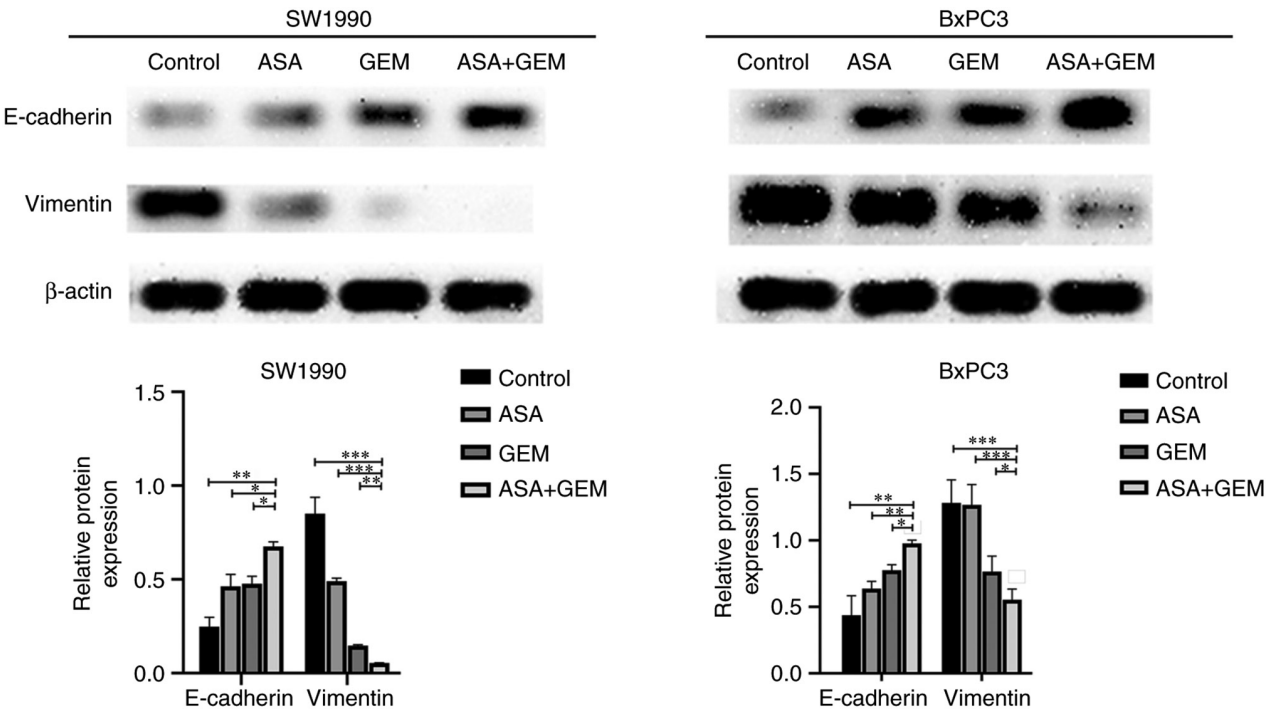


Figure 4. ASA combined with GEM significantly increases the expression of E-cadherin and decreases the expression of vimentin. SW1990 and BxPC-3 cells were treated with ASA (2 mmol/l) and/or GEM (1 mg/l) for 24 h and the levels of the epithelial-mesenchymal transition-associated proteins E-cadherin and vimentin were determined via western blotting using β-actin as a loading control. All experiments were performed in triplicate and the data shown represent the mean ± SD. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. ASA, aspirin; GEM, gemcitabine.

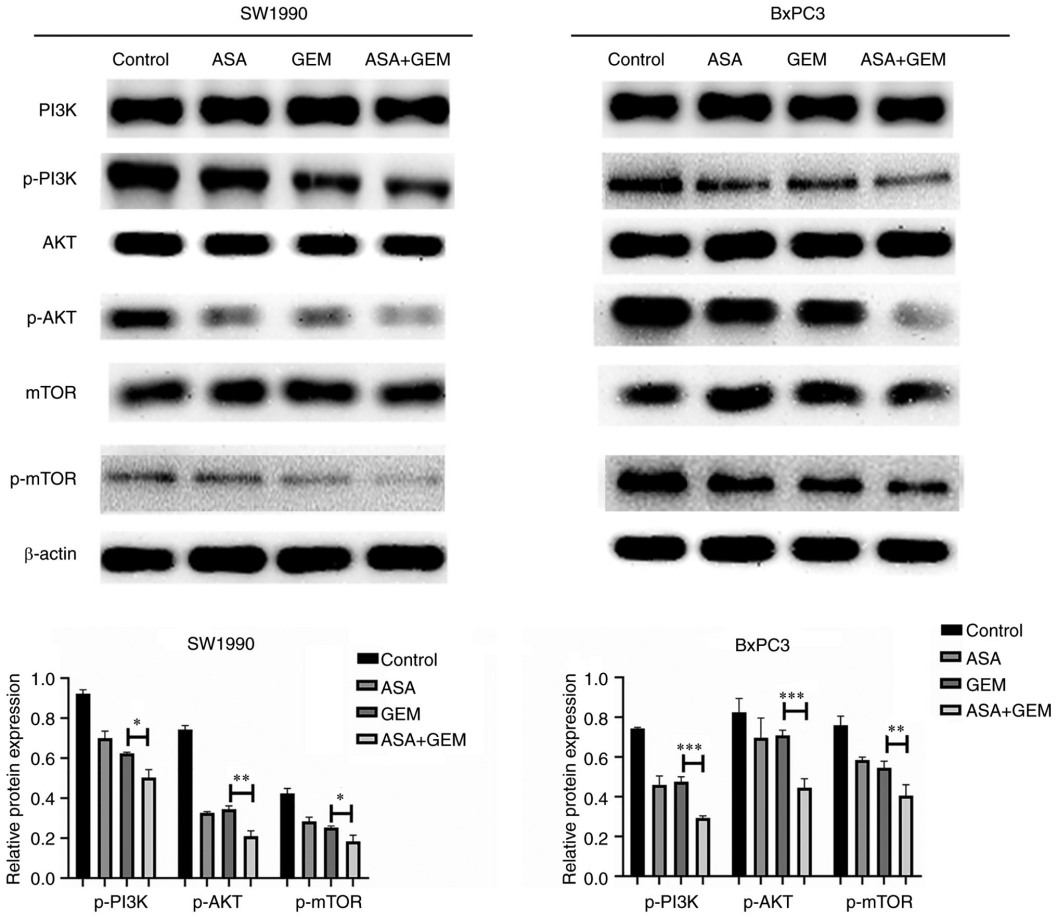


Figure 5. ASA combined with GEM downregulates PI3K/AKT/mTOR signaling. SW1990 and BxPC-3 cells were treated with ASA (2 mmol/l) and/or GEM (1 mg/l) for 24 h and the levels of PI3K, AKT, mTOR, p-PI3K, p-AKT and p-mTOR were determined via western blotting with β-actin included as a loading control. All experiments were performed in triplicate and the data shown represent the mean ± SD. $^{*}$P<0.05, $^{**}$P<0.01 and $^{***}$P<0.001. ASA, aspirin; GEM, gemcitabine; p-, phosphorylated.

whether ASA increases the efficacy of gemcitabine in pancreatic cancer. In addition, the possible underlying mechanism was investigated.

Previous research has addressed the role of EMT in tumor cell drug efficacy (42). Therefore, research into the mechanisms of EMT in pancreatic cancer is necessary to facilitate the development of new therapeutic strategies (43). The present study showed that ASA increased the expression level of E-cadherin and decreased that of vimentin in SW1990 and BxPC-3 cells, indicating that ASA increased the efficacy of gemcitabine in pancreatic cancer cells via the inhibition of EMT.

The PI3K/AKT pathway is among the most frequently mutated in human cancer, and multiple genetic events have been described that lead to activation of the PI3K/AKT/mTOR pathway in cancer (44). Inhibitors of AKT/mTOR pathways, including rapamycin and LY294002, are able to inhibit EMT progression in pancreatic cancer (45). Previous research has shown that reactive oxygen species (ROS) regulate EMT (46). ROS have roles in cellular integrity and cell death, and several anticancer drugs have been shown to induce high levels of ROS and reduce AKT/mTOR signaling (47-49). The present study suggested that ASA increased the efficacy of gemcitabine in pancreatic cancer via the downregulation of PI3K/AKT/mTOR signaling, that is, by reducing PI3K, AKT and mTOR phosphorylation, which is consistent with a previous study which demonstrated that gemcitabine inhibited cancer growth, metastasis and EMT in pancreatic cancer cells with involvement of the PI3K/AKT/mTOR pathway (50). The present study demonstrated that the combination of ASA and gemcitabine exerted a remarkable inhibitory effect on cell proliferation and markedly promoted apoptosis in SW1990 and BxPC-3 cell lines via a mechanism involving the PI3K/AKT/mTOR signaling pathway.

The difference in response rates between the two cell lines merits discussion. Previous literature supports a difference in response rates between the BxPC-3 and SW1990 cell lines; specifically, when treated with gemcitabine, the half-maximal inhibitory concentration ($IC_{50}$) of the SW1990 cell line was reported to be 6.27 mmol/l while that of the BxPC-3 cell line was 4.01 mmol/l (51). In the present study, preliminary MTT experiments indicated that the $IC_{50}$ of ASA was 1.93 mmol/l in SW1990 cells and 2.26 mmol/l in BxPC-3 cell lines. According to a study conducted by Lin *et al* (52), PANC-1 cells treated with 2 mmol/l ASA for 24 h had a growth suppression rate of ~40% compared with untreated cells, which is similar to the findings for the cell lines used in the present study. According to these data, it was decided to use a 2 mmol/l concentration of ASA in the current study. However, a 2 mmol/l concentration appears to be a little higher than that used in certain other studies. The optimum concentration of ASA for use in pancreatic cancer is unclear and may differ from that in other cancers.

The present study found that low-dose ASA combined with gemcitabine significantly reduced cell proliferation and migration, increased apoptosis, increased the expression of E-cadherin and suppressed that of vimentin when compared with gemcitabine alone. When ASA plus gemcitabine combination therapy was compared with ASA or gemcitabine alone, the results revealed a significant downregulation of activation of the PI3K/AKT/mTOR pathway in SW1990 and BxPC-3 cells, as indicated by effective reductions in the phosphorylation of PI3K, AKT and mTOR. These data suggested that the complementary effect of ASA plus gemcitabine therapy in pancreatic cancer may occur by attenuation of EMT and the downregulation of PI3K/AKT/mTOR signaling. These findings are similar to those observed in a previous study, in which ASA inhibited the EMT and migration of oncogenic KRAS-expressing non-small cell lung carcinoma cells via downregulation of the E-cadherin repressor Slug (53).

In summary, the results of the present demonstrate that ASA increased the efficacy of gemcitabine in pancreatic cancer via the inhibition of cell proliferation and migration and the induction of apoptosis. The study also reveals that ASA increased the ability of gemcitabine to inhibit the PI3K/AKT/mTOR signaling pathway and reverse the EMT in pancreatic cancer cells, which is a new insight. The results suggest that ASA combined with gemcitabine has the potential to be a promising therapeutic option for patients with pancreatic carcinoma.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Authors' contributions**

HZ conceived and designed the study. XY performed the experiments. KX analyzed the data. YS made contributions to the interpretation of the data. All authors read and approved the final version of the manuscript. KX and YS 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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