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ORIGINAL ARTICLE

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Overexpression of Bax sensitizes human pancreatic cancer cells to apoptosis induced by chemotherapeutic agents

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Abstract *Purpose:* Bax plays an important role in the regulation of apoptosis induced by chemotherapy and other stimuli. We therefore investigated the role of Bax in drug-induced apoptosis in human pancreatic cancer cells. *Method:* A tetracycline-inducible retroviral expression vector bearing the human bax-α gene was constructed. ASPC-1 cells were stably infected with this vector. The sensitivity of the transformed cell line to gemcitabine and 5-Fu was assessed. *Results:* Western blots revealed that Bax expression was enhanced in these cells by the tetracycline analogue doxycycline. Enhanced expression of Bax itself did not inhibit the growth rate of infected cells and did not influence expression of Bcl-2 and Bcl-x_L. However, it significantly increased the sensitivity of cells to the chemotherapeutic drugs gemcitabine and 5-Fu. These drugs also activated caspase-8 and caspase-3 by up to ninefold. Caspase activation and/or an imbalance in Bax and Bcl-2/Bcl-x_L expression may be the reasons for the augmentation of cytotoxicity by these drugs. *Conclusions:* The findings suggest that enhanced Bax expression may have therapeutic application in enhancing the efficacy of chemotherapy in pancreatic cancers.

Keywords Bax · Apoptosis · Pancreatic cancer · Chemotherapy · Gemcitabine

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Introduction

Human pancreatic cancer is a dismal disease with a poor prognosis and a short patient survival of approximately 4–6 months after diagnosis. It is currently the fourth or fifth most common cause of cancer-related death in Western countries, with over 60,000 deaths in both the European Community and the United States each year [1]. Because of difficulties in early diagnosis, the aggressive character of the tumors, and the lack of effective systemic therapies, over 95% of patients with pancreatic cancer die within 5 years of diagnosis. Surgical therapy for pancreatic cancer is often not curative. In addition, resistance to chemotherapy, as well as to other therapeutic strategies such as immunotherapy or radiotherapy are major obstacles to effective treatment and improvement of prognosis. 5-Fluorouracil (5-Fu) and gemcitabine are currently the mainstays of the treatment of this disease among clinical chemotherapeutic agents. However, the response rate for these drugs is only around 30% [2]. Since 5-Fu and gemcitabine act through induction of programmed cell death, or apoptosis, more effective treatment modalities may be devised with a thorough understanding of the underlying mechanisms.

Apoptosis is a cell death process that plays a critical role in mammalian development and tissue homeostasis. It has now become clear that apoptosis is also the mechanism of tumor cell death in response to a variety of chemotherapeutic agents. The Bcl-2 family of proteins plays a key role in the regulation of apoptosis. Some members of this family, including Bax, Bak, Bid, and Bik, function as proapoptotic factors, and others, including Bcl-2, Bcl-x_L, Mcl-1 and A1, function as antiapoptotic proteins. Members of the Bcl-2 family share regions of homology called BH1 and BH2, and a hydrophobic C-terminus which serves to anchor the proteins to the membrane. Two additional conserved domains, BH3 and BH4, are found only among some members of the Bcl-2 family. These domains are involved in homo- and heterodimerization of Bcl-2-like

proteins. While there are different ways in which Bcl-2 members may function, the ratio of heterodimerization between anti- and proapoptotic factors has been suggested to be a pivotal decision point for death or survival [3, 4, 5, 6].

Bax, a proapoptotic factor, contains BH1 and BH2 domains, as well as the BH3 domain which is important for heterodimerization with Bcl-2 and Bcl-x_L factors. Bax mRNA has several different splice variants, including bax-α, bax-β, bax-γ and bax-δ. The function of bax-β, bax-γ and bax-δ remain unclear. Bax-α encodes a 21-kDa protein which is capable of promoting apoptosis directly through its effects on mitochondrial function, or indirectly through lowering the apoptotic threshold in response to certain chemotherapy agents or ionizing radiation [7, 8]. Overexpression of the bax gene has been found to induce apoptotic death in glioblastoma cells, lung cancer cells, and ovarian cancer cells [8, 9, 10, 11]. Enhanced expression of bax-α may thus reduce the thresholds for apoptosis in many different tumor cell types and sensitize them to treatment [12, 13]. As a corollary to this, it has been observed that reduced Bax expression is associated with a poor response to chemotherapy and shorter survival in patients with breast cancers and colorectal carcinomas [14, 15].

Chemotherapy with gemcitabine and 5-Fu may induce apoptosis in cancer cells by regulating expression of members of the Bcl-2 family [16, 17]. Caspases can also be influenced by some chemotherapeutic agents [18, 19]. In the present study, human pancreatic cancer cells were infected with a recombinant retroviral vector expressing Bax under the control of a tetracycline-inducible promoter. Bax overexpression did not affect expression of Bcl-2 and Bcl-x_L or the activity of caspase-8 and caspase-3. However, it sensitized the cells to drug-induced apoptosis, opening new avenues to chemotherapeutic treatment of pancreatic tumors.

Materials and methods

Cells and culture conditions

The human pancreatic cancer cell line ASPC-1 (ATCC, Rockville, Md.) was incubated in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 m*M* of L-glutamine, 100 U/ml penicillin, and 100 µg/ml of streptomycin at 37°C in a humidified atmosphere containing 5% CO_2. Phoenix amphotropic cells derived from 293 cells (Nolan Laboratory, Stanford University, Stanford, Calif.) as a packaging cell line were maintained in complete DMEM under the same conditions as above. A tetracycline system-approved FBS (Clontech, Basel, Switzerland) was utilized when cells were infected with pRetro vectors.

Construction of recombinant retroviral vector and transfection

A full-length human bax-α cDNA fragment (0.6 kb) was cloned by RT-PCR from normal human kidney tissue into pGEM-T Easy vector (Promega, Catalys, Wallisellen, Switzerland). After DNA sequence confirmation, the bax-α cDNA was excised and inserted into the inducible expression retroviral vector pRetro-ON (Clontech) at the NotI site to generate pRetro-bax. This vector has a tetracycline-regulated transactivation system, which can be activated by tetracycline or doxycycline. Sense orientation of the bax-α cDNA insert in the retroviral vector was verified by restriction digestion and DNA sequencing.

pRetro-bax or vehicle control vector (pV) were transfected into packaging cells or Phoenix amphotropic cells by the calcium phosphate precipitation technique. Briefly, 2.5×10^6 packaging cells were seeded in a 10-cm culture dish overnight to reach 70–80% confluence. About 5 min prior to transfection, 25 µ*M* chloroquine was added to the medium and 10 µg of retroviral plasmid DNA per plate was added to the medium dropwise. After 24 h of incubation, the medium was replaced with fresh DMEM. Supernatant containing the virus was collected 48 h posttransfection, centrifuged, filtered, and added to plates containing ASPC-1 cells in the presence of 6 µg/ml of polybrene (Sigma, Luzerne, Switzerland). The medium was changed 24 h later, and the infected cells cultured for another 24 h. The cells were then cultured for 2 weeks in the presence of 0.8 µg/ml of puromycin (Clontech) to select stably infected clones. Individual puromycin-resistant cells were cloned, expanded, and stored frozen in liquid nitrogen.

Inducible expression of Bax

ASPC-1 cells stably infected with pRetro-bax were treated with 1 µg/ml of doxycycline (Sigma) for different time periods. Cells were then harvested and lysed in lysis buffer (1% Nonidet P-40, 20 m*M* Tris-Cl, pH 8, 150 m*M* NaCl) containing protease inhibitors (Roche Molecular Biochemicals, Rotkreuz, Switzerland). Expression of Bax protein was analyzed by Western blotting as described below.

Cellular proliferation and cytotoxicity assay

To measure the effect of Bax overexpression on growth, cultures of ASPC-1 cells alone, or stably infected with pRetro-bax or pV were grown in the presence of 1 µg/ml of doxycycline for 48 h. Cells from these precultures were seeded at 3×10^3 cells/well into 96-well plates and allowed to attach for 12 h. Growth was then followed by determining the numbers of living cells with the SRB method [20]. The results are expressed as the percentage of the viable cell count at the start of the experiment.

For cytotoxicity assays, 5×10^3 cells/well were plated in 96-well plates and incubated overnight. The medium was then replaced with fresh medium containing different concentrations of gemcitabine (Eli Lilly, Geneva, Switzerland) or 5-Fu (Sigma) and incubation continued for 72 h. Doxycycline (1 µg/ml) was present throughout the experiment. The viability of the cell lines was determined with the SRB method. Percent survival was defined as $100\times(T–T_0)/(C–T_0)$ when $(T–T_0)=0$. When the T value was less than T_0, cell killing had occurred and the cytotoxic activity was determined as $(T–T_0)/T_0$ expressed as a percentage. T is the OD_{540} value at the time-point in question and T_0 the OD_{540} value at the moment of drug addition. C indicates the OD_{540} value of the untreated control group at the time-point in question. IC_{50} values were calculated from three independent experiments.

Analysis of apoptosis by DAPI staining

For apoptotic analysis, both adherent and non-adherent cells were harvested after treatment with gemcitabine or 5-Fu and inspected for cellular morphology by microscopy. DNA damage characteristic of apoptosis was identified by staining with 4′,6-diamidino-2-phenylindole (DAPI). Briefly, cells were harvested, washed, and fixed in 3.7% formaldehyde at room temperature for 15 min. After treatment with RNase A, samples were stained with 1 µg/ml of DAPI (Sigma) in PBS for 15 min at room temperature, rinsed in PBS, and analyzed using a Leica DM RXE fluorescence microscope (Leica, Wetzlar, Germany). Apoptotic cells were defined by

the condensation of nuclear chromatin, fragmentation, or margination to the nuclear membrane.

Western blotting

Cells were lysed in 250 m*M* NaCl, 50 m*M* Hepes, pH 7, 0.1% Nonidet P-40,1× proteinase inhibitor cocktail (Roche, Basel, Switzerland). Protein concentration was determined with the BCA protein assay (Pierce Chemical Company, Rockford, Ill.). Equal amounts of cellular protein (20 μg/sample) were electrophoresed on 12% SDS-polyacrylamide gels and transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Zurich, Switzerland). Depending on the experiment, the membranes were first incubated with the following polyclonal rabbit antibodies: anti-human Bax, anti-human Bcl-2, and anti-human Bcl-x_L (Santa Cruz Biotechnology, Santa Cruz, Calif.). For signal detection, the blots were then incubated with peroxidase-coupled goat anti-rabbit immunoglobulin (Amersham Pharmacia Biotech). Enhanced chemiluminescence (ECL, Amersham Pharmacia Biotech) reagents were used to detect the signals according to the manufacturer's instructions.

Caspase-8 and caspase-3 activity assays

Caspase-8 and caspase-3 activities were measured using an Apo-Alert colorimetric assay kit (Clontech) according to the manufacturer's instructions. To induce apoptosis, cells were treated with 200 n*M* gemcitabine or 10 μ*M* 5-Fu for 24 h. Subsequently, 2×10^6 cells were lysed and incubated with the substrates for 1 h at 37°C in reaction buffer. The absorbance of samples was then measured spectrophotometrically at a wavelength of 405 nm. The results are expressed as the fold induction of treated samples compared to untreated controls.

Results

Inducible expression of Bax in infected ASPC-1 cell lines

To overexpress Bax in a controlled fashion, the bax-α gene was cloned into a retroviral expression vector under the control of a tetracycline promoter. ASPC-1 cells were stably infected with this vector, called pRetro-bax. Expression of Bax could be induced by the addition of 1 μg/ml of doxycycline, a tetracycline analogue. Figure 1 shows a Western blot with the relative expression levels of Bax at different times after induction. More than fourfold induction of Bax was observed 24 to 48 h after induction with doxycycline. Growth experiments were performed to test whether transfection and overexpression of Bax affected growth rate. Untransfected control cells, cells infected with the control vector pV, or cells infected with pRetro-bax exhibited essentially the same growth rate and final cell density (not shown). Thus, enhanced expression of Bax did not inhibit host cell growth by itself, which has also been documented for other cancer cell lines [13].

Induction of Bax expression sensitizes pancreatic cancer cells to chemotherapeutic agents

Due to the important role of Bax in apoptosis, a change in its expression level is likely to affect apoptosis. Indeed,

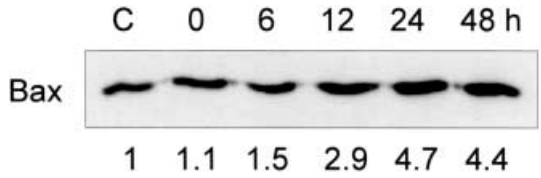


Fig. 1. Induction of Bax expression in ASPC-1 cells infected with pRetro-bax. Cells infected with pRetro-bax were cultured in the presence of 1 μg/ml of doxycycline for the times indicated. Bax expression was analyzed by Western blotting as described in Materials and methods. The expression level of Bax relative to uninfected control cells (*lane C*) is indicated below the figure

overexpression of Bax in ASPC-1 cells increased their susceptibility to gemcitabine and 5-Fu. Figure 2 shows the decrease in cell viability as a function of drug concentration following a 72-h treatment. pRetro-bax-infected ASPC-1 cells were significantly more sensitive to gemcitabine and 5-Fu than both control cell lines, pV-infected and uninfected parent cells ($P = 0.01$ for 10 n*M*

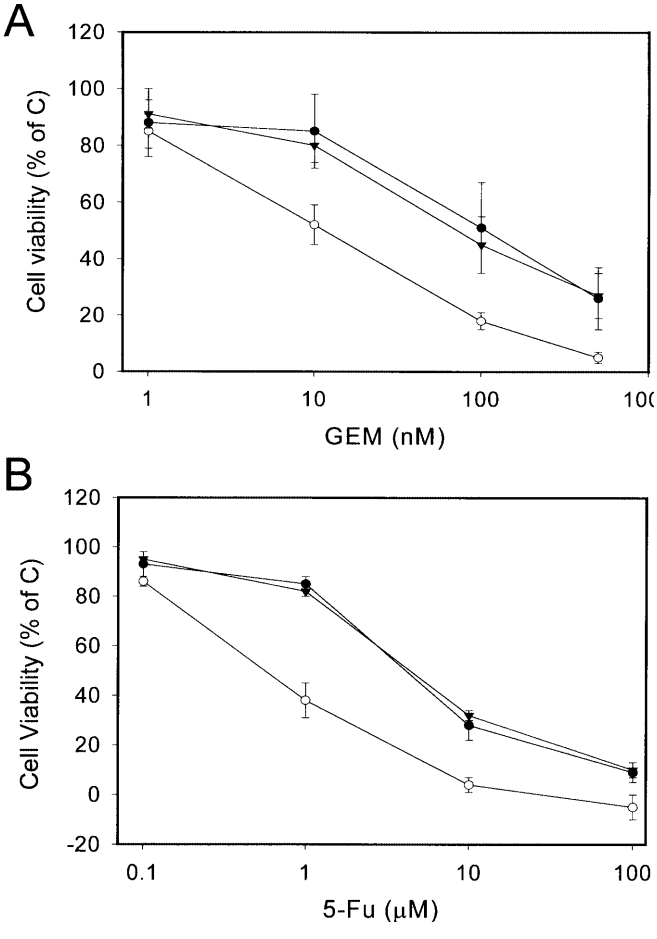


Fig. 2A, B. Cytotoxicity of gemcitabine (*GEM*) and 5-Fu in ASPC-1 cells (*solid circles*), ASPC-1 cells infected with pRetro-bax (*open circles*), or with pV vector (*solid triangles*). Cells were treated with gemcitabine or 5-Fu at the indicated concentrations for 72 h. Percent survival was determined with the SRB assay. Each point represents the mean ± SD (*error bars*) from three independent experiments

Table 1. IC_{50} values for gemcitabine and Fu-5 in different cell lines. The IC_{50} values were determined after 72 h of exposure to the drugs and are defined as the concentration causing 50% growth inhibition in treated cells compared to that in control cells. Values are means ± SD from at least three independent experiments

Cell line	Gemcitabine (n*M*)	5-Fu (μ*M*)
ASPC-1	167 ± 14	5.4 ± 0.7
pV-ASPC-1	140 ± 17	5.8 ± 0.8
pRetro-bax-ASPC-1	10.3 ± 1.4	0.7 ± 0.2

gemcitabine, $P = 0.0003$ for 1 μ*M* 5-Fu; Student's *t*-test). Lower survival of Bax-overexpressing cells was most pronounced at low drug concentrations. No significant difference was observed between pRetro-v and parent ASPC-1 cell lines. Specifically, the mean IC_{50} for gemcitabine in pRetro-bax cells was 10.3 n*M*, compared to 140 n*M* for control cells (Table 1). This corresponds to a 14-fold increase in chemosensitivity. Similarly, in 5-Fu-treated cells a 7.7-fold increase in chemosensitivity was observed for Bax-overexpressing cells compared to controls.

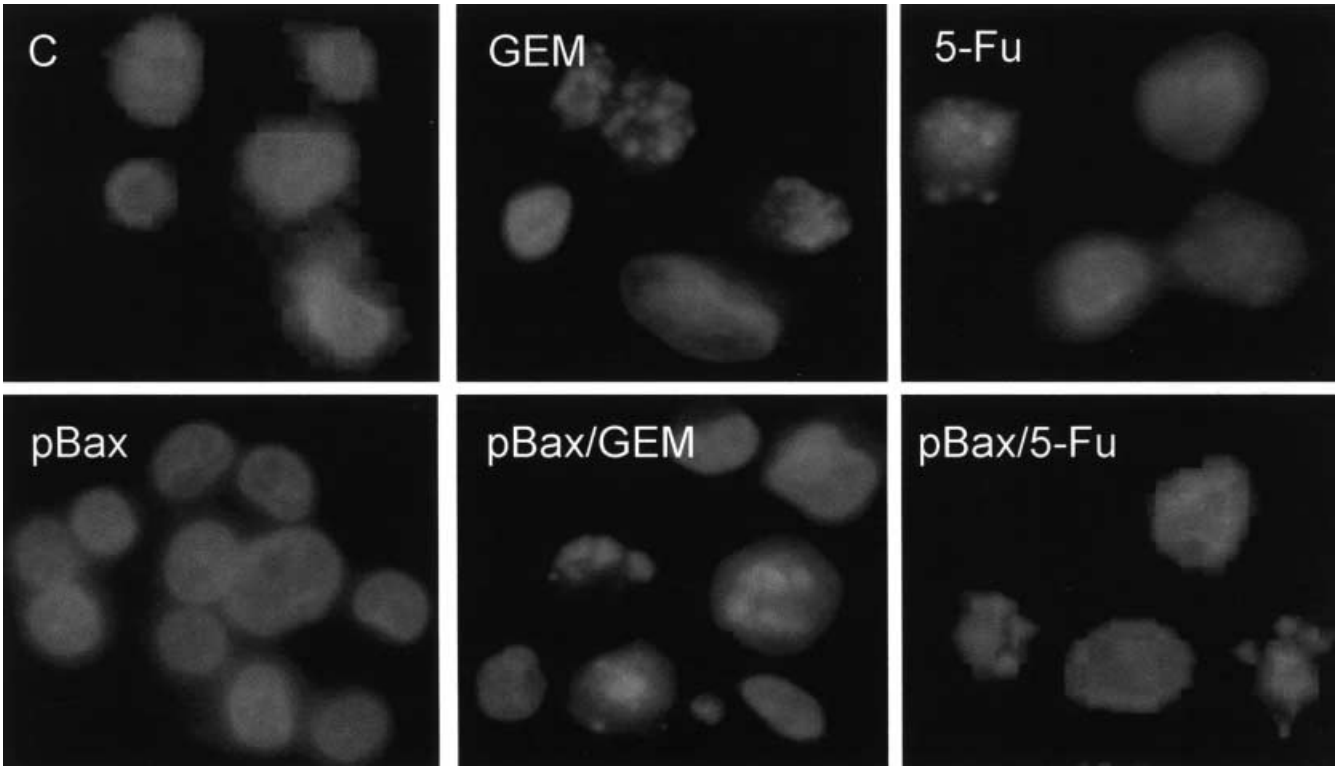
Apoptosis observation

Treatment of infected ASPC-1 cells with gemcitabine and 5-Fu resulted in morphological changes typical of apoptosis, such as cell shrinkage, rounding and detachment of the cells from the plate, as observed by phase-contrast microscopy (not shown). DAPI staining was used to visualize nucleosomal DNA damage. Nuclear fragmentation and apoptotic bodies were clearly apparent in pRetro-bax-infected cells as well as in control cells treated with gemcitabine and 5-Fu (Fig. 3). These findings show that infected as well as non-infected ASPC-1 cells died by apoptosis following treatment with gemcitabine or 5-Fu.

Expression of Bcl-2 and Bcl-x_L in ASPC-1 cells

Bcl-2 and Bcl-x_L are functionally closely related to Bax and changes in their expression levels could lead to the observed increase in cytotoxicity of gemcitabine and 5-Fu. Bcl-2 and Bcl-x_L expression was thus tested in Bax-overexpressing ASPC-1 cells by Western blotting. There was no observable change in the expression of either Bcl-2 or Bcl-x_L in any of the cell lines tested (Fig. 4A). Clearly, overexpression of Bax did not affect the expression levels of Bcl-2 or Bcl-x_L. In addition, the expression levels of Bax, Bcl-2, and Bcl-x_L were the same in cells treated with gemcitabine or with 5-Fu, compared to untreated control cells (Fig. 4B). This suggests that

Fig. 3. DAPI staining for detection of apoptotic cells. Parental and pRetro-bax-infected ASPC-1 cells were treated with gemcitabine or 5-Fu respectively for 48 h and then fixed and stained with DAPI. Morphological changes were visualized by fluorescence microscopy (*C* parental control cells without drug treatment, *GEM* ASPC-1 cells treated with gemcitabine, *5-Fu* ASPC-1 cells treated with 5-Fu, *Bax* pRetro-bax-infected ASPC-1 cells without drug treatment, *pBax/GEM* pRetro-bax-infected ASPC-1 cells treated with gemcitabine, *pBax/5-Fu* pRetro-bax-infected ASPC-1 cells treated with 5-Fu)



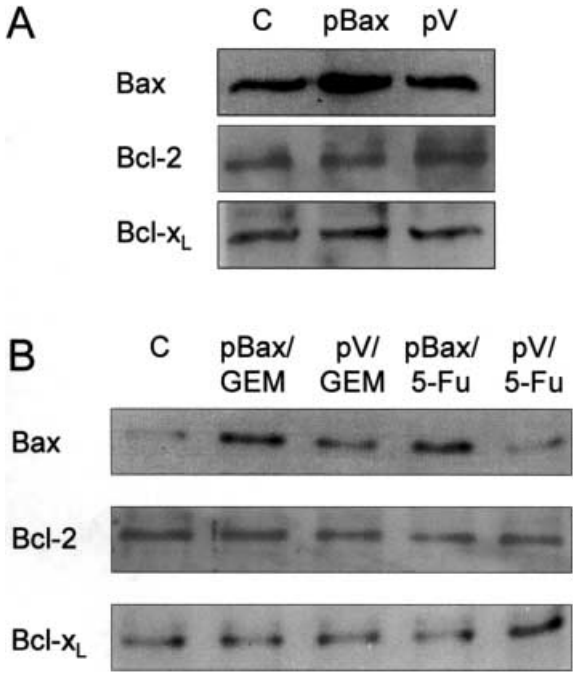


Fig. 4A, B. Effect of Bax overexpression and chemotherapeutic agents on expressions of other proteins of the Bcl-2 family. Infected cells were induced with doxycycline for 48 h (**A**). Expressions of Bax, Bcl-2 and Bcl-x_L were analyzed by Western blotting (*C* parental control, *pBax* cells infected with pRetro-bax, *pV* cells infected with pV control vector). These cells were then treated with gemcitabine (*GEM*) or 5-Fu (*5-Fu*) for 48 h (**B**)

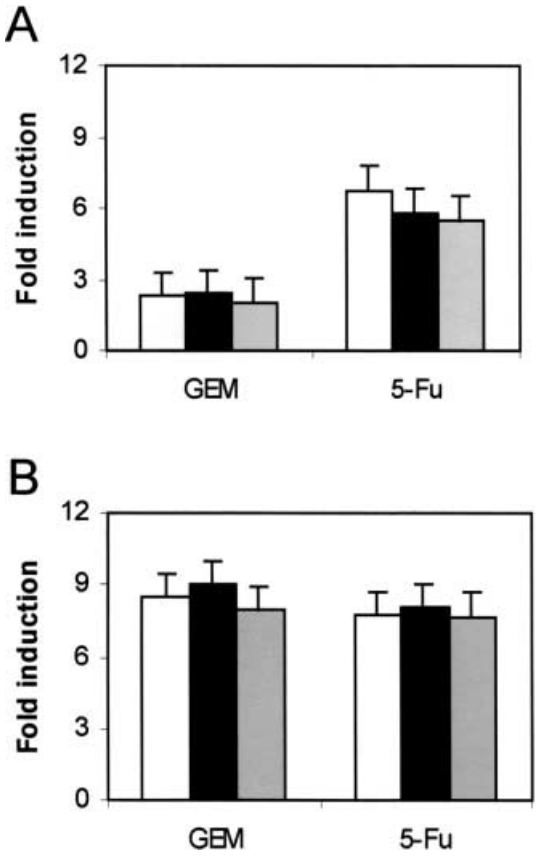


Fig. 5A, B. Caspase-8 and caspase-3 activities. ASPC-1 cells alone (*open columns*), or infected with pRetro-bax (*black columns*) or pV control vector (*shaded columns*) were treated with gemcitabine or 5-Fu for 24 h. Caspase-8 activity (**A**) and caspase-3 activity (**B**) were then measured colorimetrically as described in Materials and methods. The results are expressed as fold induction in gemicitabine-/5-Fu-treated cells compared to untreated cells. The data shown are means ± SD of three independent experiments

mechanisms other than changes in expression levels of Bcl-2 and Bcl-x_L are responsible for the observed sensitization of ASPC-1 cells by Bax overexpression.

Activation of caspase-8 and caspase-3 activity by gemcitabine and 5-Fu

It has previously been observed that chemotherapeutic agents can activate caspases [18, 19]. To gain insight into the relationship between enhanced cytotoxicity and caspase activities, caspase-8 and caspase-3 were measured in Bax-overexpressing cells treated with gemcitabine or 5-Fu (Fig. 5). 5-Fu, but not gemcitabine, augmented caspase-8 activity in all cell lines. Caspase-3, a critical downstream caspase member, was strongly activated by both gemcitabine and 5-Fu treatment. Caspase-8 and caspase-3 stimulation was the same in all three cell lines tested and thus was not influenced by the overexpression of Bax. These findings suggest that sensitization of cells by Bax overexpression does not proceed via caspase-8 and/or caspase-3 activation.

Discussion

In the present study we constructed a retroviral vector bearing the human bax-α gene under the control of a tetracycline-inducible promoter. In human ASPC-1 pancreatic cancer cells infected with this vector, the bax-α gene was effectively activated by the tetracycline analogue doxycycline leading to enhanced cytotoxicity of the chemotherapeutic agents gemcitabine and 5-Fu. This suggests that Bax overexpression could have therapeutic utility.

Bax, as a universal mediator of apoptotic cell death in the prokaryotic unicellular organism, *Escherichia coli*, and mammalian cells, has received considerable attention in recent years [21]. Upregulation of Bax, induced by transfection with the p53 gene or by introduction of bax-α cDNA, has been found to induce apoptosis in some, but not all, cancer cell lines [10, 22, 23]. The fact that the effect is not general suggests that Bax plays different roles in the induction of apoptosis in different cancer cells. Recent investigations have also shown that the sensitivity of some cancer cells to chemotherapeutic agents or irradiation therapy can be significantly increased by enhanced expression of Bax [12, 13, 24, 25, 26, 27, 28], but again, the effect does not appear to apply to all chemotherapeutic agents [29].

It is well known that pancreatic cancer resists chemotherapy or other conventional treatments. A better understanding of pancreatic cancer biology is urgently needed to develop novel treatment strategies to improve the survival of patients. In the study reported here we showed that the human bax-α gene under the control of a tetracycline-inducible promoter could be effectively overexpressed in ASPC-1 pancreatic cancer cells. Bax overexpression alone did not cause apoptosis or a significant change in growth rate of this cell line. Similar observations have been made in other cancer cells, such as the breast cancer cell line MCF-7 [13], SW626 ovarian epithelial cancer cells [30], and glioma cell lines with a mutant p53 genotype [31]. It remains unclear why Bax overexpression by itself can trigger apoptosis in some cancer cell lines, but not in others. Conceivably, different cell lines have different thresholds for Bax-induced apoptosis [29, 32]. Also, the balance of expression between Bax and Bcl-2 or Bcl-x_L is a critical index for the occurrence of apoptosis in some cell types. In our experiments, the enhancement of Bax expression did not influence the expression of either Bcl-2 or Bcl-x_L in ASPC-1 cells. Similarly, treatment with gemcitabine or 5-Fu did not change the level of expression of Bax, Bcl-2, and Bcl-x_L. The imbalance of expression between Bax and Bcl-2/Bcl-x_L in cells overexpressing Bax may thus have triggered gemcitabine- or 5-Fu-induced apoptosis in our system.

Apoptosis has been shown to be a significant mode of cell death induced by radiation or cytotoxic drugs, including gemcitabine and 5-Fu [33, 34, 35], in a variety of cancer types [2]. Proteins of the Bcl-2 family seem to be centrally involved in controlling such therapy-induced apoptosis. Caspases have also been found to be activated, but their role in the process has not been fully elucidated [18, 18, 36, 36, 37]. In our experiments both caspase-8 and caspase-3 were activated by 5-Fu treatment, while only caspase-3 was activated by gemcitabine. Caspase activation in ASPC-1 pancreatic cancer cells by gemcitabine has not previously been reported. Since there was no significant differences between Bax-overexpressing and control cells, caspase activation was apparently independent of Bax expression in these cells. The mechanism or route by which drug-induced caspase activation proceeds is of great interest and requires further investigation.

In conclusion, our study shows that overexpression of Bax in ASPC-1 pancreatic cancer cells markedly increased the sensitivity of cells to gemcitabine and 5-Fu in vitro, suggesting that Bax plays a key role in modulating apoptotic cell death in response to these anticancer drugs. Bax overexpression may thus have therapeutic application in the enhancement of chemotherapy in human pancreatic cancer.

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References

1. Parker SL, Tong T, Bolden S, Wingo PA (1997) Cancer statistics, 1997. CA Cancer J Clin 47:5–27
2. Bold RJ, Chandra J, McConkey DJ (1999) Gemcitabine-induced programmed cell death (apoptosis) of human pancreatic carcinoma is determined by Bcl-2 content. Ann Surg Oncol 6:279–285
3. Hengartner MO (2000) The biochemistry of apoptosis. Nature 407:770–776
4. Lutz RJ (2000) Role of the BH3 (Bcl-2 homology 3) domain in the regulation of apoptosis and Bcl-2-related proteins. Biochem Soc Trans 28:51–56
5. Reed JC (1998) Bcl-2 family proteins. Oncogene 17:3225–3236
6. Weinmann P, Bommert K, Mapara MY, Dorken B, Bargou RC (1997) Overexpression of the death-promoting gene bax-alpha sensitizes human BL-41 Burkitt lymphoma cells for surface IgM-mediated apoptosis. Eur J Immunol 27:2466–2468
7. Sakakura C, Sweeney EA, Shirahama T, Igarashi Y, Hakomori S, Tsujimoto H, Imanishi T, Ohgaki M, Yamazaki J, Hagiwara A, Sawai K, Yamaguchi T, Takahashi T (1997) Overexpression of bax enhances the radiation sensitivity in human breast cancer cells. Surg Today 27:90–93
8. Tai YT, Strobel T, Kufe D, Cannistra SA (1999) In vivo cytotoxicity of ovarian cancer cells through tumor-selective expression of the BAX gene. Cancer Res 59:2121–2126
9. De Feudis P, Vignati S, Rossi C, Mincioni T, Giavazzi R, D'Incalci M, Broggini M (2000) Driving p53 response to Bax activation greatly enhances sensitivity to taxol by inducing massive apoptosis. Neoplasia 2:202–207
10. Coll JL, Negoescu A, Louis N, Sachs L, Tenaud C, Girardot V, Demeinex B, Brambilla E, Brambilla C, Favrot M (1998) Antitumor activity of bax and p53 naked gene transfer in lung cancer: in vitro and in vivo analysis. Hum Gene Ther 20:2063–2074
11. Shinoura N, Yoshida Y, Asai A, Kirino T, Hamada H (1999) Relative level of expression of Bax and Bcl-XL determines the cellular fate of apoptosis/necrosis induced by the overexpression of Bax. Oncogene 18:5703–5713
12. Arafat WO, Gomez-Navarro J, Xiang J, Barnes MN, Mahasreshti P, Alvarez RD, Siegal GP, Badib AO, Buchsbaum D, Curiel DT, Stackhouse MA (2000) An adenovirus encoding proapoptotic Bax induces apoptosis and enhances the radiation effect in human ovarian cancer. Mol Ther 1:545–554
13. Wagener C, Bargou RC, Daniel PT, Bommert K, Mapara MY, Royer HD, Dorken B (1996) Induction of the death-promoting gene bax-alpha sensitizes cultured breast-cancer cells to drug-induced apoptosis. Int J Cancer 67:138–141
14. Krajewski S, Blomqvist C, Franssila K, Krajewska M, Wasenius VM, Niskanen E, Nordling S, Reed JC (1995) Reduced expression of proapoptotic gene BAX is associated with poor response rates to combination chemotherapy and shorter survival in women with metastatic breast adenocarcinoma. Cancer Res 55:4471–4478
15. Ogura E, Senzaki H, Yamamoto D, Yoshida R, Takada H, Hioki K, Tsubura A (1999) Prognostic significance of Bcl-2, Bcl-xL/S, Bax and Bak expressions in colorectal carcinomas. Oncol Rep 6:365–369
16. Li Y, Singh B, Ali N, Sarkar FH (1999) Induction of growth inhibition and apoptosis in pancreatic cancer cells by auristatin-PE and gemcitabine. Int J Mol Med 3:647–653
17. Sugamura K, Makino M, Shirai H, Kimura O, Maeta M, Itoh H, Kaibara N (1997) Enhanced induction of apoptosis of human gastric carcinoma cells after preoperative treatment with 5-fluorouracil. Cancer 79:12–17
18. Keane MM, Ettenberg SA, Nau MM, Russell EK, Lipkowitz S (1999) Chemotherapy augments TRAIL-induced apoptosis in breast cell lines. Cancer Res 59:734–741
19. Kim DG, Chung HT, Gwak HS, Paek SH, Jung HW, Han DH (2000) Gamma knife radiosurgery for brain metastases:

prognostic factors for survival and local control. J Neurosurg 93 [Suppl 3]:23–29
20. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 82:1107–1112
21. Ishibashi Y, Nishimaki K, Asoh S, Nanbu-Wakao R, Yamada T, Ohta S (1998) Pore formation domain of human pro-apoptotic Bax induces mammalian apoptosis as well as bacterial death without antagonizing anti-apoptotic factors. Biochem Biophys Res Commun 243:609–616
22. Pearson AS, Spitz FR, Swisher SG, Kataoka M, Sarkiss MG, Meyn RE, McDonnell TJ, Cristiano RJ, Roth JA (2000) Up-regulation of the proapoptotic mediators Bax and Bak after adenovirus-mediated p53 gene transfer in lung cancer cells. Clin Cancer Res 6:887–890
23. Ramondetta L, Mills GB, Burke TW, Wolf JK (2000) Adenovirus-mediated expression of p53 or p21 in a papillary serous endometrial carcinoma cell line (SPEC-2) results in both growth inhibition and apoptotic cell death: potential application of gene therapy to endometrial cancer. Clin Cancer Res 6:278–284
24. Kim HR, Luo Y, Li G, Kessel D (1999) Enhanced apoptotic response to photodynamic therapy after bcl-2 transfection. Cancer Res 59:3429–3432
25. Guo B, Cao S, Toth K, Azrak RG, Rustum YM (2000) Overexpression of Bax enhances antitumor activity of chemotherapeutic agents in human head and neck squamous cell carcinoma. Clin Cancer Res 6:718–724
26. Lee A, DeJong G, Guo J, Bu X, Jia WW (2000) Bax expressed from a herpes viral vector enhances the efficacy of N,N′-bis(2-hydroxyethyl)-N-nitrosourea treatment in a rat glioma model. Cancer Gene Ther 7:1113–1119
27. Shinoura N, Yoshida Y, Asai A, Kirino T, Hamada H (2000) Adenovirus-mediated transfer of p53 and Fas ligand drastically enhances apoptosis in gliomas. Cancer Gene Ther 7:732–738
28. Xiang J, Gomez-Navarro J, Arafat W, Liu B, Barker SD, Alvarez RD, Siegal GP, Curiel DT (2000) Pro-apoptotic treatment with an adenovirus encoding Bax enhances the effect of chemotherapy in ovarian cancer. J Gene Med 2:97–106
29. Kobayashi T, Ruan S, Clodi K, Kliche KO, Shiku H, Andreeff M, Zhang W (1998) Overexpression of Bax gene sensitizes K562 erythroleukemia cells to apoptosis induced by selective chemotherapeutic agents. Oncogene 16:1587–1591
30. Strobel T, Kraeft SK, Chen LB, Cannistra SA (1998) BAX expression is associated with enhanced intracellular accumulation of paclitaxel: a novel role for BAX during chemotherapy-induced cell death. Cancer Res 58:4776–4781
31. Vogelbaum MA, Tong JX, Perugu R, Gutmann DH, Rich KM (1999) Overexpression of bax in human glioma cell lines. J Neurosurg 91:483–489
32. Nishii K, Gibbons DL, Titley I, Papworth D, Goodhead DT, Greaves M (1998) Regulation of the apoptotic response to radiation damage in B cell development. Cell Death Differ 5:77–86
33. Oki E, Sakaguchi Y, Toh Y, Oda S, Maehara Y, Yamamoto N, Sugimachi K (1998) Induction of apoptosis in human tumour xenografts after oral administration of uracil and tegafur to nude mice bearing tumours. Br J Cancer 78:625–630
34. Tolis C, Peters GJ, Ferreira CG, Pinedo HM, Giaccone G (1999) Cell cycle disturbances and apoptosis induced by topotecan and gemcitabine on human lung cancer cell lines. Eur J Cancer 35:796–807
35. Tong D, Poot M, Hu D, Oda D (2000) 5-Fluorouracil-induced apoptosis in cultured oral cancer cells. Oral Oncol 36:236–241
36. Ferreira CG, Span SW, Peters GJ, Kruyt FA, Giaccone G (2000) Chemotherapy triggers apoptosis in a caspase-8-dependent and mitochondria-controlled manner in the non-small cell lung cancer cell line NCI-H460. Cancer Res 60:7133–7141
37. Ohtani T, Hatori M, Ito H, Takizawa K, Kamijo R, Nagumo M (2000) Involvement of caspases in 5-FU induced apoptosis in an oral cancer cell line. Anticancer Res 20:3117–3121