

## Doxycycline Induces Apoptosis in PANC-1 Pancreatic Cancer Cells

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**Abstract.** *Background:* Tetracyclines such as doxycycline are reported to possess cytotoxic activity against mammalian tumor cells, but the mechanism of their effects on cell proliferation remains unclear. *Materials and Methods:* The antitumor effect of doxycycline was investigated in human pancreatic cancer cell line, PANC-1. We also investigated the effect of doxycycline on expression of a potent proangiogenic factor, interleukin (IL)-8. *Results:* In excess of 20 µg/ml, cytotoxic effects of doxycycline were accompanied by G<sub>1</sub>-S cell cycle arrest and DNA fragmentation in PANC-1 cells. Doxycycline consistently activated transcription of p53, p21 and Fas/FasL-cascade-related genes, while reducing the expression of Bcl-xL and Mcl-1. Doxycycline (5 µg/ml) below the cytotoxic level suppressed endogenous and paclitaxel-induced IL-8 expression. In the mouse xenograft model, doxycycline treatment was shown to suppress tumor growth by 80%. *Conclusion:* These data suggest that doxycycline exerts its antitumor effect by activating proapoptotic genes, inhibiting IL-8 expression, and suppressing antiapoptotic genes.

Tetracyclines possess cytotoxic activity against several tumor cells in addition to their antimicrobial activities, for which the possibility of their use as anticancer agents is currently investigated (1, 2). The non-antibiotic property of tetracyclines was first discovered by their ability to inhibit various zinc-dependent enzymes of the matrix metalloproteinase (MMP) family, including MMP-1, -2, -3, -9, -13 and gelatinases, via multiple mechanisms (3, 4). Doxycycline, minocycline and newly developed chemically modified derivatives that do not

have antimicrobial but retain anti-MMP activities are included in this family (5, 6). Recently, the ability of tetracyclines to induce apoptosis was reported in osteosarcoma, prostatic cancer cells, and Jurkat T lymphocytes (1, 7, 8).

Doxycycline, a semi-synthetic tetracycline, characterized by the presence of a dimethylamino group in C4 and a hydroxy group in C5, can inhibit MMP expression and cell proliferation in a number of cultured cell lines and animal models (1, 7, 11). Doxycycline-mediated antiproliferative activity may be associated with the regulation of cell proliferation as well as its ability to inhibit MMP activity (7, 9). Furthermore, doxycycline induces apoptosis, decreases the invasion of tumor cells, and suppresses the metastatic potential in breast cancer and melanoma cell lines at concentrations of 5-10 µg/ml (9, 12). Thus, doxycycline has been evaluated in preclinical cancer models and entered in early clinical trials in patients with malignant diseases (13).

Pancreatic adenocarcinoma is the fifth leading cause of cancer death in North America (14), for which surgery remains the sole curative option. Unfortunately, fewer than 15% of patients with pancreatic cancer are amenable to complete surgical resection, and the recurrence rate even after a successful pancreatectomy remains high (15). Therefore, the development of more effective treatment is urgently needed to improve outcome. To investigate the therapeutic efficacy of doxycycline in pancreatic cancer, we have studied the mechanism by which doxycycline induces apoptosis. Moreover, a tumor-bearing mouse xenograft model was used to determine whether doxycycline inhibits tumor growth *in vivo*.

### Materials and Methods

**Reagents.** Oligodeoxyribonucleotides, doxycycline, tumor necrosis factor α (TNF-α) and paclitaxel were purchased from SIGMA (The Woodlands, TX, USA) and final concentration of 10 ng/ml for TNF-α or 30 µM for paclitaxel were used for the cell treatment. Stock solution of doxycycline was prepared freshly at 8 mg/ml in phosphate-buffered saline (PBS), pH 7.4 (pH was adjusted with 1 mol/l NaOH).

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Stock solutions were diluted in PBS to allow a 1% volume addition of doxycycline to experimental wells. Control cells were treated with 1% volume of PBS. Antibody for p53 immunoblotting and electromobility supershift assay was purchased from Calbiochem (San Diego, CA, USA). Radioisotopes were purchased from Amersham Pharmacia Biotech Inc. (Piscataway, NJ, USA).

**Cell culture.** The human pancreatic tumor cell line PANC-1 was obtained from Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Seiryomachi 4-1, Sendai 980-8575, Japan). Cells were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum, sodium pyruvate, nonessential amino acids, and L-glutamine. The culture was incubated at 37°C in a 5% CO<sub>2</sub> humidified atmosphere.

**Cell proliferation.** PANC-1 cells were grown at a density of 10<sup>6</sup> cells/ml in six-well Coster plates in DMEM at 37°C for 6 days in a 95% O<sub>2</sub>/5% CO<sub>2</sub> incubator. Aliquots of cells and media were removed at 2-day intervals. Cells were treated with PBS in the absence or presence of 10-40 µg/ml doxycycline. Following treatment, cells were trypsinized and counted at each time-point.

**DNA fragmentation.** DNA fragmentation in apoptotic cells was determined by gel electrophoresis. PANC-1 cells (50×10<sup>6</sup>) were treated with doxycycline for 48 h, collected and washed with PBS, followed by lysing in extraction buffer (10 mM Tris, pH 8.0, 0.1 mM EDTA, 0.5% SDS and 20 µg/ml RNase) at 37°C for 1 h. Proteinase K (4 µM) was added, followed by incubation at 50°C for 3 h. DNA was extracted using phenol:chloroform and chloroform. The aqueous phase was precipitated with 2 volumes of 100% ethanol and 1/10 volume of 3 M sodium acetate on ice for 30 min, and then the DNA pellet was washed with 70% ethanol and resuspended in 50 µl Tris-EDTA buffer. Forty micrograms of DNA were subjected to electrophoresis on 0.8% agarose gel. The gel was stained with 50 µg/ml ethidium bromide for 30 min, and then photographed.

**Cell cycle analysis.** Subconfluent cells were treated with 0-40 µg/ml doses of doxycycline for 8, 12 and 24 h. Following treatment, cells were harvested and fixed with ice-cold 80% (v/v) ethanol for 24 h. After centrifugation at 200 × g for 5min, the cell pellet was washed with PBS (pH 7.4) and resuspended in PBS containing propidium iodide (50 µg/ml), Triton® X-100 (0.1%, v/v), 0.1% sodium citrate and DNase-free RNase (1 µg/ml), followed by incubation at room temperature for 1 h. The DNA content was determined by flow cytometry using a FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA).

**Northern blot analysis.** For Northern blot analysis, total RNA was extracted using TRIZOL Reagent (Life Technologies, Inc. Gaithersburg, MD, USA) according to the manufacturer's protocol. Fifteen µg of RNA were electrophoresed on a 1% denaturing formaldehyde agarose gel, transferred to a nylon membrane in the presence of 20 × SSC, and UV cross-linked. The blots were hybridized either with random primed radiolabeled human p53 or with p21 cDNA (16). The cDNA probes were labeled with [α-<sup>32</sup>P] deoxycytidine triphosphate using a random labeling kit (Roche, Indianapolis, IN, USA) and used for hybridization. Equal loading of mRNA samples was monitored by hybridizing the same membrane filter with the cDNA probe for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) as described by Hunt *et al.* (17).

**Ribonuclease protection assay.** PANC-1 cells were treated in the presence of doxycycline. Cells were scraped and total RNA was harvested using TRIZOL reagent. A custom made Riboquant™ Multiprobe RNase Protection Assay System (Pharmingen, San Diego, CA, USA). RPA was used to detect and quantify 16 different mRNA apoptotic species expression with 2 human template probe sets. hApo-2 and hApo-3c. (The RiboQuant Multi-probe protection assay system: Pharmingen, San Diego, CA, USA). The probe set hApo-2 includes 8 *Bcl-2*-related gene templates including *Bcl-xL*, *Bcl-xS*, *Bfl-1*, *Bik*, *Bak*, *Bax*, *Bcl-2* and *Mcl-1*. The hApo-3 probe set includes 8 proapoptotic and tumor necrosis factor family-related gene templates, namely, *caspase-8*, *FasL*, *Fas*, *FADD*, *DR3*, *FAP*, *FAF* and *TRAIL*. The <sup>32</sup>P-labeled riboprobes were hybridized for 18 h with 15 µg of total RNA. The hybridized RNA was digested with RNase, and remaining RNase-protected probes were purified and resolved on denaturing polyacrylamide gels. Equal loading of mRNA samples was monitored by the two housekeeping genes *L32* and *GAPDH*.

**Animal study.** Male nu/nu mice, 6-8 weeks old, were maintained in a specific-pathogen-free animal care facility according to institutional guidelines. Xenografts of tumors were established by subcutaneous (*s.c.*) injection of 1×10<sup>7</sup> cells (suspended in PBS) into the flank. After the tumors reached an approximate volume of 100 mm<sup>3</sup> (six weeks after injection), doxycycline-containing pellets (35 mg/pellet with a timed-release of 60 days; Innovative Research of America, Sarasota, FL, USA) (n=7) or placebo pellets (n=7) were implanted *s.c.* on the other side of the xenograft. The two longest tumor diameters were measured every week, and tumor volumes were calculated according to the formula  $V=ab^2/2$  (18). Seven weeks after treatment, all mice were sacrificed and xenografts were resected and measured. Differences in tumor growth were compared by the two-way repeated measures ANOVA, and comparisons of tumor weight between the groups were made using the two-tailed Student's *t*-test. A *p*-value of <0.05 is considered significant. All of the statistical analyses were performed using StatView 5.0 (Abacus Concepts Inc., Berkeley, CA, USA).

## Results

**Doxycycline effectively inhibits cell growth in PANC-1 cells.** The effects of doxycycline at increasing concentrations on cell proliferation in human pancreatic cancer cell line PANC-1 are shown in Figure 1A. The treatment with 10 µg/ml doxycycline failed to show an inhibitory effect on cell proliferation, whereas the treatment with 20 µg/ml doxycycline resulted in up to 15% cell death on day 3, followed by complete cell death on day 6. Moreover, 40 µg/ml doxycycline exerted more enhanced cytotoxicity, leading to complete cell death 4 days after treatment. Thus, in excess of 20 µg/ml, doxycycline exerted a cell-killing effect in PANC-1 cells in a dose-dependent manner.

**The effect of doxycycline on induction of apoptosis.** To test the hypothesis that antitumor effect of doxycycline is attributable to its ability to induce apoptosis, we treated PANC-1 cells with different doses of doxycycline for 48 h. Following incubation, cells were harvested and genomic DNA was prepared to determine DNA fragmentation. DNA gel electrophoresis revealed a typical pattern of DNA

fragment ladders among the cells treated in excess of 20  $\mu\text{g/ml}$  doxycycline (Figure 1B). These results suggest that the cytotoxic effect of doxycycline may be a consequence of apoptosis induction in PANC-1 cells.

**Doxycycline arrests cells in  $G_1$ -S phase of the cell cycle.** We investigated whether doxycycline interferes with the cell cycle as a leading phenomenon of the observed apoptosis. PANC-1 cells were treated with increasing doses of doxycycline for 8, 12, and 24 h, followed by cell cycle analysis using flow cytometry. As shown in Figure 2, cells treated with 20  $\mu\text{g/ml}$  doxycycline started to arrest in  $G_1$ -S phase at 24 h. For example, at 0 h, 28% of the cell population was in the  $G_1$ -phase, and by 8, 12, and 24 h after treatment, the  $G_1$  population accounted for approximately 28%, 30%, and 48%, respectively. In contrast, treatment with PBS alone (indicated as 0  $\mu\text{g/ml}$ ) or 10  $\mu\text{g/ml}$  doxycycline treatment did not change the size of the  $G_1$  population. The cells treated with 40  $\mu\text{g/ml}$  doxycycline exhibited  $G_1$ -S cell cycle arrest as early as 12 h after treatment, and these effects continued throughout the treatment periods. An increment in the sub- $G_1$  population with apoptosis induction was not observed in any of the treatment groups within 24 h. These results suggest that the doxycycline induces cell cycle arrest at the  $G_1$ -S phase.

**Doxycycline induces the expression of  $p53$  and its downstream target  $p21$  in PANC-1 cells.** The findings of the previous set of experiments suggested that doxycycline could induce cell cycle arrest and apoptosis in PANC-1 cells. One possible mechanism by which doxycycline participates in cell cycle arrest and apoptosis is the involvement of the tumor suppressor gene  $p53$ . The activation of  $p53$  probably causes cell cycle arrest through the transcriptional up-regulation of its downstream targets including  $p21$ , and/or a transcription-independent apoptosis (16, 19). Therefore, we investigated the effect of doxycycline on the levels of  $p53$  and  $p21$ . PANC-1 cells were treated with doxycycline for 48 h. Following treatment, cells were harvested and total RNA was prepared for Northern blot analysis. As shown in Figure 3A,  $p53$  mRNA level was induced after exposure of PANC-1 cells to doxycycline. This induction was observed at as low as 15  $\mu\text{g/ml}$  of doxycycline and increased at higher doses in a dose-dependent manner. Like  $p53$  mRNA up-regulation,  $p21$  mRNA was increased in excess of 15  $\mu\text{g/ml}$  doxycycline (Figure 3A). Up-regulation of  $p53$  mRNA appeared at as early as 24 h after exposure to 20  $\mu\text{g/ml}$  doxycycline (Figure 3B), in agreement with  $G_1$ -S arrest determined by FACS analysis. Furthermore, 40  $\mu\text{g/ml}$  doxycycline treatment resulted in earlier  $p53$  induction at 12 h (Figure 3B), followed by detectable  $p21$  mRNA induction at 24 h. These transcriptional activations of  $p53$  and  $p21$  genes support the assumption of doxycycline-mediated  $G_1$ -S cell cycle arrest and subsequent induction of apoptosis.

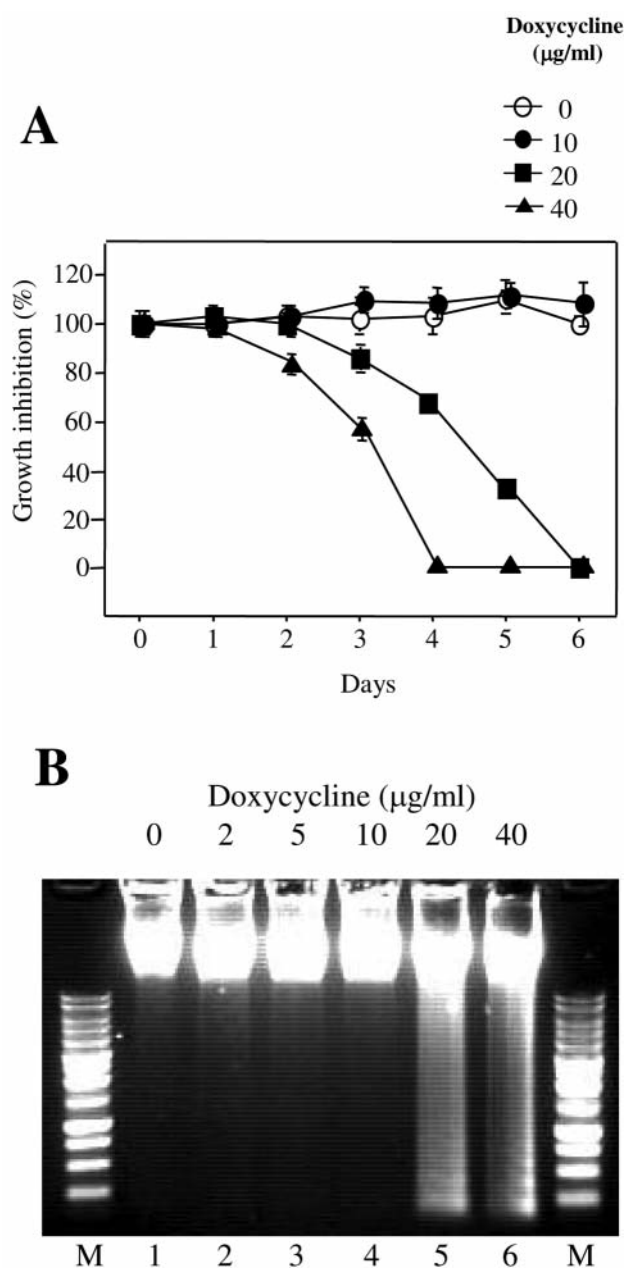


Figure 1. Growth inhibition by doxycycline in PANC-1 cells. A, PANC-1 cells were incubated with different concentrations of doxycycline. Cell growth was inhibited by doxycycline in a dose-dependent manner. Bars, SD. B, Demonstration of apoptosis by DNA fragmentation. Lane M, 1 kb DNA ladder; lane 1, DNA from control cells incubated with PBS (as shown 0  $\mu\text{g/ml}$ ) for 48 h; Lanes 2 to 6, DNA from cells cultured with increasing concentrations of doxycycline for 48 h. Note laddering in lanes 5 and 6, consistent with fragmentation of DNA.

**Doxycycline down-regulates antiapoptotic genes and induces proapoptotic genes.** Uncontrolled growth of cancer cells results from the inability of these cells to undergo cell cycle arrest and apoptosis. The phenomenon of apoptosis is regulated by

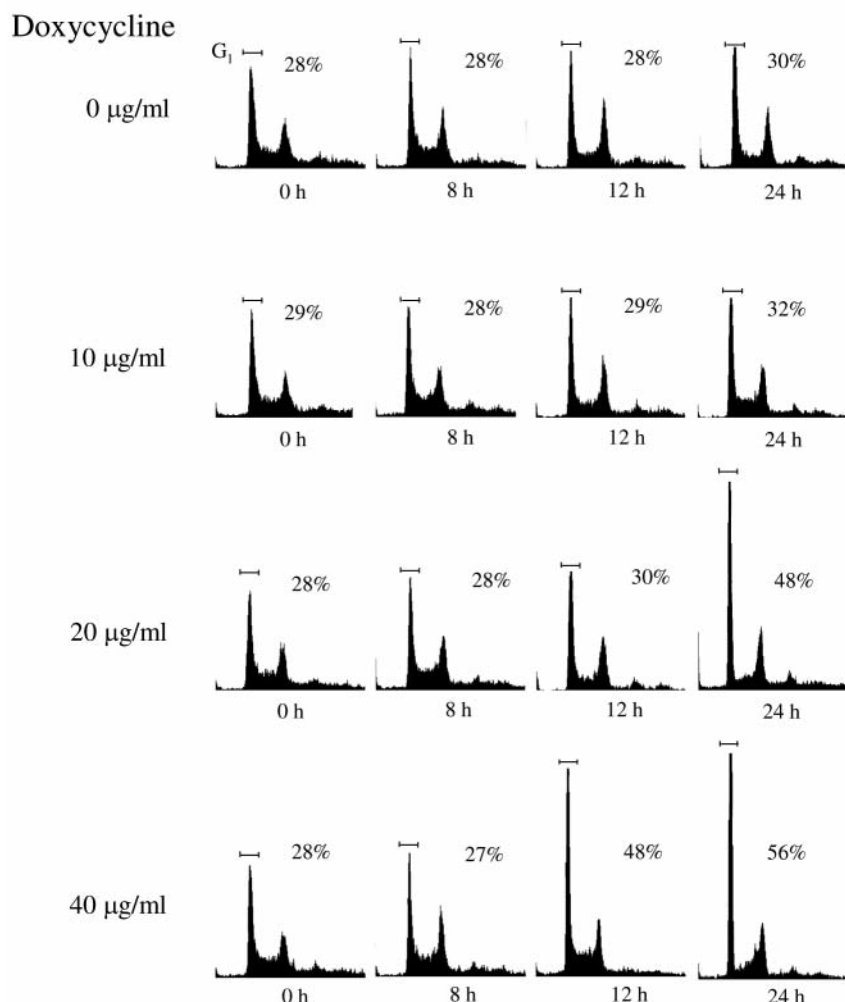


Figure 2. Doxycycline arrests cells at the  $G_1$ -S phase of the cell cycle. PANC-1 cells treated with different doses of doxycycline for 8, 12, and 24 h were evaluated by FACS analysis.

integration of both proapoptotic and apoptotic signals. Doxycycline has been reported to induce apoptosis in several cancer cell lines, but its mechanism remains largely unknown (1, 7, 20). Therefore, we investigated the effect of doxycycline on pro- and antiapoptotic gene regulation. As demonstrated in Figure 4A, doxycycline down-regulated the antiapoptotic genes including *Bcl-xL* and *Mcl-1* in a time- and dose-dependent manner. In contrast, 20 µg/ml doxycycline treatment significantly induced proapoptotic gene expression, including of *caspase-8*, *FasL*, *Fas*, *FADD*, *DR3*, *FAP*, *FAF* and *TRAIL* (Figure 4B). These results may raise the possibility that doxycycline enables cells to become highly susceptible to apoptosis by activation of proapoptotic genes and suppression of antiapoptotic genes.

**Doxycycline blocks TNF- $\alpha$ - and paclitaxel-mediated IL-8 induction.** In addition to its stimulatory effects on neutrophil migration, IL-8 has been shown to have a proangiogenic

property in tumor metastasis (21, 22). It is supposed that IL-8-mediated angiogenesis is enhanced by TNF- $\alpha$  (23, 24). Recent reports have shown that paclitaxel-resistant ovarian cancer cells overexpress IL-8, suggesting the role of this angiogenic factor in paclitaxel-resistance (25, 26). For these reasons, we determined endogenous and TNF- $\alpha$ - and paclitaxel-mediated IL-8 expression in the presence of doxycycline. Endogenous IL-8 mRNA expression was reduced by doxycycline treatment in a dose-dependent manner (Figure 5A). TNF- $\alpha$ -induced IL-8 mRNA expression peaked at 3 h (Figure 5B, lane 3), which was effectively blocked by 48 h of doxycycline-pretreatment (20 µg/ml, lane 7), resulting in delayed induction of IL-8 mRNA at 6 h (lane 8) in accordance with the elimination of doxycycline (27). The inhibition of TNF- $\alpha$ -induced IL-8 expression by doxycycline-pretreatment was observed at as low as 5 µg/ml concentration (Figure 5C, lane 3), with a dose-dependent augmentation of this effect (lanes 4 to 7). We also confirmed the



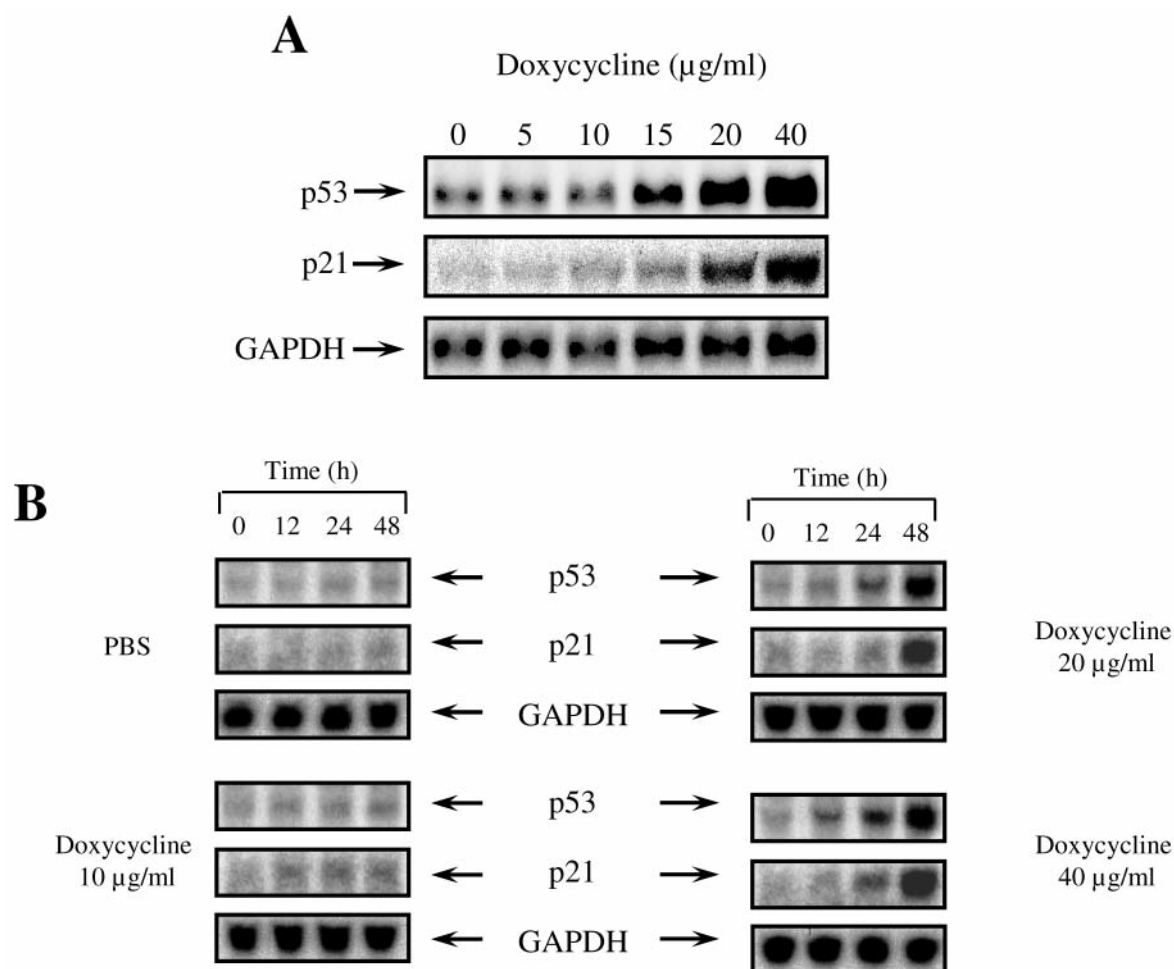


Figure 3. Increased expression of p53 and p21 mRNA by doxycycline in PANC-1 cells. **A**, Dose response: PANC-1 cells were incubated with increasing concentrations of doxycycline for 48 h. Following incubation, total RNA was prepared and Northern blot analysis for p53 and p21 was performed. GAPDH was used as a loading control. Both p53 mRNA and p21 mRNA were increased in excess of 15 µg/ml doxycycline. **B**, Time course and dose response: PANC-1 cells were cultured in the presence of no doxycycline (shown as PBS) or 10, 20 or 40 µg/ml doxycycline. Cells were harvested at each time-point, and total RNA was prepared for p53 and p21 Northern blot analyses. GAPDH was used as a loading control.

doxycycline effect on paclitaxel-mediated *IL-8* regulation. Paclitaxel induced *IL-8* expression in a time-dependent manner (Figure 5D, lanes 1-4), which was significantly blocked by doxycycline-pretreatment (lanes 6 to 8). Interestingly, 5 µg/ml doxycycline-pretreatment was sufficient to block paclitaxel-induced *IL-8* expression (Figure 5E, lane 3). These results suggest that doxycycline blocks TNF- $\alpha$ - and paclitaxel-induced *IL-8* expression.

**Antitumor effect of doxycycline in a mouse xenograft model.** Tumor growth was significantly suppressed in mice treated with doxycycline as compared to those treated with placebo pellet ( $p < 0.05$ , Figure 6C). Doxycycline treatment led to an overall reduction by up to 80% in tumor weight at the end of the experiment (Figure 6A, B and D).

## Discussion

Doxycycline has recently been shown to possess non-antibiotic properties including cytotoxic activity against several tumor types (7, 9, 12). Although the mechanism by which doxycycline exerts its apoptotic effect remains unclear (1, 7), it is proposed that doxycycline induces apoptosis through a *Fas/Fas-ligand* dependent pathway in Jurkat T lymphocytes (8). We demonstrated cytotoxic activity of doxycycline in excess of 20 µg/ml in PANC-1 cells. It is assumed that this activity of doxycycline is mediated by the induction of apoptosis, which was evidenced by DNA fragmentation. In the process of apoptotic cell death, two central pathways are known: activation of the caspase proteases and the mitochondrial pathway (28). Doxycycline induced both

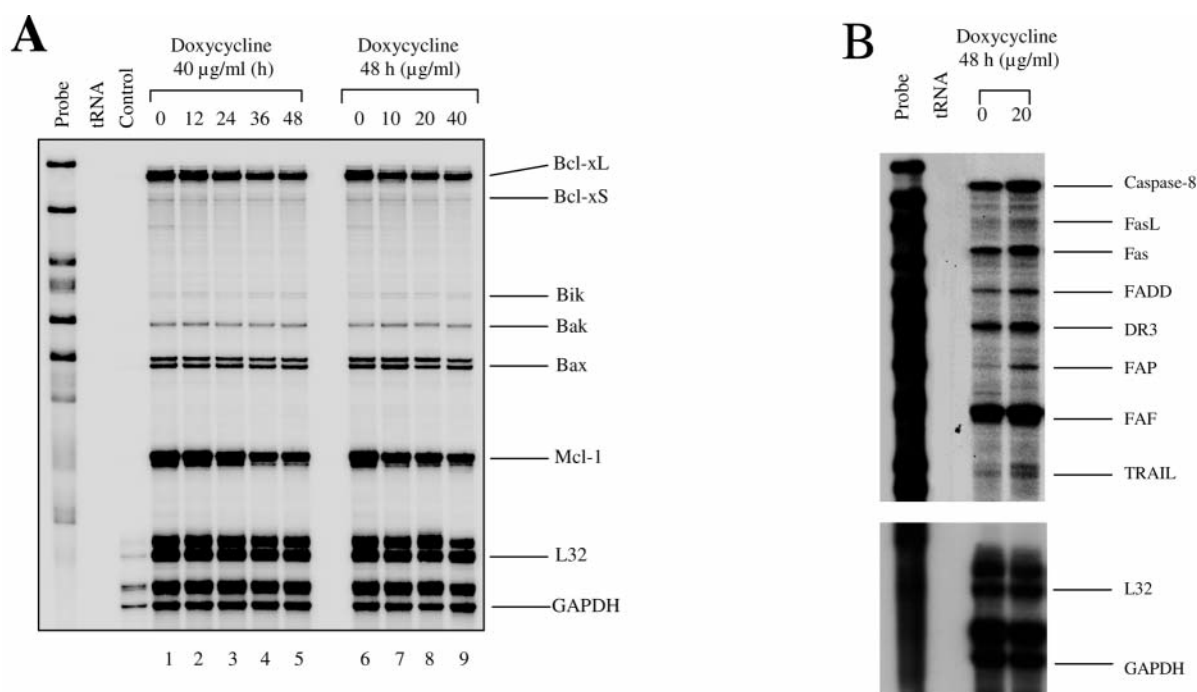


Figure 4. Ribonuclease Protection Assay. RNA samples were prepared from PANC-1 cells treated with 40 µg/ml doxycycline for the indicated times (A, lanes 1 to 5) and with increasing doses of doxycycline for 48 h (A, lanes 6 to 9). Ribonuclease Protection Assay was carried out using Riboquant™ Multiprobe RNase Protection Assay System with multiprobe template set, hAPO-2 (for A) and hAPO-3 (for B).

caspase activators such as *FADD* and members of caspase cascade including *FasL*, *Fas*, *DR3* and *caspase-8*. Regarding the mitochondrial pathway, doxycycline did not influence the level of proapoptotic factors *Bax* and *Bak*, but down-regulated antiapoptotic factors such as *Bcl-xL* and *Mcl-1*. These results suggest that activation of caspase proteases may be a major proapoptotic mechanism of doxycycline. It was previously shown that *caspase-8* and *-10* activity induced by doxycycline were responsible for induction of apoptosis in two other pancreatic cancer cell lines, T3M4 and GER (29). Our observations are consistent with these results. In the present study, we further investigated whether doxycycline inhibits growth of pancreatic cancer cells *in vivo*. Doxycycline successfully reduced tumor growth in this mouse xenograft model. This observation is the first report to show that apoptosis induction by doxycycline inhibited pancreatic cancer cell growth *in vivo*.

A variety of chemotherapeutic agents can cause some degree of DNA damage, resulting in a rapid up-regulation of *p53* protein levels by a posttranscriptional mechanism (30, 31). The outcome of the activation of *p53* is either apoptosis or cell cycle arrest, depending on the level of *p53* expression: *p53* at high levels promotes cell death by a transcriptionally independent mechanism, whereas lower levels initiate a transcriptionally controlled inhibition of cell cycle progress (32). We have previously reported that doxycycline-induced

apoptosis is a nuclear factor (NF)-κB- and *p53*-dependent in colon cancer cell line HCT116 (33). Similarly, simultaneous activation of *p53* and *p21* as well as cell cycle arrest were observed in PANC-1 cells, suggesting that a *p53*-dependent mechanism is involved in doxycycline-mediated apoptosis.

In viable cells, doxycycline is transferred into the cytoplasm and localizes in mitochondria, where it is metabolized to stable photoproducts, such as lumidoxycycline, by photodegradation, which induce mitochondrial fragmentation and alter mitochondrial membrane integrity, leading to continuous inhibition of mitochondrial protein synthesis (34). These effects of doxycycline on mitochondria are likely to depend on generation of excited-state singlet molecular oxygen ( $O_2(^1\Delta g)$ ) (35). Reactive oxygen species (ROS) and mitochondria play an important role in the induction of apoptosis under both physiological and pathological conditions. ROS induce many types of DNA damage, including single- and double-stranded DNA breaks, base and sugar modifications, DNA-protein crosslinks, and depurination and depyrimidination. This DNA damage is followed by activation of the tumor-suppressor gene *p53*, which transcriptionally regulates the expression of *Fas* on various cell types (35). Thus, ROS produced by doxycycline photoproducts could potentially be a mediator of doxycycline-mediated apoptosis.

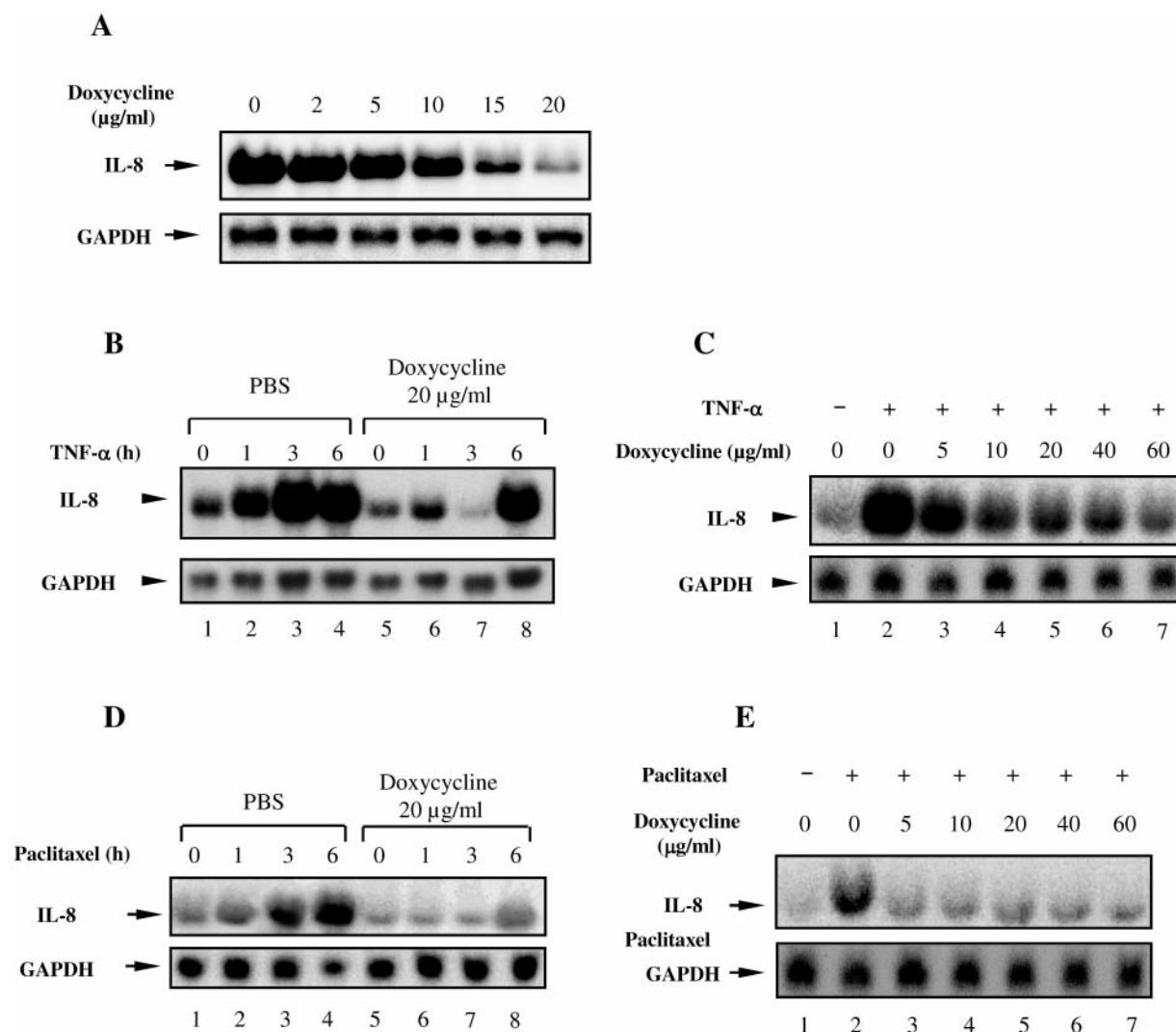


Figure 5. Doxycycline inhibits TNF- $\alpha$ - and paclitaxel-induced IL-8 mRNA expression in PANC-1 cells. A to E, Northern blots for IL-8 and GAPDH are shown. A, Endogenous IL-8 expression. PANC-1 cells were treated with increasing doses of doxycycline for 48 h. GAPDH was used to ensure approximately equal sample loading. B, PANC-1 cells were pretreated either with PBS or 20  $\mu$ g/ml doxycycline for 48 h, followed by stimulation with 20 ng/ml TNF- $\alpha$  for the indicated times. C, increasing doses of doxycycline treatment for 48 h was followed by stimulation with 20 ng/ml TNF- $\alpha$  for 3 h. D, Pretreatment either with PBS or 20  $\mu$ g/ml doxycycline was followed by stimulation with 30  $\mu$ M paclitaxel for the indicated periods. E, Increasing dose of doxycycline-pretreatment for 48 h was followed by stimulation with 30  $\mu$ M paclitaxel for 6 h.

Overexpression of chemokine/cytokine genes, including *IL-6*, *IL-8*, *monocytochemotactic protein 1 (MCP-1)* and *macrophage inflammatory protein 2 $\alpha$  (MIP-2 $\alpha$ )*, has previously been observed in a paclitaxel-resistant ovarian cancer cell line (25). Among them, *IL-6*, *IL-8* and *MCP-1* are induced by paclitaxel treatment in a paclitaxel-resistant phenotype, but not in parental cells, implying that constitutive and inducible expression of these molecules plays a crucial role in paclitaxel-resistance. In the present study, *IL-8* mRNA was inducible in PANC-1 cells after paclitaxel treatment. In contrast, both basal and paclitaxel-inducible levels of *IL-8* mRNA in doxycycline-pretreated cells were effectively

inhibited. Interestingly, inhibitory effects of doxycycline on *IL-8* synthesis for paclitaxel treatment could be achieved at a low dose (5  $\mu$ g/ml). Importantly, a daily oral dose of 800 mg, which is tolerated by most patients, can maintain serum levels of doxycycline between 5 and 10  $\mu$ g/ml. Although further investigation is required to determine whether doxycycline has synergetic properties with anticancer agents, our findings suggest that doxycycline could be used as an adjunct for the treatment of chemoresistant cancer cells.

In conclusion, doxycycline exhibited cytotoxic effects in pancreatic cancer PANC-1 cells and a mouse xenograft model. Doxycycline-mediated apoptosis seems to be involved

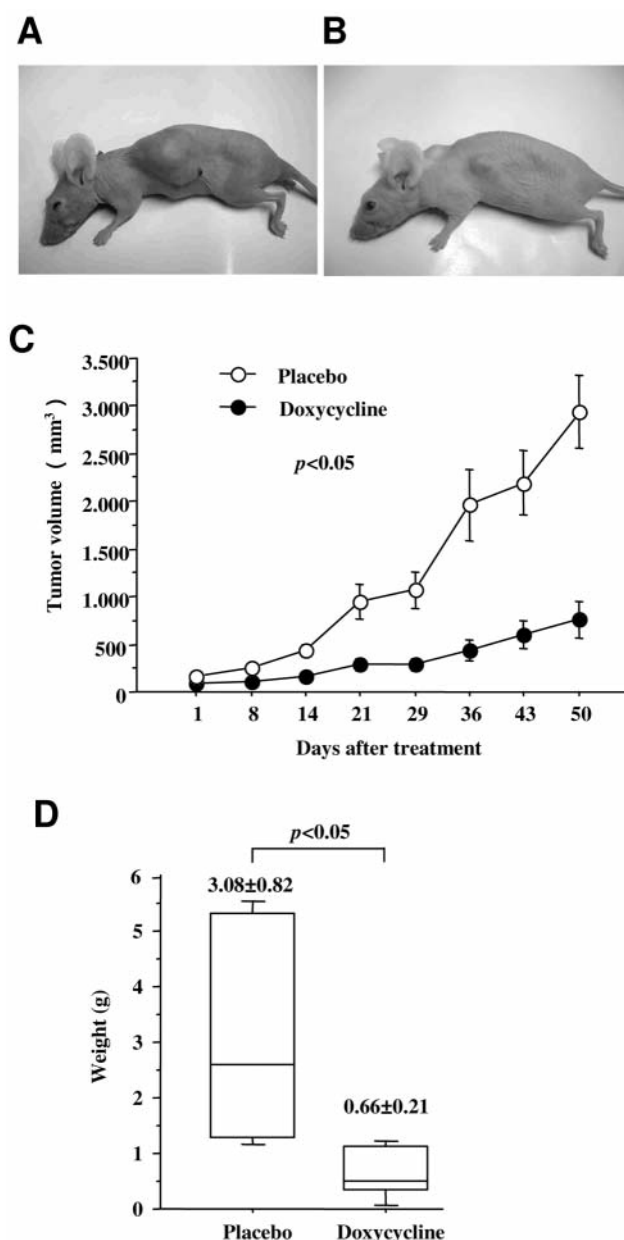


Figure 6. Effects of doxycycline on tumor growth in a nude mouse xenograft model. Overview of mice implanted with placebo (A) and doxycycline pellet (B) seven weeks after doxycycline therapy. C, Changes in tumor volume. D, Tumor xenograft weight at seven weeks after doxycycline therapy. There were significant differences in tumor volume and weight between the groups.

in several apoptotic signal pathways, including caspase activation and the *p53* signal pathways. Additionally, low-dose doxycycline blocked paclitaxel-induced *IL-8* expression. Further studies are warranted to determine the potential efficacy of doxycycline as an anticancer agent as well as an enhancer of conventional chemotherapeutic agents.

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