Interference with ATF5 Function Enhances the Sensitivity of Human Pancreatic Cancer Cells to Paclitaxel-induced Apoptosis

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Abstract. *Background: Past work has established that human glioblastomas and breast cancer cells invariably express the activating transcription factor 5 (ATF5) and that loss of function of ATF5 caused massive apoptotic death of all cancer cell lines tested. ATF5 expression and function in pancreatic cancer cells have not been investigated. Materials and Methods: Quantitative real-time/reverse transcription-polymerase chain reaction (QRT/RT-PCR), western blotting (WB), immunohistochemistry (IHC) and promoter reporter assay were used for gene expression analysis. MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay and FACS (fluorescence-activated cell sorting) analysis were used to monitor cell viability/apoptosis. Results: ATF5 is highly expressed in pancreatic cancer cells as compared with non-tumor tissues. Both paclitaxel treatment and loss of function of ATF5 elicited apoptosis of SW1990 cells. Interference with ATF5 function in SW1990 cells resulted in down-regulation of BCL-2 and up-regulation of BAX, resulting in enhanced sensitivity to apoptosis induced by paclitaxel treatment. Conclusion: ATF5 is highly expressed in pancreatic cancer cells. Targeting ATF5 significantly enhances paclitaxel-induced apoptosis in human pancreatic cancer cells. ATF5 could be an important therapeutic target for pancreatic cancer treatment.*

Pancreatic carcinoma currently remains one of the leading causes of cancer death in the United States (1). Disease in most patients is surgically unresectable at the time of diagnosis. For patients whose pancreatic carcinomas are resected, the prognosis is often very poor because of late diagnosis and the fact that metastatic pancreatic cancer easily acquires chemoresistance.

Resistance to chemotherapeutical drugs is one of the greatest obstacles in clinical treatment. Most cancer drugs induce cell apoptosis, while cancer cells may develop resistance and survive prolonged treatment (2). Paclitaxel, which is derived from the needles and bark of western yew trees, is widely used to treat a variety of solid tumors including pancreatic cancer. Apoptotic tumor cell death is commonly observed in paclitaxel therapy. However, resistance to paclitaxel is often observed in pancreatic cancer therapy (3-5). Cancer cells escape apoptosis by a number of mechanisms, among which up-regulation of anti-apoptotic genes and down-regulation of pro-apoptotic genes play an important role.

Activating transcription factor 5 (ATF5) is a member of the activating transcription factor/cyclic-AMP responsive element-binding (ATF/CREB) family of basic leucine zipper transcription factors (6). Previous studies have demonstrated that ATF5 is highly expressed in several types of cancer cells and generally poorly expressed in normal human cells and tissues (7-9). For instance, a tissue microarray immunostained for ATF5 showed that a significantly greater proportion of adenocarcinomas, transitional cell carcinomas, squamous cell carcinomas and metastatic carcinomas of various tissue origins had increased nuclear ATF5 staining when compared to non-neoplastic tissues (9).

Studies have also shown that ATF5 is down-regulated in several types of cells following growth factor deprivation, which leads to apoptosis (8, 10-12). Interference with ATF5 function induces apoptosis of these cells, including HeLa, FL5.12, and a number of glioma and breast cancer cell lines cultured in the presence of growth factors. In contrast, similar interference of ATF5 function in non-neoplastic breast cells or in non-brain tumor cells, such as mature neurons and glial cells, did not affect their survival (7-9, 12). On the other hand, regulation and function of ATF5 seem highly dependent on cell and tissue types, even for different types of cancers. For instance, ATF5 is abundantly expressed in liver cells and down-regulated in hepatocarcinomas. Forced expression of

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ATF5 in hepatocarcinomas blocks cell-cycle progression at the G_2/M transition (13, 14). We previously identified B-cell CLL/lymphoma 2 (BCL-2) as an essential mediator for the pro-survival function of ATF5 in breast cancer and glioblastoma cells, and provided direct evidence that the cell type-specific function of ATF5 derives from differential regulation of downstream targets by ATF5 (8).

Here, we investigated ATF5 expression in pancreatic cancer cells and malignant pancreatic carcinomas as compared with non-malignant pancreatic cells and tissues. This study implicated ATF5 as a significant player in chemoresistance of pancreatic carcinomas and identified for the first time that BAX is an ATF5-repressed gene.

Materials and Methods

Cell lines and culture. Human pancreatic cancer cell lines Capan-2, SW1990 and ASPC-1 were obtained from the cell bank at the Shanghai Institute of Cell Biology, Chinese Academy of Sciences, China.

Plasmids, transfection and paclitaxel treatment. The pLeGFP-C1 and pLeGFP-C1-FLAG-dnATF5 (d/n ATF5) plasmids and transfection were described previously (15). For paclitaxel (Yangtze River Pharmaceutical Co., Ltd., Taizhou, Jiangsu, China) treatment, non-transfected or post-transfected (9 h) SW1990 cells were re-fed with fresh culture medium. Thirty-six hours post-transfection, cells were treated with PACLITAXEL at the indicated doses and for the indicated times.

Human pancreatic specimens. All 46 patients who provided samples for this study had undergone surgery at the Department of General Surgery, the Affiliated Hospital of Qingdao University Medical College, China, between January 2010 and March 2011. The mean age of the study population was 60 years (range from 38-74 years) with 26 males (57%) and 20 females (43%). The initial histological grade of the pancreatic cancer specimens was 21 well/moderately-differentiated and 25 poorly-differentiated. The corresponding normal pancreatic tissues were obtained 5-7 cm away from the area of tumor. None of the patients had received chemotherapy before surgery. All the paraffin-embedded specimens were provided by the Department of Pathology of the Affiliated Hospital of Qingdao University Medical College.

All human materials used in this study were sampled at the Department of General Surgery, the Affiliated Hospital of Qingdao University Medical College, which only maintains information on the patients for statistical analyses. The research activity was determined not to require formal Institutional Review Board (IRB) review, according to the policies of the institution and the provisions of applicable national regulations, as specified by the Ministry of Health's national Biomedical Research Ethics Committee.

Immunohistochemistry. The paraffin-embedded human pancreatic resection slides were stained for ATF5 expression. Paraffin was removed by heating sections at 60˚C for 1-2 h, followed by three incubations in 100% xylene for 5 min each. Subsequent incubations were performed in 100, 75, 50 and 0% ethanol for 5 min each. Specimens were then microwaved in 1 l of a sodium citrate buffer (pH 6.0) for 45 min for heat-mediated antigen retrieval. A solution of 1% hydrogen peroxide in methanol (v/v) was used to block endogenous peroxidase activity before transferring the sections into phosphate-buffered saline (PBS) (pH 7.2). The slides were incubated at room temperature for 1 h with the primary antibody (anti-ATF5, Abcam, Cambridge, MA, USA) at 1:200 dilution. Peroxidase activity was developed in 0.5% (v/v) 3,3'-diaminobenzidine hydrochloride in PBS containing 0.03% hydrogen peroxide for 2 min. Then the slides were rinsed in tap water, dehydrated, placed in xylene, and mounted at room temperature. The same protocol was used for immunostaining of control slides except that the primary antibody was omitted, showing the absence of non-specific staining in each experiment.

Semiquantitative immunoreactive scoring was calculated based on the percentage of positively stained cells relative to the total number of recognizable cells in randomly selected fields. When 20%, or more, ATF5 (for cytoplasmic staining) of the tumor cells exhibited immunoreactivity, the result was interpreted as positive. All slides were evaluated for immunostaining without any knowledge of the clinical outcome or other clinicopathological data.

Western blotting, reverse transcription/quantitative real-time PCR, luciferase reporter assay, MTT assay and flow cytometry analysis. These were performed as previously described (11, 14). The primers used were as follows: for ATF5 detection, 5'-AAGTCGGCGGC TCTGAGGTA -3' as forward and 5'-GGACTCTGCCCGTTCC TTCA-3' as reverse; for BAX, 5'-TGCTTCAGGGTTT CATCCAG-3' and 5'-GGCGGCAATCATCCTCTG-3'; for BCL-2, 5'-CGACTTTGCAGAGATGTCCA -3' and 5'-ATGCCGGTTC AGGT ACTCAG-3'; and 5'-TGGAACGGTGAAGGTGACAG-3' and 5'-GGCTTTTAGGATGGCAAGGG-3' were used for the detection of β-actin as an internal control. All real-time PCR products were visualised on an agarose gel containing ethidium bromide to confirm the correct amplicon size. For flow cytometric analysis of apoptosis, cells were grown to confluency in a 6-well plate. The cells were then trypsinized and collected by centrifugation, and were treated with both 7-aminoactinomycin D (7-AAD) and annexin V-PE (Abcam, Cambridge, MA, USA) for 15 min. Fluorescence flow cytometric analyses of apoptosis were performed using a Guava EasyCyte Mini instrument (MILLIPORE Guava technologies, Billerica, MA, USA). Gating was adjusted using 7-AAD staining with dot plots displaying FL3-7-AAD on the y-axis and FL2 annexin V-PE on the x-axis. More than 5,000 events were collected for each sample.

Statistical analysis. Determination of statistical significance was carried out with two-tailed Student's *t*-test between two groups and the chi-square test. All experiments with statistical analyses were performed at least three times, and data are expressed as the means±standard deviation (SD). A p-value of <0.05 was considered to be statistically significant. These analyses were performed using the PASW 18.0 software (SPSS Inc., Chicago, IL, USA).

Results

Expression of ATF5 in human pancreatic cancer cell lines and tumors. Previous studies have demonstrated that ATF5 is highly expressed in several types of cancer cell, including human breast cancer and human and rodent glioblastomas (7-9, 16), and that the level of expression correlates with disease

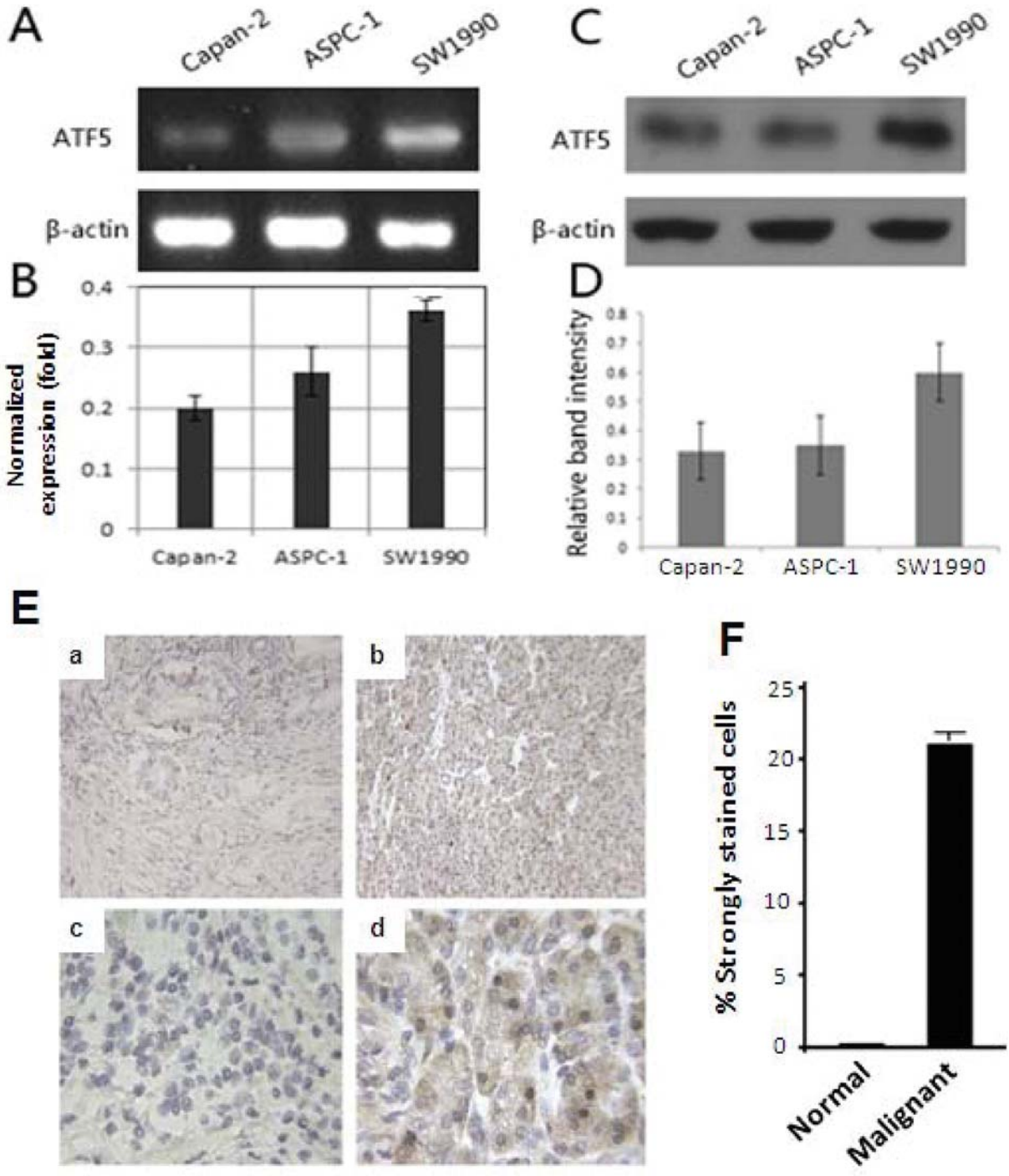

Figure 1. *Comparison of ATF5 expression in human pancreatic cancer cell lines and normal and tumor pancreatic tissues. A: RT-PCR detection of ATF5 expression in human pancreatic cancer cell lines (Capan-2, SW1990 and ASPC-1). β-Actin was used as a loading control. B: Quantitative real-time PCR analysis of the same samples used in (A). At least three independent experiments were performed. Data are presented as means±SD. C: Western blotting detection of ATF5 expression in Capan-2, SW1990 and ASPC-1 cells. Equal amounts of cell extracts were used in all lanes. D: Western blotting films from three independent experiments as in (C) were scanned and the relative density of protein bands was calculated using β-actin as a loading control. E: The immunostaining for ATF5 is more uniform and intense in tumors (b, d) than in comparable non-neoplastic tissues (a, c). Magnification is ×200 for (a, b) and ×400 for (c, d). F: Quantification analysis of percentage of cells in normal and malignant pancreatic tissue specimens that were strongly stained for ATF5. Percentage of cells strongly stained with ATF5 were calculated as 100× the number of strongly-positive cells/total number of cells counted in a representative field.*

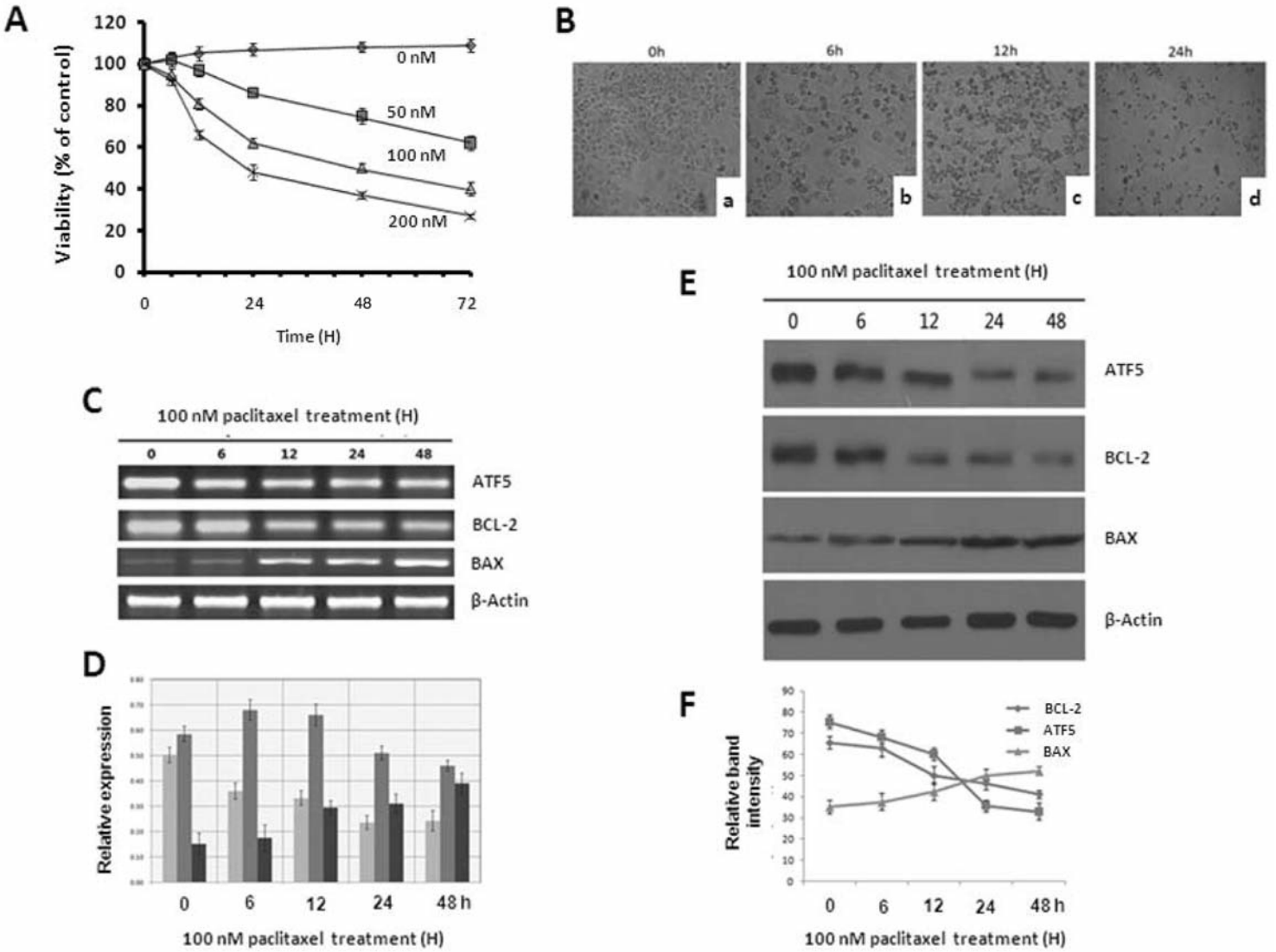

Figure 2. *Effect of paclitaxel treatment on SW1990 cell survival and on expression of ATF5, BCL-2 and BAX. A: The effect of paclitaxel on the survival of SW1990 cells as determined by MTT. SW1990 cells were treated with paclitaxel at the indicated concentrations and time periods. MTT analyses were performed as described in Methods. B: Morphological changes of SW1990 cells in response to paclitaxel treatment. a) Untreated SW1990 cells; b-d) SW1990 cells treated with paclitaxel for indicated times. Magnification: ×100. C: RT-PCR analysis of ATF5, BCL-2 and BAX expression in SW1990 cells treated with paclitaxel (100 nM) for the indicated times. β-actin was used as control. D: Quantitative real-time PCR analysis of the same samples used in (C). At least three independent experiments, as in (C), were performed. β-Actin was used as control. E: Western blotting analysis of ATF5, BCL-2 and BAX in SW1990 cells treated as in (C). β-Actin was used as loading control. F: Western blotting films from three independent experiments shown in (E) were scanned and the relative density of protein bands for ATF5, BCL-2 and BAX was calculated using β-actin as a loading control.*

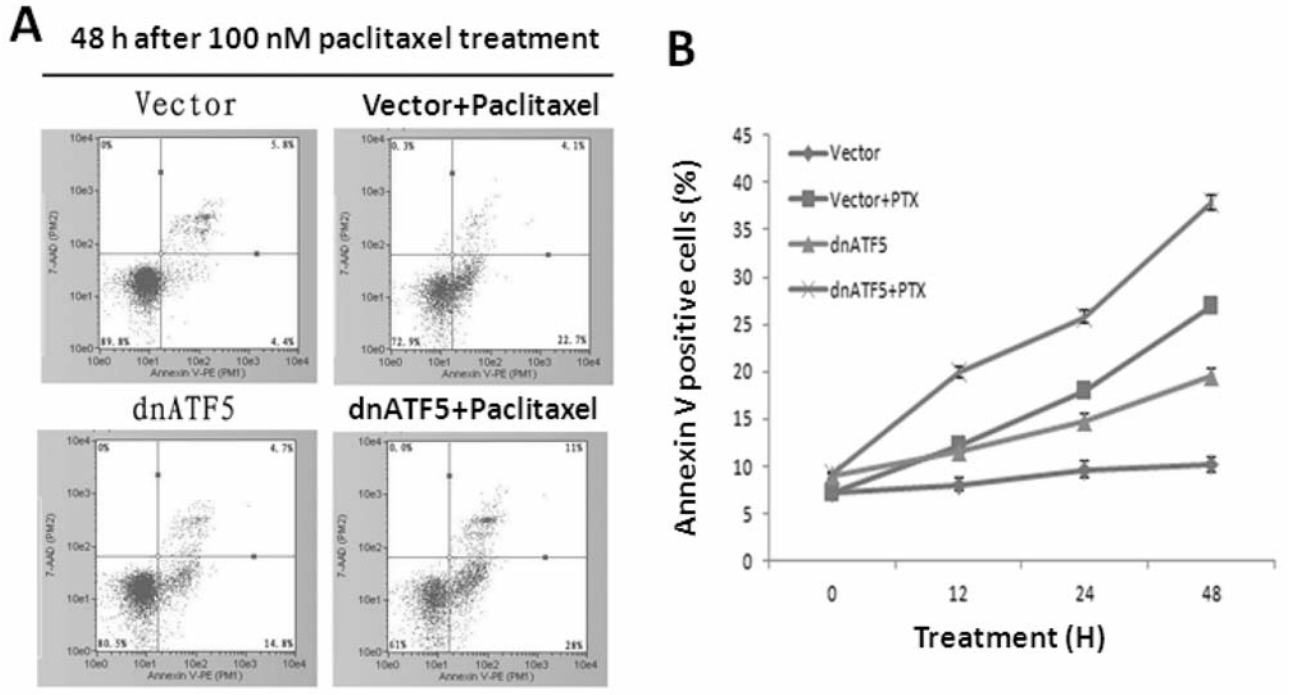

Figure 3. *Apoptotic analysis of SW1990 cells transfected with vector-empty or expressing dnATF5 and treatment with or without paclitaxel. A: SW1990 cells transfected with vector-empty (Vector) or expressing dnATF5 and treated with or without paclitaxel (100 nM) for 48 h were analyzed for annexin V/7-AAD staining by flow cytometry. Events in each of the four quadrants are as follows: Lower left, viable cells; lower right, cells in the early-to mid-stages of apoptosis; upper right, cells in the late-stages of apoptosis; upper left, mostly nuclear debris. B: The apoptotic rates of the SW1990 cells treated in (A) were assessed at the indicated time periods by annexin V/7-AAD staining.*

prognosis (7, 9, 17, 18). To examine the expression of ATF5 in human pancreatic cancer cells, mRNA and whole cell protein were prepared from three pancreatic cancer cell lines, Capan-2, SW1990 and ASPC-1, and ATF5 expression was determined by RT-PCR, real-time quantitative PCR (QRT-PCR) and western blot analyses. As shown in Figure 1A-D, ATF5 was detected in all three cell lines, both at mRNA and protein levels. In particular, the level of ATF5 mRNA and protein in SW1990 cells was the highest among the three cell lines and therefore SW1990 was chosen for further analyses.

We next examined ATF5 expression in malignant human pancreatic carcinomas by immunostaining, using normal pancreatic tissues obtained 5-7 cm away from the tumor area as controls. Positive ATF5 immunostaining was seen in 41/46 (89%) of the pancreatic carcinoma specimens. In contrast, positive ATF5 staining was observed in only 2/15 of the normal pancreatic tissues (Figure 1E). In addition, ATF5 staining was more prevalent in malignant pancreatic carcinomas than in surrounding normal pancreatic tissues. The population of cells that were highly stained with ATF5 was over 21% in malignant pancreatic carcinomas tissues as compared with virtually 0% in non-malignant pancreatic tissues (Figure 1F). Taken together, these data indicated that ATF5 expression is elevated in malignant human pancreatic carcinomas and in pancreatic cancer cell lines as compared with non-malignant pancreatic cells and tissues.

Effect of paclitaxel treatment on cell viability and expression of ATF5. To determine the role of elevated ATF5 expression in the chemoresistance of pancreatic cancer cells, we first examined the survival profile of SW1990 cells in response to paclitaxel treatment. SW1990 cells were treated with paclitaxel at different dosages and time periods and cell survival was determined by the MTT assay. As shown in Figure 2A, the viability of SW1990 cells subjected to paclitaxel treatment decreased rapidly in a paclitaxel dose-dependent manner. Fewer than 40% of cells survived when treated with 200 nM of paclitaxel for 24 h, while the half maximal inhibitory concentration (IC_{50}) determined at 48 h of treatment was 115 nM (Figure 2A and B). Therefore, the paclitaxel of 100 nM for 48 h was the condition used for additional analyses.

We next examined the effect of paclitaxel on the expression of ATF5 in SW1990 cells. As shown in Figure 2C and D, *ATF5* mRNA expression was significantly down-regulated 6 h after paclitaxel treatment and was maintained

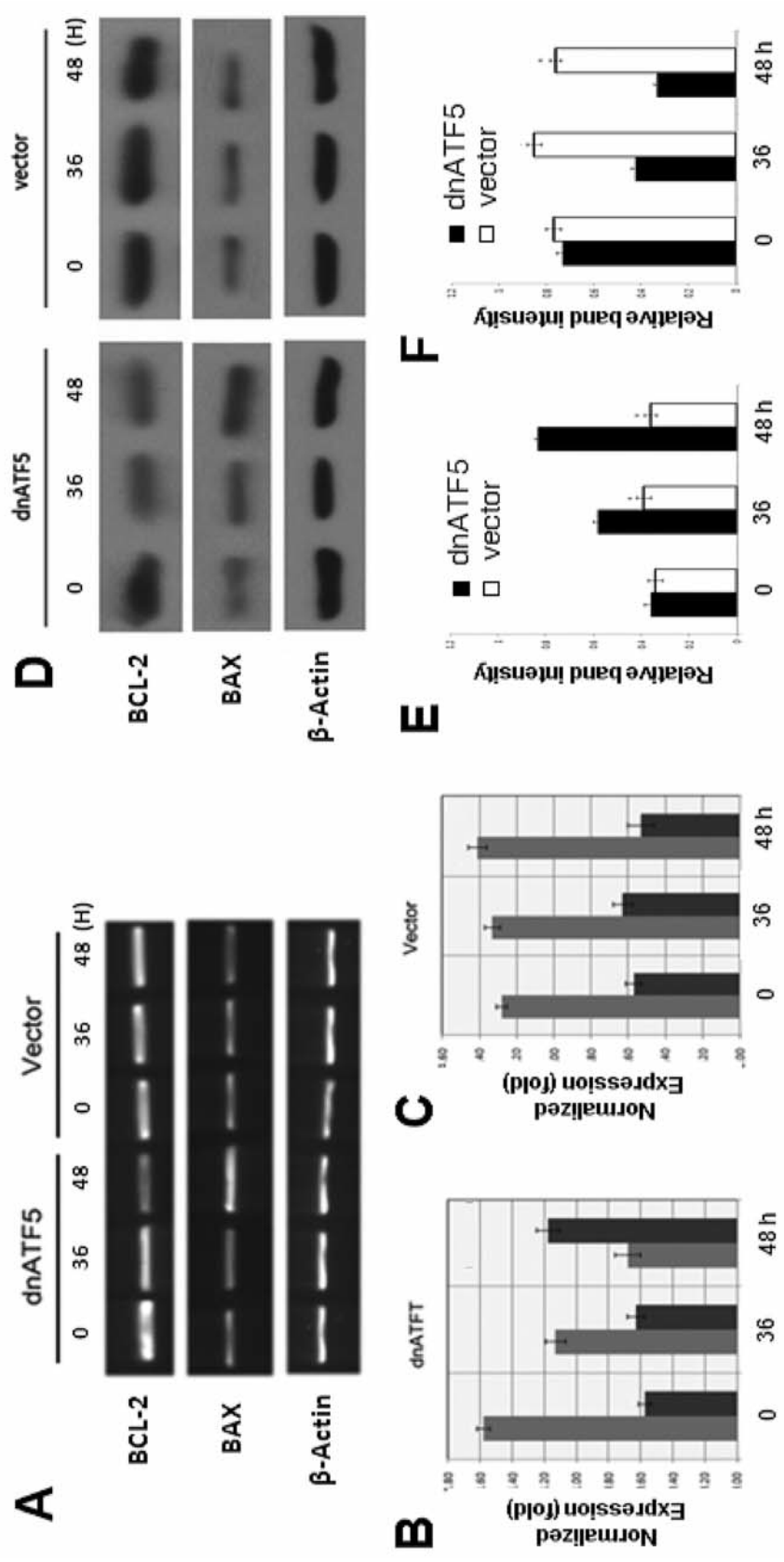

Figure 4. *Interference of ATF5 function leads to down-regulation of BCL-2 and up-regulation of BAX in SW1990 cells. A: RT-PCR analysis of BCL-2 and BAX expression in SW1990 cells transfected with vector-empty (vector) or expressing dnATF5 (dnATF5). β-Actin was used as a control. B and C: Quantitative real-time PCR analysis of BCL-2 (B) and BAX (C) expression in SW1990 cells transfected as in (A). At least three independent experiments were performed. β-Actin was used as a control. Data are presented as means±SD. D: Western blotting analysis of BCL-2 and BAX in SW1990 cells transfected with vector-empty (vector) or expressing dnATF5 (dn ATF5). β-Actin was used as a control. E and F: Western blotting films from three independent experiments in (D) were scanned and the relative density of protein bands for BCL-2 and BAX was calculated using β-actin as a loading control. Expression of dnATF5 in transfected cells was monitored by western blotting analysis, which is shown in the insert in (F). *$p<0.05$, vector vs. dnATF5.*

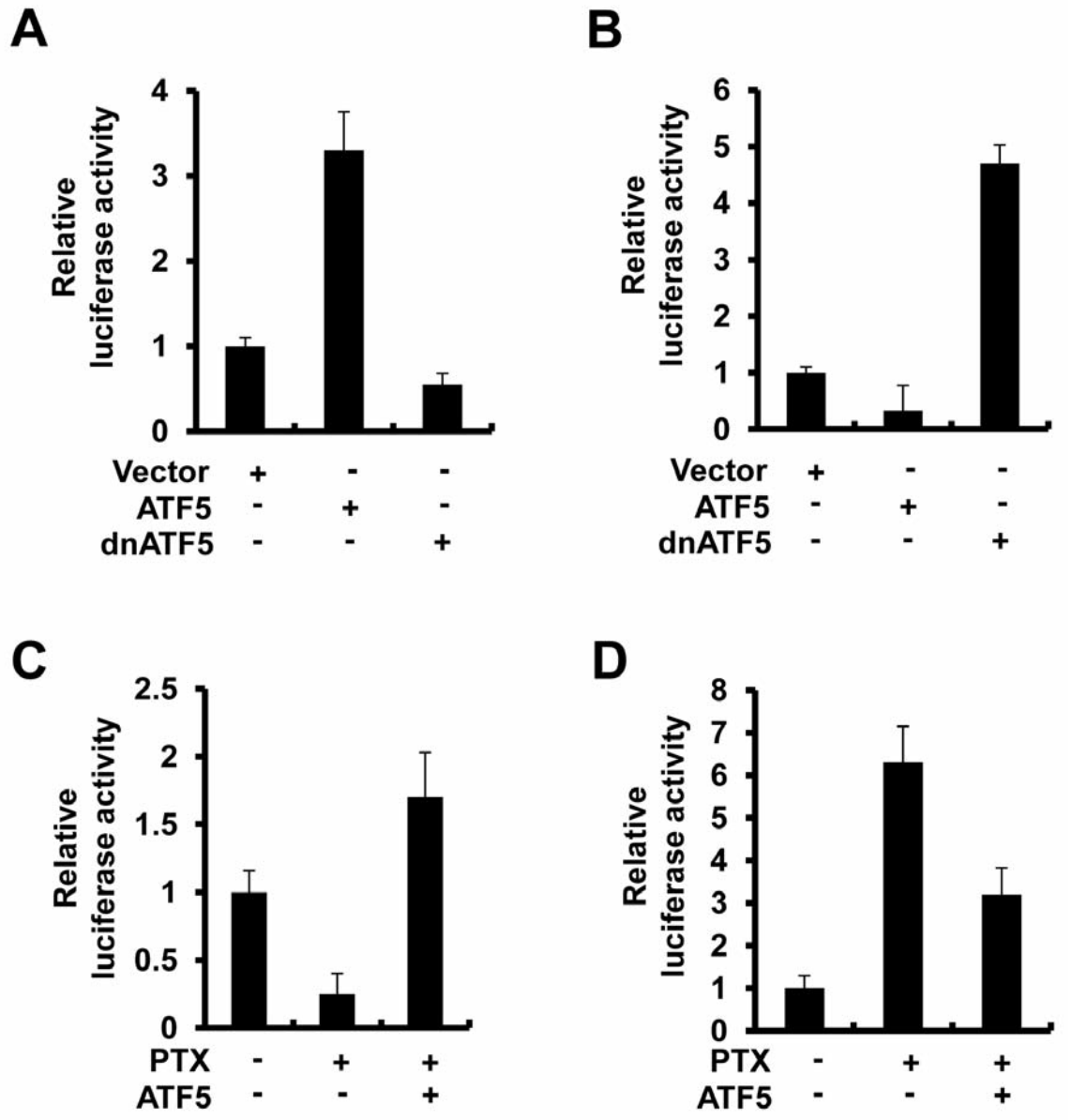

Figure 5. *ATF5 mediates paclitaxel-dependent transcriptional up-regulation of BCL-2 and down-regulation of BAX in SW1990 cells. A and B: SW1990 cells were co-transfected with the indicated constructs and renilla, and BCL-2 (A) or BAX (B) promoter luciferase reporter for 48 h. Relative luciferase activity was determined after correction with renilla activity. At least three independent experiments were performed. Results are presented as means±SD. C and D: SW1990 cells were co-transfected with vector (–) or ATF5 (+), renilla, and BCL-2 (C) or BAX (D) promoter luciferase reporter for 24 h. Cells were then treated with (+) or without (–) paclitaxel (100 nM) for another 24 h before luciferase reporter assay, as in (A). At least three independent experiments were performed. Results are presented as means±SD.*

down-regulated for at least 48 h. Similarly, ATF5 protein levels were accordingly down-regulated (Figure 2E and F). These data demonstrate that paclitaxel treatment rapidly down-regulates ATF5 expression in SW1990 pancreatic cancer cells and suggest that down-regulation of ATF5 is pre-requisite to paclitaxel-induced apoptosis in this cell line.

Paclitaxel treatment of SW1990 cells leads to down-regulation of BCL-2 and up-regulation of BAX. We have previously reported that BCL-2 is regulated by ATF5 in breast cancer and glioblastoma cells (8). To examine whether BCL-2 is also down-regulated in SW1990 cells in which ATF5 is down-regulated in response to paclitaxel treatment, we performed RT-PCR, QRT-PCR and western blotting analyses for BCL-2 in parallel with those for ATF5. As

shown in Figure 2C-F, BCL-2 mRNA and protein levels were similarly down-regulated in SW1990 cells treated with paclitaxel. Interestingly, *BCL-2* mRNA expression became markedly down-regulated 12 h after paclitaxel treatment, which occurred a few hours after the down-regulation of ATF5 (Figure 2C and D). This is consistent with BCL-2 being a regulated target of ATF5 and supports a similar finding that we made in a previous study using breast cancer and glioblastoma cells (8). We additionally found that BAX, an apoptotic member of the BCL-2 family, was up-regulated at both mRNA and protein levels following paclitaxel treatment (Figure 2C-F). Thus, paclitaxel treatment of SW1990 cells, which promotes down-regulation of ATF5, evokes simultaneously down-regulation of anti-apoptotic BCL-2 and up-regulation of the pro-apoptotic BCL-2 family member BAX.

Interfering with the function of ATF5 promotes apoptosis of human pancreatic cancer cells and increases paclitaxel-induced cell death in vitro. Down-regulation of ATF5 and ATF5-dependent BCL-2 and up-regulation of BAX in SW1990 cells in response to paclitaxel treatment suggested that ATF5 plays a role in the chemosensitivity of SW1990 cells. To examine whether ATF5 function affects paclitaxel-induced cell death of SW1990 cells, we performed FACS analysis on SW1990 cells that were transfected with empty vector empty or expressing dnATF5 and then treated with paclitaxel (100 nM), using annexin V/7-AAD as the staining marker. As shown in Figure 3, elevated cell death was observed in SW1990 cells transfected with empty vector and treated with paclitaxel (compare Vector+paclitaxel with Vector control), as expected. Similarly, cells transfected with dnATF5 and treated with vehicle (dnATF5) also displayed increased cell death, confirming that ATF5 is required for survival of SW1990 cells. In addition, cells transfected with dnATF5 and treated with paclitaxel (dnATF5+paclitaxel) exhibited significantly higher rates of cell death, indicating increased sensitivity of SW1990 cells to paclitaxel-induced apoptosis, when ATF5 function is lost in the cell. These data support the conclusion that interference with ATF5 function in SW1990 cells promotes apoptosis and sensitizes the cells to apoptotic stimulation induced by paclitaxel treatment.

Expression of BCL-2 and BAX is dependent on ATF5 activity in SW1990 cells. To determine whether ATF5 activity directly affects the expression of BCL-2 and BAX in pancreatic cancer cells, we performed RT-PCR, QRT-PCR and western immunoblotting analyses for BCL-2 and BAX mRNA and protein expression levels in SW1990 cells, transfected with a vector-empty, or expressing dnATF5. As shown in Figure 4A-C, in contrast to the control vector-transfected cells, in which the expression of both BCL-2 and BAX remained unchanged, expression of BCL-2 was significantly down-regulated, while expression of BAX was markedly up-regulated 48 h after transfection of dnATF5. As expected, the protein levels of BCL-2 and BAX were down-regulated and up-regulated, respectively, in response to the expression of dnATF5 (Figure 4D-F). Taken together, these data indicate that the increased apoptosis and sensitivity of SW1990 cells results from down-regulation of BCL-2 and up-regulation of BAX promoted by paclitaxel-induced ATF5 loss-of-function.

Ectopic expression of ATF5 blocks paclitaxel-induced transcriptional down-regulation of BCL-2 and up-regulation of BAX in SW1990 cells. To further determine that ATF5 regulates BCL-2 and BAX transcriptionally in SW1990 cells, we performed promoter luciferase analysis. SW1990 cells were co-transfected with a BCL-2 or BAX promoter-luciferase construct with an empty vector or one expressing ATF5 or dnATF5, and luciferase activity was determined 48 h later. As shown in Figure 5A, the BCL-2 promoter was activated by more than 3-fold in response to ATF5 overexpression and reduced by 50% in response to expression of dnATF5. In contrast, BAX promoter activity was inhibited by ATF5 and up-regulated by dnATF5 (Figure 5B). As shown in Figure 5C, while paclitaxel treatment down-regulated BCL-2 promoter activity, as expected, overexpression of ATF5 not only abrogated the paclitaxel-induced BCL-2 promter down-regulation but in fact elevated the promoter activity. In contrast, paclitaxel up-regulated the BAX promoter activity and ATF5 abrogated the paclitaxel-induced BAX promoter up-regulation (Figure 5D).

Discussion

Our study demonstrates that the ATF/CREB family transcription factor ATF5 is highly expressed in human pancreatic carcinomas and pancreatic cancer cell lines, as compared with non-cancerous human pancreatic cells and tissues (Figure 1). We showed, using pancreatic cancer cell line SW1990, that ATF5 is markedly down-regulated when cells are treated with the anticancer drug paclitaxel and that interference with ATF5 function leads to apoptotic death of SW1990 cells (Figure 2). In revealing the mechanism of the pro-survival function of ATF5 in pancreatic cells, we not only found that BCL-2 is down-regulated in SW1990 cells in response to ATF5 interference, which is consistent with our previous findings of similar regulation in breast cancer and glioblastoma cells (8), but we also showed, to our knowledge for the first time here, that expression of BAX, which is a pro-apoptotic BCL-2 family member, is up-regulated in SW1990 cells in response to ATF5 interference (Figures 2, 4 and 5). In addition, we showed that ectopic expression of ATF5 completely reversed paclitaxel-induced transcriptional down-regulation of BCL-2 and up-regulation

of BAX in SW1990 cells (Figure 5). These data provide new insights into the function and mechanism of action of ATF5 in human pancreatic cells and the mechanism of cell death induced by paclitaxel, which will be useful for therapeutic intervention for pancreatic cancer.

Although ATF5 expression appears grossly elevated in human pancreatic carcinoma cells, as compared with normal pancreatic cells in both the intensity of staining (Figure 1E) and in the percentage of strongly stained cells (Figure 1F), not all cells in the tumors were positive for ATF5 staining. This is reminiscent of the staining patterns of ATF5 in glioblastomas and breast cancer tissues reported previously (7, 9). The reason for this heterogeneity is currently unknown.

Previous studies have demonstrated that BCL-2 and myeloid cell leukemia sequence 1 (MCL-1), the latter being another pro-survival BCL-2 family member, are regulated by ATF5 and mediate the pro-survival function of ATF5 in different types of cells (8, 18). Our work showing that interference with ATF5 function simultaneously down-regulates BCL-2 and up-regulates BAX expression in SW1990 pancreatic cancer cells reinforces the notion that BCL-2 family members are among the major targets of ATF5 that mediate its pro-survival function. Because cancer cells frequently overexpress BCL-2 and down-regulate BAX (19), and modulation of BAX and MCL-1 is often involved in tumor-selective apoptosis (20), our data establishing ATF5 regulation of BCL-2 and BAX in SW1990 cells point to a critical role of ATF5 in paclitaxel-induced cell death of SW1990 cells and reveal a potential role of ATF5 in the development of chemoresistance of pancreatic cancer cells to paclitaxel treatment. A previous report showing that paclitaxel regulates a subtype of T-cells by modulating the ratio of BCL-2 and BAX (21) seems to be in agreement with such a mechanism.

Paclitaxel belongs to the most successful group of anticancer drugs ever developed and has been US FDA-approved for the treatment of various types of cancers. Nevertheless, the development of resistance of cancer cells and the severe side-effects in patients require further understanding of its anticancer mechanism and development of additional drugs that can be used along with it. Our current study demonstrating that ATF5 plays a role in paclitaxel-induced cell death of SW1990 pancreatic cancer cells suggests that targeting ATF5 could be an effective means of increasing paclitaxel efficacy and combating paclitaxel resistance of cancer cells. Interestingly, paclitaxel-induced apoptosis has been associated with inhibition of autophagy in several types of cancer cells (22, 23), while ATF5 is known to suppress autophagy through the mammalian target of rapamycin (mTOR) pathway (24). Therefore, regulation of autophagy by ATF5 could be a major mechanism involved in cell death promoted by paclitaxel and in the development of resistance to paclitaxel by cancer cells.

In summary, our study demonstrates that ATF5 is highly expressed in pancreatic cancer cells and plays an essential role in pancreatic cancer cell survival. We also showed that BCL-2 and BAX are downstream targets of ATF5 that mediate the pro-survival function of ATF5 in SW1990 pancreatic cancer cells. Our study also revealed that ATF5 is down-regulated by paclitaxel in SW1990 pancreatic cancer cells and is involved in paclitaxel resistance of SW1990 pancreatic cancer cells. These results have significant implications for therapeutic intervention for pancreatic cancer and particularly for the treatment of pancreatic cancer with paclitaxel.

Competing Interests

Authors declare no competing interest.

Author Contributions

Conception and design of the project: B.W., D.Q., and D.X.L.; acquisition, analysis and interpretation of data: M.H., D.Q., L.L., L.Z., X.S., D.X.L.; drafting of the manuscript: B.W., D.Q., D.X.L.

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